

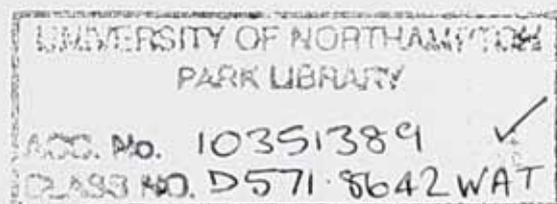


Plant-flower visitor interactions in the Sacred Valley of Perú

Submitted for the Degree of Doctor of Philosophy
at the University of Northampton

2008

Stella Watts



ABSTRACT

The structural organisation of species-rich plant-pollinator networks is important to understanding their ecology and evolution and is essential for making informed conservation and restoration decisions. This thesis reports on a study located at different altitudes in nine tributary valleys of the Sacred Valley, Vilcanota Highlands, Perú. The assemblages of flower visitors were described and the plant-flower visitor matrices were analysed and compared to those found from other montane systems. Additionally, the thesis also addressed how the European honeybee (*Apis mellifera*) fits into these communities.

Previous studies have predicted that abundance, diversity, and importance of hymenopterans as pollinators decrease with increasing altitude, where they are replaced by Lepidoptera and Diptera. Contrary to other temperate montane areas, Hymenoptera were more diverse at higher elevations. Diptera was the most abundant functional group overall but did not significantly increase in abundance with altitude as predicted. Species richness of visited plants reached a maximum at the highest altitudes.

Using ordination analysis, hummingbirds, honeybees, flies and beetles were identified as major functional groups of flower visitors with significantly different visitation profiles. Nestedness analysis revealed that the plant-flower visitor networks had a similar structure to other published networks, consisting of core groups of generalist plants and animals which interacted with one another and with specialised flower visitors and plants, respectively. The core species varied in identity between valleys, but were usually the species in greatest abundance, implying that the networks were abundance structured. In addition, 85% of the interactions were observed only in single valleys. This context specificity may have implications for the conservation of plant-pollinator interactions in the Sacred Valley.

Comparisons of the pollinator efficiencies of honeybees, hummingbirds, native bees and moths to *Duranta mandonii* (Verbenaceae) demonstrated significant variation among flower visitors in rates of visitation, pollen removal ability and contribution to fruit set. This variation was not correlated: hummingbirds were by far the most frequent visitors but removed virtually no pollen and did not contribute to fruit set. Despite the taxonomic diversity of flower visitors, the main pollinators were large native bumblebees and honeybees. Results highlighted the importance of measuring efficiency components when documenting plant-pollinator interactions, and also demonstrated that visitation rates may give little insight into the relative importance of flower visitors.

Overall, the study showed that *Apis* was the most generalist flower visitor and a dominant core species within networks. However, although *Apis* visited a relatively large proportion of the flora compared to native taxa, they only intensively utilised a small proportion of available plant species. No evidence was found from the surveys to suggest that honeybees used interference competition and displaced other species. It was suggested that because specialised rare species are frequently dependent on a core of generalist taxa honeybees may play an important role for the possibilities of rare species to persist. However, perhaps the greatest threat to biodiversity and the persistence of plant-flower visitor communities in the Sacred Valley is from the destruction and fragmentation of habitats and from facilitative interactions between native and alien plants, mediated through visitation from honeybees.

For my late Mother and Father who gave me a love of natural history; particularly my Father who even in his final year, still selflessly encouraged me to return to my beloved Perú to fulfil my dreams.

Also for Jose Oh in Belize who inspired me to take this journey by introducing me to flora and fauna of this amazing country, where I observed my first hummingbird and became addicted!

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to my supervisor, Jeff Ollerton for his support, guidance and encouragement throughout the six years; for always being available, especially through the latter stages and for useful discussions and suggestions. I also thank Janet Jackson who came aboard the supervisory team during the last three years and was able to provide many useful suggestions from a landscape perspective. Thanks are also due to my external advisor Nick Waser who made invaluable comments regarding the *Duranta mandonii* chapter. Thanks also to Mary Price and Nick Waser for interesting discussions regarding hummingbird pollination.

In Perú I would also like to express my sincere gratitude to the staff and undergraduates from Universidad Nacional de San Antonio Abad del Cusco, Perú for making me welcome. Without their support and the collaboration of the National Institute of Natural Resources (INRENA) I would not have been able to complete this research. I thank Alfredo Tupayachi and Natividad Raurau (Herbario Vargas) for plant identification and Eric Yabar, Orlinto Aguilar, Claus Rasmussen, Barry Walker, Javier Llaxacondor and the Asociación de Apicultura de Urubamba for sharing information. I am greatly indebted to my field assistants: Federico Argandoña, Carmen Aparicio, Celia Zuñiga, Carlos Calvo, Rossemeri Cuéllar, Juan Flores, David Huamán, Marcela Herrera, Ramon Ipanaque, Karin Nuñez, Barioska Quispe, Jeff Ollerton, Fatty Ramirez, Javier Saldivar and Adrian Stott. I thank my cook Rosa Duran and Helbert Duran for logistical support. Permission to undertake fieldwork was granted by the Director of the National Institute of Natural Resources (INRENA) - Permit numbers: 008799 and 0001982. I would also like to extend my appreciation to the communities of the Sacred Valley for permission to undertake fieldwork on their land.

Beyond the Academic world I would like to thank my dear sisters Alison and Vanessa and their families for unconditional support, particularly through testing times. Special thanks are also due to my friends Gary Knibbs and Stephen Humphreys for their encouragement and belief in me, particularly for supporting me through the final part of my PhD work. Without their continued moral and financial support throughout the six years, I would not have been able to complete this research. I would also like to thank my friends

Michael Dawson and Max Effrem for support and funding in the early years. I also thank my friends Azucena, Nell, Ruth, Clive, Graham, David, Max, Lucy, Sofia, Helbert, Molly and Jane for encouraging me throughout the duration of this project.

I am grateful to the staff and students from the School of Applied Sciences, University of Northampton who have provided encouragement and support over the years. Thanks go to Duncan McCollin, Ian Livingstone, Paul Phillips and David Wilson. I appreciate the moral support of fellow members of the Landscape and Biodiversity Group, Lizzie Cant, Paul Clarkson, Louise Cramer, Lutfor Rahman, Adrian Stott and Sam Tarrant. I am also grateful to Adam Killick for counting pollen grains. I also wish to extend my thanks to many SCAPE colleagues, in particular, Scott Armbruster, Anke Dietzsch, Carl Duffy, Bente Ericksen, Jose Gómez, Roosa Leimu, Tommy Lennartsson, Marcos Méndez, Anders Nielsen, Miguel Rodriguez-Gironés, Martina Stang and Ørjan Totland for fruitful discussions.

Finally, special thanks are due to Petr Šmilauer, University of South Bohemia, Czech Republic, for providing invaluable help with statistical analysis using Canoco, and to Robin Someš at Pisces Conservation Limited.

This PhD was self-funded with the financial support of The British Ecological Society, Idea Wild, The Biodiversity Trust, The Anglo Peruvian Society and The Leslie Church Bursary Fund.

TABLE OF CONTENTS

Chapter One: Introduction	1
Chapter breakdown with aims and objectives	3
Early pollination studies	7
Patterns in species diversity along an altitudinal gradient	8
The effects of the introduced European honeybee (<i>Apis mellifera</i>) on native ecosystems	15
Structure in pollination webs	28
Characterising generalisation and specialisation	34
Biodiversity and conservation status of the study region	37
Study sites	47
Chapter Two: Plant-flower interactions in the Sacred Valley: patterns of diversity and abundances along an elevational gradient	60
Introduction	60
Methods	66
Results	72
Discussion	95
Conclusions	112
Chapter 3: Structure in plant-flower interactions assemblages: linking functional groups of visitors and plants using Canonical Correspondence Analysis	114
Introduction	114
Methods	117
Results	124
Discussion	143
Summary	152
Chapter 4: Structure of a plant-flower network in the Sacred Valley Perú	153
Introduction	153
Methods	157
Results	159
Discussion	194

Summary	214
Implications for conservation	215
Chapter 5: Pollinator effectiveness of native and non-native flower visitors to an Andean shrub, <i>Duranta mandonii</i> (Verbenaceae)	216
Introduction	216
Materials and Methods	218
Results	223
Discussion	233
Conclusions	236
Chapter 6: Conclusions	237
Conservation implications	251
Opportunities for future work	257
References	260
Appendices	279
Appendix I	279
Appendix II	282
Appendix III	285
Appendix IV	286

LIST OF TABLES AND FIGURES

Chapter One: Introduction	1
Table 1.1 Studies focusing directly or indirectly on the impacts of honeybees on native flora and fauna	17
Table 1.2 Definitions for different concepts of specialisation and generalisation proposed in the literature	36
Table 1.3 Number of plant and vertebrate species and endemism, and percentage of global totals for the 25 leading biodiversity hotspots	37
Table 1.4 Holdridge Life Zones sampled in this present study, with descriptions of the vegetation and prominent plant species	40
Table 1.5 Threatened plant and bird species identified in the study region	46
Chapter Two: Plant-flower interactions in the Sacred Valley: patterns of diversity and abundances along an elevational gradient	60
Table 2.1 Total number of flower visitors in each of the nine valleys	75
Table 2.2 Results of a randomization test using a Shannon -Wiener index with 10 000 random partitions	78
Table 2.3 Results of a randomization test using a Shannon -Wiener index with 10 000 random partitions	80
Table 2.4. Results of a randomization test using a Shannon -Wiener index with 10 000 random partitions	84
Table 2.5 Visited plant species richness per altitudinal band	85
Table 2.6 Kruskal Wallis analysis of variance of differences in median number of individuals of flower visitor taxa per altitudinal band in all nine valleys	86
Table 2.7 Spearman's rank correlations of abundances of <i>Apis</i> versus each functional group, (1-5) in all valleys	89
Table 2.8 Pearson and Spearman's rank correlations of rank abundances of <i>Apis mellifera</i> versus each functional group analysed separately for individual altitudes	89
Table 2.9 <i>G</i> – test for differences in proportion of plant species visited by <i>Apis mellifera</i> versus each taxon across altitudinal bands 1-5. Numbers are percentages	91
Table 2.10 Ranking for the three most important plant species visited by honeybees, bumblebees and native bees	93

Table 2.11 Observations and outcomes of encounters between <i>Apis</i> and other flower visiting taxa	94
Chapter 3: Structure in plant-flower interactions assemblages: linking functional groups of visitors and plants using Canonical Correspondence Analysis	114
Table 3.2 Eigen values, gradient length for all four axes of ordinations by detrended correspondence analysis (DCA)	125
Table 3.3 Summary of results of a constrained ordination by canonical correspondence analysis (CCA) of the plant flower visitor data	129
Table 3.4 Partial CCA to test the significance of the second ordination axis	130
Table 3.5 Partial CCA to test the significance of the third ordination axis	130
Table 3.6 Partial CCA to test the significance of the fourth ordination axis	131
Table 3.7 Summary of Canonical correspondence analyses selecting functional groups of pollinators as the only environmental variables	132
Table 3.8 Canonical correspondence analyses selecting functional groups of pollinators as the only environmental variables	134
Table 3.9 Summary of canonical correspondence analyses selecting functional groups of pollinators as the only environmental variables and valleys as covariables	139
Table 3.10 Canonical correspondence analyses selecting functional groups of pollinators as the only environmental variables and valleys as the covariables	141
Table 3.11 Summary of Canonical correspondence analyses selecting <i>Apis</i> as the only environmental variable	142
Chapter 4: Structure of a plant-flower network in the Sacred Valley Perú	153
Table 4.1 Comparison of network characteristics for the nine plant-flower visitor communities of the Sacred Valley	161
Table 4.2 Quantitative measures of the nine plant-flower visitor communities of the Sacred Valley	162
Table 4.3 Comparisons of network characteristics for the nine plant-flower visitor communities using pooled data across five altitudinal zones	163
Table 4.4 Rankings of core species of plants according to 10% of the relative linkage (l_n) scores	184
Table 4.5 Rankings of core species of flower visitors according to 10% of the relative linkage (l_m) scores.	185

Table 4.6 Rankings of core species of plants according to 10% of the relative linkage (l_n) scores. Data pooled data across five altitudinal zones	188
Table 4.7 Rankings of core species of flower visitors according to 10% of the relative linkage (l_m) scores. Data pooled data across five altitudinal zones	189
Table 4.8 Summary statistics of published sub-alpine and alpine pollination networks	196
Table 4.9 Plant species visited by floral larcenists	201
Chapter 5: Pollinator effectiveness of native and non-native flower visitors to an Andean shrub, <i>Duranta mandonii</i> (Verbenaceae)	216
Table 5.1 Fruit set levels in flowers of <i>Duranta mandonii</i> following treatments	227
Table 5.2 Number of fruits set for each treatment in flowers of <i>D. mandonii</i>	229
Table 5.3 Pollinator efficiency rankings across the different plant fitness components measured for each taxon visiting <i>D. mandonii</i>	230
Table 5.4 Pearson and Spearman Rank* correlations of visitation rate between <i>Apis mellifera</i> and other taxon	231
Table 5.5 Observations and outcomes of encounters between <i>Apis mellifera</i> and other flower visiting taxa	232
Chapter 6: Conclusions	237
Table 6.1 Some of the most serious threats to plant-flower visitor communities and biodiversity in the Sacred Valley	255
Chapter One: Introduction	1
Figure 1.1 Potential effects of honeybee foraging on native flora and fauna, and some of the possible mechanisms by which they may disrupt native plant-pollinator interactions	16
Figure 1.2 Main structural features of plant-animal interaction matrices	29
Figure 1.3 Map showing the location of Cordillera de Vilcanota Perú.	38
Figure 1.4 Typical habitats encountered in the study area	41
Figure 1.7 Schematic diagram representing the characteristics of each of the nine tributary valleys in terms of different habitats encountered along an elevational gradient from 2900-4100m	50
Figure 1.8 Left hand side of Chicon showing pine plantations	51

Figure 1.9 Views of Chicon showing transect route in red	52
Figure 1.10 View of Mantanay from the valley floor near the village of Yanahuara	53
Figure 1.11 View of Mantanay from 3500m showing areas of landslide activity	53
Figure 1.12 Pumamarca	54
Figure 1.13 Choquebamba	55
Figure 1.14 Poques	55
Figure 1.15 Aerial view of Pumamarca and Choquebamba showing cultivated Inca terraces, the Inca fortress of Pumamarca and <i>Eucalyptus</i> plantations	56
Figure 1.16 View of Tiaparo and Piscacucho	57
Figure 1.17 View of Tiaparo and Piscacucho	58
Figure 1.18 Views of Tiaparo showing in red the route for transect 3 at approximately 3100-3200m	58
Figure 1.19 Transect 1 at Tiaparo and marking transect 5 in elfin forest at 3752m	58
Chapter Two: Plant-flower interactions in the Sacred Valley: patterns of diversity and abundances along an elevational gradient	60
Figure 2.1 Species richness of different functional groups of flower visitors. <i>Apis</i> and Vespidae were grouped with Hymenoptera	74
Figure 2.2 Proportions of different functional groups of flower visitors and plants species visited in the Sacred Valley	74
Figure 2.3 Plot of heterogeneity for species and morphospecies of visitors observed across nine valleys	76
Figure 2.4 Plot of heterogeneity for visited plant species observed across valleys	76
Figure 2.5 Shannon-Weiner's H' diversity indices for species of flower visitors recorded in each valley	78
Figure 2.6 Shannon-Weiner H' diversity indices for those plant species visited by flower visitors in each valley	80
Figure 2.7 Shannon-Weiner H' diversity indices for all flower visitor pooled and each functional group of flower visitors	83
Figure 2.8 Mean number of individuals (\pm SD) per transect in each altitudinal band	87

Figure 2.9 Proportion of plant species visited by each taxon per altitude	92
Chapter 3: Structure in plant-flower interactions assemblages: linking functional groups of visitors and plants using Canonical Correspondence Analysis	114
Figure 3.1A Scatter plot based on detrended correspondence analysis (DCA) of plant species according to their flower visitor profiles displaying the major variation in species composition in the Sacred Valley	126
Figure 3.1 B DCA scatter plot of all the environmental variables. Qualitative variables altitude and valleys were transformed into binary dummy variables and were plotted as centroids into the ordination diagram	127
Figure 3.2 Species-environment biplot diagram of plants and functional groups of flower visitors based on the first two axes of canonical correspondence analysis	133
Figure 3.3 Species-environment biplot diagram of plants and functional groups of flower visitors based on axes two and three of canonical correspondence analysis	136
Figure 3.4 Species-environment biplot diagram of plants and functional groups of flower visitors based on axis three and four of canonical correspondence analysis	137
Figure 3.5 Ordination diagram of plant species according to their flower visitor profiles based on the two first axes of a partial canonical analysis	140
Chapter 4: Structure of a plant-flower network in the Sacred Valley Perú	154
Figure 4.1 Presence /absence matrices showing low, high, and a maximally packed matrix of a perfectly nested system	155
Figure 4.2 The maximally packed plant-flower matrix for Huaran	165
Figure 4.3 The maximally packed plant-flower matrix for the valley of Yanacocha	167
Figure 4.4 The maximally packed plant-flower matrix for the valley of Chicon	169
Figure 4.5 The maximally packed plant-flower matrix for the valley of Mantamay	171
Figure 4.6 The maximally packed plant-flower matrix for the valley of Pumamarca	173
Figure 4.7 The maximally packed plant-flower matrix for the valley of Choquebamba	174
Figure 4.8 The maximally packed plant-flower matrix for the valley of Poques	176
Figure 4.9 The maximally packed plant-flower matrix for the valley of Tiaparo	177
Figure 4.10 The maximally packed plant-flower matrix for the valley of Piscacucho	179

Figure 4.11 Mean \pm SD linkage level (l_n) scores for plant species and flower visitors (l_m) for each valley	181
Figure 4.12 Number of plant interactions recorded in each valley for <i>Apis</i> and <i>Bombus funebris</i> and <i>Bombus melaleceus</i> combined	181
Figure 4.13 Network characteristics for each valley	186
Figure 4.14 Core hummingbird species	190
Figure 4.15 Morphology of core species <i>Baccharis salicifolia</i>	191
Figure 4.16 Average number of visits to plant species with open access, tubular, bell, flag and open tube morphology	192
Figure 4.17 Average number of visitor species to plant species with open access, tubular, bell, flag and open tube morphology	193
Chapter 5: Pollinator effectiveness of native and non-native flower visitors to an Andean shrub, <i>Duranta mandonii</i> (Verbenaceae)	216
Figure 5.1 Nectar volume, concentration and sugar content for flowers of <i>Duranta mandonii</i>	224
Figure 5.2 Flower visitation rate expressed as mean percentage of visits per minute for thirteen census periods	225
Figure 5.3 Mean percentage of visits (\pm SD) per minute for each taxon	226
Figure 5.4 Mean (\pm SD) number of pollen grains remaining from <i>Duranta mandonii</i> anthers following single visits to flowers	228
Figure 5.5 Average number of seeds set in flowers of <i>Duranta mandonii</i>	229

CHAPTER ONE

Introduction

Plant-pollinator interactions have a pervasive influence on the dynamics of communities and play a fundamental role in maintaining biodiversity and ecosystem functioning (Constanza *et al.* 1997; Kearns and Inouye 1997; Kremen *et al.* 2002). In the majority of terrestrial ecosystems biotic pollination is a ubiquitous ecological interaction. Pollination by insects and other arthropods is a requirement of more than 90% of angiosperms (Ollerton 1999; Memmott *et al.* 2004; Ollerton *et al.* 2006a). Bees, butterflies, moths, wasps, beetles and other invertebrates are critically important to the reproduction and survival of many cultivated and wild plants species (Allen-Wardell *et al.* 1998). The Hymenoptera, which include an estimated 20,000 named bee species worldwide, are the most important pollinators of angiosperms both in terms of the behavioural adaptations shown by some groups and in terms of the large proportion which act as flower visitors (Roubik 1989; Proctor *et al.* 1996; Ollerton 1999). Moreover, bees are unique in that they rely almost entirely on floral resources throughout their life cycle (Minkley and Roulston 2006). On the whole, the insect order Lepidoptera has the closest association with flowering plants; the larvae of moths and butterflies feed on the leaves, stems and flower heads, the adults are dependent upon nectar and pollen and thus are important pollinators of a wide range of plant species (Ollerton 1999). Diptera (the two winged flies) are often considered opportunistic, inefficient flowers visitors, unlikely to transfer pollen between conspecific plants but their importance as pollinators is under-appreciated (Kearns 1992; Kearns and Inouye 1994; Ollerton 1999). The Coleoptera is the largest insect order, with 350,000 named species (one fifth of all known life forms – (Morell 1999). Although beetles can be inactive and infrequent visitors to flowers in cool temperate climates, some temperate and tropical plants are mainly or exclusively beetle pollinated (Ollerton 1999).

Without a doubt, insects are the most important animal pollinators, but also of significance is pollination by vertebrates such as birds, bats and non-flying mammals (Proctor *et al.* 1996). Of these groups, birds play a significant role in plant reproduction in

many parts of the world, particularly in tropical and subtropical habitats (Proctor *et al.* 1996). In the Neotropics, hummingbirds pollinate a large proportion of plants, and in southern Australia, nectarivorous birds (honeyeaters) are prominent pollinators (Stiles 1975; Feinsinger and Colwell 1978; Feinsinger *et al.* 1987; Paton 2000). Although many studies have focused on hummingbirds, sunbirds and honey creepers, certain plant species rely exclusively on passerine birds (perching birds and parrots) for cross pollination (Allen-Wardell *et al.* 1998). Also noteworthy are large flying foxes, which are considered keystone pollinators for a growing number of plant species in Samoa (Cox and Elmqvist 2000).

Ecosystem services provide significant benefits to humankind and the plant-pollinator route is a highly important means for the flow of energy within terrestrial ecosystems (Ollerton 1999; Kremen *et al.* 2002). For example, animals (bees in particular) are directly or indirectly essential pollinators for 15-30% of food production, and pollination services are worth an estimated \$US 114 x 10⁹ per year world wide (Constanza *et al.* 1997). Furthermore, in the United States alone, crop pollination by the honeybee *Apis mellifera* is estimated to be worth 5-14 billion dollars a year (Kremen *et al.* 2002). The destruction and fragmentation of natural habitats through agricultural intensification has led to a reported decline in pollinator diversity, raising fears of a major global "pollination crisis" (Allen-Wardell *et al.* 1998; Cox and Elmqvist 2000; Kremen and Ricketts 2000; Paton 2000; Roubik 2000; Memmott and Waser 2002; Traveset and Richardson 2006). Major forces to the disruption of natural biotic pollination processes also include pesticide and herbicide use, and the introduction of non-native honeybees and other invasive species resulting in increased extinction of local populations and entire species (Allen-Wardell *et al.* 1998; Kremen and Ricketts 2000; Paton 2000; Roubik 2000). However, Ghazoul (2005) suggested that perceptions regarding such declines are based largely on reports of a reduction in crop-pollinating honeybees in North America, and bumblebees and butterflies in Europe, but elsewhere native pollinator communities showed mixed responses to environmental change.

Chapter breakdown with aims and objectives of the thesis

Recently a revival of community studies has flourished, treating the whole plant-pollinator community as a network of interactions (Waser and Ollerton 2006). Large scale community studies of plant-pollinator interactions along an altitudinal gradient are non-existent in tropical America and still rare in South America as a whole. Since the structural organisation of species-rich plant-pollinator networks is important to understanding their ecology and evolution and is essential for making informed conservation and restoration decisions, this research will set out to characterise the architecture of the plant-flower visitor networks of the Sacred Valley. The region is recognised as a global hotspot for diversity (Myers *et al.* 2000), the combination of diverse, endemic flora and fauna and the existence of honeybees in this valley system means that this area offers an excellent opportunity to study plant-pollinator interactions.

The overall objective of this research was to describe the assemblages of flower visitors, to identify the structural properties of the plant-flower visitor matrices and consider possible implications for the conservation of plant-pollinator interactions in the Sacred Valley. Additionally, the thesis also addressed how the European honeybee (*Apis mellifera*) fits into these communities.

A brief summary of some of the research questions and hypotheses generated are included in the following chapter descriptions:

Chapter 1 - Introduction -: provides historical summaries of some important pollination studies, an overview of themes in the literature relevant to the project, background information on the biodiversity of the study region and site descriptions.

- Diversity of pollinators and their role in maintaining biodiversity and ecosystem functioning
- The historical background of important pollination studies
- Community wide patterns in plant-pollinator interactions
- The effects of the introduced European honeybee (*Apis mellifera*) on native ecosystems
- Structure in pollination webs

- Characterising generalisation and specialisation
- Study region and conservation status
- Background information on the Andean flora and fauna
- Site descriptions and maps

Chapter 2: examines community wide patterns in plant-flower visitor interactions over an altitudinal range of 1106m in nine tributary valleys of the Sacred Valley of Perú. The main aims are to examine the effects of altitude on the composition of visitor assemblages and how species diversity and abundance of flower visitors differs among valleys. Previous studies have predicted that abundance, diversity, and importance of hymenopterans as pollinators decrease with increasing altitude, where they are replaced by Lepidoptera and Diptera. The chapter addresses the following questions:

1. Does species diversity of functional groups of flower visitors differ among valleys?
2. Does species diversity of visited plants differ among valleys?
3. How does the flower visitor activity within the Sacred Valley compare with other high altitude montane and alpine systems?
4. What role do honeybees play in these communities?

In addition, the following predictions were generated and tested to explore these questions:

- Prediction 1: Diversity of functional groups of flower visitors will decline with altitude
- Prediction 2: Species richness of visited plants will decline with altitude
- Prediction 3: The abundance of different functional groups of flower visitors will vary predictably with altitude
- Prediction 4: Native bees decline proportionately in the presence of *Apis*
- Prediction 5: The proportion of plant species utilised by *Apis* compared to native flower visitors will vary with altitude
- Prediction 6: The proportion of plant species visited by both native bees and honeybees will overlap by less than a third
- Prediction 7: *Apis* actively displaces other species when foraging

Chapter 3: Continuing the theme of structure and pattern in the plant-flower visitor assemblages, this chapter aims to probe for structural patterns of plant-pollinator interactions by using multivariate analysis as a complement to nestedness analysis. Multivariate analysis of visitation data was performed by using the program CANOCO 4.5. The following questions are addressed:

1. Is there a difference in the foraging profiles between functional groups of flower visitors?
2. Is there a difference in the foraging profiles between functional groups of flower visitors once the compositional variability explained by valleys is removed?
3. Is there a difference in foraging profiles between *Apis* and other functional groups of flower visitors?

Chapter 4: focuses in more detail on the structure of these interaction webs for each of the nine communities. The main aims of this chapter are:

1. To examine the assemblages for indication of nested patterns and identify the possible processes that might generate these features
2. To explore the topological features and patterns of specialisation and generalisation and to determine whether the patterns are consistent across valleys
3. To determine which species and functional groups of plants and flower visitors dominate the network core, and whether their positions are constant across the nine valleys
4. To examine if there is a correlation between relative abundance of a given species and its degree of generalisation within each network
5. To establish where *Apis* fits into this network of interactions and whether this position is constant across the nine valleys

Chapter 5: focuses on a single species and pollinator effectiveness of native and non-native flower visitors to an Andean shrub, *Duranta mandonii* (Verbenaceae). The following questions are addressed:

1. Which native flower visitors of *Duranta mandonii* are the most efficient in terms of visitation rate, pollen deposition and pollen removal?
2. Are honeybees as efficient as the native pollinators of *D. mandonii*?

3. Is there evidence that honeybees compete with native flower visitors for floral resources, to the detriment of the native fauna?
4. Does the interaction between *D. mandonii* and the introduced *A. mellifera* have a negative effect on the reproductive success of the plant?

Chapter 6: consolidates and summarises the findings from the entire investigation, making recommendations for sustaining or restoring these plant-flower visitor communities and also discusses the limitations of this study and the possible areas for future study.

Early pollination studies

The scientific study of pollination of flowers by animals was first recognised by Professor Joseph Gottlieb Kölreuter (1733-1806). Kölreuter produced a series of publications on plant sexuality between 1761 and 1766, beginning with "*Voläufige Nachricht*" (Proctor *et al.* 1996; Waser 2006). These works involved hybridisation experiments and systematic observations at single plants. Kölreuter's studies on the plant families Cucurbitaceae, Malvaceae and Iridaceae showed that insect visits were necessary for successful pollination, suggesting that this important service probably applied to the majority of plants (Proctor *et al.* 1996). Following on from Kölreuter's works in 1793, the founder of the systematic study of plant-insect interactions, Christian Konrad Sprengel (1793) concluded that many flowers are fertilised by multiple species of insects (Proctor *et al.* 1996; Waser 2006). Sprengel's conclusions focused far more on floral characteristics than insects, involving a few hundred native species in mostly natural conditions, whereas Kölreuter adopted a more experimental approach on a comparatively smaller number of plant species (Waser 2006).

Later important developments to the study of pollination came from Charles Darwin (1809-1882) which included a monograph on the fertilisation of Orchids, observations on the pollination mechanism in *Primula* flowers (1862) and the classic *Origin of Species* (1859) (Proctor *et al.* 1996). Although Darwin introduced the concept of adaptation by natural selection and placed the study of pollination in its modern evolutionary framework, the major contributions are credited to Hermann Müller and Paul Knuth (Waser 2006). In 1873, Müller published a compendium with details of flower and pollinator characteristics for 400 plant species, whilst Knuth published a *Handbook of Flower Pollination* in three volumes between 1898 and 1905, which included observations of flower visitation to 6385 species of plants worldwide (Waser 2006). Other contributions for the classifications of flowers during the nineteenth century included a comprehensive textbook on floral biology in 1867 by Friedrich Hildebrand (Proctor *et al.* 1996). More influential was the scheme introduced by the Italian botanist Federico Delpino (1868-1875), who also provided detailed records of pollination, including bird pollination (Proctor *et al.* 1996; Waser 2006). Delpino devised two distinct classification schemes for categorising flowers into adaptational groups, according to floral traits such as colour, scent, reward, shape and size. Seventy-five years later these two schemes were merged by Vogel (1954) and further modified by van der Pijl (1961), Faegri and van der

Pijl (1996) and Baker and Hurd (1968), into what we now term “pollination syndromes” (cited in Waser 2006).

In recent decades, developments in ecology, genetics, evolution and cytology have brought the study of biotic pollination into the mainstream of biological research (Proctor *et al.* 1996). Currently, approaches in the field of biotic pollination range from field and laboratory techniques to sophisticated procedures to analyse genetic diversity within and between populations using mathematical models (Waser and Ollerton 2006). More recently, the study of interaction networks has stimulated a resurgence of interest in community studies which evaluate patterns among plants and pollinators and identify the potential mechanisms which account for such patterns (Waser and Ollerton 2006). This chapter continues by describing some of the common global trends in plant-pollinator assemblages and how they vary with elevation.

Patterns in species diversity along an altitudinal gradient

Plants

Evaluations of tropical vegetation have shown that floristic composition changes with altitude and that species diversity generally decreases with increasing elevation (Lieberman *et al.* 1996). These trends have been described throughout the tropics; for example, in Mexico, Vázquez and Givnish (1998) analysed the composition, structure and diversity of plant communities along a 1000m altitudinal transect from dry forest to cloud forest. They found that plant species richness decreased sharply with altitude, and per sample, the number of species and genera and families declined linearly with elevation. In Costa Rica, Lieberman *et al.* (1996) also examined the structure and composition of tropical forest along an elevational transect, by sampling plots from 30m to 2600m. These authors found that plant species richness, species diversity and number of families progressively decreased with altitude and was lowest at the summit of Volcan Brava and that composition varied continuously with elevation.

Gentry (1988) reviewed trends in plant community composition and diversity of Neotropical forests by considering latitude, altitude, precipitation, edaphic conditions and comparing trends with those from other continents. Along an altitudinal gradient in the

Tropical Andes, Gentry (1988) found a sharp decrease in diversity between 1500m to near the tree line above 3000m, although no sites were sampled from the Andean foothills between 600 and 1500m. Gentry (1988) concluded that diversity and floristic composition was highly predictable based on geographical and environmental factors, with plant community diversity reaching a maximum in tropical lowland regions with high annual rainfall, intermediately infertile soils and low dry-season stress. Large differences in plant species composition and diversity with altitude may often be related to temperature differences, nutrient availability, decreases in plant growth and habitat turnover, slope and aspect (Jacquemyn *et al.* 2005).

It has been suggested that environmental parameters such as altitude, temperature, precipitation, wind speed and the particular geological substrates associated with species richness may affect the composition of pollination types, wind pollination (anemophily), insect pollination (entomophily) and self pollination (autogamy) (Kühn *et al.* 2006 and references therein). Wind pollination has been found to increase with altitude and latitude in moist temperate forests and to decline with plant species richness, while entomophily increases with plant species richness (Whitehead 1968; Regal 1982, cited in Kühn *et al.* 2006). High levels of autogamy are predicted in alpine habitats above the timber-line, where plants deal with progressively deteriorating conditions and unfavourable temperatures and weather conditions for biotic pollination to occur (Arroyo *et al.* 2006).

If the environmental and biogeographic trends for plant communities reported above are the general rule, it may be expected to find predictable gradients in species diversity and composition of plants and flower visitors along an elevational gradient.

Pollinators

A number of montane community-level studies from around the world have demonstrated a general trend of declining bee species richness and a greater importance of flies, butterflies and hummingbirds with increasing elevation (Cruden 1972; Arroyo *et al.* 1982; Warren *et al.* 1988; Kearns 1992; McCall and Primack 1992; Hingston and McQuillan 2000; Medan *et al.* 2002). These studies revealed that at high elevation sites, flies and butterflies pollinated a greater proportion of the flora, whilst bees declined in importance and pollinated proportionately fewer plant species and hummingbirds replaced bees. Some studies have also shown that pollinator abundance and diversity progressively declined above the tree

line (Arroyo *et al.* 1982; Totland 1993; Arroyo *et al.* 2006). Based on a survey of 134 plant species in the high Andes of central Chile, Arroyo *et al.* (1982) found that flower visitation rates were generally low and that from the lower subandean zone to the upper alpine zone (elevation range of 1400m), rate of flower visitation progressively declined by more than half. Arroyo *et al.* (1982) attributed this altitudinal turnover in major groups of pollinators, and the decline in visitation rates with elevation, to lower temperature, increased cloud cover and differences in life histories and thermoregulatory biology between butterflies, bees, birds and flies.

Distinct patterns of altitudinal variation in species richness among flower visiting insects in high altitude montane systems have also emerged in the literature. In the Andean zone of central Chile, Arroyo *et al.* (1982) found that fly species richness remained constant moving from the subandean zone into the cushion-plant zone, but changes in taxonomic composition among families were found. For example, Tachinidae remained important in the cushion-plant zone, but Bombilliidae abundant in the sub Andean scrub zone, were replaced by Sarcophagidae. In the upper subnival zone however, fly species richness decreased abruptly (Arroyo *et al.* 1982). In the Colorado Rocky Mountains changes across elevation in the distribution of dipteran families and species were also apparent. Species richness of Bombilliidae also declined with elevation, whereas tachinid and muscoid flies increased along the elevational gradient (Kearns 1992). The most important Diptera family in the sub-alpine zone in New Zealand was Tachinidae, followed by Syrphidae and Muscidae (Primack 1983), whereas, in two alpine sites in the Andes of Mendoza, Argentina, the most species rich families of flies were Syrphidae, Tachinidae and Bombilliidae (Medan *et al.* 2002).

In contrast with many temperate montane systems, a unique feature of the Andean zone of central Chile is the very high bee diversity and abundance of bee pollination systems. Arroyo *et al.* (1982) reported more than 50 species of bees with Megachilidae, Anthophoridae and Colletidae being the most important families. Over 90% of bees in the Andean zone occurred in the lower subandean zone between 2200-2600m. They proposed that the extraordinarily rich bee fauna of this region is a result of a number of climatic and biogeographic factors. The gradual characteristic of the ecotone which connects the Andean zone to the lower Mediterranean sclerophyllous woodland vegetation (where Andean bee diversity is greatest) facilitates the upwards migration and colonisation of bees. Above 2600m

however, not only do bees diminish rapidly in numbers, but fewer families and bee species are represented and above 3100m only five species of bees remained (Arroyo *et al.* 1982). At high alpine sites in Argentina, Medan *et al.* (2002) described assemblages of flower visitors to plants but observed a much more moderate decrease in bees and wasps; at the highest altitudes they found twice as many bee species as in Chile. In contrast to many temperate montane environments, bees were the main pollinators in the high Andes of Venezuela. In these high páramos, the most common bees present are large *Bombus* spp.; smaller halictids and colletids were restricted to periods of warm and sunny conditions above 3500m or to lower elevations (Berry and Calvo 1989).

In marked contrast with the abrupt drop in species richness of bees and flies with altitude, in Chile butterfly species richness did not change from one extreme of the Andean zone to the other. In fact, nine species of butterflies were still recorded in the highest subnival zone compared to twelve species in the subandean region (Arroyo *et al.* 1982). A similar pattern was also found by Pojar (1974) in the subalpine meadows of British Columbia; Lepidoptera, particularly skipper butterflies, were important. An increase of lepidopteran pollinators was also observed by Medan *et al.* (2002) in Argentina. However, a trend of increasing importance of butterflies as flower visitors may not be representative of lepidopteran distributions worldwide. For example, Warren *et al.* (1988) analysed data from two subalpine meadows in Utah and from a variety of other montane sites, and found that the relative contribution of butterflies was variable; at three locations relative species richness did increase, but at two of the sites in Utah and in Costa Rica, relative species richness was higher at the lower elevational sites.

In the Andean alpine zone of Central Chile, Arroyo *et al.* (1982) compared three elevationally distinct zones (subandean scrub: 2200-2600m; cushion-plant zone: 2700-3100m; subnival feldfield: 3200-3600m). They estimated that for the entire flora of the Andean alpine zone, hymenopterans (mainly bees) pollinated 40%, butterflies 19% and flies 37%. Although they observed a decline in species richness for flies and butterflies with increasing elevation, the proportions of plant species these functional groups pollinated actually increased. The general trend with altitude for plants pollinated by bees was a decrease in the number of visiting bees per plant species. For example, *Phacelia secunda* was pollinated by 20 bee species at the lowest elevation, but only five species of bees in the subnival. When the entire

Andean zone was considered, all lepidopteran species visited at least one plant species, but in the subnival zone, some species visited up to 14 subnival butterfly pollinated species. In the cushion- zone, dipterans pollinated a much greater proportion of plants than butterflies or bees. Moving up into the subnival zone, over 60% of all plant species were pollinated by flies (Arroyo *et al.* 1982).

Compared to other regions of the world, the native bee fauna of New Zealand is depauperate in both numbers of species and families (Donovan 1980). At sub-alpine sites on the South Island of New Zealand, native bee abundance is more affected by weather than most other groups of insects, although during sunny weather bees are important flower visitors and often forage in great abundance (Primack 1983). Similarly, in the sub-alpine forests of Tasmania, bee assemblages were particularly depauperate during the coolest months of the year (Hingston 1998).

In accord with the trend of a decline in species richness with increasing elevation is an increase in the altitudinal range of species. Stevens (1992 and references therein) hypothesised that species inhabiting high elevation habitats have larger altitudinal ranges than those of lowland areas with high species richness. Stevens (1992) also suggested that the Rapoport's rule (i.e. a trend whereby latitudinal ranges of species become smaller at lower latitudes) could be broadened to elevational distributions of species. Patterns of species distribution and species richness have been moulded by natural selection to favour those individuals that are behaviourally or physiologically capable of tolerating the extreme diurnal climatic fluctuations found at higher elevations (Stevens 1992). Terborgh (1971) studied the elevational distributions of Andean birds in the Cordillera Vilcabamba, Perú, to explore the factors determining their distribution. He also reported that at high elevation sites birds had larger altitudinal ranges than those at lower elevational sites. Andean bird distributional patterns and limits were attributed to a combination of ecotones (habitat discontinuities), competitive exclusion and the gradually changing physical and biological conditions along the elevational gradient (Terborgh 1971).

A prominence of hummingbird pollination at high altitudes has been documented in a variety of studies (Cruden 1972; Feinsinger 1983; Kessler and Krömer 2000; Borgella *et al.* 2001). In Mexico, Cruden (1972) compared pollinator efficiency between hummingbirds and bees at three different elevations and suggested that during the rainy season, with favourable

flight conditions, bird and bees were equally as effective as pollinators. However, during the rainy season and at higher elevations, birds were more effective pollinators because they were able to forage in cloudy and rainy conditions. In contrast, in localities subject to prolonged cloudiness, the poor flight conditions forced bees to either remain in their nests or forage elsewhere (Cruden 1972). Similarly, in the Andean forests of Bolivia, Kessler and Krömer (2000) found that ornithophily was the dominant pollination mode at high elevations and moist regions, and bromeliads (particularly terrestrial species of *Puya*) were the most important resource for hummingbirds (Kessler and Krömer 2000).

Weather conditions influence the flight activity of bees and the cost of body temperature regulation (Roubik 1989). For example, a study of bee activity during three flowering seasons in an apple orchard showed that bee species from the families Andrenidae and Halictidae and the honeybee *Apis mellifera*, had differing responses to light intensity, temperature and humidity. Honeybees responded more to changes in humidity, whereas solitary bees were active under a smaller range of conditions, but were most active in adverse conditions (Roubik 1989 and references therein). It has also been noted that bumblebees and the Africanized honeybee continue foraging during light rain, larger bees move relatively slowly and tend to remain closer to the ground, whereas some stingless bee species cease foraging completely (Roubik 1989). McCall and Primack (1992) concluded that in three contrasting plant communities (deciduous meadow in Massachusetts; Mediterranean scrub in South Africa and alpine tundra in New Hampshire) the most important variables influencing visitation rates were seasonality, weather and flower shape.

The ranges of some Andean Lepidoptera species are more closely related to physiographic factors rather than altitude, particularly the availability of small exposed summits (Arroyo *et al.* 1982). Descimon (1986) also noted some Andean lepidopterans favoured boulders and rocks, whilst several species belonging to the Andean *Rhopalocerca* remained in sheltered places or low to the ground during windy conditions, whereas most species of the Peruvian puna only fly during the dry season (Descimon 1986).

Seasonal fluctuations in species richness, abundance and composition of male euglossine bees at a premontane site and three lowland sites were investigated by Ackerman (1983). Species compositions of male euglossines, relative abundance, species phenological profiles and dominance ranks were similar among lowland sites. In contrast, the upland

locality differed from lowland sites in species composition, rank order of dominance and timing of seasonal fluctuations in bee abundance. Furthermore, some of the bee species resident in both lowland and upland sites exhibited different phenological behaviour. Given the differences among sites, Ackerman (1983) argued that communities of male euglossine bees should only be similar within restricted geographical areas, with similar vegetation, climate and topography.

In summary, community level studies along altitudinal gradients are characterised by a trend toward a dominance of flies among insect flower visitors, a decline in bee species richness and a greater importance of butterflies and flies, and a progressive decline in pollinator abundance and diversity above the tree-line. Altitudinal turnover in major groups of pollinators and a decline in visitation rates are attributed to thermoregulatory factors, and spatiotemporal fluctuations in pollinator populations. The composition of pollinators may also be influenced by temperature, precipitation, wind speed, geology and land use (Kühn *et al.* 2006). Coincident with this decline with species richness with increasing elevation is an increase in the altitudinal range of some species (i.e. Terborgh 1971; Stevens 1992).

The effects of the introduced European honeybee (*Apis mellifera*) on native ecosystems

The European honeybee (*Apis mellifera*) has been introduced to almost every country in the world including the high Andes of Perú. In recent years studies have been undertaken to determine the extent to which *Apis* negatively impacts on native plants and their associated pollinators, though it is extremely difficult to demonstrate that honeybees are competitors of native bees (Goulson 2003). Foraging by honeybees has variable impacts on the native fauna and flora depending on the amount of resources removed and on the plant species visited (Butz Huryn 1997; Horskins and Turner 1999). Figure 1.1 shows possible mechanisms by which honeybees may disrupt native interactions. Honeybees are likely to negatively impact the reproductive success of native plant populations if they affect the quality and quantity of the pollen transferred among plants, resulting in reduced seed set (Traveset and Richardson 2006). Reduced seed set may occur by pollen not being transferred to stigmas or by incompatible pollen transfer, and plants populations that are usually seed limited are the most likely to be vulnerable (Horskins and Turner 1999; Traveset and Richardson 2006). Any changes in the seed set of native plant populations within a community may result in long-term ecological change, but such changes would be extremely difficult to identify, particularly among the much greater environmental changes that are currently occurring (Goulson 2003).

In some countries, honeybees have been found to compete with native fauna for resources, are inferior pollinators, and may alter the foraging behaviour and abundance of native flower visiting fauna (Paton 1990; Butz Huryn 1997; Gross and Mackay 1998; Goulson 2003). Additionally, several studies have observed declined abundances of native visitors on blossoms when honeybees were present in large numbers (Roubik 1978; Schaffer *et al.* 1979, Ginsberg 1983; Schaffer *et al.* 1983; Paton 1993; Aizen and Feinsinger 1994) and others have shown that honeybees vary widely in their efficiency as pollinators of native plants (Percival 1974; Paton 1995; Butz Huryn 1997; Freitas and Paxton 1998; Rymer *et al.* 2005). A number of studies which focused directly or indirectly on honeybee impacts on native fauna, with indications from the authors for resource competition are presented in Table 1.1.

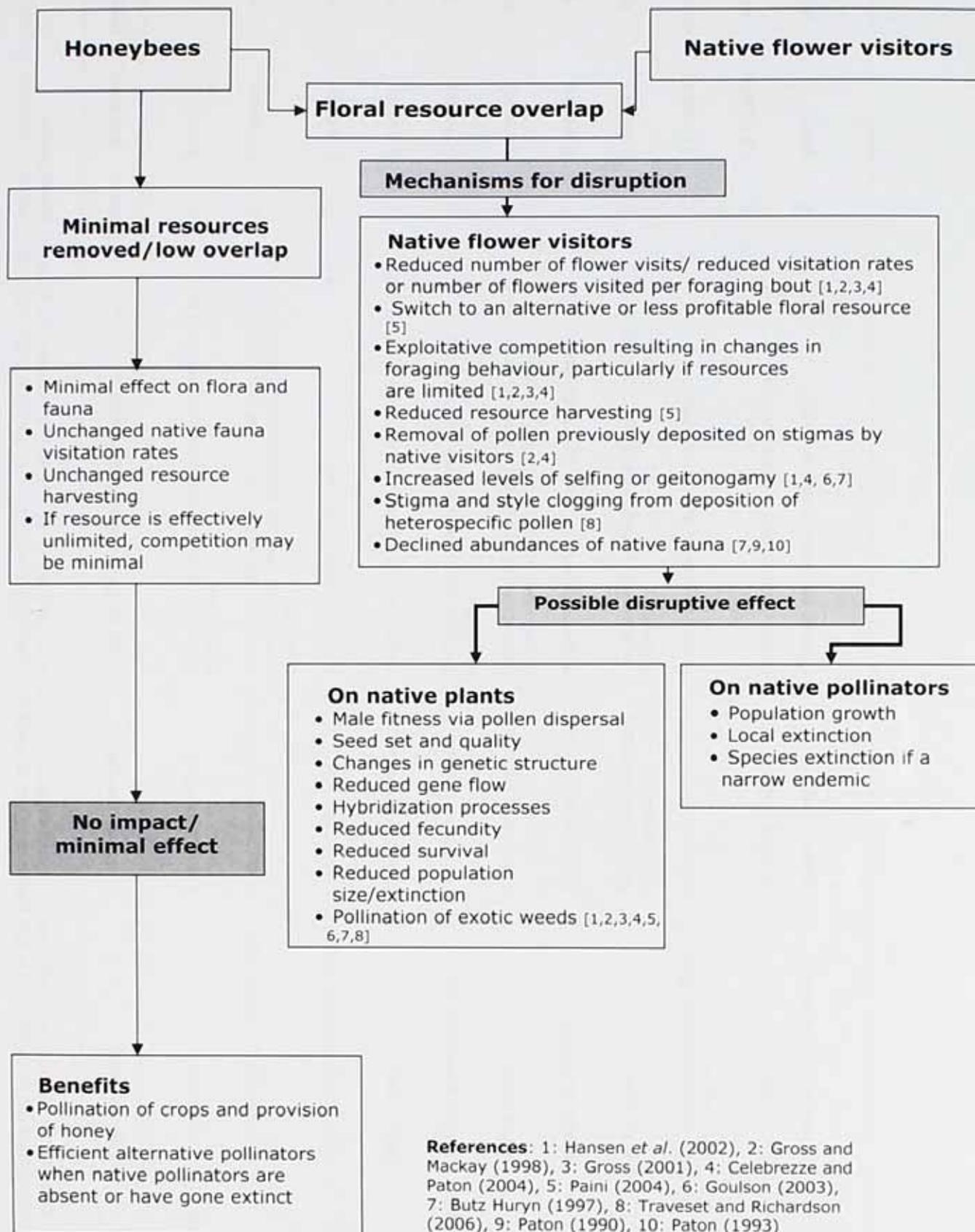


Figure 1.1. Potential effects of honeybee foraging on native flora and fauna, and some of the possible mechanisms by which they may disrupt native plant-pollinator interactions. Adapted from Butz Huryn 1997; Paini 2004; Traveset and Richardson 2006. References in parentheses.

Table 1.1. Studies focusing directly or indirectly on the impacts of honeybees on native flora and fauna. Indication from authors of impact: negative impact; NI: no impact; NO; C: conditional impact dependent on certain conditions; U: Unclear. Sugden *et al.* (1996) interpretation given in parentheses

Country	Study animal	Plant species	Type of evidence and approach	Sites	Duration	Impact
Australia Gross and Mackay (1998)	Native bees	<i>Melastoma affine</i>	Visitation rates, pollen deposition, fruit/seed set, interactions	1	5 years	NI
Paton (1993)	Honeyeaters and native bees	<i>Callistemon rugulosus</i>	Visitation, pollen harvest, nectar consumption, interactions changes in bird territory size, fruit production	2	6 months	NI
Paton (1990)	Honeyeaters and native bees	<i>Callistemon</i> and <i>Eucalyptus</i>	Pollen and nectar removal	1	6 months	NI
Gross (2001)	Native bees	<i>Dillwynia juniperina</i>	Visitation rates, pollen supplementation, fruit set	2	6 month	NI
Horskins and Turner (1999)	76 invertebrate species	<i>Eucalyptus costata</i>	Visitation rates, pollen loads and resource utilisation	1	3 months	NI
Rymer <i>et al.</i> (2005)	Native bees	4 species of <i>Perisoonia</i>	Behaviour and movement, visitation rates, plant reproduction success, breeding systems	8	4 months	NI/C
Celebrezze and Paton (2004)	Eastern Spinebills	<i>Brachyloma ericoides</i>	Foraging behaviour, fruit set experiments	4	5 months	NI
Mauritius Hansen <i>et al.</i> (2002)	White-eyes	<i>Sideroxylon cinereum</i> , <i>S. puberulum</i>	Visitation rates, nectar production, pollination efficiency	1	4 months	NI/C
Jamaica Percival (1974)*	Hummingbirds, butterflies, solitary bees	100 plant species	Nectar and pollen resource overlap	1	5 months?	NI/C(U)
USA Ginsberg (1983)	Native bees	29 plant species	Forager abundance, resource overlap	1	18 months	NI (C)
Schaffer <i>et al.</i> (1979)	<i>Bombus</i> spp., <i>Xylocopa</i>	<i>Agave schottii</i>	Forager abundance, resource level	6	days	C (C)

Table 1.1 continued

Country	Study animal	Plant species	Type of evidence and approach	Sites	Duration	Impact
Schaffer <i>et al.</i> (1983)	<i>Bombus</i> , <i>Xylocopa</i> and solitary bees	<i>Agave schottii</i>	Forager abundance	1	36 days	C (C)
Marlin and LaBerge (2001)	Native bees	24 plant species	Re- surveyed the native bee fauna of Carlinville Illinois after 75 years		24 months	NO
Thomson (2006)	<i>Bombus</i> spp.	19-26 plant species	Forager abundances, plant use and niche overlap, introduction of <i>Apis</i> colonies	3	12 months	NI/C
Panama, Peru, California Roubik (2001)	59 native bee species	-	Comparisons of annual variability	5	2-4 years 17-21 years	NI
French Guiana Roubik (1983)	<i>Melipona</i> , Africanized <i>Apis mellifera</i>	200 plant species	Controlled introduction and removal of African honeybee colonies and brood dynamics	2	8 months	NO (C)
Roubik (1980)	<i>Melipona</i> , <i>Trigona</i>	-	Sugar bait experiments, forager abundance, hive introduction and resource overlap	1	4 months	NI(C)
New Caledonia Kato and Kawakita (2004)	Native bees, Coleoptera Lepidoptera, Aves, Diptera	99 plant species	Observations of flower visitors, evaluation of behaviour and pollen attachment on insects	31	21 days	NI
Canary Islands, Tenerife Dupont <i>et al.</i> (2004)	16 species of native insects	<i>Echium wildpretii</i>	Introduction of 1393 hives, visitation and seed set	17	77 days	NI/C
Dupont <i>et al.</i> (2003)	Native bees, Lepidoptera, Aves, Diptera	11 plant species	Observations, forager abundance	1	1 month	NI
England Forup and Memmott (2005)	<i>Bombus</i> spp.	6 heath land plant species	Forager abundances	19	1 month	NI/C

Table 1.1 continued

Country	Study animal	Plant species	Type of evidence and approach	Sites	Duration	Impact
Brazil Menezes Pedro and Camargo (1991)	Native bees	184 plant species	Relative forager abundance, floral resource overlap and phenology	1	6 months	N (U)
Argentina Aizen and Feinsinger (1994)	Native insects	16 plant species	Fruit set, seed set and pollen loads	5	6 months	NO/C
New Zealand Donovan (1980)	Native bees	-	Literature review on floral overlap and observations	-	-	N (U)
India Atwal and Sharma (1971)	<i>Apis indica</i>	-	Hive introduction, hive numbers, behaviour	-	4 years	NI (U)
Mexico Villanueva and Roubik (2004)	European <i>Apis mellifera</i> <i>ligustica</i>	22 plant species	Resource use/ competition, re- introduction of 30 European bee colonies in a habitat saturated by the hybridised African and European honeybees	2	3 years	N/C
Germany Steffan-Dewenter and Tschamtkke (2000)	Native wild bees	57 plant species	Resource overlap, species richness and abundance	15	5 months	NO

Notes: Percival (1974)*: unclear of duration, the study only mentions the dry season December to April.

The competition issue

Native Australian plants and their pollinators have largely evolved in the absence of social bees and some authors contend that honeybees have had a detrimental effect on the biota (Paton 1993; Gross and Mackay 1998). *Apis mellifera* was first introduced into Australia in 1822 (New 1997) and honeybees were maintained in hives for crop pollination and honey production (Horskins and Turner 1999). The spread of feral honeybees has led to the establishment of feral colonies and honeybees are now ubiquitous in most habitats within Australia (Horskins and Turner 1999). In recent years, there has been considerable scientific and political debate over the effects of honeybees in the Australian environment. Conflicts have arisen between the apiary industry and concerned conservationists over the potential detrimental effects to native biota (New 1997; Sugden *et al.* 1996). Pollination services from honeybees are estimated to be worth four billion Australian dollars per year (Sugden *et al.* 1996). There still remains much controversy regarding the impact of honeybees from feral and managed hives on the native Australian biota (Paton 1990; Paton 1993; Butz Huryn 1997; Gross and Mackay 1998; Paton 2000; Goulson 2003; Paini 2004). In some areas of Australia it has been reported that honeybees have a negative effect, but in others their effects may be neutral or in some cases beneficial. According to Paton (2000) introduced honeybees provide an effective and much needed pollination service to the winter-flowering *Banksia ornata*, due to a lack of native nectarivorous birds. However, some studies may give a biased view on the potential impacts from honeybees on plant-pollinator systems in Australia, as many have focused on bird-pollinated systems. The high diversity of native bees, with some 2000 species, compared to a 100 or so nectarivorous native bird species, suggests that bees are important pollinators of a broad range of native Australian plant species (Gross and Mackay 1998 and references therein).

Pollinator exclusion experiments were undertaken by Celebrezze and Paton (2004) to assess whether honeybee pollination was as effective as pollination by honeybees and native birds together in *Brachyloma ericoides* (Epacridaceae). The authors found that the exclusion of birds but not honeybees from these small flowers resulted in significantly lower seed set. Native birds contributed significantly to fruit set, despite higher visitation rates and more inter-plant movement by honeybees. Fruit set following exposure to honeybees and birds was low and may have been limited by pollen-harvesting honeybees, where it was shown that honeybees removed pollen from more than half of all flowers daily. They suggested that this

behaviour could reduce the effectiveness of subsequent visits by nectar-collecting honeybees or birds (Celebrezze and Paton 2004). Indeed, Paton (1990) reported that in other native Australian plant species, such as *Eucalyptus*, *Callistemon* and *Correa*, honeybees removed substantial quantities, (up to 90%) of pollen and nectar. Also in Australia, Gross and Mackay (1998) found that honeybees reduced the fitness of *Melastoma affine* by actively removing previously deposited pollen from stigmas. Honeybees may also alter plant population structure resulting in a different pattern of pollen transfer to that of native pollinators (Goulson 2003). Several studies have reported that honeybees make fewer interplant movements than other flower visitors such as birds and therefore promote greater levels of selfing or geitonogamy (Heinrich and Raven 1972; Hansen *et al* 2002; Traveset and Richardson 2006).

In South Australia, Paton (1993) showed that honeybees altered pollination rates by removing pollen from flowers of *Correa reflexa*, affecting the amounts subsequently transferred by native New Holland Honeyeaters. Additionally, on inflorescences of *Callistemon rugulosus*, when honeybee activity was high, visitation by honeyeaters was reduced significantly and birds altered their foraging by avoiding the flowers most extensively used by honeybees.

Potential negative impacts from honeybees

All too often studies regarding the potential detrimental impacts from honeybees on native pollinators have focused on visitation rates, floral resource overlap or resource harvesting and any observed negative interaction has been construed as a detrimental impact (Paini 2004). According to Paini (2004), such conclusions may not be justified because for competition to occur there must be an overlap of floral resource, with both species harvesting both pollen and nectar from the same plant species. Furthermore, even if both honeybees and native insects and birds visit the same flowers, competition may be absent if honeybees fail to affect visitation rates or if floral resources are not limiting (Paini 2004). Some of the global studies regarding the potential negative impacts from honeybees are discussed below.

Resource overlap by honeybees

Given that honeybees are broadly polylectic (able to collect and use pollen from most genera and species of many plant species) they visit resources utilised by a wide range of native species (Goulson 2003; Cane and Sipes 2006). According to plant lists, it is estimated that worldwide honeybees visit close to 40,000 plant species and within any one geographic region up to a hundred or more (Butz Huryn 1997; Ollerton and Liede 1997; Goulson 2003 and references therein). Butz Huryn (1997) proposed that although honeybees have been recorded visiting many plant species, they often use less than a third of the available flora and only a limited subset are used intensively. However, she also suggested that resource overlap may vary widely ranging from hardly any overlap with some species to as much as 83% overlap. This was also supported by Steffan-Dewenter and Tscharnkte (2000) who compared resource overlap between honeybees and wild bees on calcareous grasslands in Germany and found that honeybees used about a third of all flower visiting melittophilous plant species visited by wild bees. In addition, Menezes Pedro and Camargo (1991) also found that of the 33% of plants visited by honeybees in Brazil, half of them were only occasionally visited and 65% of visits were to only nine plant species. Kwak and Bekker (2006) also observed that honeybees visited 41% of the 135 plants species surveyed in the Netherlands, but they only occurred in large numbers on eight of the species. Memmott and Waser (2002) analysed historical records for a community in central USA and found that honeybees visited 47% of the total flora, whilst on the Canary Island of Tenerife, on New Caledonia and Santa Cruz, the proportion of plant species visited was 52%, 63% and 88% respectively (Dupont *et al.* 2003; Kato and Kawakita 2004).

Interspecific competition

Interspecific competition is one of the most controversial topics in ecology and although laboratory experiments have shown the potential for it in simple systems, it is difficult to demonstrate in the field (Sugden *et al.* 1996). One of the most common failings in studies investigating potential competitive effects from honeybees has been the lack of replication of sites, small sample sizes and data interpretation (Paini 2004). A further confusing factor is that evolutionary forces acting in the past means that extinctions may have already occurred, therefore evolution and the tendency towards equilibrium may mask potential competitive effects (Sugden *et al.* 1996). Since honeybees visit resources utilised by a wide range of

native species, substantial overlap may lead to competition if resources are limiting. Interference competition arises when one organism physically excludes the other from a part of the habitat and thus from the resources that could potentially be exploited there (Begon *et al.* 1990). Exploitive competition is considered to be any detrimental effect on an organism which leads to a reduction in resources by other, competing organisms (Begon *et al.* 1990).

Interference competition

Several studies have reported that honeybees reduced the visitation rates of native flower visitors by means of exploitive or interference competition (Schaffer *et al.* 1979, 1983; Butz Huryn 1997; Gross 2001; Hansen *et al.* 2002; Dupont *et al.* 2003). In southern Arizona, direct interference was not thought to play an important role in a plant pollinator community and honeybees, bumblebees and carpenter bees were often observed feeding together on the same inflorescences (Schaffer *et al.* 1979). Although occasional jostling by honeybees at flowers has been reported (Roubik 1991; Gross and Mackay 1998), aggression between honeybees and native bees is considered rare and relatively unimportant (Butz Huryn 1997).

In 1969, the hive robbing by *Apis mellifera* on colonies of the native *Apis indica* in India resulted in excessive absconding by *A. indica*. A similar development was observed in Japan, where the indigenous honeybee *Apis cerana* was out-competed by the introduced *A. mellifera*. As a consequence, *A. cerana* was displaced to remoter areas where *A. mellifera* colonies were not established (Atwal and Sharma 1971). This interaction has been widely cited in the literature as evidence of interference competition. However, although the introduction of *A. mellifera* in Japan may have contributed to the decline in population abundances of *A. cerana*, a significant number of beekeepers opted to switch to *A. mellifera* because the species is easier to manage (Butz Huryn 1997).

Exploitative competition

Hansen *et al.* (2002) investigated the effects of honeybees on endemic nectarivorous birds in Mauritius and found most bird visitation occurred early in the morning and ceased when honeybee foraging activity depleted nectar standing crops. In Australia, Gross (2001) investigated the effects of introduced honeybees on native visitation and fruit set in *Dillwynia juniperina* and showed that honeybees visited slightly more flowers per plant than native

bees, and native bee visitation was significantly negatively correlated with honeybee foraging at the same bush. Although the potential for an impact was suggested, the impact on native bee fitness could not be determined without further studying brood levels (Gross 2001).

Evidence indicative of exploitative competition was also documented in southern Arizona, among *Apis mellifera* and native bees visiting *Agave schottii* (Schaffer *et al.* 1979, 1983). Honeybees preferentially exploited the most productive habitats and restricted foraging activity to peak nectar availability, thus reducing the standing crop of nectar to native bees. Bumblebees were most abundant in intermediate quality patches, whilst carpenter bees used the least productive habitats. Over the long term however, the authors could not categorically state that honeybees depressed the abundance of native bees.

According to Gross (2001) there are no documented cases in Australia of the complete displacement of a pollinator as a result of the introduction of species, but there are cases where the abundance of nectar feeding birds has been reduced by the presence of honeybees (Paton 1993; Gross and Mackay 1998). For instance, Paton (1993) found when honeybee numbers were increased by the experimental placing hives next to patches of *Callistemon*, adult male New Holland honeyeaters increased their territory size by displacing juveniles and females from adjacent territories. Paton (1993) suggested these competitive interactions could potentially affect honeyeaters' long-term survival because the disproportionate loss of females could affect population dynamics more than if both sexes were equally displaced. Gross and Mackay (1998) concluded that honeybees are an unwelcome introduction in montane tropical rainforest systems in Australia. In comparison with native bees, honeybees were inferior pollinators of *Melastoma affine* and in 91% of interactions between native bees and honeybees, native bees were disturbed from foraging at flowers by honeybees. They also noted that honeybees sometimes aggressively excluded native bees from flowers by pulling them from the stamens of flowers.

Globally, studies concerning the competitive impacts of introduced honeybees on native flora and fauna have concentrated on indirect measurements such as resource overlap, change in visitation rates of native flower visitors, changes in abundance of native fauna in the presence of honeybees, and changes in the level of resource harvested. Table 1.1 summarises a variety of studies from around the world which highlight the conflicting opinions and conclusions of the authors as to how strongly an impact was demonstrated. The studies

selected use indirect and direct measurements. Included are oceanic islands where pollinators may be more vulnerable to honeybee introductions owing to small population sizes, and studies from Australia, where compared to Europe and the Americas, honeybees are a relatively new introduction (Roubik 2000; Ghazoul 2005). Also included are areas of the world in which the Africanised honeybee (*Apis mellifera scutellata*) has rapidly expanded (Spivak 1992).

Some general conclusions can be drawn from the studies presented in Table 1.1, and some of these investigations listed have been examined in more detail above. Firstly, many of the studies which suggested a competitive impact from honeybees were dependent on certain conditions. Oceanic islands all reported the potential for competition and are perhaps the most susceptible to invasion from honeybees owing to low species diversity, small population sizes and little potential of recovery through immigration (Ghazoul 2005). Secondly, those studies conducted in Australia did not provide clear cut evidence for negative impacts, although the greatest potential for honeybee impacts may be in environmentally sensitive ecosystems such as montane tropical rainforest (Gross and Mackay 1998; Goulson 2003). Finally, those studies which used direct measurements of survival, fecundity and population density showed no evidence of an impact from honeybees; for example no noticeable changes in abundance were observed in Panama, after the arrival of the Africanised honeybee (Wolda and Roubik 1986). This finding was later endorsed in Panama, where inter-annual variation in abundance of euglossine bees over a period of 21 years did not show any aggregate trend (Roubik 2001). Over a longer time period, the native bee fauna in the Carlinville area of Illinois USA remained remarkably intact after being re-sampled 75 years later (Marlin and LaBerge 2001).

Paini (2004) reviewed 28 studies investigating potential honeybee impacts worldwide and suggested that a common failing was the lack of replication of sites, citing 68% using two sites or fewer. In agreement with this, 59% of the studies listed in Table 1.1 included two or fewer sites. However, a number of studies presented in Table 1.1 also have been conducted over longer periods, spanning from five to 21 years, to a resurvey after 75 years had elapsed (e.g. Wolda and Roubik 1986; Gross and Mackay 1998; Marlin and Laberge 2001; Roubik 2001).

Although studies investigating indirect measurements may be useful in detecting the potential for competition between honeybees and native bees, Paine (2004) argued that only by using direct measurements such as fecundity, individual survival or population size can negative impacts be properly assessed. The author concluded that many of the studies reviewed were compromised by low replication, poor interpretation and confounding factors. Similarly, Sugden *et al.* (1996) reviewed 26 studies focusing on honeybee competition with other native bee species and pointed out that the validity in proving honeybee competition was dependent on certain assumptions and conditions. Among the studies they included, none of the authors completely dismissed the possibility of resource based competition, despite some results suggesting the contrary. They argued that ecological studies rarely provide indisputable proof for resource competition and that the most rigorous analysis would consider most of the studies they included as flawed and incapable of providing useful conclusions. They suggested a more sensitive rationale for interpretation would be to assign values to each of the studies they listed relative to the contribution in supporting (>0) or rejecting (<0) their null hypothesis that honeybees have no influence on other species. Although Sugden *et al.* (1996) recognised that using a quantitative value may be meaningless, they interpreted their scores as evidence that honeybees under some circumstances may impact other bees.

Butz Huryn (1997) also reviewed the ecological impacts of introduced honeybees and concluded that their effects seemed to be relatively subtle. Although the presence of honeybees may modify foraging abundance and behaviour on native fauna, no studies have shown a negative impact on population abundances of any native animals or plants. Furthermore, studies which claim increased abundances of honeybees on flowers when compared to native fauna are often confounded with human landscape modification (Steffan-Dewenter and Tschamtkke 2002).

As Goulson (2003) has pointed out, showing overlap in floral resource use does not necessarily demonstrate that competition is occurring, therefore predicting the outcomes of honeybee introductions remains highly problematic (Thomson 2006). For example, many of the studies presented in Table 1.1 are compromised by low replication of sites and sampling, short duration, lack of control sites where honeybees were absent, and 41% focused on only one or two plant species. Furthermore, estimating bee abundances either in flight or at flowers is potentially challenging because honeybees are wide ranging, exhibit behavioural flexibility,

and bee abundance fluctuates seasonally and among years (Ackerman 1983; Wolda and Roubik 1986; Minkley and Roulston 2006; Thomson 2006).

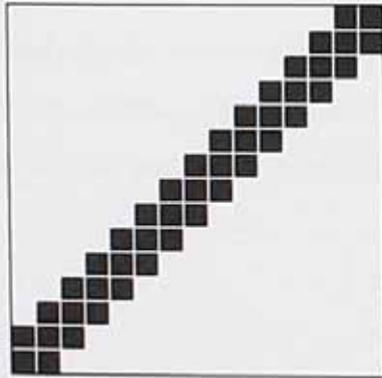
From the studies outlined above, it is apparent that the impact of honeybees on native fauna and flora is still controversial; some would argue that direct evidence for resource competition is still absent (Butz Huryn 1997; Steffan-Dewenter 2002), others maintain that honeybees can disrupt and affect the fitness of native species (Paton 1990; Gross and Mackay 1998), while others argue that there is still a scarcity of research which addresses competitive interactions using multiple methods (Paini 2004; Thomson 2006). A recent example using multiple measures is described below, highlighting how observational measures to predict competitive effects are limited.

In California, Thomson (2006) assessed the competitive effects of *Apis mellifera* on bumblebees by using multiple experimental and simultaneously applied monitoring approaches. Based on measures commonly applied to infer competition, she used three indirect measures: observations of resource overlap, observational data on spatial and temporal correlations and density manipulation experiments by introducing *Apis* colonies into three sites. For all three measures, her results showed patterns that were consistent with competitive effects. At the colony level, the experimental data were far more reliable than either of the observational approaches in predicting competition. The correlative data were highly variable, resulting in trends in different directions during different months. Although the experimental data for both foragers and colonies revealed significant competitive impacts, the correlative data did not detect this and rarely showed a significant negative association between *Apis* and *Bombus*. Of the two observational measures, niche overlap was judged to be a better predictor. However, the author was unable to test the expectation that colony fitness declines in years with high niche overlap because only three years of data were available. Thomson (2006) suggests that great caution should be taken when assessing impacts on the basis of temporal or spatial correlations between invasive and native species and that even limited experimental studies may be a far more useful approach.

Structure in pollination webs

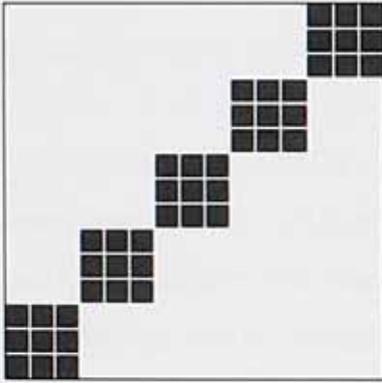
Historically, most pollination studies focused on a single plant or pollinator species, while investigations aimed at the level of the entire community were much rarer (Waser 2006). Recently a revival of community studies has flourished, treating the whole plant-pollinator community as a network of interactions (Waser and Ollerton 2006). Many plant-pollinator networks show regular structural patterns, with a high degree of internal organisation, regardless of the type of network (Jordano *et al.* 2006 see Figure 1.2). Furthermore, most plant-flower visitor and other mutualistic webs are highly nested (Bascompte *et al.* 2003; Jordano *et al.* 2006; Ollerton *et al.* 2007a). In nested assemblages, a core of generalist species interact with one another, while specialist species interact mainly with generalists. Identifying the processes which generate these patterns is relevant for our understanding of how species coevolve, and for maintaining biodiversity and ecosystem stability (Jordano *et al.* 2003; Thompson 2006).

The nestedness theory was originally developed in the context of island biogeography to characterise the distribution of species on island archipelagos or within fragmented landscapes (Patterson 1987; Cutler 1991; Atmar and Patterson 1993). Within sets of islands, biotas show a nested subset pattern if the species present on a depauperate island are a proper subset of those on richer islands (Patterson 1987). More recently advances in evaluating the various measures of nestedness have been developed using multivariate statistical methods, including randomisation procedures and the application of appropriate null models (Wright and Reeves 1992; Atmar and Patterson, 1993; Bascompte *et al.* 2003; Vázquez and Aizen 2004; Lewinsohn *et al.* 2006; Santamaría and Rodríguez-Gironés 2007). The more recent work has shown that most mutualistic networks are significantly nested among plants and seed dispersers (Jordano 1987; Bascompte *et al.* 2003), plants and pollinators (Bascompte *et al.* 2003; Dupont *et al.* 2003; Jordano *et al.* 2003; Ollerton *et al.* 2003; Vázquez and Aizen 2004; Nielsen and Bascompte 2007), anemonefish and their hosts (Ollerton *et al.* 2007a); marine cleaning symbiosis between marine reef animals (Guimarães *et al.* 2006), regardless of the type of network studied and the ecological setting (i.e. abiotic, biotic, terrestrial or aquatic habitats) (Guimarães *et al.* 2006). According to some authors, nestedness may be a feature of most, or possibly all, mutualistic associations (Ollerton *et al.* 2007a; Bascompte *et al.* 2003, 2006).



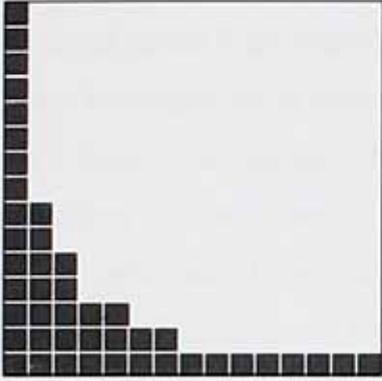
A. Gradient

A gradient appears within a matrix as a diagonal band stretching along the main diagonal. The width of the diagonal band is dependent on the ratio of animals to plants and by the degree of overlap among a given fauna (Lewinsohn *et al.* 2006).



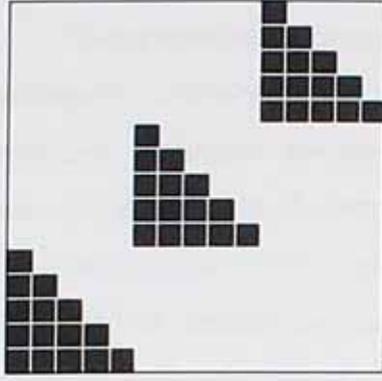
B. Compartmented

Compartmented assemblages appear in ordered interaction matrices as distinct clusters of cells.



C. Nested

In a perfectly nested matrix the data will fill the matrix so that interactions will be packed into the upper and left corner, ordered in a way to minimise unexpected species absences and presences. Presences of species in a given column will be a proper subset of those preceded in columns with a higher number of presences, so that the interactions of the more specialised species tend to be nested within the interactions already observed among more generalised species



D. Combined

Combined assemblages appear in interaction matrices as an obvious combination of compartmented and nested structures. Thus, the species within each block are themselves nested, forming nested compartments (Lewinsohn *et al.* 2006)

Figure 1.2. Main structural features of plant-animal interaction matrices (A-D). Realised interactions correspond to shaded squares and absent interactions to white squares. Animal species are represented by rows and plants as columns. Adapted from Lewinsohn *et al.* (2006).

Characteristically, nestedness has a number of salient features: firstly it generates highly asymmetric interactions, with a tendency for specialist plants to interact with the most generalist pollinator species. Secondly, nestedness organises the community cohesively around a central core of generalist plant and animal species which interact among themselves (Bascompte *et al.* 2003; Jordano *et al.* 2006). Finally, interactions among species are not distributed at random nor compartmentalised, but instead show a high degree of internal organisation (Bascompte *et al.* 2003; Jordano *et al.* 2006).

Plant-pollinator interaction matrices follow certain regular patterns; they are typically sparse and reveal high heterogeneity; the majority of species have few interactions, but a few species are much more connected than expected by chance alone (Fortuna and Bascompte 2006). Many mutualistic data sets fit a power-law relationship where the number of interactions increases with network size, while the percentage connectivity decreases with network size, and larger communities tend to be more loosely connected than smaller communities (Fonseca and Leighton 1996; Olesen and Jordano 2002; Bascompte *et al.* 2003). Olesen and Jordano (2002) analysed 29 complete plant-pollinator networks which encompassed a wide range of latitudes and altitudes, and suggested that the level of connectedness in communities decreased with increasing species richness in a similar manner reported previously by Jordano (1987).

Dupont *et al.* (2003) investigated a complete network of plants and their associated flower visitors in a subalpine desert on the island of Tenerife, Canary Islands and found a significantly asymmetric nested structure; specialised locally rare plants tended to be visited by generalised locally abundant visitors, and specialised locally rare visitors tended to forage on generalised, locally abundant plants. Generalisation level of a species was significantly correlated with local abundance for flower visitors, thus species interacting with many other species tended to be the most abundant in the community. In the grasslands of KwaZulu-Natal, South Africa, Ollerton *et al.* (2003) used a similar approach with flower visitor and pollinator data sets generated from insect visits to asclepiads, and also found significantly nested and patterns, correlated with insect abundance.

Null models to study patterns of specialisation in 17 plant-pollinator networks from around the world were developed by Vázquez and Aizen (2006). They suggested that specialisation between pairs of interacting species was not exceptional and that both extreme generalisation and extreme specialisation was a common feature of many networks. They attributed these structural patterns to community characteristics such as species richness, community size, the number of interacting species, and number of interaction partners, particularly their frequency of interaction. Finally, they suggested that the observed asymmetry in most data sets may be partly a function of species abundance in the community and to random interactions between individuals rather than species, and to sampling bias, influenced by data aggregation. In agreement, Ollerton and Cranmer (2002) showed that sampling effort has a significant effect on observed levels of plant ecological specialisation and suggested that this may also apply when estimating numbers of discrete pollination systems, that is, the diversity of pollinators which service a plant at a higher taxonomic level (e.g. fly pollinated or bird pollinated) referred to as functional specialisation by Fenster *et al.* (2004).

The proposal that asymmetric specialisation in interaction webs is generated by coevolutionary processes has strong links to the “forbidden interaction” hypothesis which proposes that connectivity decreases because interactions are prevented by a mismatch of biological attributes of species (Jordano *et al.* 2003, 2006; Santamaría and Rodríguez-Gironés 2007). Certain interactions may not arise if the phenology of a plant and pollinator are not synchronised, if functional groups of pollinators show a certain preference to sugar content or concentration, or choice of flower colour. Additionally, a mismatch in morphology between plant and pollinator may provide a barrier to reaching the reward (Proctor *et al.* 1996; Jordano *et al.* 2006; Santamaría and Rodríguez-Gironés 2007).

Jordano *et al.* (2006) illustrated that the forbidden interaction concept by analysing a hummingbird-plant network from an original subset of data by Snow and Snow (1972). Out of a possible 522 pair-wise interactions, only 185 were realised. They attributed 29% to habitat uncoupling and the vertical zonation between hermits and non-hermit hummingbird species (sub-family Phaethorninae and family Trochilidae respectively). For example, most understory hermits are considered to be high-reward traplining foragers that visit flowers such as *Heliconia* species (Stiles 1975; Feinsinger and Colwell 1978). In contrast, many of the non-hermit hummingbirds typically foraged in the sub canopy (Snow and Snow 1972). Only a

small proportion of forbidden interactions were due to a mismatch in morphology between flower and hummingbird species, such as length of corolla tube, colour preference and the reward offered.

Factors structuring plant-pollinator webs

Two possible mechanisms to explain the topological properties of plant-pollinator networks have been proposed: complementary traits and traits that act as a barrier to exploitation (Santamaría and Rodríguez-Gironés 2007). Complementary traits are defined as the similarity between the reward that the plant has to offer and the resource that a flower visitor seeks, and these traits determine whether or not a pair-wise interaction will occur. In the second mechanism, barrier traits determine whether species pairs interact dependent on the ability of the pollinator to reach the reward offered. The authors investigated the implication of these proposed mechanisms by applying simple models to plant-pollinator interaction networks and comparing their results with data from 37 real-world networks. The models included from one to four complementary or barrier traits, and mixed models based on two of each of the traits. The authors also incorporated two variations of a neutral interaction model (i.e. random interactions among individuals are proportional to their relative abundances, proposed by Vázquez and Aizen 2004). Ninety-five per cent of the real-world models were significantly nested and most modelled networks were also highly nested. The complementarity and barrier models had contrasting effects on the characteristics of the networks. Santamaría and Rodríguez-Gironés (2007) showed that with complementarity models plant-pollinator pairs specialised on each other, producing highly connected networks of low nestedness. Conversely, barrier models resulted in low connectance and perfectly nested networks because specialist plants tended to associate with pollinators that had access to a diverse array of alternative resources. The authors suggested that the mixed models provided the best fit to the real community data since complementary traits would diminish the trend towards extreme nestedness produced by barrier models, whilst barrier models would reduce the low connectance and high dependency of random effects generated by complementary models.

The factors promoting asymmetry were examined by Stang *et al.* (2006) by focusing on the impact of morphological size constraints (nectar holder depth and width) and species abundances in a Mediterranean insect-flower interaction web. Actual field data were

compared with predictions from Monte Carlo simulations including different combinations of the possible factors structuring the web. Results showed that the interaction web was significantly asymmetrical, both nectar-holder depth and species abundance produced asymmetry, but was stronger when based on both factors. Although both parameters were good predictors for the number of interaction partners, only nectar spur depth was able to predict the degree of asymmetry for certain species. Stang *et al.* (2006) also found that although abundance produced asymmetry, species specific predictions about the degree of generalisation of the interaction partners could not be made. The study revealed that morphological size threshold (nectar-holder depth and width) was a good predictor for the level of ecological generalisation within the community as a whole, and emphasised the importance of including morphological traits when characterising generalist and specialist species. These authors concluded that more studies were required to fully understand the factors that promote asymmetry such as the role of sampling intensity, flowering phenology, nectar quantity and the energetics of the individual flower visitors involved. Nevertheless, they claimed that observed asymmetric patterns alone would not equal reproductive susceptibility and extinction risks for plant species because to a large extent, the observed asymmetry resulted from abundance patterns and morphological constraints (Stang *et al.* 2006).

Nested patterns have implications for understanding community assembly, co-evolution and the conservation of pollination ecosystem services and biodiversity (Memmott *et al.* 2004; Jordano *et al.* 2006). Asymmetrical interactions and the presence of a core of taxa with a high density of interactions will not only affect the robustness of the mutualistic network, but may also provide pathways for the persistence of rare species (Jordano 1987; Bascompte *et al.* 2003; Jordano *et al.* 2006). For example Bascompte *et al.* (2003) showed that specialised species frequently depended on a core of generalist species and suggested that this core of taxa may drive the evolution of the entire community. Memmott *et al.* (2004) explored potential extinction patterns in two large plant-pollinator networks by simulating the removal of pollinators at random from the most generalised to the most specialised, and proposed that the loss of a core of generalist key species represented a serious threat to pollination networks. However, Ollerton *et al.* (2003, 2007a) have argued that present day nested patterns may have been generated by a process of natural “ecological filtering” that have largely removed most specialist-specialist interactions from communities, resulting in a structure that may be ecologically robust to environmental perturbations.

Characterising generalisation and specialisation

The long standing view that pollination systems tend toward specialisation has been challenged over the past decade (Ollerton 1996; Waser *et al.* 1996; Hingston and McQuillan 2000; Johnson and Steiner 2000). The latest reviews show that plant-flower visitor networks are much more generalised than was previously thought and that the topological properties of networks are typically asymmetrical (Jordano 1987; Olesen and Jordano 2002; Jordano *et al.* 2006). Many researchers now agree that although specialised pollination systems do exist, extreme one to one specialisation is very rare (Waser *et al.* 1996; Olesen and Jordano 2002; Herrera 2005; Petanidou and Potts 2006) and that most pollination associations fall along a continuum between generalisation and specialisation (Waser *et al.* 1996; Johnson and Steiner 2000). Although, Vázquez and Aizen (2004) suggested that asymmetric specialisation could be more common than previously thought. They proposed that asymmetric specialisation in species interactions could be explained by random interactions among individuals rather than species, sampling biases, data aggregation and adaptive consequences of specialisation. Vázquez and Aizen (2004) further argued that a limitation to the recent generalisation/specialisation debate is the lack of null hypotheses with which to contrast the observed patterns of specialisation. They advocated a null model approach to ascertain if either extreme generalisation or extreme specialisation was unusually common in nature.

In order to assess the degree of generalisation and specialisation in plant-flower visitor networks it is important to use appropriate definitions that are evolutionally and ecologically relevant (Vázquez and Aizen 2004). Multiple definitions have been proposed in the literature for the words “specialisation” “generalisation” in the context of pollination biology. Armbruster *et al.* (2000) and Ollerton *et al.* (2007b) discussed these inconsistencies and the confusion it has generated. To illustrate this point, some of the multiple definitions for specialisation and generalisation proposed in the literature are presented in Table 1.2. Armbruster *et al.* (2000) stressed the importance of distinguishing between “evolutionary specialisation” (a process) and “ecological specialisation” (a state) because in their opinion, this distinction may lead to misunderstandings regarding the importance of evolutionary specialisation in angiosperm evolution, also discussed in Ollerton (1996). Moreover, Ollerton *et al.* (2007b) emphasised the significance of developing a broader conceptual framework for understanding how plant-pollinator interactions evolve along a continuum of ecological generalisation to specialisation and that researchers interested in the biology of generalist

flowers should use more exact terms such as ecological, functional and phenotypic generalisation as it allows for a greater accuracy and better understanding of plant-pollinator interactions.

Since pollination efficiency and plant fecundity was not measured in this thesis (with the exception of the *Duranta mandonii* (Verbenaceae), all visiting taxa are referred to as flower visitors. Thus, the definition of ecological generalisation and specialisation refers to the number of flower visitors with which a plant interacts, or the number of plant species utilised by different species of insects and birds. I use the term functionally generalised or specialised to refer to the diversity of pollinators which service a plant at a higher taxonomic level (e.g. family or above) see Chapter 3. I use the term phenotypically generalised or specialised to refer to the adaptations exhibited by a flower, mainly flower morphology and type and amount of rewards (Ollerton *et al.* 2007b).

Table 1.2. Definitions for different concepts of specialisation and generalisation proposed in the literature. Direct quotes have been used to avoid confusion regarding interpretation

Term	Definition
Specialisation	Successful pollination by a small number of species of animals (Armbruster <i>et al.</i> 2000)
Adaptive specialisation	Related to the "most effective pollinator principle" proposed by Stebbins (1970) (cited in Armbruster <i>et al.</i> 2000). As a result of the selection exerted by pollinators, flowers of most plants are expected to be visited mainly by a limited group of highly effective pollinators (Armbruster <i>et al.</i> 2000; Gómez and Zamora 2006 and references therein)
Fundamental specialisation	The potential interactions that would lead to positive fitness for a given species under any possible ecological circumstances; thus, fundamental specialisation ultimately depends on the background of a species (Vázquez and Aizen 2006)
Fundamental generalisation	The number of potentially effective pollinators with which a plant may interact (Ollerton <i>et al.</i> 2007b)
Evolutionary specialisation	A relative concept; the process of evolving in the direction of increasing specialisation. Evolution from pollination by more animal species to pollination by fewer species or functional groups (Armbruster 2000; Fenster <i>et al.</i> 2004)
Ecological specialisation	A state that refers to having only a few similar species of pollinators at the present time (Fenster <i>et al.</i> 2004)
Ecological generalisation/ specialisation	The number of effective pollinators with which a plant interacts, i.e. how many species of pollinators are involved in the relationship (Ollerton <i>et al.</i> 2007b)
Functional specialisation/ generalisation	Refers to the diversity of pollinators which service a plant at a higher taxonomic level (e.g. family or above) and is usually expressed in relation to that taxon, for example "fly pollinated" or "bird pollinated" (Ollerton <i>et al.</i> 2007b)
Functional specialisation	Plants that are specialised onto a particular functional group of pollinators (e.g. hummingbird pollinated) (Fenster <i>et al.</i> 2004)
Phenotypic generalisation/ specialisation	Adaptations exhibited by a flower (e.g. radial or zygomorphic symmetry, specialist rewards, complex scents, specific timing of anthesis), often in relation to functional specialisation (Ollerton <i>et al.</i> 2007b)
Realised specialisation	The actual specialisation attained under a particular ecological context (Vázquez and Aizen 2006)
Realised generalisation	The number of effective pollinator species which interact with a plant and affect its fitness, within the setting of geographical and temporal variability in community context (Ollerton <i>et al.</i> 2007b)
Adaptive generalisation	The evolution of generalisation mediated by selection exerted by pollinators is possible in some cases (Gómez and Zamora 2006)
Non-adaptive generalisation	Non-adaptive generalisation occurs when pollinators do not constitute real selection agents (Gómez and Zamora 2006)
Generalised flowers	Those flowers with an open floral design with typical rewards, which are both attractive and accessible to most flower visitors (Minkley and Roulston 2006)

Biodiversity and conservation status of the study region

The high-altitude flora of the Andes is the most species rich of any tropical mountains, and in Perú over 40% of Andean tropical forests still remain (Myers *et al.* 2000; Hughes and Eastwood 2006). Conservation International designated the Tropical Andes as one of 25 global biodiversity “hotspots”, with an extraordinarily high concentration of endemic species (Alonso *et al.* 2001) (Table 1.3). The region contains the highest proportion of the world’s endemic plant (7.4%) and vertebrate (6%) species and has been identified as a target area for conservation efforts (Alonso *et al.* 2001; Fjeldså 2002a).

Table 1.3. Number of plant and vertebrate species and endemism, and percentage of global totals for the 25 leading biodiversity hotspots

Hotspot	Plant species	Endemic plants (global %)	Bird species	Bird endemism	Vertebrate species	Endemic Vertebrates (global %)
Tropical Andes	45,000	6.7	1,666	677	3,389	5.7
Mesoamerica	24,000	1.7	1,193	251	2,859	4.2
Caribbean	12,000	2.3	668	148	1,518	2.9
Brazil’s Atlantic Forest	20,000	2.7	620	181	1,361	2.1
Choc/Darien/Western Ecuador	9,000	0.8	830	85	1,625	1.5
Brazil’s Cerrado	10,000	1.5	837	29	1,268	0.4
Central Chile	3,429	0.5	198	4	335	0.2
California Floristic Province	4,426	0.7	341	8	584	0.3
Madagascar	12,000	3.2	359	199	987	2.8
Eastern Arc / Coastal Forests of Tanzania/Kenya	4,000	0.5	585	22	1,019	0.4
Western African Forests	9,000	0.8	514	90	1,320	1.0
Cape Floristic Province	8,200	1.9	288	6	562	0.2
Succulent Karoo	4,849	0.6	269	1	472	0.2
Mediterranean Basin	25,000	4.3	345	47	770	0.9
Caucasus	6,300	0.5	389	3	632	0.2
Sundaland	25,000	5.0	815	139	1,800	2.6
Wallacea	10,000	0.5	697	249	1,142	1.9
Philippines	7,620	1.9	556	183	1,093	1.9
Indo-Burma	13,500	2.3	1,170	140	2,185	1.9
South-Central China	12,000	1.2	686	36	1,141	0.7
Western Ghats/Sri Lanka	4,780	0.7	528	40	1,073	1.3
SW Australia	5,469	1.4	181	19	456	0.4
New Caledonia	3,332	0.9	116	22	190	0.3
New Zealand	2,300	0.6	149	68	217	0.5
Polynesia/Micronesia	6,557	1.1	254	174	342	0.8

Vertebrate groups are birds, mammals, reptiles and amphibians, excluding fish. Source: Myers *et al.* (2000).

The Cordillera Vilcanota spans an altitudinal range from 2700 to over 5200 metres above sea level, and extends north approximately 80km and then west for 40km (Tupayachi 2005). The mountain chain is geographically positioned in the transition/interaction zone sandwiched between the Andes and the Amazon (Figure 1.3). The eastern side of the Andes in the Department of Cuzco includes the two disjunct cordilleras north of the Apurímac River, known as the Vilcabamba and Vilcanota (Stattersfield *et al.* 1998). The isolation of this segment of the Peruvian Andes has created particular biogeographic, climatic and physiographic conditions that have resulted in a unique and highly endemic flora and fauna (Tupayachi 2005). Three distinct geological zones have been identified in Patacancha Valley and other surrounding valleys of the Vilcanota Highlands. The upper zone located between Patacancha and Maracocha contains Cambrian deposits of aluminium, calcite and silicate. The mid to lower valley between Maracocha and Ollantaytambo is characterised by Ordovician and Devonian sandy clay sediments. The valley floor itself is located geologically within an area of early Cambrian volcanic sediments (Casagli *et al.* 2006). The other major geological feature of the region is the Vilcabamba Batholith, a large intrusive formation of white granites, granodiorites and basalts (Casagli *et al.* 2006).



Figure 1.3 Map showing the location of Cordillera de Vilcanota Perú. (Source Google Earth 2008)

The study region is best known for the Peruvian Expeditions of Yale University and the National Geographic Society, under the direction of Professor Hiram Bingham, which resulted in more than fifty publications and monographs (Bingham 1922). Other historical works include the bird collections made by Sclater and Salvin in 1873, Whitley in 1868, Kalinowski in 1894, Berlepsch and Stolzmann in 1906, Heller in 1915, Chapman in 1916, and Watkins in 1917 (Chapman 1921). More recently Terborgh (1971) studied the distributional patterns of birds in the Cordillera Vilcabamba. In 1989, Conservation

International Perú (CI-Perú) was created to work in important regions for biodiversity conservation and in 2001 CI-Perú conducted a Rapid Assessment Program, a biological and social assessment of the Cordillera de Vilcabamba (Alonso *et al.* 2001)

Andean flora

Botanical collections from the Vilcanota mountain range have yielded 145 families, 460 genera and 871 plant species (Tupayachi 2005). Above or near the treeline, small stands of *Polylepis* (Rosaceae) woodlands are distributed from elevations of 3800 to 5000 metres (The Association of Andean Ecosystems (ECOAN) 2004). These woodlands are considered a distinctive vegetation type and are particularly important for *Polylepis*-adapted birds (Stattersfield *et al.* 1998; Fjeldså 2002a). The World Conservation Monitoring Centre has listed all species of *Polylepis* as conservation dependent, and the Cusco-Urubamba region has also been identified as a key area for bird endemism (Wege and Long 1995; Stattersfield *et al.* 1998; Fjeldså 2002b; Servat *et al.* 2002). Galiano *et al.* (2000) consider the Department of Cusco to be the centre of diversity for the genus *Polylepis*, containing six of the ten species listed for Perú (Tupayachi 2005). The Cordillera Vilcanota also contains up to nine life zones, each one characterised by a distinct vegetation type (Holdridge 1967; Tupayachi 2005) (Table 1.4). Habitat types and some of the plant species surveyed are presented in Figures 1.4 and 1.5.

Other Andean insect fauna

International peer reviewed publications regarding the ecology and diversity of other insect orders such as Diptera, Tachinidae, Coleoptera and Lepidoptera are still rare for this region. For example, the Lepidopteran fauna of elfin forests in Perú is poorly known (Alonso *et al.* 2001). A list of 80 species of Arctiidae and 17 of Sphingidae have been recorded in the Historic Sanctuary of Machu Picchu (Grados 2002). The Association of Andean Ecosystems (ECOAN) has recorded seven orders of arthropods, distributed among 41 families in *Polylepis* forests.

Table 1.4. Holdridge Life Zones sampled in this present study, with descriptions of the vegetation and prominent plant species

Life Zone	Vegetation Characteristics
Subtropical montane thorn steppe	Corresponds to vegetation found from the valley floor (2700m) up to 3200m. Dominated by deciduous shrubs and abundant annual herbs. The vegetation is also characterised by small leguminaceous trees and large cacti such as <i>Opuntia ficus-indica</i> . In this altitudinal zone the main activity is agriculture, primarily maize cultivation, fruit production and horticulture. Mean temperatures vary between 15°C to 18°C and mean annual precipitation is 460mm.
Subtropical montane dry forest	Found between 3000m to 3400m. The vegetation is characterised by spiny shrubs such as <i>Duranta mandonii</i> , with many <i>Agave</i> spp., <i>Puya</i> spp. and Asteraceae (e.g. <i>Baccharis</i> spp., <i>Ageratina</i> spp., <i>Senecio</i> spp.). This zone is also associated with high land slide activity.
Subtropical humid montane forest	Found at elevations between 3500m to 3800m. Annual precipitation is 580mm and average temperatures range from 6°-12° C. The vegetation is characterised by a mixture of small trees and shrubs. Many of the trees are confined to the edge of rivers, (referred to as either gallery forest or riparian thickets). Important trees include <i>Escallonia resinosa</i> , <i>Vallea stipularis</i> , <i>Alnus acuminata</i> and <i>Myrcianthes oreophylla</i> . Epiphytes, mosses, ferns and vines are also prominent along river edges. The exposed ridge tops are characterised by the absence of trees and densely stunted vegetation referred to as elfin forest.
<i>Polylepis</i> woodlands	<i>Polylepis</i> stands frequently occur in sheltered ravines, along water courses and on boulder slopes, between 3700m to 4860m. The composition of woodlands varies according to altitude; between 3700m to 4200m <i>Polylepis besseri</i> and <i>P. racemosa</i> dominate. Some of the most important plant species associated with <i>Polylepis</i> woodlands are: <i>Escallonia myrtilloides</i> , <i>Escallonia resinosa</i> , <i>Gynoxis</i> spp., <i>Duranta armata</i> , <i>Saracha punctata</i> , <i>Buddleia montana</i> and <i>Myrcianthes oreophylla</i> .
Subtropical pluvial sub-alpine puna	Covers the upper altitudes between 4000m and 4400m. The average annual rainfall is 1500mm and average temperatures range from 3° to 6°C. The vegetation is often mire like, with spongy layers of mosses, lichens and bunchgrass.

Sources: Fjeldså and Krabbe 1990; Kessler and Krömer 2000; Servat et al. 2002; Parkswatch Perú 2004; Tupayachi 2005.

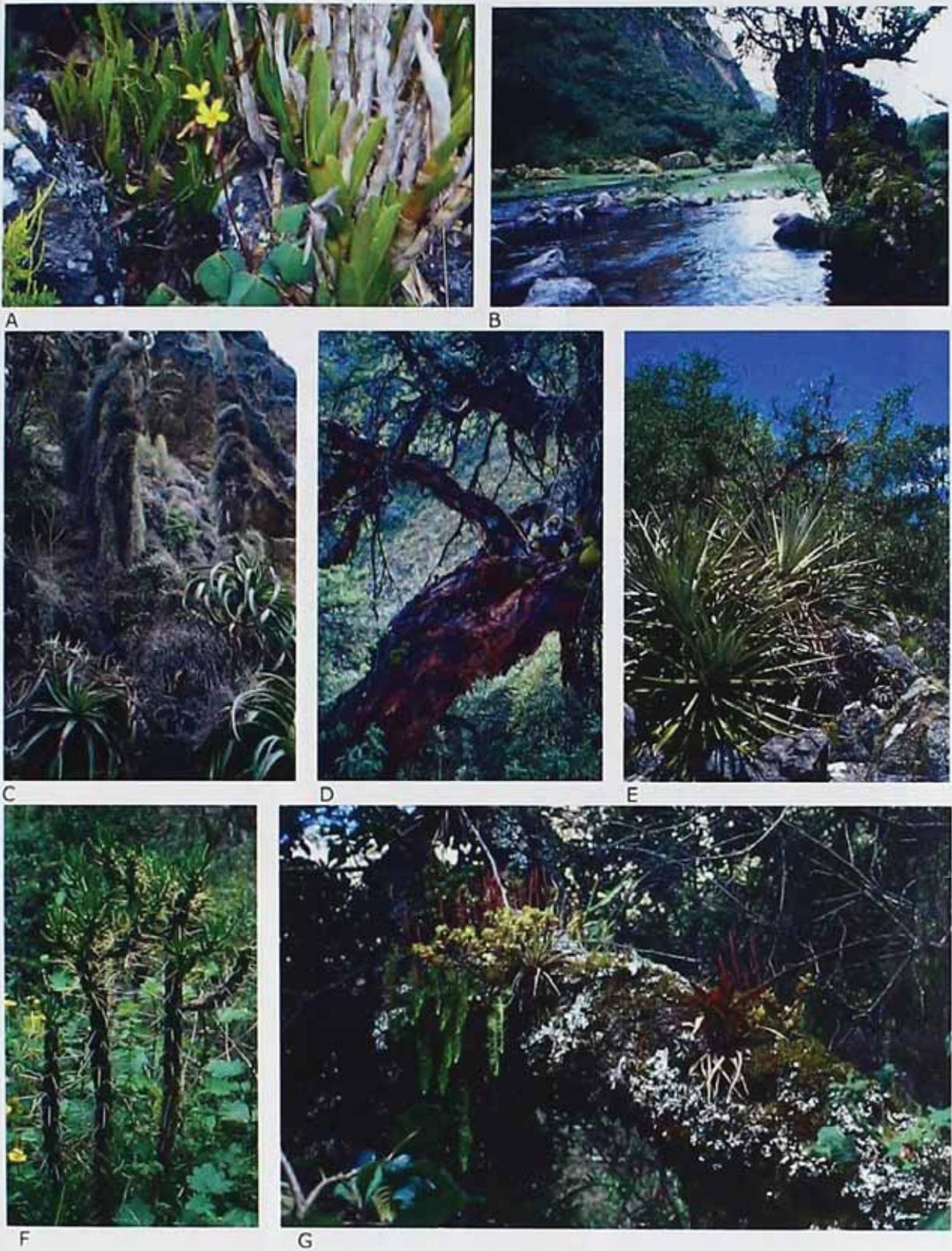


Figure 1.4. Typical habitats encountered in the study area. (A) *Oxalis* sp. growing on scree slope; (B) Riparian; (C) Bromeliads covered in *Tillandsia* sp.; (D) *Polylepis* stands; (E) steep rocky slopes characterised by spiny shrubs such as *Duranta mandonii*, with many *Agave* sp. and *Puya* sp.; (F) landslide areas colonised by cacti; (G) tree branches laden with mosses, lichens and ferns. Photos by Jeff Ollerton.



A



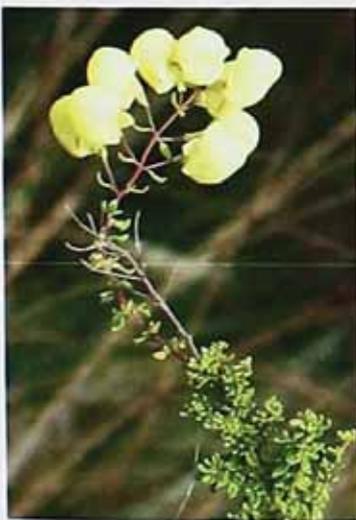
B



C



D



E



F



G

Figure 1.5. Seven of the plant species surveyed: (A) *Barnadesia horrida*, (B) *Oreocallis grandiflora*, (C) *Epidendrum* sp., (D) *Lupinus* sp., (E) Fabaceae sp. (F) *Passiflora tripartita*, (G) *Minthostachys spicata*. Photos by the author.

Andean bee fauna

Bees (Apoidea) are distributed in most biogeographic zones; bee diversity and abundance is greatest in warm, xeric regions, and unusually, bee diversity per km² peaks in the subtropics (latitude 30°-40°) rather than the tropics (Michener 1979). Presently there are an estimated 20,000 bee species with valid names from specimens in museum collections from around the world, although it has been suggested that twice this number may be more realistic (Roubik 1989). The high tropical Andes from Venezuela, Colombia, Ecuador, Perú and Bolivia are among the least known bee faunas of the world. Colombia has been more comprehensively studied than any of the other Andean countries, whilst detailed check lists from Perú, Bolivia and Ecuador are still entirely lacking (Gonzalez and Engel 2004).

Preliminary field surveys conducted by Gonzalez and Engel (2004) from the Andean regions of Venezuela to Perú yielded 131 species from 33 genera, representing all five extant bee families, i.e. Andrenidae, Apidae, Colletidae, Halictidae and Megachilidae. In Perú, 14 species of *Bombus* (bumblebees-Hymenoptera: Apidae) have been listed (Rasmussen 2003). In the Cordillera of Vilcabamba (a neighbouring mountain range of the Vilcanota Highlands) a Conservation International Rapid Assessment Program (Alonso *et al.* 2001) collected 102 species and 10 families of Hymenoptera (bees and wasps). The family Bethyridae was the most diverse with 34 species, followed by Apidae (23 species) and Halictidae (14 species).

Gonzalez and Engel (2004) have identified two general distribution patterns for the Andean bee fauna of Colombia, Venezuela, and Perú. The first is a group of bee species distributed from the lowlands to the highlands (e.g. *Bombus* spp., *Trigona* spp., and the European honeybee *Apis mellifera*). The second is a group with a distribution restricted to high elevations. The majority of Andean bee species occur in cloud forests and only a quarter of species reach high altitude páramo-puna ecosystems. To date Gonzalez and Engel (2004) report that *Bombus (Funebribombus) funebris*; Smith (1854) is the only well known species restricted to the Páramo (Colombia, Venezuela and Ecuador) (Rasmussen 2003). However, in the Sacred Valley, *Bombus funebris* and *Bombus (Robustobombus) melaleucus*; Handlirsch (1888) are not restricted to puna zones and inhabit the entire altitudinal range (SW personal observation).

Apis mellifera in the Sacred Valley

In comparison with other South American countries, there is a lack of any significant data on Peruvian beekeeping, at either the national, regional or local level (Kent 1989). Much of the information reported below was obtained by personal communications. In Perú, before the introduction of the European honeybee (*Apis mellifera*) some five hundred years ago, the only source of honey came from stingless bees in the genera *Melipona* and *Trigona* exploited by the Incas (Javier Llaxacondor, personal communication 2001). In the Sacred Valley the black Italian subspecies *Apis mellifera ligustica* (Spinola) *Apis mellifera carnica* (Pollman) and *A. mellifera caucasica* (Hellmich and Rinderer 1991), is used extensively by beekeepers for pollination services. The majority are part-time and hobby beekeepers and only 5% are professional (Kent 1989). According to the association of beekeepers in Urubamba, Department of Cusco, a total of 390 hives are owned within the Sacred Valley, with an average of ten hives per keeper.

The presence of the African honeybee was first recorded in the early 1970s and Peruvian honeybees now show extensive hybridisation with the African derived *Apis mellifera scutellata*, particularly at intermediate and high elevations (Kent 1989; Quezada-Euán *et al.* 2003). In the highland rainforest of the east side of the Andes 11% of colonies have honeybee hybrids. The rates of admixture between African derived and resident European populations depend on the altitude of the region (Quezada-Euán *et al.* 2003). No information was available from the beekeepers maintaining colonies in the Sacred Valley regarding possible honeybee hybridisation.

Hummingbird fauna

The avifauna of Perú is one of the most species-rich in the world, with more than 1678 species of resident and migrant birds recorded (Wege and Long 1995). Perú is ranked second in the Americas for numbers of threatened birds, of which 7% are endemic, 13% have restricted ranges and 4% are threatened. A total of 89 Key Areas for threatened birds have also been identified (Wege and Long 1995). Perú is ranked third in the Americas for hummingbird (Trochilidae) diversity; over a hundred species have been recorded, of which fifteen are endemic (Johnsgard 1997). The Cusco-Urubamba region supports three restricted-range hummingbirds: White-tufted Sunbeam (*Aglaeactis castelnaudii*), Olivaceous Thornbill

(*Chalcostigma olivaceum*) and Bearded Mountaineer (*Oreonympha nobilis*). The IUCN Red List categorises these three hummingbird species as having a status of *Least Concern* (taxa which do not qualify for Near Threatened or Conservation Dependent (Stattersfield *et al.* 1998). Additionally, all the remaining hummingbird species observed in this present study are categorised as species with a status of Least Concern (Table 1.5).

The Historic Sanctuary of Machu Picchu

The Historic Sanctuary of Machu Picchu is located in the highest part of the eastern central Andes, above the Rio Urubamba, 70km northwest of the city of Cusco. The Sanctuary was established in 1981, under Law (Supreme Resolution) DS 001-81 to protect the ancient citadel of the Incas and the Inca trail. The Sanctuary is located in the Selva Alta zone, and covers a total of 32,592 hectares of which 30,000 hectares are cloud forest (World Conservation Monitoring Centre 1999; ParksWatch Perú 2004). Biological diversity within the protected area is a conservation priority; according to Holdridge (1967) nine distinct life zones have been identified and biodiversity is greater than neighbouring ecosystems. The Management Committee of Machu Picchu, formed in 2001, has created seven zones; Strict Protection Zone; Wildlife Zone; Tourism and Recreation Zone; Special Use Zone; Restoration Zone; Historic-Cultural Zone and a Buffer Zone (ParksWatch Perú 2004). The Buffer Zone includes settlements within the Sacred Valley, such as Piscacucho and Chillca; areas which are included in this present study. Due to the close proximity of the limits of the Historic Sanctuary of Machu Picchu, these sites contain some flora with restricted distributions, such as the Andean shrub *Oreocallis grandiflora* or bird species such as the hummingbird *Leucippus viridicauda*, which inhabits humid forests or forest edges at the lowest elevations at Machu Picchu (Walker 2005). Threatened flora and fauna of the region is presented in Table 1.5.

Table 1.5. Threatened plant and bird species identified in the study region

Plants	Birds
<i>Polylepis racemosa</i> (cd)	Royal Cinclodes (<i>Cinclodes aricomae</i>) (CR)
<i>Polylepis pepeii</i> (cd)	White-browed Tit-spinetail (<i>Leptasthenura xenothorax</i>) (EN)
<i>Polylepis subsericans</i> (cd)	Ash-breasted Tit-tyrant (<i>Anairetes alpinus</i>) (EN)
<i>Polylepis microphylla</i> (cd)	Giant Conebill (<i>Oreomanes fraseri</i>) (CR)
<i>Fuchsia apetela</i> (T)	Andean Condor (<i>Vultur gryphus</i>) (V)
<i>Myrcianthes oreophylla</i> (V)	White-tufted Sunbeam (<i>Aglaeactis castelnaudii</i>) (lc)
<i>Duranta armata</i> (LR/nt)	Olivaceous Thornbill (<i>Chalcostigma olivaceum</i>) (lc)
<i>Alnus acuminata</i> (T)	Bearded Mountaineer (<i>Oreonympha nobilis</i>) (lc)
	Taczanowskii Tinamou (<i>Notoprocta taczanowskii</i>) (VU)
	Sparkling-Violetear (<i>Colibri coruscans</i>) (lc)
	Green-and-white Hummingbird (<i>Leucippus viridicauda</i>) (lc)
	White-bellied Hummingbird (<i>Leucippus chionogaster</i>) (lc)
	Andean Hillstar (<i>Oreotrochilus estella</i>) (lc)
	Giant Hummingbird (<i>Patagona gigas</i>) (lc)
	Shinning Sunbeam (<i>Aglaeactis cupripennis</i>) (lc)
	Great Sapphirewing (<i>Pterophanes cyanopterus</i>) (lc)
	Swordbill Hummingbird (<i>Ensifera ensifera</i>) (lc)
	Tyrian metaltail (<i>Metallura tyrianthina</i>) (lc)
	Black-tailed Trainbearer (<i>Lesbia victoriae</i>) (lc)
	Green-tailed Trainbearer (<i>Lesbia nuna</i>) (lc)
	<i>Coeligena violifer</i> (lc)
	<i>Eriocnemis luciani</i> (lc)

Letters in parentheses indicate conservation status according to IUCN Red List categories. EN: Endangered, V: Vulnerable, lc: least concern, cd: conservation dependent, LR/nt: near threatened, CR: critically endangered

Study sites

The study was carried out in the Cordillera del Vilcanota in nine tributaries (microcuencas) of the Sacred Valley of the Incas (Figure 1.6). This region lies 60km north of the city of Cusco. Surveys were conducted between the villages of Pisac, Ollantaytambo and Chillea, in the provinces of Calca and Urubamba, Department of Cusco. The study sites spanned an area of ca. 60km in length along the Urubamba river, from Huaran to the eastern limits of the Historical Sanctuary of Machu Picchu at Piscacucho, situated between ($13^{\circ} 13' S$, $72^{\circ} 2' W$ and $13^{\circ} 12' 42' S$, $72^{\circ} 21' 41' W$). In the Central Andes, variation in local precipitation is influenced by the following factors: the strength and occurrence of El Niño Southern Oscillation (ENSO) events; the location and intensity of the Bolivian cell; the 'Bolivian high'; sea-surface temperatures of the equatorial Pacific; the location and magnitude of equatorial Atlantic and Amazonian convection; and the level of insolation (Chepstow-Lusty *et al.* 2003). The Peruvian Andes experience a monsoonal climate, characterised by prevailing westerly winds which reverse during December, January and February (wet season). During the wet season ("estación lluviosa"), strong insolation creates a deep convective updraft over the highlands, drawing up moist easterly winds from the Amazon (Chepstow-Lusty *et al.* 2003). The dry season ("estación seca") corresponds to the Southern Hemisphere winter (April to November) when monthly precipitation is very low and minimum temperatures during June and July fall to $0^{\circ}C$ (Sarmiento 1986). The average temperature for Calca and Urubamba at the valley floor is $14.3^{\circ}C$ and mean annual rainfall is 459mm (Tupayachi 2005). During August, the Sacred Valley also experiences moderate westerly winds reaching a maximum velocity of 2-3 metres per second (SW personal observation; Guadalupe *et al.* 2002). Descriptions of each valley are presented in the following section. A schematic representation of the main characteristics of the vegetation encountered along the elevational gradient and indication of the presence of plantations, Inca terraces and honeybee hives for each of the nine valleys is presented in Figure 1.7.



Figure 1.6. Aerial view of the Sacred Valley from Pisac to Chillca. (Source Google Earth 2008)

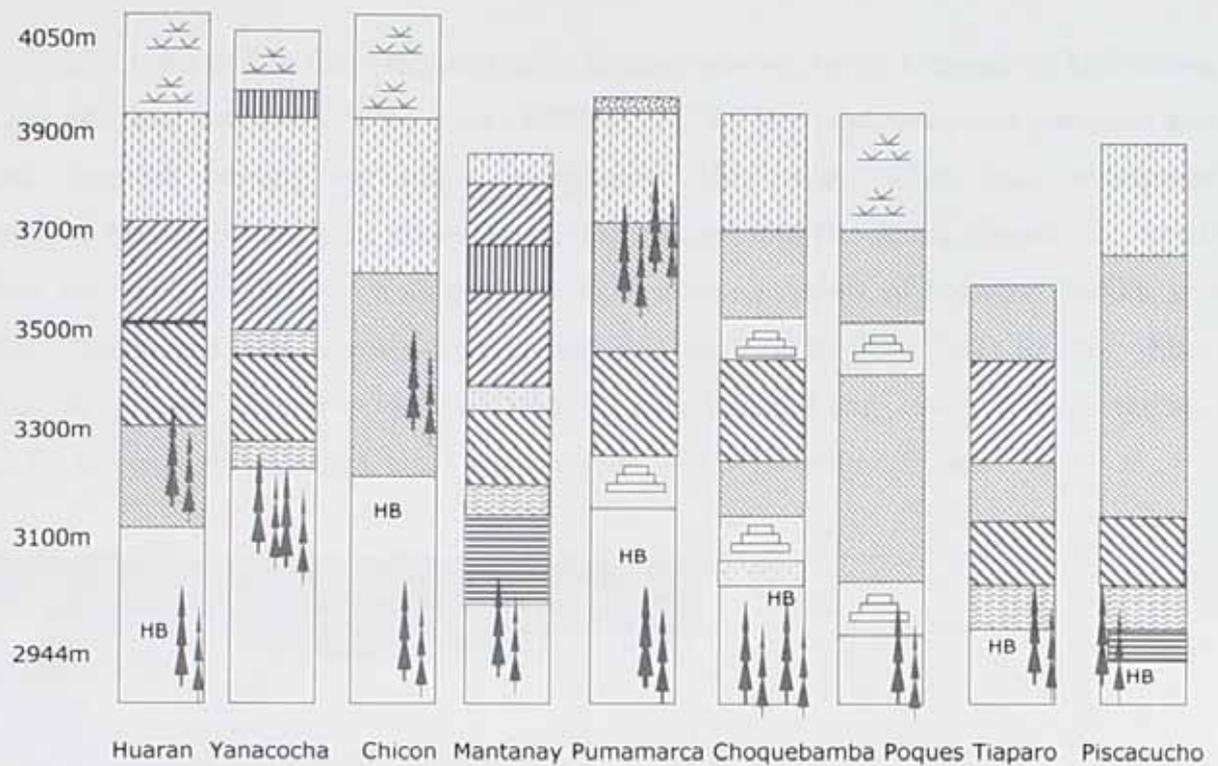
The nine tributary valleys

Huaran

Huarán is situated in the quebrada of Cancha Chanca, Province of Calca, between the villages of Calca and Yucay (13° 13'S, 72° 2'W). Livestock farming and agriculture are prominent at both the lowest elevations and highest life zones. Between 3900m-4200m, numerous corrals known as "Canchas" are present, which give this community its name (ECOAN 2004). Huaran contains important stands of *Polylepis besseri* and *P. subsericans*, with 39 associated plant species (Servat *et al.* 2002). Anthropogenic pressures include extraction of *Polylepis* wood by rural communities, because these forests constitute the only source of native fire wood and building materials. Other pressures include overgrazing, soil erosion which can result in landslides, and the establishment of *Eucalyptus* plantations which supply 70% of the firewood in the Sacred Valley (Chepstow-Lusty and Winfield 2000; Servat *et al.* 2002). The elevational range for transects surveyed at Huaran were between 3200m to 4050m.

Yanacocha

Yanacocha is situated at Huayocari, bordering the Provinces of Calca and Urubamba (13°17' S, 71 °59' W). Anthropogenic pressures mainly include livestock farming, agriculture, overgrazing and widespread planting of *Eucalyptus* in the lower elevations. Yanacocha contains *Polylepis* forests with some of the largest concentrations of populations of *P. besseri* and *P. subsericans*, and has 47 associated plant species. A report of the flora of Yanacocha listed 510 plant species, 270 genera and 96 plant families (Tupayachi 2005). Additionally, (Servat *et al.* 2002) reported 60 species birds, of which half were associated with *Polylepis*. Yanacocha has been identified as an Endemic Bird Area (EBA B27) for the White-browed Tit-Spinetail (*Leptasthemura xenothorax*) and the Ash-Breasted Tit-tyrant (*Anairetes alpinus*) (Wege and Long 1995). The elevational range for transects surveyed at Yanacocha was between 3250m to 3900m.



Key to habitats

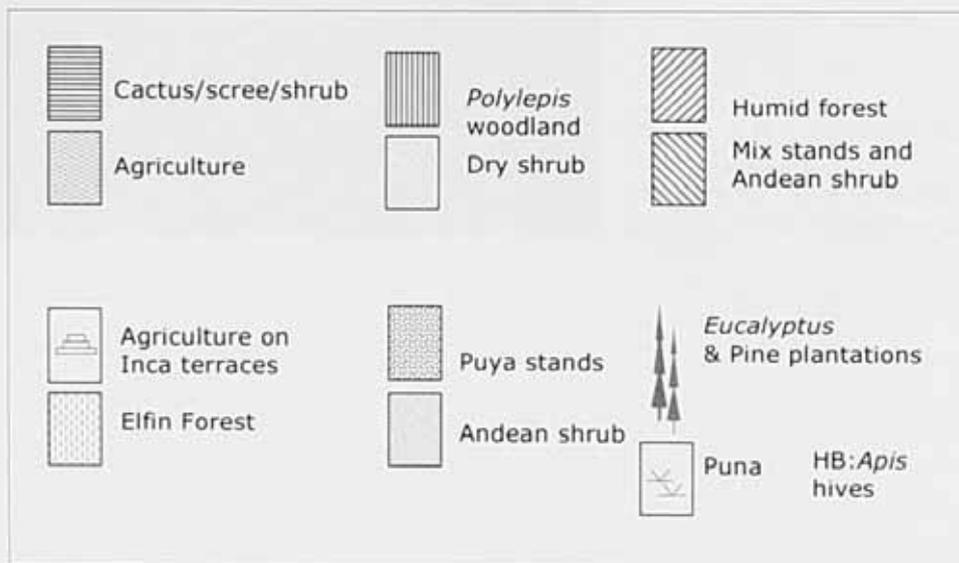


Figure 1.7. Schematic diagram representing the characteristics of each of the nine tributary valleys in terms of different habitats encountered along an elevational gradient from 2944-4050m. Also indicated are the presence of Inca terraces, *Eucalyptus* and pine plantations and honeybee hives

Chicon

Chicon is located in the valley ending at Urubamba town in the Province of Urubamba, and lies 5km east-north-east of the town ($13^{\circ}13' S$, $72^{\circ} 07' W$). Anthropogenic pressures also include livestock farming, agriculture, overgrazing. This valley suffers from widespread planting of *Eucalyptus* and pine plantations in the low and mid elevations (Figure 1.8). Small patches of *Polylepis-Gynoxis* woodland are located at elevations of between 3800m and 4500m. Chicon has been identified as an Endemic Bird Area (EBA B27) for the white-browed tit-spinetail (*Leptasthenura xenothorax*) (Wege and Long 1995). The elevational range for transects surveyed at Chicon was between 3100m to 4000m (Figure 1.9).



Figure 1.8. Left hand side of Chicon showing pine plantations. All photos by the author unless otherwise stated.



Figure 1.9. Views of Chicon showing transect route in red from 3100 to puna at 4000m

Mantanay

Mantanay is located near the village of Yanahuara in the Province of Urubamba ($13^{\circ}12'S$; $72^{\circ}5'W$) (Figure 1.10). Livestock farming, agriculture and overgrazing are also evident in this valley, although there are no *Eucalyptus* plantations. Mantanay contains the largest expanse of *Polylepis* forest in the Vilcanota highlands. Fragments of *P. racemosa* and *P. subsericans* are distributed between 3800m and 4700m. As in Huaran, anthropogenic pressures include extraction of *Polylepis* wood by rural communities, because these forests constitute the only source of fire wood for the nearby populations of Huacahuasi, Huilloc, Patacancha, Rumira Sondormayo, Chupani, and Chalhuacocha (ECOAN 2004). Other pressures include the burning of *Puya* spp. and *Polylepis* and frequent landslide activity during the wet season (SW personal observation) (Figure 1.11). The elevational range for transects surveyed at Chicon was between 3300m to 4000m.



Figure 1.10. View of Mantabay from the valley floor near the village of Yanahuara (source: Google Earth 2007).



Figure 1.11. View of Mantabay from 3500m showing areas of landslide activity

Pumamarca, Choquebamba and Poques

Pumamarca, Choquebamba and Poques are located near the Patacancha Valley approximately 10km north of Ollantaytambo ($13^{\circ}13'52''\text{S}$, $72^{\circ}14'17''\text{W}$) in the province of Urubamba (Figure 1.12, 1.13 and 1.14). The study sites are associated with Inca and pre-Inca remains, including the fort of Pumamarca (Chepstow-Lusty and Winfield 2000; Chepstow-Lusty *et al.* 2003). The landscape in the lower parts is characterised by Inca terraces which are still cultivated today and are important for grazing cattle and horses (SW personal observation) (Figure 1.15). In 1997, the government of Perú initiated the planting over more than 100 million *Eucalyptus* trees, mainly in the *Polylepis* zone (Chepstow-Lusty and Winfield 2000). Widespread planting of exotic species such as *Eucalyptus* and pine is prevalent within the whole of the Sacred Valley from the valley floor up into higher life zones. Indeed, Pumamarca contains a large *Eucalyptus* plantation at over 3500m. The native tree *Alnus acuminata* has a restricted distribution in the Patacancha Valley, surviving only as a few individuals strewn in steep ravines and along water courses (Chepstow-Lusty and Winfield 2000). This tree was found growing along the rivers at these sites, and is considered as an important resource for the hummingbird species which visited the flowers of *Passiflora* species growing within it (SW personal observation). The elevational range for transects surveyed within the Patacancha Valley are as follows: Pumamarca 3200m to 3900m; Choquebamba 3200 to 3900m; Poques 3130m-3900m.



Figure 1.12. Pumamarca (source Google Earth 2007)



Figure 1.13. Choquebamba (source: Google Earth 2007)



Figure 1.14. Poques (Source: Google Earth 2007)



Figure 1.15. Aerial view of Pumamarca and Choquebamba showing cultivated Inca terraces, the Inca fortress of Pumamarca and the *Eucalyptus* plantations (Source: Google Earth 2007)

Tiaparo and Piscacucho

Tiaparo and Piscacucho are situated between the village of Chillca and Km 82 of the Road from Cusco ($13^{\circ} 12' 42''$ S, $72^{\circ} 21' 41''$ W) (Figure 1.16). The sites mark the limits of the Historic Sanctuary of Machu Picchu, and they are located in the Buffer Zone. Both locations are also characterised by ancient overgrown terraces which have now been abandoned. Exotic plantations have not been established in this area; *Eucalyptus* and occasional pines tend to be restricted to the valley floor. These valleys also contain dense forests of "Unca" (*Myrcianthes oreophila*), a cloud forest species recorded from disturbed areas. The World Conservation Monitoring Centre considers this tree as Vulnerable according to IUCN Red list of threatened species (Table 1.5). At the highest altitudes the flora is characterised by elfin forest with many *Oreocallis grandiflora* shrubs (Figure 1.17). The elevational range for transects surveyed at Tiaparo was 2944m to 3700m and between 3011m to 3752m for Piscacucho (Figure 1.18 and 1.19).



Figure 1.16. View of Tiaparo and Piscacucho (centre) from the village of Chillca (Source Google Earth 2007)



Figure 1.17. View of Tiaparo (left) and Piscacucho (right)



Figure 1.18. Views of Tiaparo showing in red the route for transect 3 at approximately 3100-3200m



Figure 1.19. Transect 1 at Tiaparo (left) and marking transect 5 (right) in elfin forest at 3752m.

This thesis starts off by examining community-wide patterns in plant-flower interactions along an elevation gradient the Sacred Valley. Chapter 2 addresses a number of key questions related to trends in species diversity and abundance and how it differs among valleys and altitudes. The chapter examines the generality of the prediction that the altitudinal distribution patterns among functional groups of flower visitors in the Sacred Valley are typical of pollinator distributions in other high altitude environments. Additionally the Chapter addresses how the introduced honeybee (*Apis mellifera*) fits into these communities.

CHAPTER TWO

Plant-Flower Visitor Interactions in the Sacred Valley: Patterns of Diversity and Abundance along an Altitudinal Gradient

Introduction

Patterns of species diversity is a topic which has stimulated much discussion among botanists, ecologists and evolutionists in an attempt to discover general rules concerning the structure, diversity and functioning of terrestrial plant communities (Richerson and Lum 1980 and references therein). The early literature includes evaluations of plant community composition and structure along environmental gradients (summarised by Whittaker 1956), the classification of vegetation types according to life zones (Holdridge 1967) and the application of island biogeography in relation to elevational gradients (MacArthur 1972 cited in Stevens 1992). Assessments of tropical vegetation have shown that floristic composition changes with altitude and that species diversity generally decreases with increasing elevation (Lieberman *et al.* 1996). These marked trends have been described throughout the tropics; for example, in Mexico, Vázquez and Givnish (1998) analysed the composition, structure and diversity of plant communities along a 1000m altitudinal transect from dry forest to cloud forest. They found that plant species richness decreased sharply with altitude, and per sample, the number of species and genera and families declined linearly with elevation. Gentry (1988) reviewed how species richness in plant communities changed on different environmental and geographical gradients. On an altitudinal gradient in the tropical Andes, he found a linear decrease in plant diversity from 1500m to the upper limit of forest above 3000m. However, even close to the tree-line, montane tropical forests were shown to be as equally diverse as the most diverse temperate forests. According to Gentry (1988) with a few notable exceptions (e.g. tropical West Africa and the upper Amazon of Perú), equivalent forests in the Americas, tropical Africa, and tropical Australasia, are similar in plant species richness and floristic composition, but vary considerably in their structure (Gentry 1988).

Along an altitudinal gradient species diversity generally tends to decrease with elevation, although this pattern is not universal for all high mountains (Diamond 1988).

For example, in Ecuador Keating (1999) investigated the species composition and diversity of grass and shrub páramos and found that with increasing vertical distance below the ridge top of the Cordillera Oriental, cumulative species richness increased sharply until the shrub páramo was reached. Also in South America, along the western Andes of northern Chile, plant diversity increases from middle to high elevations, after which it only decreases from high elevations onwards. Diamond (1988) proposed that the most important contributory factors controlling species diversity along an elevational gradient were changes in habitat structural complexity, productivity and area gradient.

Numerous studies from around the world have revealed a reduction in insect diversity, pollinator abundance and activity in high montane and alpine environments. Some of these studies have demonstrated a general trend of declining bee species richness and a greater importance of flies, butterflies and hummingbirds with increasing elevation (Cruden 1972; Arroyo *et al.* 1982; Warren *et al.* 1988; Kearns 1992; McCall and Primack 1992; Hingston and McQuillan 2000; Medan *et al.* 2002). Furthermore, some of these studies revealed that at high elevation sites, flies and butterflies pollinated a greater proportion of the flora, whilst bees declined in importance and pollinated proportionately fewer plant species, and hummingbirds replaced bees. Hummingbird pollination at high altitudes has been documented as prominent in a variety of studies (Cruden 1972; Wolf *et al.* 1976; Snow and Snow 1980; Feinsinger 1983; Kessler and Krömer 2000; Borgella *et al.* 2001). Altitudinal turnover in major groups of pollinators and a decline in visitation rates are attributed to thermoregulatory factors and spatiotemporal fluctuations in pollinator populations. The composition of pollinators may also be influenced by temperature, precipitation, wind speed, geology and land use (Kühn *et al.* 2006). Coincident with this decline with species richness with increasing elevation is an increase in the altitudinal range of some species (Terborgh 1971; Stevens 1992).

Some studies conducted in montane and alpine areas have documented an increase in insect pollination limitation with altitude (Campbell 1987; Arroyo *et al.* 1982; Berry and Calvo 1989). Other reported trends include an increase in pollinator body size with elevation (Malo and Baonza 2002), or an increase in the longevity of individual flowers with altitude (Arroyo *et al.* 1982; Fabbro and Körner 2004). However in Chile, Arroyo *et al.* (2006) surveyed populations of *Chaetanthera euprasiodes* (Asteraceae) and showed that

there were no consistent differences between populations or elevational trends in breeding system, rates of selfing, longevity of flowers and floral morphology.

Ollerton *et al.* (2006b) investigated the geographical variation in diversity and specificity of pollination systems in different latitudinal and altitudinal zones and found that communities at different altitudes did not vary significantly in the mean number of pollination systems. These authors found that communities from three altitudinal zones overlapped considerably in their proportional composition of plants associated with the various pollination systems and suggested that any elevational effects were masked by latitudinal effects. Communities at low altitudes exhibited the least association with fly pollination and communities at high altitude showed a weaker association with fly pollination than did those at mid-altitude. They suggested that on the whole, altitude had little influence on the range and proportions of pollination systems. However, the tropical communities reviewed were all at low elevations (low: up to 300m above sea level, mid: 300-1900m and high: 2000m to 3000m), whereas many of the studies mentioned above where flies were dominant among insect flower visitors were not from montane habitats in the tropics and commenced at much higher elevations rather than from sea level, and incorporated a variety of elevational ranges.

Over the past few years, there has been a resurgence of interest in community studies which evaluate structural patterns among plants and pollinators and identify the potential mechanisms which account for such patterns (Waser and Ollerton 2006). Large scale community studies of plant-pollinator interactions which consider all the visiting insects and birds along an altitudinal gradient are non-existent in tropical America and are still rare in South America as a whole, with fewer than 20 publications in the world. This chapter describes the assemblages of insect and bird visitors to plants in nine tributary valleys of the Sacred Valley to determine if functional groups of flower visitors vary quantitatively in abundance and diversity between valleys and if they also vary quantitatively in abundance and diversity with increasing altitude. The results will then be compared with other altitudinal community studies from various localities around the world. A preliminary reconnaissance of the chosen study sites within the Sacred Valley revealed that honeybees were present at all locations. The literature has presented vast amounts of circumstantial evidence suggesting that the introduced European honeybee (*Apis mellifera*, hereafter *Apis*) competes for resources with the native flower visitors. Therefore the study area offered an

excellent opportunity to collect honeybee data in an area that has not been previously investigated. This chapter addresses the following questions:

1. Does species diversity of functional groups of flower visitors differ among valleys?
2. Does species diversity of visited plant species differ among valleys?
3. How does the flower visitor activity within the Sacred Valley compare with other high altitude montane and alpine systems?
4. What role do honeybees play in these communities?

To explore these questions the following predictions were generated and tested:

- Prediction 1: Diversity of functional groups of flower visitors will decline with altitude

Community level studies along elevational gradients are characterised by a trend toward a dominance of flies among insect flower visitors, a decline in bee species richness and a greater importance of butterflies, flies and hummingbirds, and a progressive decline in pollinator diversity above the tree-line (e.g. Cruden 1972; Arroyo *et al.* 1982; Warren *et al.* 1988; Kearns 1992; McCall and Primack 1992; Hingston and McQuillan 2000; Medan *et al.* 2002).

- Prediction 2: Species richness of visited plants will decline with altitude

Plants diversity falls with increasing altitude in the tropics (Gentry 1988) despite the fact that seasonality is similar between tropical lowlands and highlands (Richerson and Lum 1980). If the environmental and biogeographic trends for plant communities reported above are the general rule, it may be expected to find predictable gradients in species diversity and composition of plants and flower visitors along an elevational gradient.

- Prediction 3: The abundance of different functional groups of flower visitors will vary predictably with altitude

Many montane community studies from around the world have revealed that pollinator abundance and flower visitation rates declines with increasing altitude and that

flower visitation rates are generally low (e.g. Arroyo *et al.* 1982; Warren *et al.* 1988; Totland 1993; Hingston and McQuillan 2000; Medan *et al.* 2002).

Potential impacts of the introduced European honey bee (*Apis mellifera*)

Honeybees have the potential adversely to affect the reproductive success of native plant communities by reducing the amount of pollen available to native pollinators, reducing seed set by displacing native pollinators, or altering pollen flow between plants (Horskins and Turner 1999). Potential negative impacts from honeybees include the overlap in resource use by honeybees and native bees, negative relationships between the abundance of honeybees and native bees and in some cases birds; and interference competition by aggressive exclusion. In order to test effects of *Apis* in these valleys the following predictions were generated:

- Prediction 4: Native visitation will be significantly negatively correlated with honeybee foraging

Honeybees are a successful introduced species with a near global distribution and are now abundant over large areas where they naturally did not occur (Goulson 2003). The high requirements for nectar and pollen of their large colonies compared to wild solitary bees, combined with a remarkably efficient communication system, enable honeybees rapidly to exploit profitable patches of flowering plants (Steffan-Dewenter and Tschamtkke 2000). Several studies have shown a decrease in abundance of native bees in the presence of honeybees foraging on the same plant species (Roubik 1983; Schaffer *et al.* 1983).

- Prediction 5: The proportion of plant species utilised by *Apis* compared to native flower visitors will vary with altitude

Along an altitudinal gradient it has been shown that flies and butterflies grow in importance, pollinating a greater proportion of the flora with altitude (e.g. Arroyo *et al.* 1982; Warren *et al.* 1988). Conversely, at higher elevations bees decline in importance and pollinate proportionally fewer plant species along the same gradient. Additionally, honeybees visit resources utilised by a wide range of native species (Goulson 2003).

- Prediction 6: The proportion of plant species visited by both native bees and honeybees will overlap by less than a third

It is estimated that worldwide honeybees visit close to 40,000 plant species and within any one geographic region up to a hundred or more (Butz Huryn 1997; Goulson 2003). Therefore the potential for disruption of plant-pollinator interactions could be large. Studies which have considered resource overlap have reported that the proportion of total flora utilised by honeybees can range from 33% up to as much as 88% (i.e. Donovan 1980; Menezes Pedro and Camargo 1991; Memmott and Waser 2002; Dupont *et al.* 2003; Kato and Kawakita 2004 and Kwak and Bekker 2006). However, Butz Huryn (1997) argues that honeybees often use less than a third.

- Prediction 7: *Apis* actively displaces other species when foraging

Interference competition arises when honeybees occur in high densities and displace native bees and birds from floral resources by their physical presence. Although occasional jostling by honeybees at flowers has been reported (Roubik 1991; Gross and Mackay 1998), aggression between honeybees and native bees is considered rare and relatively unimportant (Butz Huryn 1997).

Methods

Transects

The study was conducted in the Cordillera del Vilcanota mountain range in the Sacred Valley of the Incas. Surveys were conducted in nine tributary valleys or “microcuencas”, between the towns of Calca, Ollantaytambo and Chillca (Figure 1.6 Chapter 1). A team of up to eight students from the Universidad de San Antonio Abad del Cusco (UNSAAC) with expertise in botany, entomology and ornithology were selected to assist with fieldwork. In order to minimise observer biases, preliminary surveys of transects were undertaken in each valley to fully train fieldworkers and to assess the quality and accuracy of taxonomic identification. A total of 90 transects (10 per valley) were sampled during the dry season between April and October 2002. In total, 90 person-hours of observations were carried out. Additional observations were made throughout the year from December 2001 to November 2002. The distributions of different life zones varied with altitude across valleys and were difficult to distinguish into discrete zones, therefore five altitudinal bands were surveyed instead. Each transect was subdivided at each altitude into two 500 x 3m sampling areas, running parallel either side of established trails. Transects were marked with 10 points at intervals of 50m, corresponding to the following mean altitudes: (1) 3147-3235m, (2) 3351-3424m, (3) 3464-3558m, (4) 3653-3746m, (5) 3846-4003m. The topography of the mountain chain dictated where transects started and finished, and whether they were orientated horizontally across or vertically up the valleys. The lowest transect commenced at 2944m and the highest transect terminated at 4050m, giving an altitudinal range of 1106m.

The order in which each transect was walked in each of the valleys was determined using random numbers (1-5). Shade temperature and relative humidity was measured prior to and after each census period using a temperature probe and a pocket hygrometer and altitude was measured using an altimeter. It was decided not to present the temperature and humidity data, since insect and bird abundance was not correlated with these variables. During each one hour observation period, two people walked each transect on either side.

Observers were timed with stop watches to walk without stopping at a pace 12 minutes to every 50m.

In each transect the following data were recorded: the identification of flower visitor and the number of individuals observed foraging on each plant species, the identification of the plant species being visited, whether animals foraged for nectar or pollen and if encounters between flower visitors resulted in displacement. Individual flower visitors were only recorded at each plant species and were not subsequently followed to different plant species. No independent measure for the abundance of plants was calculated. Instead abundance of plant species was measured as a surrogate based on the number of visits by insects and birds.

For comparative purposes, a sampling method was designed to obtain data on flower visitor activity at different times of the day and at different elevations. Surveys were undertaken during the following time periods: 08:00-09:00, 10:00-11:00, 12:00-13:00, 14:00-15:00, and 16:00-17:00, allowing for one hour to reach the next transect. Due to changeable weather conditions and the different microclimates experienced in these valleys, transects were surveyed in windy, overcast, sunny and misty conditions. In some valleys the sun rose over the mountains later, therefore surveys started at 09:00 when the first diurnal insects were active. It was only possible to survey transects once per valley, due to logistic constraints in undertaking the fieldwork in these remote localities.

All insects observed were captured with a standard butterfly net while foraging and deposited individually into labelled plastic bags. A morphospecies approach was used to classify insects. Morphospecies do not involve the identification of species; instead taxa are separated based on external morphological traits by non-specialists (Oliver and Beattie 1995; Derraik *et al.* 2002). When a morphotype was a frequent visitor to a plant species, several specimens were captured. At the end of each day, insects were placed in a killing jar with a few drops of ethyl acetate and left for two hours. The larger specimens were sorted by an experienced entomologist to order, then further sorted into morphospecies (Oliver and Beattie 1995) and pinned in the field. The remainder of the collection was divided into two groups of specimens, the first contained numbered vouchers and the second contained a random subsample of specimens from each morphospecies. These collections were later examined in the laboratory by Professor Erick Yabar (Department of Entomology,

UNSAAC). The specimens were subsequently pinned, where possible specimens were identified to species level using a dissecting microscope and keys, and by comparing vouchers with the reference collection at UNSAAC. It was not possible to match any of the Tachinidae morphospecies to taxonomic species because published keys were not available.

Functional taxonomic groups of flower visitors (*sensu* Fenster *et al.* 2004; Ollerton *et al.* 2007b) were identified as follows: Diptera were divided as Syrphidae, Tachinidae, and all other Diptera. Hymenoptera were divided as all other bees, *Bombus* spp., Vespidae and *Apis*. Hummingbirds were identified in the field using the field guide *Birds of the High Andes* (Fjelds  and Krabbe 1990). Voucher specimens of insects have been retained at the Faculty of Biological Sciences at the University of San Antonio Abad, Cusco, Per . Plant specimens were collected, coded as vouchers, and pressed in the field. Vouchers of all the plants are kept in the Herbarium Vargas (UNSAAC). It was not possible to identify some of the plants to species level, therefore those specimens were categorised into morphospecies.

Statistical Analyses

Prediction 1: Diversity of functional groups of flower visitors will decline with altitude

Prediction 2: Species richness of visited plants will decline with altitude

To compare the diversity of flower visitors and visited plants along the altitudinal gradient and diversity among valleys, Shannon-diversity indices were calculated for functional groups using the software Species Diversity and Richness version 4 (Seaby and Henderson 2006). Shannon-Wiener index rather than Simpson's index was chosen to characterise diversity because it takes into account richness and evenness, but is weighted towards rarer species (Krebs 1994).

After calculating the Shannon-diversity indices for functional groups of flower visitors, a standard bootstrap method for estimating the upper and lower 95% confidence intervals was undertaken for each sample (functional groups of flower visitors in each

separate valley) and for pooled samples across all valleys. Variance H' values for each index were then used to calculate standard errors.

Prediction 3: The abundance of different functional groups of flower visitors will vary predictably with altitude

To compare differences in the median number of functional groups across each altitude, data were pooled across nine valleys and were tested using a Kruskal-Wallis one-way analysis of variance. Statistical analysis was performed using SPSS 11.5 for Windows (2006, SPSS Inc, Chicago, IL, USA).

Potential impacts from *Apis*

Prediction 4: Native visitation will be significantly negatively correlated with honeybee foraging

To test the prediction that native visitation will be significantly negatively correlated with honeybee foraging abundance, correlations of abundances of *Apis* versus each functional group at each altitudinal band (1-5) were calculated and for rank abundances of *Apis* versus each insect order/bird family in each valley. Data were pooled from all nine valleys for both analyses. Pearson correlations were used when data were normally distributed; Spearman rank correlations were used when data were non-normally distributed. Hemiptera and Vespidae were relatively rare and therefore excluded from statistical analysis. Statistical analysis was performed using SPSS 11.5 for Windows (2006, SPSS Inc, Chicago, IL, USA). Means are presented as \pm 1SD.

Prediction 5: The proportion of plant species utilised by *Apis* compared to the native flower visitors will vary with altitude

To determine if there were differences in the proportion of plant species visited by each functional group across altitudinal bands, a G – test of independence was calculated. Only the most frequent visitors were included in this analysis, since rarer groups (especially Vespidae and Hemiptera) were only recorded in some samples. To determine if there were differences in the proportions of plant families visited by each functional group of

pollinators along the altitudinal gradient, a G – test of independence was also calculated. These were calculated using a spreadsheet.

Randomisation procedures to test for significant differences between indices

By itself, a diversity index often gives little more insight than species number, so no formal inferences can be made (Seaby and Henderson 2006). According to Solow (1993), when such an index is calculated it is important to assess the significance of observed differences in community structure. Solow (1993) described a simple randomisation test which can be utilised in conjunction with any measure of community structure based on species abundances such as diversity indices. The advantage of this test is that it makes no assumptions that all species in the community are observed (Solow 1993). Therefore, to assess the differences in observed diversity, randomisation tests were calculated for flower visitors and visited plants in each valley and for functional groups of flower visitors across altitudes, pooled from nine valleys. The randomisation test re-samples 10,000 times from a distribution of species abundances produced by a summation of the two samples (Solow 1993). A summary of the procedure outlined from the manual Species Diversity and Richness Version 4 is presented below (Seaby and Henderson 2006).

1. The diversity of each of the samples was calculated and then differences between these indices (delta) were calculated.
2. The two samples were tested for a significant difference in their index and were then combined together to form a single joint sample.
3. The individuals in this joint sample were then randomly assigned to two samples, each of which had the same number of individuals as the actual two samples.
4. The diversity index for each of these generated samples was then calculated and the difference between these indices (delta) was stored.
5. 10,000 random assignments and calculation of delta were undertaken.
6. The observed value of delta was compared against the observed distribution of delta values generated at random to determine if the observed p - values for the

difference between the indices of the two samples could have been generated by random chance.

Sampling effort: Heterogeneity test

To determine whether sampling effort was sufficient to estimate total species richness in the Sacred Valley, heterogeneity tests were calculated for flower visitors and visited plants using Species Diversity and Richness Version 4. This test is more appropriate for species abundance, rather than presence-absence data (Seaby and Henderson 2006). If the expected curve rises more steeply from the origin then heterogeneity is greater than could be explained by random sampling error alone (Seaby and Henderson 2006).

Prediction 6: The proportion of plant species visited by both native bees and honeybees will overlap by less than a third

To determine if the proportion of plants visited by honeybees and native bees overlapped, the number of plant species shared by both taxa was calculated as percentages.

Prediction 7: *Apis* actively displaces other species when foraging.

To ascertain if the presence of honeybees resulted in active or passive displacement of native fauna, frequencies of encounters between honeybees and other flower visiting taxa were calculated from visitation data recorded in each valley.

Results

Plant communities

From April to October 2002, 1583 visits were observed to flowers of 114 plant species belonging to 38 families (see Table 2.1, Appendix I). Forty species (36%) belonged to the Asteraceae family, followed by Fabaceae (7 spp., 6%) and Lamiaceae (4 spp., 3.5%), (unidentified plant species were not included in the calculations). Asteraceae was the most heavily visited family and received 60% of total visits, followed by the Lamiaceae (14%) and Myrtaceae (6%). Small shrubs belonging to the Andean genus *Baccharis*, *Ageratina* and *Aristeguietia* were the most frequently visited members of the Asteraceae and also the most common plant species. These montane, páramo and sub-páramo shrubs are characterised by a brush-like flower head, consisting of small, long slender corolla tubes. More than half the plant species visited in the Sacred Valley were represented by only one or two plant individuals (Appendix I). The scrub layer associated with small patches of *Polylepis* woodlands which were included in some transects were: *Gynoxys* sp., *Satura boliviana*, *Ageratina sternbergiana*, *Baccharis* sp. The most common tree species in the communities visited were *Myrcianthes oreophila* and *Escallonia resinosa*. The vegetation characteristics of the study area are presented in Table 1.4 (Chapter 1), in total the altitudinal gradient surveyed passed through five life zones.

Animal communities

In total 144 morpho-species from five insect orders, and seven species of hummingbirds as were recorded as flower visitors (Appendix II, III and IV). Of the insects specimens collected, only five were identified to species level. Potential pollinators were classified into 11 functional groups as follows: *Apis*, *Bombus* spp., all other bees, Vespidae, all other Diptera, Syrphidae, Tachinidae, Trochilidae, Lepidoptera, Coleoptera and Hemiptera. Since pollinator efficiency was not measured, all visitors are referred to as flower visitors. From the material collected, only 20 insect families were identified (Appendix II). Dipterans were the most abundant flower visitors and comprised 48% of the total flower visitors of which Syrphidae comprised of 17% and Tachinidae 4% (Figure 2.1). Hymenopterans were the second most abundant insect order, comprising 33% of the total

flower visiting fauna. Of the Hymenoptera, *Apis* dominated the bee fauna (26%); only 6% of the individual flower visitors were native bees.

All other Diptera (i.e. excluding Syrphidae and Tachinidae) dominated the visitor assemblage in six of the nine valleys, whilst, Hymenoptera were more prominent at Chicon, Yanacocha, Tiaparo and Mantanay (Table 2.1). Coleoptera and Trochilidae were similar in abundances (8% and 6% respectively) although hummingbirds may have been underrepresented due to the transect census method undertaken. Hummingbirds were easily disturbed from foraging and did not tend to visit plants within the sampling area, remaining instead on the periphery. This may explain why during the census period, only 9 out of the 15 species of hummingbirds previously identified in the vicinity of the study areas were recorded visiting plants within the transects.

The flower-piercer *Diglossopsis cyanea* was only observed in one transect and on one occasion, therefore this bird was not included in any of the analyses. Hemiptera and Vespidae were uncommon flower visitors and were not observed in every valley. Lepidoptera, including diurnal moths, only constituted 4% of the total fauna. Although Lepidoptera only comprised of a small fraction of the total fauna, butterflies were comparatively a species rich group with 15 species recorded among 67 individuals. The most frequently observed butterfly was *Metardaris cosinga* (Hesperiidae) which at Yanacocha reached relatively high local abundances (34 individuals). The most species rich functional groups were Dipterans (all other Diptera, Tachinidae and Syrphidae) and a large proportion of these flower visiting flies were members of the muscoid group (Appendix II). Although Coleoptera were a small component of the total flower visitor fauna, beetles were ranked second in terms of species richness with 28 species between 126 individuals (Figure 2.1). The most important beetle families were: Merilidae, Bruchidae, Chrysomelidae, Coccinellidae and Curculionidae. The Apidae was the family with the highest proportion of bee species and the two species of Hemiptera observed belonged to families Cicadellidae and Lygaeidae. As mentioned above, although hummingbirds are ranked comparatively quite low, this group was almost certainly under represented.

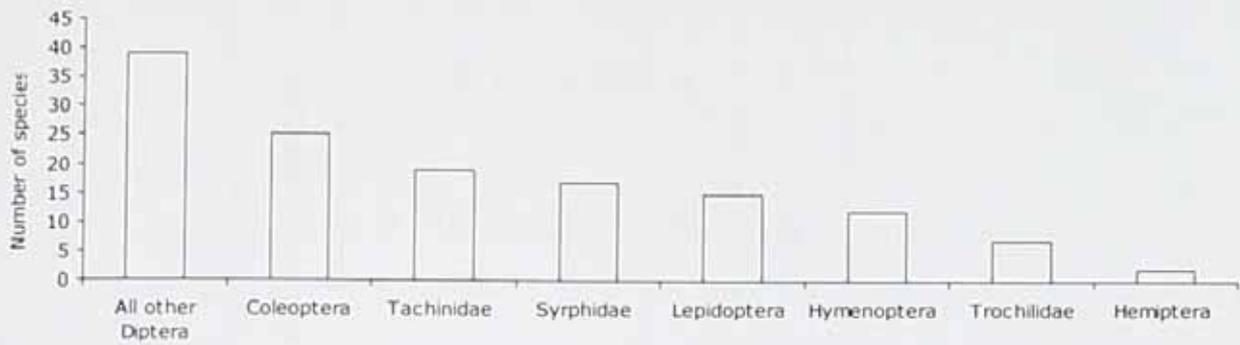


Figure 2.1. Species richness of different functional groups of flower visitors. *Apis* and Vespidae were grouped with Hymenoptera.

The proportion of the total flora utilised by each functional group

Compared to the rest of the flower visiting taxa, honeybees visited a relatively large proportion of the total flora surveyed in the Sacred Valley (13%) and were ranked joint fourth with Coleoptera (Figure 2.2). However, it should be noted that many of the plant species surveyed were only visited occasionally by one insect order or by one individual and that comparing the proportions of plants per altitudinal band may be more representative of the whole community (as calculated in Figure 2.9. and Table 2.9).

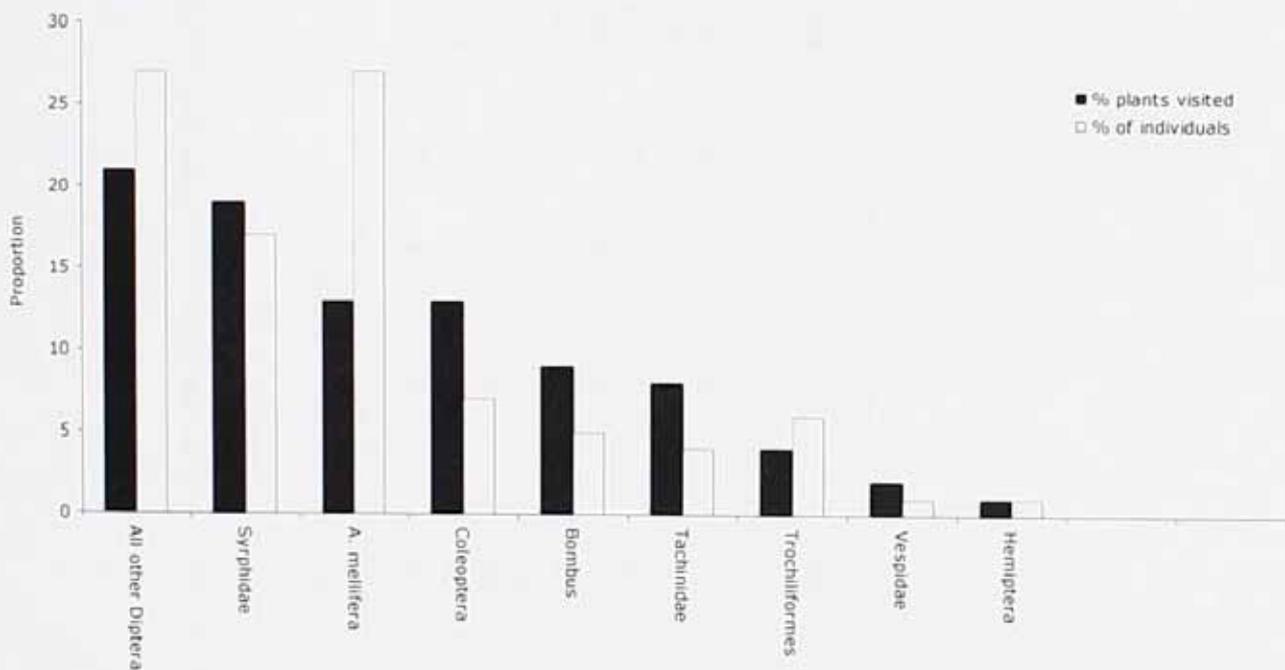


Figure 2.2. Proportions of different functional groups of flower visitors and plants species visited in the Sacred Valley. Data were pooled across nine sites and calculated as percentages from visits to 114 plant species.

Table 2.1. Total number of flower visitors in each of the nine valleys

	<i>Apis</i>	Lepidoptera	<i>Bombus</i>	Bees	Diptera	Syrphidae	Tachinidae	Coleoptera	Trochilidae	Hemiptera	Vespidae	Total
Huaran	40	0	3	0	4	8	1	0	26	0	0	82
Yanacocho	79	41	0	1	111	25	11	4	3	0	2	277
Chicon	104	4	5	1	26	21	4	10	33	0	0	208
Mantanay	47	5	20	2	12	32	21	5	19	0	0	163
Pumamarca	24	2	21	3	55	20	0	41	0	0	1	167
Choquebamba	7	0	19	1	86	27	3	12	1	15	3	174
Poques	29	5	10	0	55	41	4	34	0	0	3	181
Tiaparo	74	2	0	3	47	34	5	6	4	0	1	176
Piscacucho	24	14	4	0	22	61	14	16	0	0	0	155
Total	418	67	82	10	419	260	61	126	91	15	8	1583

Species accumulation curves and the measure of sampling effort

Heterogeneity plots for number of visited plant species and the number of species of flower visitors are presented in Figures 2.3 and 2.4. Species accumulation curves for both visitors and visited plants did not reach an asymptote, suggesting that sampling effort in the Sacred Valley was not sufficient to observe all species. Both Coleman curves were above the observed acquisition curves, suggesting high sample heterogeneity.

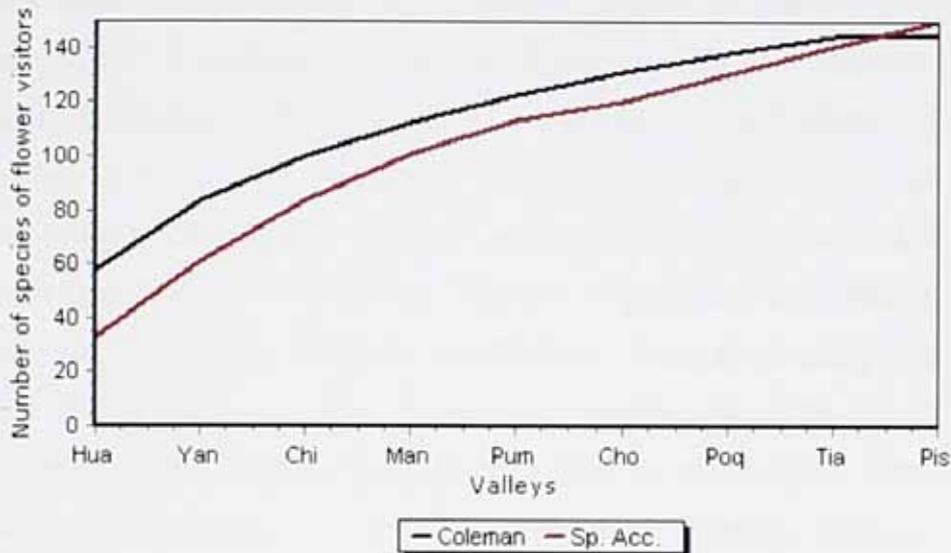


Figure 2.3. Plot of heterogeneity for species and morphospecies of flower visitors observed across nine valleys. Observed species accumulation curve in red, and expected Coleman accumulation curve in black.

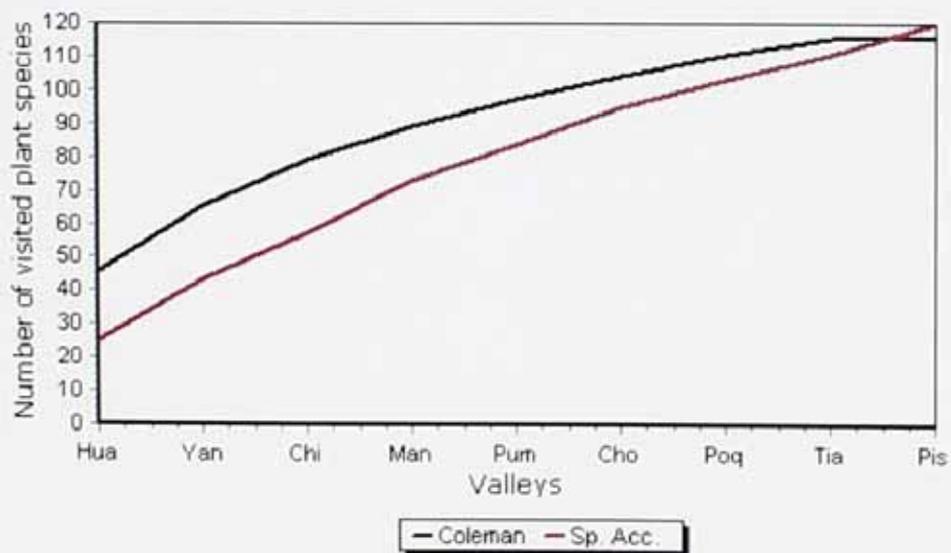


Figure 2.4. Plot of heterogeneity for visited plant species observed across nine valleys. Observed species accumulation curve in red, and expected Coleman accumulation curve in black.

Diversity of flower visitors between valleys

The Shannon-Wiener diversity index for flower visitors varied from 3.18 in Poques to 1.96 in Huaran (Table 2.2, Figure 2.5). Those valleys which recorded the highest index values were Poques, Piscacucho, Choquebamba and Pumamarca. The least diverse valleys were Huaran, Chicon and Tiaparo. Species diversity and evenness of flower visitors was significantly different between valleys (Table 2.2). Huaran was highly significantly different to all of the valleys, with the exception of Chicon and Tiaparo. Whilst diversity in Yanacocha was highly significantly different to Chicon and Tiaparo, no difference was found with the remaining valleys. Diversity in Chicon was highly significantly different with most of the valleys geographically located far away, with the exception of Tiaparo. This result could be explained in part by the most frequently recorded species they share in common (e.g. *Apis*, *Aglaeactis cupripennis*, *Colibri coruscans*). Mantabay was significantly different to all of the valleys with the exception of Yanacocha. Species diversity in Pumamarca, Choquebamba and Poques was not significantly different between sites. These three valleys recorded the highest number of flower visitor species in common (ranging from 19-22). This result is not surprising since these valleys are located very close to one another. However, Tiaparo and Piscacucho are also situated close to one another, but diversity between sites was highly significantly different.

Table 2.2. Results of a randomization test (Solow 1993) using a Shannon-Wiener index with 10 000 random partitions. *P* values for a two sided test estimate the probability that visitor diversities between valleys are equal. For a summary of methods see statistical analysis section. (*P* ≤ 0.05) indicated in bold

Yan	0.0001							
Chi	0.3500	0.0001						
Man	0.0030	0.1380	0.0395					
Pum	0.0001	0.7790	0.0001	0.0280				
Cho	0.0001	0.4970	0.0001	0.0079	0.6820			
Poq	0.0001	0.1660	0.0001	0.0026	0.2260	0.4350		
Tia	0.2870	0.0001	0.8930	0.0451	0.0001	0.0001	0.0001	
Pis	0.0001	0.5350	0.0001	0.0051	0.5506	0.8230	0.6229	0.0001
Valley	Hua	Yan	Chi	Man	Pum	Cho	Poq	Tia

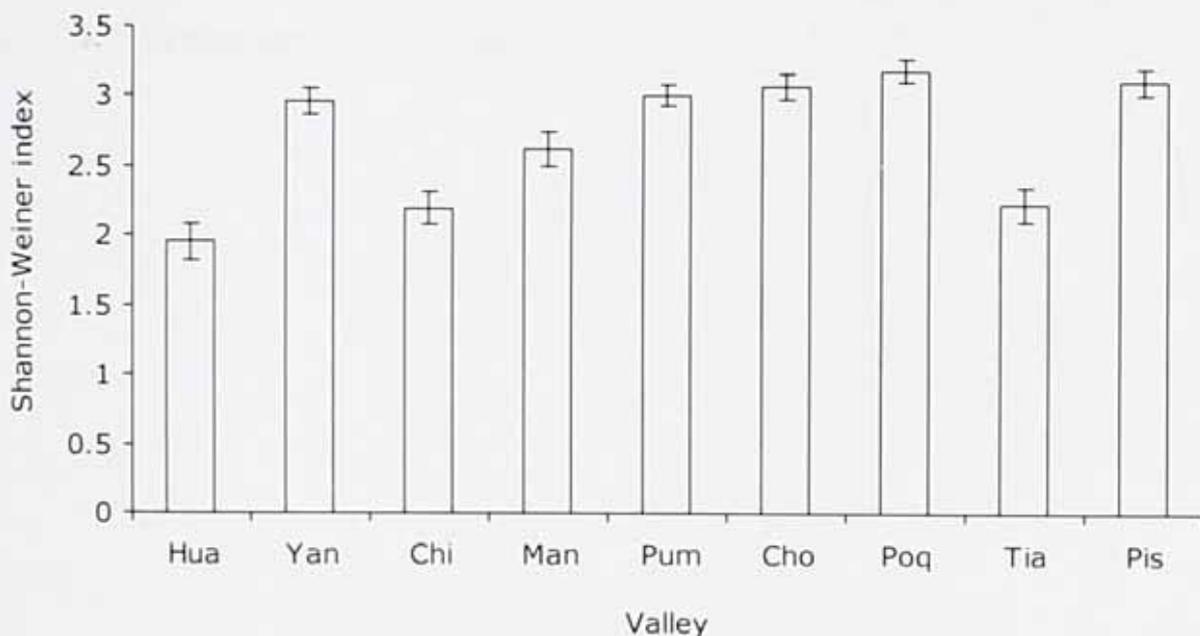


Figure 2.5. Shannon-Wiener H' diversity indices for species of flower visitors recorded in each valley. Error bars (\pm SE). Indices were calculated using data according to morphospecies and where possible to species level. Valleys; **Hua**, Huaran; **Yan**, Yanacocha; **Chi**, Chicon; **Man**, Mantanay; **Pum**, Pumamarca; **Cho**, Choquebamba, **Poq**, Poques; **Tia**, Tiaparo; **Pis**, Piscacucho.

Diversity of visited plant species

The Shannon-Wiener diversity index for visited plant species varied from 2.70 (Choquebamba) to 1.63 (Huaran) (Figure 2.6). Those valleys which recorded the highest index values were Choquebamba, Poques and Mantanay. The least diverse valleys were Huaran, Yanacocha and Pumamarca. Plant species diversity was significantly different in many of the valleys (Table 2.3). Huaran was highly significantly different to Mantanay, Choquebamba and Poques and marginally different to Pumamarca, Tiaparo and Piscacucho. No differences were found between those valleys situated closest to Huaran (e.g. Yanacocha and Chicon). Chicon recorded similar diversities to Tiaparo and Piscacucho and was highly significantly different to Mantanay, Choquebamba and Poques. Species diversity in Mantanay was significantly different in all valleys, except for Choquebamba and Piscacucho. Diversity between Pumamarca and Choquebamba and Pumamarca and Poques was marginally significantly different, whereas species diversity in Choquebamba and Poques was equal, with 12 plant species in common. Similarly, diversity between Tiaparo and Piscacucho was the same, with 10 plant species in common.

Table 2.3. Results of a randomization test (Solow 1993) using a Shannon-Wiener index with 10 000 random partitions. *P* values for a two sided test estimate the probability that diversities between valleys are equal. For a summary of methods see statistical analysis section. (*P* < 0.05) indicated in bold

Yan	0.3190							
Chi	0.5870	0.1820						
Man	0.0001	0.0007	0.0094					
Pum	0.0630	0.3540	0.0330	0.0100				
Cho	0.0001	0.0035	0.0100	0.1184	0.0200			
Poq	0.0010	0.0007	0.0062	0.0410	0.0200	0.7260		
Tia	0.0360	0.2840	0.6530	0.0473	0.5760	0.0393	0.0302	
Pis	0.0310	0.0052	0.2970	0.2410	0.5768	0.1813	0.1190	0.5991
Valley	Hua	Yan	Chi	Man	Pum	Cho	Poq	Tia

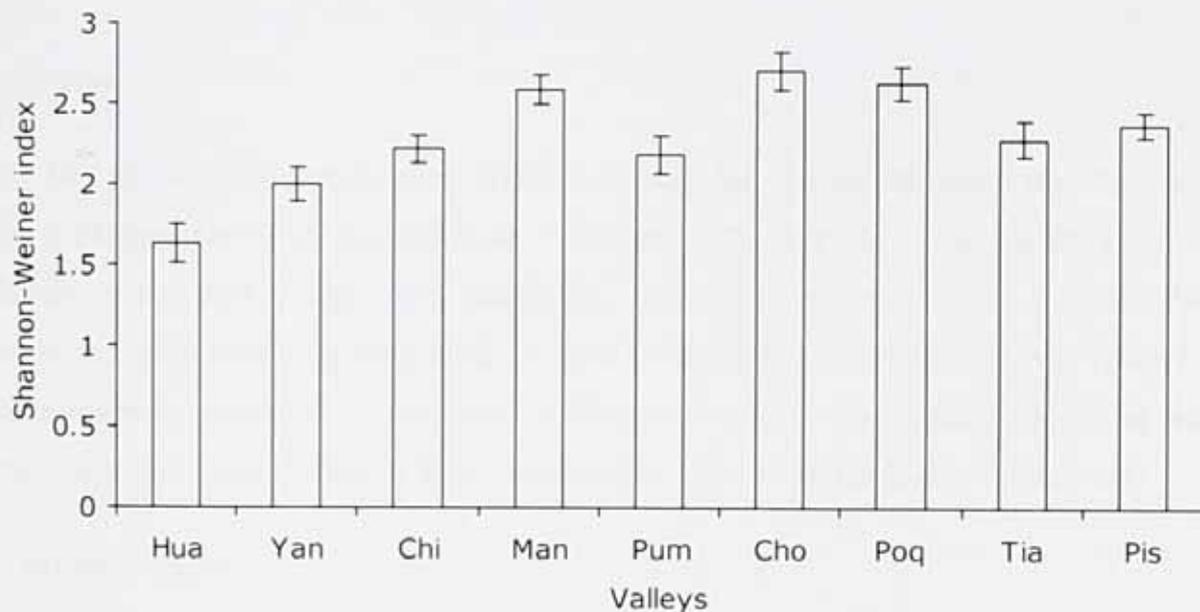


Figure 2.6. Shannon-Wiener H' diversity indices for visited plant species in each valley. Error bars (\pm SE). Indices were calculated using data of visited plant species identified to the species level in the majority of cases. Valleys; **Hua**, Huaran; **Yan**, Yanacocha; **Chi**, Chicon; **Man**, Mantanay; **Pum**, Pumamarca; **Cho**, Choquebamba; **Poq**, Poques; **Tia**, Tiaparo; **Pis**, Piscacucho.

Prediction 1: Diversity of functional groups of flower visitors will decline with altitude

All flower visitors

The Shannon-Wiener diversity index for all flower visitors among altitudes varied from 3.40 (altitude 5) to 2.84 (altitude 1) (Figure 2.7). Overall species diversity did vary significantly with altitude and was greatest at the three highest elevations. Altitude 1 was highly significantly different to all altitudes except for altitude 2, and altitude 2 was significantly different from altitude 5 (Table 2.4). A general trend showed that above 3464m diversity between the three highest elevations remained the same, but the highest altitudes were significantly different to the lowest altitudes. Those functional groups of flower visitors which varied significantly with altitude are described in detail below:

Syrphidae

The Shannon-Wiener diversity index for Syrphidae among altitudes varied significantly from 2.14 (altitude 5) to 1.42 (altitude 1) (Figure 2.7). Altitude 1 was highly significantly different to altitudes 4 and 5 and marginally different to altitudes 2 and 3 (Table 2.4). In general, a similar trend was evident for Syrphid diversity compared to all flower visitors when pooled together; above 3351m (altitude 2) diversity between the remaining altitudes was the same, whilst the lowest altitudes were significantly different to the highest elevations.

All other Diptera

The Shannon-Wiener diversity index for all other Diptera among altitudes varied from 2.52 (altitude 2) to 1.92 (altitude 3) (Figure 2.7). Dipteran morphospecies diversity varied significantly among altitudes and was highest at altitude 2, followed by altitude 5 and lowest at altitude 3 (Table 2.4). Altitude 2 was highly significantly different to altitude 3 and marginally different to altitude 4, whereas altitude 4 was highly significantly different to altitude 5. Dipteran morphospecies diversity peaked at altitude 2, dipped at altitudes 3 and 4 and peaked again at the highest altitude.

Lepidoptera

The Shannon-Wiener diversity index for Lepidoptera among altitudes varied from 1.64 (altitude 3) to 0.35 (altitude 3) (Figure 2.7). It was not possible to calculate an index for altitude 5 since only one individual butterfly (*Metardaris cosinga*) was recorded. Lepidopteran morphospecies diversity varied significantly among altitudes and was highest at altitude 3 (Table 2.4). Altitude 4 was highly significantly different from altitude 3 and marginally different to altitudes 1 and 2. The trend for butterflies indicated that diversity was highest at the lower altitudes, and above 3653m diversity decreased sharply.

Hymenoptera

The Shannon-Wiener diversity index for Hymenoptera among altitudes varied from 1.07 (altitude 4) to 0.57 (altitude 2) (Figure 2.7). Bee diversity varied significantly with altitude and peaked at altitude 4. Excluding altitude 4, diversity between all remaining elevations was the same (Table 2.4).

Trochilidae

The Shannon-Wiener diversity index for Trochilidae among altitudes varied from 1.07 at altitude 5 to 0.57 at altitude 4 (Figure 2.7). Hummingbird diversity varied significantly among altitudes, although only marginally (Table 2.4). A general trend showed that at 3464m and above, diversity between the three highest elevations varied, whilst at the three lowest elevations, diversity between altitudes remained the same.

With the exception of hummingbirds, the above results revealed highly significant differences among altitudes for each functional group. Species diversity did change with altitude in these Peruvian valley systems; therefore Prediction 2 was accepted.

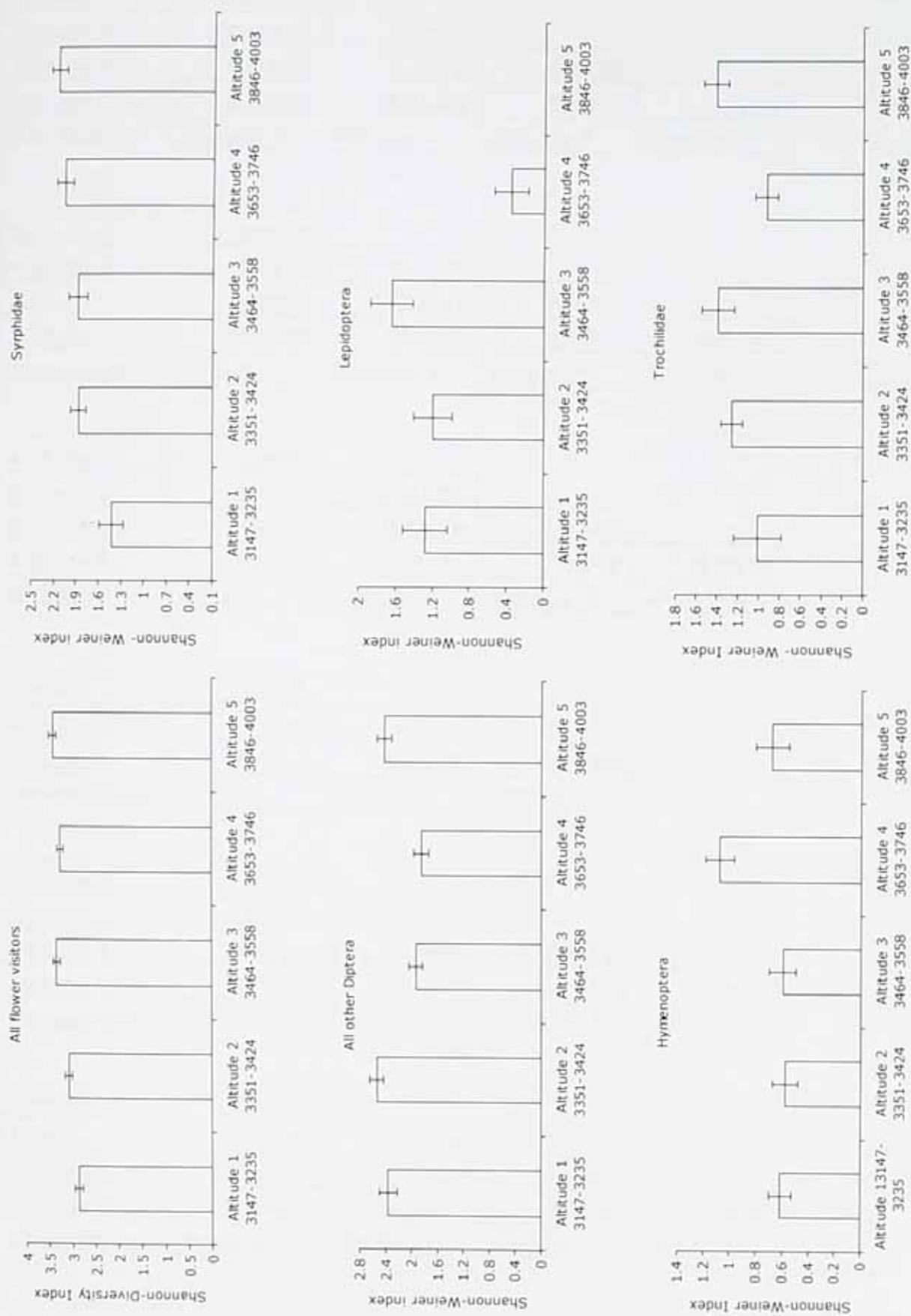


Figure 2.7. Shannon-Wiener H' indices for all flower visitor and each functional group of flower visitors. Data pooled across altitudes. Error bars (\pm SE)

Table 2.4. Results of a randomization test (Solow 1993) using a Shannon-Wiener index with 10 000 random partitions. *P* values for a two sided test estimating the probability that diversities of between altitudes are equal. Data pooled across altitudes. (*P* ≤ 0.05) indicated in bold. Marginal values (*P* < 0.10) indicated in italics

Altitude 2	0.1330			
Altitude 3	0.0029	<i>0.0929</i>		
Altitude 4	0.0108	0.2372	0.5132	
Altitude 5	0.0002	0.0160	0.4971	0.1744
All visitors	Altitude 1	Altitude 2	Altitude 3	Altitude 4

Altitude 2	<i>0.0656</i>			
Altitude 3	<i>0.0577</i>	0.9590		
Altitude 4	0.0014	0.2631	0.3377	
Altitude 5	0.0002	<i>0.0656</i>	0.1870	0.6149
Syrphidae	Altitude 1	Altitude 2	Altitude 3	Altitude 4

Altitude 2	0.4049			
Altitude 3	0.3360	0.0009		
Altitude 4	0.0492	0.0120	0.6449	
Altitude 5	0.8160	0.6117	0.0249	0.0072
Diptera	Altitude 1	Altitude 2	Altitude 3	Altitude 4

Altitude 2	0.8531		
Altitude 3	0.3720	0.2831	
Altitude 4	<i>0.0606</i>	0.0375	0.0069
Lepidoptera	Altitude 1	Altitude 2	Altitude 3

Altitude 2	0.8106			
Altitude 3	0.9388	0.9478		
Altitude 4	0.0069	0.0036	0.0118	
Altitude 5	0.7158	0.5774	0.6591	0.0301
Hymenoptera	Altitude 1	Altitude 2	Altitude 3	Altitude 4

Altitude 2	0.7251			
Altitude 3	0.4670	0.556		
Altitude 4	0.9163	0.1477	<i>0.0809</i>	
Altitude 5	0.5856	0.3851	0.9198	<i>0.0645</i>
Trochilidae	Altitude 1	Altitude 2	Altitude 3	Altitude 4

Prediction 2: Species richness of visited plants will decrease with altitude

Visited plant species richness increased significantly with altitude (Pearson $r = 0.97$, $P = 0.006$, $N = 5$) and reached a maximum at altitude 5. Similarly, the number of visited plant families also peaked at the highest altitude. Contrary to expectation, a trend of diminishing species richness among visited plant species and families along the elevational gradient was not detected, therefore Prediction 3 was rejected.

Table 2.5. Visited plant species richness per altitudinal band

	Altitude 1	Altitude 2	Altitude 3	Altitude 4	Altitude 5
Mean altitude (m)	3147-3235	3351-3424	3464-3558	3653-3746	3846-4003
Number of visited plant species	38	44	42	48	52
Number of visited plant families	16	18	15	15	20

Prediction 3: The abundance of different functional groups of flower visitors will vary predictably with altitude

The mean and median number of individual flower visitors recorded per transect in each altitudinal band is shown in Figure 2.8. The results showed that the data were highly skewed with a large spread around the means and medians. Kruskal Wallis analysis of variance for the number of individuals per altitudinal band revealed that only *Apis* abundance varied statistically with altitude (Table 2.6). Honeybees were the most abundant flower visitor at the two lowest altitudes and declined with altitude. Lepidoptera were absent from altitudes three and only one individual was recorded at altitude 5. In general, butterflies were rare; the relatively high median and mean values recorded for Lepidoptera was attributed to the seasonal emergence of adult butterflies at Yanacocha. Rare species in terms of relative abundances (all other bees, Vespidae and Hemiptera) were not seen at every altitude and two orders were absent at altitude 3. In summary, flies did not significantly increase in abundance with altitude and dominate the flower visiting fauna at the highest elevation as predicted. Of the Hymenoptera, only honeybees predominated at lower elevations and became rarer in the

insect assemblages along a gradient of increasing elevation. In contrast, *Bombus* spp. was found at every elevation and mean and median numbers were highest at altitude 4. Thus, for the majority of functional groups of flower visitors (with the exception of honeybees) abundances of functional groups did not vary with altitude, therefore Prediction 3 is rejected.

Table 2.6. Kruskal Wallis analysis of variance of differences in median number of individuals of flower visitor taxa per altitudinal band in all nine valleys

	<i>df</i>	χ^2	<i>P</i>
<i>Apis</i>	4	10.4	0.03
<i>Bombus</i> sp.	4	2.19	0.70
All other bees	4	4.15	0.38
Lepidoptera	4	0.74	0.94
All other Diptera	4	1.59	0.81
Syrphidae	4	1.35	0.85
Tachinidae	4	2.53	0.63
Coleoptera	4	1.14	0.88
Trochilidae	4	5.33	0.25

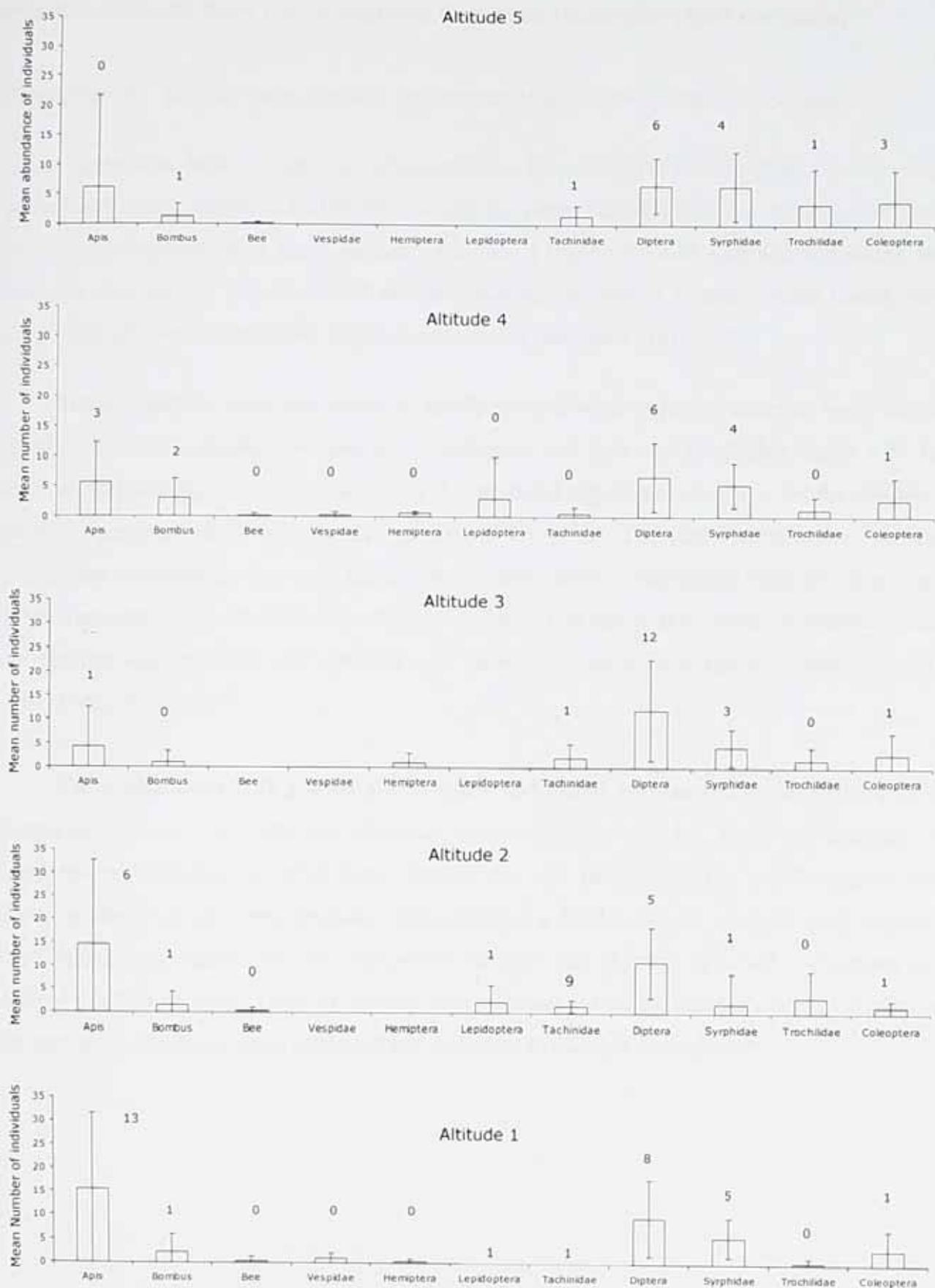


Figure 2.8. Mean number of individuals (\pm SD) per transect in each altitudinal band (1-5). Median values are shown above each column. Transects correspond to the following mean altitudes: (1) 3147-3235m, (2) 3351-3424m, (3) 3464-3558m, (4) 3653-3746m, (5) 3846-4003m.

Possible impacts from the introduced European honeybee (*Apis mellifera*)

Prediction 4: Native bees decline proportionately in the presence of *Apis*

Spearman rank correlations of abundances between *Apis* and each functional group pooled across all valleys and altitudinal bands are presented in Table 2.7. Strong significant positive correlations were found between *Apis* and Lepidoptera and *Apis* and Syrphidae and between *Apis* and all other bees. Of all the functional groups of flower visitors tested, only Coleoptera showed a significant negative association with *Apis* (Table 2.7).

Similar patterns were also found at the finer level when separate analyses were carried out for individual altitudes for *Apis* and Lepidoptera and *Apis* and Syrphidae (Table 2.8). Of the five altitudes tested, only altitude 2, and 3 revealed significant results, whereas altitude 5 showed marginal significant results at the $P < 0.1$ level. Pearson's correlations showed Tachinidae and *Bombus* spp. were highly statistically positively correlated with *Apis* at altitude 3, whereas Syrphidae showed a significant relationship at the $P < 0.05$ level. Spearman's rank correlations indicated that only *Bombus* spp. showed a significant negative relationship with *Apis* at only at altitude 5.

The results from both pooled altitudes and individual altitudes indicated that for most functional groups, as honeybee numbers increased, so too did mean abundances of Lepidoptera, Syrphidae, all other bees, *Bombus* spp. and Tachinidae. This result suggests that these functional groups were probably responding to a third factor. In contrast, only negative correlations were found between abundances of *Apis* and *Bombus* spp. and Coleoptera and only at the $P < 0.05$ level. Thus, in general, native flower visitor abundances did not decline in the presence of honeybees as predicted and therefore Prediction 4 is rejected.

Table 2.7. Spearman's rank correlations of abundances of *Apis* versus each functional group, (1-5) in all valleys

<i>A. mellifera</i> versus	All altitudes pooled	
	r_s	<i>P</i>
Lepidoptera	0.348	0.019
<i>Bombus</i> sp.	0.022	0.885
All other bees	0.356	0.016
All other Diptera	-0.109	0.475
Syrphidae	0.380	0.010
Tachinidae	0.228	0.132
Coleoptera	-0.304	0.042
Trochilidae	0.203	0.181

N = 45. Bold entries indicate *P* values < 0.05

Table 2.8. Pearson and Spearman's rank correlations of rank abundances of *Apis* versus each functional group analysed separately for individual altitudes. Only those altitudes with significant values are presented

<i>A. mellifera</i> versus	Altitude 2		Altitude 3		Altitude 5	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	r_s	<i>P</i>
Lepidoptera	0.886	0.001	0.4440	0.2310	-	-
<i>Bombus</i> sp.	-0.237	0.540	0.0951	0.0001	-0.6600	<i>0.0530</i>
All other bees	0.152	0.697	-0.3350	0.3780	0.6000	<i>0.0880</i>
All other Diptera	0.614	0.540	-0.3350	0.3780	0.3220	0.3980
Syrphidae	0.531	0.141	0.6850	0.0420	0.1850	0.6340
Tachinidae	-0.380	0.313	0.9020	0.0010	0.6050	<i>0.0840</i>
Coleoptera	<i>0.068</i>	0.863	-0.2390	0.2680	-0.3920	0.2970
Trochilidae	0.122	0.755	0.2680	0.4860	-0.0570	0.8860

N = 9. Bold entries indicate *P* values < 0.05; italic entries indicate *P* values < 0.1

Prediction 5: The proportion of plant species utilised by *Apis* compared to the native fauna will vary with altitude

The proportion of native plant species visited by *Apis* compared to some of the native fauna did vary with altitude, therefore Prediction 5 is accepted (Figure 2.9). No significant differences were found between Hymenoptera; all other bees, *Bombus* spp. and honeybees utilised proportionally similar numbers of species with altitude (Table 2.9). Along the altitudinal gradient, honeybees used a relatively small fraction of the total flora (range 13%-32%) (Table 2.9). The rankings between the proportions of flowers visited by honeybees compared to bumblebees remained the same throughout the altitudinal range, except for altitude 4, where bumblebees visited a higher proportion of plant species than honeybees (Table 2.9). Honeybees also visited a relatively high proportion of plant species at the highest elevations. Although small native bees were not recorded at altitude 3, they visited a relatively constant proportion of plant species along the gradient and visited more plant species at altitudes 2 and 4. Of the dipterans, Tachinidae were the only functional group that did not significantly differ from honeybees. Similarly, no significant differences were found between the proportion of plants used by honeybees and butterflies. Significant differences in the proportion of plant species visited across altitudinal bands however, were found between *Apis* and all other Diptera, *Apis* and Syrphidae, *Apis* and Coleoptera, and *Apis* and Trochilidae (Table 2.9, Figure 2.9).

A major elevational trend indicated that the relative proportion of plant species visited by flies (all other Diptera + Syrphidae + Tachinidae) varied between groups with elevation. All other Diptera utilised the highest proportion of plants of all the pollinators, and reached a maximum at altitude 1 and 3 (49%). Syrphidae visited the most plant species at altitudes 2 and 4. The observed trends for all the taxa (except honeybees, hummingbirds and all other Diptera) showed that the proportion of plant species visited diminished at the highest elevation in comparison with that of altitude 4. Conversely, Syrphidae and Trochilidae visited the highest proportion of plants species at altitudes 4 and 5 respectively and for all other Diptera, the proportion of plant species visited was the second highest at altitude 5. Hummingbirds tended to visit the highest percentage of plants above 3500m and visited the most at altitude 5. Honeybees visited more plant species at lower elevations, whereas bumblebees peaked at altitudes 2 and 4. Overall, Lepidoptera visited a small fraction of the total flora, the highest proportions they visited were recorded at the lowest elevations. Vespidae and Hemiptera were

absent from altitudes 3 and 5 and visited a small proportion of the flora, therefore they were not included in analysis (Table 2.9). Coleoptera visited the highest percentage of plant species at altitude 1 (23%) and peaked again at altitude 4 (24%). Although differences were found between groups in terms of plant species being visited, the proportion of plant families utilised by different functional groups did not vary with altitude ($G = 4.14$; $df = 4$; $P = 1.00$).

Table 2.9. G - test for differences in proportion of plant species visited by *Apis* versus each taxon across altitudinal bands 1-5. Numbers are percentages. Significant differences between groups ($P = > 0.05$) indicated in bold. $Df = 4$. Only the most abundant visitors were included in the analysis. $N = +1$ for all other bees for analysis

Altitude	<i>Apis</i>	<i>Bombus</i>	Bees	Lepidoptera	Diptera	Syrphidae	Trochilidae	Tachinidae	Coleoptera
5	18	6	4	2	45	34	22	8	12
4	13	21	6	6	38	42	11	13	23
3	21	12	0	7	49	30	14	9	9
2	30	21	4	16	32	37	9	19	9
1	32	16	6	8	39	26	8	8	24
	G	6	7.1	6	6	13.2	15	5.7	14.8
	P	0.2	0.13	0.2	0.02	0.01	0.01	0.22	0.01

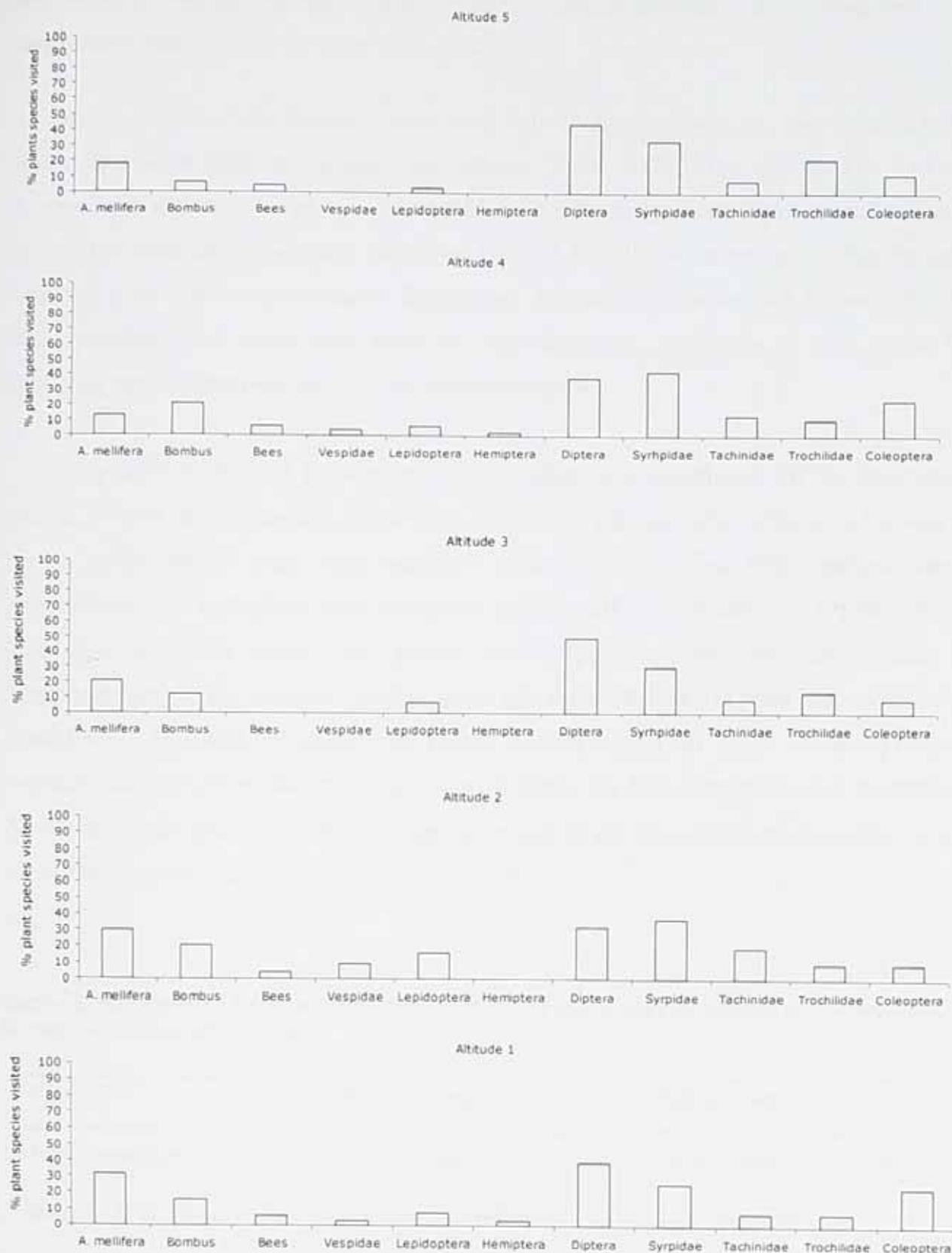


Figure 2.9. Proportion of plant species visited by each taxon per altitude. Transects correspond to the following mean altitudes: (1) 3147-3235m, (2) 3351-3424m, (3) 3464-3558m, (4) 3653-3746m, (5) 3846-4003m.

Prediction 6: The proportion of plant species visited by both native bees and honeybees will overlap by less than a third

In the whole of the Sacred Valley, 418 individual honeybees, 82 bumblebees and 10 native bees were observed visiting plant species (Table 2.11); 31% of a total of 114 plant species were visited by honeybees compared to 26% by native bees. Plant species visited by both native bees and honeybees comprised only 14.4%; 11.2% were only visited by native bees and 2.3% were only visited by honeybees. Additionally, those plant species visited by both honeybees and native bees were the most abundant; consisting of 53% of the total flowering plants surveyed, thus Prediction 6 is accepted.

Only 2.6% of the 114 plant species in this study were introduced. Of the plant species visited by both honeybees and native bees, only one plant species (*Melilotus alba*) was non native, whilst native bees were exclusive visitors to the alien plant species *Lamium amplexicaule* and honeybees were exclusive visitors to the introduced tree *Prunus serotina*. The most important nectar and pollen source for honeybees and native bees was *Minthostachys spicata*, whereas *Jungia rugosa* was the most frequent plant species utilised by bumblebees. *Baccharis buxifolia* was ranked second across all bees, whilst *Myrcianthes oreophila* was ranked as the third most visited plant for both honeybees and bumblebees. Native bees were uncommon flower visitors throughout the Sacred Valley, but were observed to visit *Barnadesia horrida* for pollen (Table 2.10).

Table 2.10. Ranking for the three most important plant species visited by honeybees, bumblebees and native bees

	<i>Apis</i>	<i>Bombus</i> spp.	Native bees
1	<i>Minthostachys spicata</i>	<i>Jungia rugosa</i>	<i>Minthostachys spicata</i>
2	<i>Baccharis buxifolia</i>	<i>Baccharis buxifolia</i>	<i>Baccharis buxifolia</i>
3	<i>Myrcianthes oreophila</i>	<i>Myrcianthes oreophila</i>	<i>Barnadesia horrida</i>

Prediction 7: *Apis* actively displaces other species when foraging

The survey of 90 transects yielded very few observations where *Apis* used interference competition when foraging. Of the 1583 visits, only 14 encounters were recorded where *Apis* physically displaced the native fauna from flowers (Table 2.11). *Bombus* spp. were the only bees observed physically displacing *Apis*. In the majority of interactions honeybees were passive, so therefore Prediction 7 is rejected.

Table 2.11. Observations and outcomes of encounters between *Apis* and other flower visiting taxa

<i>Apis</i> with	Total No. of encounters	No. of displacements <i>Apis</i> loses	No. of displacements <i>Apis</i> wins
<i>Bombus</i> spp.	10	4	6
Lepidoptera	1	0	1
All other Diptera	3	0	3
Syrphidae	4	0	4

Discussion

Plant communities

The plant communities in the Sacred Valley visited by insects and birds were dominated by shrubs and herbs of the Asteraceae family, particularly plants belonging to the Andean genus *Baccharis*. This pattern is consistent with the nearby Patacancha Valley where 33% of the flora consists of members of the Asteraceae (Chepstow-Lusty *et al.* 1996) and with other studies of montane plant communities in Venezuela (Berry and Calvo 1989), Mexico (Vázquez and Givnish 1998), the Andes (Gentry 1988), Utah, (Collins *et al.* 1983), South Africa and USA (McCall and Primack 1992) and Argentina (Medan *et al.* 2002). The varied floral morphology of the Asteraceae attracted a wide range of insects and bird species and provided one of the main floral resources in the community. *Myrcianthes oreophila* (Myrtaceae) was also prominent; this tree produced large floral displays, consisting of hundreds of easily accessible open brush flowers, attracting a diverse array of flower visitors. Plants from the Fabaceae, Onagraceae and Lamiaceae families were also important floral resources for bees. Some plants associated with *Polylepis* woodlands such as *Gynoxys longifolia* were important for species of hummingbirds. Plant communities and animal assemblages are discussed in more detail in Chapters 3 and 4.

Animal assemblages: functional groups

Diptera were the most abundant functional group of flower visitors, followed by Hymenoptera, Coleoptera, Trochilidae and Lepidoptera (Table 2.1). These findings mirror that of the hierarchy of functional groups (with the exception of hummingbirds) reported by Medan *et al.* (2002) (see Appendix IV). Considering flower visitor activity within the whole of the Sacred Valley, altogether the results were most consistent with Smith (1975), Primack (1983), Warren *et al.* (1988); Medan *et al.* (2002) (Rio Blanca). In New Zealand, Primack (1983) found that Dipterans, in particular Tachinidae and Syrphidae were the most prominent functional groups of pollinators. Tachinid flies were considered especially important pollinators because they foraged in cold and rainy weather. In the Sacred Valley, Syrphidae and Tachinidae were also observed foraging in windy and cooler conditions. Of the Diptera,

all other flies were the most abundant flower visitors followed by Syrphidae, whereas Tachinidae were only minor visitors. Dipterans (all other flies, Syrphidae and Tachinidae) all visited a relatively high proportion of plant species (Figure 2.2). Abundances of Coleoptera were similar to many of those studies presented in Appendix IV, which contrasted with results reported by Primack (1983). In New Zealand, Coleoptera were not considered to be important pollinators at sites since they were present in low densities and did not move between flowers. In the Sacred Valley, beetles visited a relatively high proportion of plant species at all altitudes and were present in comparable densities to many of the other functional groups of flower visitors (Figure 2.9). Although their status as pollinators was not measured, this may be comparable with the situation in KwaZulu-Natal (South Africa) where chafer beetles were common flower visitors to a wide range of plant species at an elevation of ca. 1100m (Ollerton *et al.* 2003).

Lepidoptera were not considered to be an important functional group of flower visitors since they visited few plant species and only accounted for small fraction (4%) of the total visitor fauna (Figure 2.1). This finding is consistent with three other montane systems (e.g. Primack 1983; Warren *et al.* 1988; Medan *et al.* 2002), but is in marked contrast with the plant-pollinator assemblages in Chile, where butterflies were important pollinators of 24% of the flora (Arroyo *et al.* 1982). Similarly, butterflies also comprised high proportions of the total visitor fauna in California (17%) and Argentina (20.9%) (Moldenke 1975; Medan *et al.* 2002) (Appendix IV).

Compared to Chile, Hymenopterans were less diverse and abundant in the Sacred Valley. This finding is not surprising, since the lower Mediterranean sclerophyllous scrub in Chile is a notable region of high bee diversity (Moldenke 1975, Arroyo *et al.* 1982; Medan *et al.* 2002). Nevertheless, Hymenoptera still accounted for 33% of the pollinator assemblage and collectively visited 27% of all plant species surveyed. In the Sacred Valley the majority of bees were introduced honeybees (26%), followed by bumblebees, whereas in Chile, Arroyo *et al.* (1982) only recorded one species of *Bombus* and honeybees were not prominent. In Argentina, honeybees were not the dominant bee species and were only observed at Río Blanca (Medan *et al.* 2002). Hemiptera were uncommon flower visitors within the Sacred Valley which is consistent with other floras (Primack 1983).

The relative contribution of hummingbirds to the whole plant–pollinator assemblage was likely to be an under representation because a total of 91 individuals were recorded, representing only 6% of the total fauna. During the census period only 7 out of 15 species of hummingbirds previously identified in the study area were observed visiting plants within transects. Although line transects may be a suitable census technique for recording insect activity, this sampling method may have resulted in biases for bird species. For example, most of the hummingbird species in this study were highly territorial and remained close to their chosen resource, and some species such as *Ensifera ensifera* were trap-lining altitudinal migrants, moving great distances up and down the valleys (Fjeldså and Krabbe 1990; Walker 2005). Moreover, hummingbirds were easily disturbed from foraging when the sampling was undertaken. Faunal composition and the relative abundance of hummingbird species may be affected by a number of parameters such as their morphological-behavioural attributes, available resources and the distributional limits of a particular bird species (Terborgh 1971; Feinsinger and Colwell 1978; Borgella *et al.* 2001). Indeed, eleven species of hummingbirds observed in the study area are considered *Polylepis* specialists. These small patches of forests are distributed between 3600m and 4500m (Fjeldså 2002a; Servat *et al.* 2002).

Based on prior observations throughout the year, hummingbirds may be an important functional group of pollinators for some plant species in this valley system, in contrast with pollinator assemblages at higher latitudes in the southern hemisphere, where hummingbird diversity is much lower (e.g. Arroyo *et al.* 1982; Medan *et al.* 2002 -see Appendix IV). However, the actual status of each functional group as pollinators could not be assessed without further rigorous studies on the pollination efficiency of individual species within these groups (see Chapter 5). Since flower-visitor fauna may be highly variable in composition between years (Petanidou and Potts 2006) and visitation by flies and Andrenid bees above the tree-line can be highly erratic in plant populations and among years (Arroyo *et al.* 2006), sampling would be necessary over many years to draw definitive conclusions.

Morphospecies and their accuracy

It should be recognised that the use of higher-taxon levels as surrogates for the number of real species could potentially bias estimates of total flower visitor richness in this present study. There has been strong debate regarding the usefulness and application of the morphospecies approach to estimate species richness of invertebrates (Derraik *et al.* 2002 and references therein). Nevertheless, since accurate species level identification of specimens from large collections is often impractical, morphospecies may be effective in the initial sorting stage, before specimens are examined by specialist taxonomists (Kerr *et al.* 2000).

Oliver and Beattie (1995) suggested that for some environmental monitoring, invertebrate morphospecies can be used as surrogates for real species, and that non-specialists with minimal training can provide precise estimates of species richness without compromising scientific accuracy. However, the accuracy of morphospecies separation has been shown to vary greatly among different invertebrate groups (Derraik *et al.* 2002). Correct separation by non-specialists of one taxonomic species to one morphospecies may be of limited use due to lumping species (classifying more than one species as a single morphospecies) or splitting species (separating one species into more than one morphospecies) (Oliver and Beattie 1995; Kerr *et al.* 2000; Derraik *et al.* 2002). High levels of lumping and splitting tends to occur when species are sexually dimorphic, when size and colour differ between sexes or when particular families show a diverse array of colour patterns. This can result in either species overestimation or underestimation of true species numbers (Kerr *et al.* 2000; Derraik *et al.* 2002). Nevertheless, since the outcome of morphospecies separation may not be suitable for all taxa, overall, both lumping and splitting may act as a balancing effect (Oliver and Beattie 1995; Derraik *et al.* 2002). For example, a case study conducted in New Zealand by Derraik *et al.* 2002 with Araneae, Coleoptera, and Lepidoptera, demonstrated that species underestimation for Coleoptera was compensated for by the overestimation for Lepidoptera and Araneae.

Underestimation of species richness may also occur with some arthropod orders, even with those that are taxonomically well known. For example, in Area de Conservación Guanacaste, Costa Rica, Burns *et al.* (2008) used DNA bar codes to identify cryptic species of skipper butterflies (Hesperiidae), previously reached by traditional means and to provide evidence to the existence of unsuspected species. They found that for the ecologically widespread neotropical skipper butterfly *Perichares philetus*, DNA barcoding separated this

species into a panneotropical complex of four cryptic species. Moreover, Burns *et al.* (2008) suggested that it is plausible that this complex includes still more species.

Given the above findings, the total estimation of 137 morphospecies of insects reported here may not necessarily be a true reflection of total species richness of flower visitors in the Sacred Valley. However, the flora of this region is taxonomically well known and the majority of plant specimens were identified as named species by the Herbarium.

Species richness and diversity of visited plant species and flower visitors

A comparison of species richness and evenness for visited plant species and flower visitors revealed high heterogeneity amongst some of the valleys and remarkable homogeneity amongst others (Figure 2.6). Diversity, composition and abundance in pollinator availability have been shown to vary markedly across sites and between years (Herrera 1988). In Spain, Herrera (2005) surveyed *Lavandula latifolia* populations and found that the measures implemented to estimate pollinator diversity were prone to suffer from artefacts due to sampling effort, pollinator visitation frequency, or a combination of both. Thus, it was not surprising that high heterogeneity and notable homogeneity amongst valleys was found. Observed species diversity is complex and may be the outcome of many contributory factors such as seasonality, predation, herbivory, disturbance and environmental predictability (Diamond 1988). Since diversity indices are closely related to sample size and spatiotemporal structure of the assemblages under investigation (Richerson and Lum 1980), the Shannon-Wiener Diversity indices reported here may only have reflected community diversity at the time of sampling.

There was some evidence that valleys geographically close to each other, such as Pumamarca, Choquebamba and Poques, tended to be similar in diversity and shared many of the same species. However, a comparison of valleys located more than 40km apart such as Huaran and Tiaparo showed that species diversity did not differ significantly and these valleys tended to share the most common and wide-ranging species. Interestingly, Yanacocha showed the highest species richness and abundances of flower visitors but was not the most diverse valley. This was attributed to the higher dominance of Diptera and hence the community

structure of flower visitors in Yanacocha was less uniform in composition than in Poques and Piscacucho.

An interesting feature of both data sets was the homogeneity between Pumamarca, Choquebamba and Poques for flower visitor diversity and Choquebamba and Poques for visited plant diversity. The slightly higher H' values recorded for visited plants and animals in these valleys is likely to be influenced by their orientation. For example, according to Holdridge (1967), sites situated in the lower part of the Patacancha Valley near Ollantaytambo correspond to Sub-Tropical Lower Montane Thorn Steppe and were the only valleys orientated west rather than north east. These inter-Andean valleys are often dry zones caused by a rain shadow effect (Young 1992). Some of the plant species observed in this locality are typically associated with Sub-Tropical Lower Montane Thorn Steppe and were not observed in other valleys. Piscacucho on the other hand is located on the edge of a transitional zone, within a few kilometres of the park boundaries of the Historic Sanctuary of Machu Picchu, where biological diversity is higher than neighbouring ecosystems (Galiano 2000). This could possibly explain why this valley recorded the second highest H' value. Also noteworthy from additional observations was the presence of the hummingbirds *Eriocnemis luciani* and *Coeligena violifer* which were only recorded at Piscacucho.

Possible explanations for the observed species diversity patterns

The two main factors that threaten the existence of pollinator diversity are habitat loss and fragmentation (Kearns and Inouye 1997, Kremen and Ricketts 2000). Anthropogenic pressures such as the widespread planting of *Eucalyptus*, overgrazing, the cultivation of crops, and the burning and cutting of *Polylepis* forests (Chepstow-Lusty *et al.* 1996; Chepstow-Lusty and Winfield 2000; Servat *et al.* 2002; Revilla *et al.* 2003) are all likely to have contributed to the observed patterns of diversity. Agricultural intensification may disrupt the interactions between native bees and their resources, resulting in a significant reduction in species diversity and abundance (Gathmann and Tschardt 2002; Kremen *et al.* 2002). Fire parameters have also been suggested by some authors to explain a substantial proportion of the variation in Andean plant communities (Keating 1999 and references therein). Finally, along an altitudinal gradient from the moist tropics through to montane cloud forest and then elfin and páramo grasslands at higher elevations, habitat physiognomy varies considerably and structural complexity decreases monotonically (Diamond 1988).

Other important determinants for the observed differences in diversity between sites were likely to be local microclimate, topographic relief, slope aspect, slope gradient, high landslide activity and the pattern of inter-annual variability in ground conditions (Fjeldså 2002a). According to Diamond (1988), in order to explain altitudinal gradients of species diversity, it is important to consider variables such as habitat structural complexity, productivity, and area of the sites studied. The distribution of available area with elevation depends on the of mountain form considered. For example, on conical shaped mountains such as those in the New Guinea highlands, area declines continuously with altitude, resulting in a decrease in species diversity. However, in comparison, the Peruvian and Ecuadorian Andes are more trapezoidal in form with a broad plateau at high altitudes (Altiplano), so therefore the maximum area may be at the highest elevations rather than at sea level (Diamond 1988).

In Perú, Yensen and Tarifa (2002) found that the diversity of small mammal communities was correlated with high rainfall. On the small scale, Richerson and Lum (1980 and references therein) found that habitat heterogeneity was an important factor in terms of the structural heterogeneity of vegetation for both birds and animals. Along an elevational gradient in the Cordillera Vilcabamba highlands of Perú, Terborgh (1971) showed that the distributional patterns of birds varied among trophic groups such as nectarivorous hummingbirds, frugivorous toucans and insectivorous antbirds. He suggested that in the closely packed bird fauna of the Andes, competition determined twice as many distributional limits than environmental discontinuities (ecotones), whereas gradually changing physical and biological conditions along the gradient accounted for approximately half of the distributional limits of bird species.

Sampling effort

The Sacred Valley up to the limits of the Historical Sanctuary of Machu Picchu and within the National Park, are areas well known for this unique flora and fauna and high levels of endemism (Stattersfield *et al.* 1998; Fjeldså 2002b; World Conservation Monitoring Centre 1985; ParksWatch Perú 2004). As expected, this was reflected in the diversity indices (Figure 2.5). For ecological data, Shannon-Wiener indices typically range from 1.5 to 3.5 and rarely exceed 4 (Seaby and Henderson 2006). To my knowledge, only values above 7 (for the number of tree species in the Peruvian Amazon) have been recorded by Gentry (1988). Given

that in tropical habitat surveys many taxa may never reach a clear asymptote (Gotelli and Colwell 2001), it was not surprising that neither of the species accumulation curves for visited plants and flower visitors showed sampling effort to be sufficient to typify fully the variability and diversity of community composition of the Sacred Valley (Figures 2.3 and 2.4). These findings are in agreement with the view of Ollerton and Cranmer (2002) that tropical community pollination studies may suffer from under sampling and as a consequence the real diversity of flower visitors and the plant species they visit may be far greater than predicted.

Nevertheless, a shortcoming of the data was the lack of replication and the use of higher-taxon levels as surrogates for the number of real species, which could have biased estimates of total flower visitor richness in this present study. Although specimens were identified by experts, the estimation of 137 morphospecies was probably conservative and may not be representative of species diversity for the whole valley. Given that the flora of this region is taxonomically well known and the majority of plant specimens were identified as named species by the Herbarium, visited plant species richness was thought to be accurate. However, it should be also be recognised that there was no independent measure for the abundance of plants in this study; instead abundance was estimated based on the number of visits. Given that the abundance of plants was not likely to be consistent along transects and among valleys, the results may be biased by variation in sampling intensity. Ideally, mean floral abundance per transect for each plant species should have been measured. The consequences of variation in sampling intensity for the data will be further discussed in Chapter 4.

Altitudinal trends in the abundances of different functional groups

Although the data showed a large spread around the means and medians per altitudinal band, with the exception of honeybees none of the functional groups varied significantly with elevation (Figure 2.8, Table 2.6). Honeybees were more abundant and tended to forage in higher densities at lower altitudes, nearer to where hives were located. It has been hypothesised that the foraging radius of honeybees may limit population sizes and that the mean number of honeybees should decline with increasing distance from the nearest apiary (Steffan-Dewenter and Tschardtke 2000). The results did suggest a decline in honeybee numbers away from hives, in agreement with Steffan-Dewenter and Tschardtke (2000). In contrast, Pyke and Balzer (1985) found that honeybee densities did not diminish with increasing distance to colonies, although these authors also found conflicting results

depending on the experiments they conducted. In the Sacred Valley, beekeepers' hives were usually located near farms and dwellings lower down the valleys, although in many cases honeybees were also observed at the highest elevations suggesting that honey bees may have been tracking the most profitable patches of floral resources (Visscher and Seeley 1982).

Diptera (all other Diptera and Syrphidae) were prominent at all altitudes, particularly at altitudes 3 and above, but their proportions did not change significantly with elevation (Table 2.6). Both Syrphidae and all other Diptera remained very constant across the highest altitudes, whilst Tachinidae were fairly constant across all altitudes. These observations corresponded to those of Medan *et al.* (2002) who also found a lack of a significant decrease with altitude, and contrasts with other alpine areas where dipterans were the most important flower visitors (e.g. Pojar 1974; Smith 1975; Pleasants 1980; Arroyo *et al.* 1982; Primack 1983; Warren *et al.* 1988; see multivariate analysis of Ollerton *et al.* 2006b). At two sites in the Norwegian alpine, Totland (1993) found that flies almost exclusively dominated the flower visitor assemblage at both sites and in both years and showed strong associations with flowers with open access morphology.

Compared with the rest of the taxa in the Sacred Valley, butterflies were rare; the relatively high numbers recorded at altitude 4 were attributed to observations from Yanacocha and to a single species *Metardaris cosinga* (Hesperidae). However, despite the small fraction of the total fauna observed, butterflies were a relatively species rich group with 15 species recorded among 67 individuals. Lepidopteran diversity varied significantly among altitudes and was highest at altitude 3, in accordance with Warren *et al.* (1988) who also found that at sites in Utah and Costa Rica, the relative contribution of butterflies was variable. A greater importance of Lepidoptera with increasing elevation alluded to by some authors (e.g. Pojar 1974; Moldenke 1975; Arroyo *et al.* 1982; Medan *et al.* 2002) was not apparent. Only one individual butterfly was recorded at the highest elevation. The rarity of butterflies in the Sacred Valley may partly be explained by the cold, foggy, cloudy and windy conditions in which some surveys were undertaken. A major adverse factor in the high Andes are strong winds. Descimon (1986) reported that several species belonging to the Andean *Rhopalocerca* remained in sheltered places or low to the ground during windy conditions. In addition, the ranges of some Andean Lepidoptera species are more closely related to physiographic factors rather than altitude, particularly the availability of small exposed summits (Arroyo *et al.* 1982). According to Shapiro (1992), the high Andes have a depauperate Lepidoptera fauna

compared to the tropical lowlands and none of the orcal (above the tree line páramo and puna vegetation) butterfly faunas, particularly those of the tropical Andes can be considered to be well known. Estimations of butterfly diversity and abundance in the tropical Andes has been confounded by the unsystematic collections methods by transient visitors, often in inappropriate seasons, and many habitats have never been collected at all. The seasonal component of butterfly diversity in the high Andes is very poorly understood and in Perú, the Lepidopteran fauna of elfin forests is also virtually unknown (Shapiro 1992; Alonso *et al.* 2001). Therefore, it is likely that the rarity of butterflies observed in the Sacred Valley was a result of weather conditions, under sampling and a depauperate Lepidoptera fauna within the Vilcanota highlands.

Possible explanations for the observed patterns of abundances of bees

The rarity of small native solitary bees within the whole valley system was not surprising. In the Venezuelan Andes, Berry and Calvo (1989) also found that above 3500m small Halictidae and Colletidae bees were restricted to warm and sunny periods. A number of factors may have influenced and explained the distributional patterns observed for small solitary bees. Firstly, some solitary bees are short-lived and may not have been detected during surveys (Heinrich 1975; Torchio 1987). Similarly, some solitary bees produce one generation per year, whilst others produce biannual generations (Torchio 1987; Minkley and Roulson 2006). In Chile, Arroyo *et al.* (1982) found that small bees diminished in numbers as the subnivél zone was reached. These authors attributed changes in pollinator composition along an altitudinal gradient to the different ecological responses by insects to summer daytime temperatures and their over-wintering habits. Flies and butterflies are known to engage in relatively long periods of solar basking, thereby reducing their dependence upon endothermy (Warren *et al.* 1988), whereas most bees expend more energy on brood feeding and nest warming (Arroyo *et al.* 1982). However, because the Peruvian Andes are situated closer to the equator, thermoregulation may not be as significant in explaining the observed patterns during the dry season. For example, in the Andean Cordillera of central Chile, Arroyo *et al.* (1981) recorded mean maximum and minimum temperatures at 2500m of between 12.2 °C and 3.9 °C and snow was observed above 2700m. Contrastingly, mean maximum and minimum temperatures recorded in this present study were higher (16 °C - 7.1° C respectively) with snow falling above 5000m.

Habitat fragmentation, changes in habitat use and agricultural practices may lead to the disruption of plant-pollinator interactions leading to a decline in species richness and abundance (Kremen *et al.* 2002; Memmott and Waser 2002; Steffan-Dewenter *et al.* 2002; Klein *et al.* 2003). Some habitat fragmentation studies have shown that local landscape destruction affects solitary bees more than social bees (Steffan-Dewenter *et al.* 2002). Since the abundance of ground-nesting bees depends on the availability of nesting sites, changes in the habitat matrix may determine the resulting structure of bee communities (Steffan-Dewenter and Tschardt 2000; Cane 2001; Steffan-Dewenter *et al.* 2002). The nests of large native *Xylocopa* species were found in the walls of Incan terraces at Choquebamba and Poques, and numerous solitary bee nests were detected in sandy cliffs close to the village of Yanahuara in Mantabay. This finding suggests that native bees are potentially diverse and abundant in the Sacred Valley, also supported by collections in the Natural History Museum and Department of Entomology (UNSSAC). The low abundance of small solitary native bees observed was likely to be related to the timing of sampling and weather conditions. High Andean valleys experience particular micro-climates with strong and rapid temperature fluctuations (Fjelds  2002a; Cahill and Matthysen 2007). Abundance and diversity is also determined by spatial variability, plant longevity and phenologies, the life-histories of the bees and sampling methods (Michener 2002; Minkley and Roulson 2006). Anecdotal observations in the tropical Andes of Colombia showed that when fog and low cloud prevailed, bees often stayed in their nests for days, limiting foraging times down to an hour or a few minutes (Gonzalez and Engel 2004). In a revision of bees in the Andean genus *Chilicola*, Michener (2002) also reported that in cold, windy, cloudy and foggy conditions, these small bees remained in their nests. In 1942, Benoist was the first to realise that collecting *Chilicola* with a net from flowers was not a successful way of sampling, but instead found that specimens could be obtained from pithy stems regardless of the weather (Michener 2002 and references therein). Furthermore, many of the specimens collected from the South American Andes were from dead stems of plants from the Asteraceae family (Michener 2002). Therefore, using this sampling approach may have greatly increased estimations of abundance and diversity of small solitary bees, but would not have supplied flower interaction data.

The lower number of bumblebees compared to honeybees could be explained by a major difference in foraging behaviour between these two species. According to Forup and Memmott (2005), since honeybees are able to communicate to each other where the most profitable patches of resources are located, sampling honeybees at flowers is more indicative of the

importance of a particular site to the colony rather than the population size in the area. On the contrary, bumblebees are more closely tied to nesting sites and individual bumblebees respond to changes in the nectar store. Consequently, sampling bumblebees at flowers should be a good indication of their local population abundance (Forup and Memmott 2005).

An important consideration to bear in mind is that the valleys were sampled insufficiently over just one season, therefore the data may not be representative of insect and bird abundance patterns during and between different seasons and years. Spatio-temporal variation in abundance of many functional groups of pollinators may be significant over seasons and between consecutive years, (Heinrich 1976; Ackerman 1983; Eckhart 1992; Roubik 2001; Minkley and Roulston 2006; Thomson 2006). Therefore, bees and other insect orders should be sampled frequently or continuously in order to accurately measure both yearly and seasonal changes (Roubik 2001).

Diversity with altitude

The trend towards a decrease in diversity and abundance of Hymenoptera with increasing altitude, and an increasing contribution of Diptera and Lepidoptera reported by many authors was not evident in this study (Moldenke 1975; Arroyo *et al.* 1982; Primack 1983; Warren *et al.* 1988 and references therein), but was in accordance with Gómez *et al.* (2007) who found no effect on pollinator richness and diversity with altitude in the Sierra Nevada mountains of Spain. These authors showed that plant populations differed in the degree of generalisation (plants were visited by more than a 100 insect species) and generalisation was correlated with pollinator abundance and plant population size, but not with altitude or habitat.

Contrary to many other montane studies, Hymenoptera were more diverse at the highest elevations. As a group Hymenopterans contributed to more than a quarter of the total visitor fauna, consistent with Medan *et al.* (2002). Those bees that could be identified belonged to the families Apidae and Halictidae, in accordance with Gonzalez and Engel (2004) who also showed that these two families contained the greatest proportion of species found at high altitudes from Colombia, Venezuela, Ecuador and Perú. However, many of the specimens of wasps and bees were undetermined. Bumblebees and honeybees were wide-ranging and inhabited the whole altitudinal gradient surveyed. This result supports the assumption that both bumblebees and honeybees have large foraging ranges and that foraging distances depend on

the abundance of profitable forage (Visscher and Seeley 1982; Beekman and Ratnieks 2000; Steffan-Dewenter and Kühn 2003; Westphal *et al.* 2003; Thomson 2006).

According to Gonzalez and Engel (2004) *Bombus funebris* is the only well known Andean bee species restricted to the páramo in Ecuador and puna in Perú, and *Bombus melaleceus* had not been found above 2400m in Colombia. Additionally, Rasmussen (2003) described *Bombus melaleceus* as uncommon within its distributional range (from Costa Rica to Venezuela and parts of Perú) with an altitudinal range of between 1000-3500m. Conversely, in the Sacred Valley, the altitudinal distributions of *Bombus funebris* and *Bombus melaleceus* were found to be far ranging; these species were not just restricted to puna habitats and *Bombus melaleceus* was observed foraging above 4000m (SW personal observation).

Those flies that could be identified were members of the muscoid group and included the families Muscidae, Sarcophagidae, Tachinidae and Anthomyiidae. Muscoid flies were the most abundant and diverse fly genera observed, whilst the most abundant syrphid fly genera were *Eristalis*, *Toxomerus* and *Platychierus*. Muscoidea have been listed as important pollinators in high altitude systems (Primack 1983; Kearns 1992; Kearns and Inouye 1994), whilst fungus gnats (Sciaridae and Mycetophilidae) have been reported as principle pollinators of *Listera cordata* (Orchidaceae) and *Scoliopus bigelovii* (Liliaceae) in coastal redwoods of California (Mesler *et al.* 1980) and many *Ceropegia* spp. (Apocynaceae – Ollerton. personal communication).

Altitudinal trends in species richness of plants visited by different functional groups of flower visitors

A trend of diminishing species richness among visited plants species and families with elevation was not apparent in the Sacred Valley. However, this investigation only considered visited plants rather than species richness per se. As discussed previously, species diversity is likely to be the outcome of many factors (Diamond 1988). Notable regularity of changes in species richness of vascular plants with altitude has been shown to be related in a predictable way to environmental conditions such as precipitation, soil fertility and the rate of tree turnover (Gentry 1988; Vázquez and Givnish 1998). Additionally, it is well established that bird, insect and possibly lizard and rodent diversity is correlated with aspects of plant diversity (Terborgh 1971; Murdoch *et al.* 1972; Servat *et al.* 2002). The lack of a decline in

diversity of visited plant species suggested that visited plant species diversity could be correlated with flower visitor diversity. Furthermore, the majority of the plants surveyed in this present study were not faced with the impoverished environmental and climatic conditions experienced in other alpine ecosystems where the tree-line commences at much lower elevations, the growing season is much shorter and where pollen limitation is a factor (e.g. Arroyo *et al.* 1982). In contrast, at the highest elevations potential pollinator availability was still relatively high in relation to lower altitudes. In the Sacred Valley, only in puna habitats at elevations between ca. 3900m up to 5000m do plants show adaptations to withstand intense radiation, seasonal drought, low temperatures, diurnal freezing and thawing, or periods with snow (Van der Hammen and Cleef 1987; Fjeldsá 2002a). Additionally the observed pattern could in part be attributed to facilitation and habitat heterogeneity above ca. 3500m and the general decrease from anthropogenic pressures (see Figure 1.7 Chapter 1). It has been proposed that multiple-species floral displays may facilitate pollination by attracting a greater number and variety of pollinators (Ghazoul 2006). Indeed, some of the most important floral resources for flower visitors at higher altitudes were mass-flowering plant and tree species with large floral displays such as *Minthostachys spicata*, *Baccharis buxifolia*, *Myrcianthes orephila*, *Jungia rugosa* and *Aristeguietia discolour*. Arroyo *et al.* (1981) investigated the phenological patterns of ninety seven plant species between 2320-3550m in the Andes of Central Chile and reported that the duration of flowering tended to be longer, and individual flowers remained open twice as long at the highest elevations. In accordance with Arroyo *et al.* (1981), flowers of *Passiflora tripartita*, *Barnadesia horrida*, *Duranta mandonii* and many other members of the Asteraceae also remained open for long periods (SW personal observation).

Relationship between *Apis* and native flower visitor numbers

The results indicated that as honeybee numbers increased, so too did mean abundances of Lepidoptera, Syrphidae, all other bees, *Bombus* spp. and Tachinidae. This suggests that these functional groups were probably responding to other factors. In contrast, the only negative correlations were found between abundances of honeybees and bumblebees (but only at altitude 5) and between honeybees and beetles from data pooled across all altitudes. Thus, in general, native flower visitor abundances did not decline in the presence of honeybees. This finding could be interpreted as evidence for no impact on native flora and fauna. However, these results were confounded by low replication, lack of control sites where honeybees were

not present and lack of information on native bee abundance and diversity before the introduction of *Apis* in the Sacred Valley. Furthermore, a negative impact has been defined by Paini (2004) as one which reduces the individual fitness and long-term survival of native bees. Since these data were collected over one season and observations were based on indirect measures, nothing could be inferred on native bee survival, fecundity or population density (Paini 2004). As Thomson (2006) has indicated, great caution should be taken when interpreting data from observational measures to predict competitive effects. For example, Thomson (2006) assessed the possible competitive impacts of honeybees on bumblebees by using three indirect measures: observations of resource overlap, observational data on spatial and temporal correlations and density manipulation experiments by introducing honeybee colonies. Despite some results being consistent with competitive effects, the correlative data were highly variable, resulting in trends in different directions during different months. Although the experimental data for both foragers and colonies suggested significant competitive impacts, the correlative data failed to predict the effects observed in the experimental study (Thomson 2006). Furthermore, anecdotal reports of increased densities of flower visiting honeybees compared with native visitors may be confounded by landscape modifications brought about by humans (Steffan-Dewenter and Kühn 2003). Since abundance data were collected over a short time scale, and no experimental manipulations at the colony-scale were undertaken, no definitive conclusions could be reached about the ultimate impacts of honeybees on the reproductive success of these plant and animal communities. The role of honeybees in these communities will be further explained in Chapters 3 and 4.

Altitudinal trends in the proportion of plant species visited by *Apis* and each functional group of flower visitors

Few studies have compared altitudinal trends in the proportions of plant species utilised by different taxa. Along the altitudinal gradient, the proportion of plant species visited by honeybees, hummingbirds, flies, syrphids and beetles varied significantly with altitude, whilst small native bees and bumblebees did not (Figure 2.9). Honeybees used a relatively small fraction of the total flora, ranging from 13% to 32%, but also visited a relatively high proportion of plant species at the highest elevations (Table 2.9). This finding contrasts with the high temperate Andes of central Chile, where bees pollinated only 13% of the subnival flora compared with 68% of the subandean flora (Arroyo *et al.* 1982). Dipterans (all other Diptera

and Syrphidae) visited the highest proportion of plant species and reached a maximum at altitudes 1 and 3 (49%) and those proportions remained consistently high with altitude. Conversely, Tachinidae fluctuated along the altitudinal gradient, utilised more plant species at altitude 2, and those proportions were significantly different to the other dipteran flower visitors (Table 2.9). At each elevation, dipterans (all other Diptera and Syrphidae) visited a higher proportion of the flora than any of the other functional groups of flower visitors and this was more pronounced above 3653m. Although it was not possible to determine the efficiency of flies as potential pollinators, this finding is similar to the distributional patterns described by Arroyo *et al.* (1982), in which dipterans pollinated a greater proportion of the flora than either bees or butterflies as altitude increased. Honeybees visited a higher proportion of plant species at lower altitudes, which also coincided with their higher abundances closer to hives.

Hummingbirds visited a greater proportion of plant species at the highest altitudes, in contrast with Chile where the estimated percentage of the total flora pollinated by hummingbirds was only 1.2%, and between 2700 and 3100m no birds were recorded (Arroyo *et al.* 1982). Overall, butterflies visited a small fraction of the total flora in the Sacred Valley, with the highest proportions recorded at the lowest elevations. This finding is in marked contrast with those Arroyo *et al.* (1982) where in the Andean zone in central Chile, the proportions of plant species pollinated by butterflies actually increased with elevation. In the lower subandean zone, butterflies pollinated 18% of the flora mainly belonging to the Asteraceae, compared to 31% of the flora in the cushion-plant and upper subnival zones, more than twice the number pollinated by bees (Arroyo *et al.* 1982). Coleoptera visited the highest percentage of plant species at altitude 1 (23%) and peaked again at altitude 4 (24%), in contrast with Chile where beetles visited five plant species and constituted an estimated 2.4% of the total flora. Although differences were found between groups in terms of plant species being visited, the proportion of plant families utilised by different functional groups did not vary with altitude.

Along the elevational gradient, honeybees exploited a relatively large proportion of the total flora sampled compared with the rest of the taxa. However, many of the plant species visited were by one individual, or one insect order and the majority of observations were on a limited subset of those species. Nevertheless, this altitudinal trend was consistent with much of the literature and with the view that honeybees only intensively forage on a small proportion

of available plant species (Menezes Pedro and Camargo 1991; Butz Huryn 1997; Steffan-Dewenter and Tschamntke 2000). For example, Menezes Pedro and Camargo (1991) concluded that the impact of the Africanized honeybee on the native bee community in southeast Brazil must be minimal because they only visited 33% of the total flora sampled and 50% of these plants were visited by less than five individuals. Over a three year period, Thomson (2006) found that niche overlap in plant use by *Apis* and *Bombus* varied substantially and was highest at the end of the summer during periods of resource scarcity. Given that only 14.4% of the total flora surveyed was visited by both native bees and honeybees and visitation rates were generally low, the potential for competitive impacts was probably low. Furthermore, the probable effects of honeybees on the native flora and fauna is dependent on whether resources are limited and on the amount of nectar and pollen removed (Butz Huryn 1997). As discussed earlier, because sampling was undertaken over a short time scale and pollinator efficiency was not measured, caution should be taken when interpreting these results. Visitation profiles and resource overlap will be further explored in Chapter 3.

Interference competition

Strikingly, there was little evidence to suggest that honeybees used interference competition and displaced other species when foraging. Of the 1583 visits recorded only 14 encounters resulted in honeybees physically displacing the native fauna from flowers (Table 2.11). Bumblebees were the only insect species observed displacing honeybees which is not surprising owing to their comparatively large size. Interestingly, neither active nor passive displacement of native fauna by honeybees was observed during visits to *Duranta mandonii* (see results and discussion in Chapter 5). Although occasional jostling by honeybees at flowers has been reported (Roubik 1991; Gross and Mackay 1998), aggression between native bees and honeybees is generally considered to be rare and relatively unimportant (Butz Huryn 1997). Likewise, observations reported here are in agreement with the view that honeybees are not aggressive (e.g. Schaffer *et al.* 1979; Ginsberg 1983; Roubik 1991 and references therein reviewed by Butz Huryn 1997). However, it should be noted that honeybees frequently engaged in aggressive displacement of smaller insects whilst robbing the flowers of *Nicotiana glauca* (SW personal observation). *Nicotiana glauca* was not included in this study because it was only found growing lower down on the valley floor (< 2800m). This finding is in agreement with the view that that honeybees use interference competition during nectar robbing, predominately during a nectar dearth (Butz Huryn 1997).

Conclusions

To my knowledge, no other large scale community studies have examined plant–flower visitor interactions along an altitudinal gradient in the tropical high Andes of Perú. The goal of this chapter was to compare flower visitor abundance and diversity along an elevational gradient and determine whether the distributional patterns observed conformed with the expectation that pollinator abundance and diversity experienced progressive declines with altitude. Despite the limitations regarding sampling protocols (the use of morphospecies, lack of replications and the abundance measure used to estimate plant diversity), nevertheless, this study revealed some novel relationships between these unique flora and fauna and showed noteworthy similarities, yet some important differences with other high altitude montane studies. Additionally, since transects were sampled in all weather conditions except heavy rain, important information was provided regarding visitation rates from flower visitors able to forage in less favourable conditions.

A general trend of diminishing species richness among visited plant species and families with elevation was not apparent. The animal assemblages in the Sacred Valley were most similar in abundances to those in the Andes of Mendoza, Argentina (Medan *et al.* 2002). Although the results suggested that the animal communities were dominated by Diptera, followed by Hymenoptera, mean abundance of most functional groups of flower visitors did not vary statistically with altitude. The trend towards a decrease in diversity and abundance of Hymenoptera with increasing altitude, and an increasing contribution of Diptera and Lepidoptera was not evident in this study. Contrary to other temperate montane areas, Hymenoptera were more diverse at the highest elevations. This was perhaps not surprising since the Tropical Peruvian Andes are situated closer to the equator, where thermoregulation and climate may not be as significant in explaining species diversity. Furthermore, species diversity and abundance is expected to be dependent on many extrinsic and intrinsic factors such the presence of co-flowering plant species, relative plant abundance and differences in flight distances among taxon (Gómez *et al.* 2007). It was also suggested that habitat loss and fragmentation were important determinants of the observed patterns of species diversity.

The chapter has provided novel information on the ecology and distributional limits of two species of bumblebees; *Bombus funebris* and *Bombus melaleucus*. The rarity of small native solitary bees within the whole valley system was probably influenced by a number of

factors. Nests found in Inca terraces and sandy cliffs, coupled with collections at UNSSAC, suggested that the native bee fauna was diverse. Sampling by collection from pithy stems of Asteraceae may have provided a more realistic estimate of species diversity and abundance than by collection with nets. Similarly, this investigation also provided important information regarding the ecology of endemic hummingbird species and the importance of plant communities associated with *Polylepis* woodlands.

Detecting and measuring the outcomes of the competitive effects of honeybees remains highly problematic and great caution should be taken when interpreting data from observational measures to predict such competition (Thomson 2006). Nevertheless, despite sampling over one dry season and purely using observational measures, the results were relatively consistent for the various components measured. Honeybees were a dominant part of the flower visitor assemblages and occurred in relatively high densities throughout the Sacred Valley. However, the data showed that only negative correlations were found between abundances of honeybees and bumblebees, and only at altitude 5, and between honeybees and beetles. Thus, on the whole, native flower visitor abundances did not decline in the presence of honeybees. Although *Apis* visited a relatively large proportion of the total flora surveyed in comparison to the rest of the taxa, honeybees only intensively utilised a small proportion of available plant species. Furthermore, resource overlap by honeybees and native bees was low, and little evidence was found to suggest that honeybees used interference competition and displaced other species when foraging. Despite the suggestion of a potential impact, in the absence of field experiments using direct multiple methods to assess how honeybees impact on the reproductive success of native plant communities, no definitive conclusions could be reached.

CHAPTER THREE

Structure in Plant-Flower Interaction Assemblages: Linking Functional Groups of Visitors and Plants using Canonical Correspondence Analysis

Introduction

One of the oldest pursuits in community ecology has been to examine patterns in species assemblages in order to understand their structure and dynamics (Patterson and Brown 1991). Previous work has identified the various ways in which interactions are structured. For example, Lewinsohn *et al.* (2006) examined assemblages of plants and animal species linked by interactions such as pollination, frugivory and herbivory. They advocated that instead of testing assemblages exclusively for one particular pattern, whether compartmentalised or nested (see Chapter 4), a more comprehensive approach within a broader framework should assist in the efficiency of detecting actual pattern in real, species-rich communities. Moreover, these authors argued that using more than one approach is worth considering for alternative and seemingly complementary analytical and exploratory tools. Plant animal interaction patterns can be analysed either as matrices, as a bipartite graph for the analysis of food webs, or as multivariate sets using correspondence analysis (Lewinsohn *et al.* 2006). Canonical Correspondence Analysis (CCA) is a widely used multivariate ordination method that can be applied to understand the relationships between community composition and environmental factors (Ter Braak and Verdonschot 1995). Typical community composition data consist of the abundance or the occurrence of species at a series of sites, and data relating to a number of environmental variables measured at the same sites (Ter Braak 1986). Ordination uses a conceptual model in which sites and/or species are arranged in multidimensional spaces along environmental gradients (Palmer 1993). Canonical correspondence analysis has an advantage over other techniques since it is designed to extract synthetic environmental gradients from ecological data sets and gives an automated interpretation of the ordination axes and performs well with skewed species distributions and hence it is robust to violations of assumptions (Ter Braak 1986; Ter Braak and Verdonschot 1995). Moreover, the ability to factor out covariables and test statistical significance using Monte Carlo permutation tests further expands the effectiveness of CCA

(Palmer 1993). The program CANOCO 4.5 (Ter Braak and Šmilauer 2002) has the advantage that it has an option to perform combined analyses, including analysis of nested data (subplots within plots - Ter Braak 1986).

Since the European honeybee (*Apis mellifera*) has also been introduced in Perú, and has been shown in other countries to visit resources utilised by a wide range of native species (Goulson 2003), visitation profiles of each of the functional groups of flower visitors were examined for indications of foraging overlap between *Apis* and native flower visitors. CCA was used to examine the structure of flower-visitor communities within the Sacred Valley to determine whether there were differences in the visitation profiles by pooling the flower visitor communities by functional groups. Fenster *et al.* (2004) defined functional groups of pollinators as functionally similar taxa such as long-tongued flies that behave in similar ways on a flower and exert similar selection pressures on floral traits. These authors defined plant species as being specialised in their pollination systems if they were successfully pollinated by a small subset of functional groups of pollinators. Fenster *et al.* (2004) re-analysed the Robertson (1929) data using frequency of visits as a measure of the relative potential importance of different functional groups of flower visitors to pollination. From the original papers cited in Robertson (1929) for which frequency data were included, Fenster *et al.* (2004) found that 150 plant species were pollinated by one functional group, and 59 were pollinated by two functional groups. They concluded that about three quarters of the plant species exhibited specialisation onto functional groups.

In Tasmania, Hingston and McQuillan (1998) investigated the foraging profile of the introduced bumblebee *Bombus terrestris* to determine whether it had the potential to compete with the native flower-visiting fauna. They summarised visitor profiles by carrying out ordinations using the program PATN and plotted plant species according to the presence or absence of each anthophilous taxon on their flowers. Hingston and McQuillan (1998) found *Bombus terrestris* were highly polylectic and overlapped with all insect families, all species of birds and all bee subgenera. They also found that bumblebees foraged earlier in the day than other insects and were efficient in their foraging behaviour, suggesting that this species had the potential to have a major impact on Tasmanian ecosystems. However, overlap in visitation profiles between species does not necessarily demonstrate that competition is occurring and depends on whether floral resources are limited and on the amount of resources removed from the plant species visited (Butz Huryn 1997; Goulson

2003; Paine 2004). A similar ordination approach was applied by using CCA, however, abundance data rather than presence and absence data was used because it has the advantage that it gives a better assessment of how species fall into groups according to the strengths of their interactions (Dicks *et al.* 2002). It should be stressed that the objectives were not to look for associations of plant species into pollination syndromes (categorising flowers according to suites of shared floral traits) with similar visitor profiles, but instead to compare the foraging profile of honeybees with those of the native fauna, and to explore where they were strongest. Therefore, data matrices did not include information on floral traits in relation to plant species.

Aims and objectives

This study aimed to probe further for structural patterns of plant-pollinator interactions by using multivariate analysis. The following questions were addressed:

1. Is there a difference in the foraging profiles (typical plants species being visited) between functional groups of flower visitors? If functional groups (as defined by Fenster *et al.* 2004) are important for driving floral evolution and community interaction structure, this suggest that there should be differences in the visitation profiles of these functional groups within the communities.
2. Are there significant differences between the foraging profiles of functional groups of flower visitors after the compositional variability of valleys has been explained?
3. Is there a difference in foraging profiles between *Apis* and other functional groups of flower visitors? In Chapters 2 and 4 it was demonstrated that honeybees utilised many of the same plant species compared to other functional groups and species of flower visitors, therefore it was predicted that by using canonical correspondence analysis the resulting ordinations should endorse this finding.

Methods

Data analysis

The same visitation data from the 90 transects described in Chapter 2 were subjected to multivariate analyses to examine associations between plant species based on flower visitor profiles. Firstly, a straight forward approach was chosen by quantifying beta-diversity using Detrended Correspondence Analysis (DCA) with Hill's scaling, using log transformed data. The differences between the scores of any two sites on the first axis of the DCA represent a measure of species turnover between these two sites (Ter Braak and Šmilauer 2002). Secondly, canonical correspondence analysis (CCA) ordinations were performed on the species abundance data using CANOCO 4.5 (Ter Braak and Šmilauer 2002). Flower visitors were categorised into functional groups as follows: *Apis*, Trochilidae, Coleoptera, Hemiptera, *Bombus*, Bee, Syrphidae, Tachinidae, Diptera, Vespidae and Lepidoptera. Only plant species with a frequency ≥ 3 visits were included in the analysis.

Species data file

A primary matrix (specified in CANOCO terminology as the species data file) for plants and flower visitors was first constructed using an Excel spreadsheet representing 43 plant species, 11 functional groups, 234 samples and 500 occurrences (non-zero values) over 45 sampling locations. The primary data set contains the response variables and represents the plant-flower visitor community structure (Lepš and Šmilauer 2003). Each separate row in the matrix corresponded to each combination of sampling location and taxonomic group and plant species. In other words, a single location (i.e. M1) would be represented by a variable number of rows equal to the number of flower visitor taxa observed at that particular location, and values in individual cells corresponded to abundances of flower visitors. Therefore, the independent units of evidence to address the questions outlined above would be the spectrum of plant species typically being visited by a flower visitor at a particular location. Visitor profiles (plant species typically visited by each taxa) would then be predicted (using the environmental variables specified in a constrained ordination) by pollinator identity and by altitude, at which the observation took place.

Environmental data file

The secondary matrix represented the explanatory variables used as environmental variables and/or covariables (i.e. flower visitors, valleys and altitude). It should be pointed out that traditionally, environmental variables (also called predictors) in CANOCO refers to any explanatory variable and these are more often real measurements of environmental properties, but are not always (Šmilauer personal communication). Predictors can be quantitative, semi-quantitative or nominal (Lepš and Šmilauer 2003). Pollination ecologists tend to use CCA most often to infer pollination systems based on floral traits whereby explanatory variables typically include flower shape, flower colour, flower abundance, nectar volume and nectar concentration (e.g. Dicks *et al.* 2002; Potts *et al.* 2003; Martins and Batalha 2006). In this approach however, plants were plotted in ordination space according to the presence or absence of each functional group on their flowers. The second matrix contained the same rows as the first matrix, but the columns were coded for the sampling design information (i.e. which combination of flower visitor referred to which valley and altitude). For the nominal variables (i.e. 11 functional groups of flower visitors, five altitudes and nine valleys) dummy variables were coded as either 1 or 0 corresponding to flower visitor identity, valley identity and altitude. For example in Table 3.1, the first four dummy variables indicate which functional group is considered at that particular row. The next two rows identify the altitude of the sampling point and the last two identify the valley in which the observation took place.

Table 3.1. Example of part of the primary data matrix from Mantaray for altitude 1 (top). Values represent abundances and correspond to each combination of sampling location, taxonomic group and plant species. Secondary data matrix showing the environmental variables coded as dummy variables and which combination of visitor referred to which valley and altitude (bottom)

	Tar Cyn	Esc res	Min spi	Bar hor	Dur man	Myr ore	Bac sal	Bac bux	Ast 2
M1- AM	19	1	0	0	0	0	0	1	0
M1-BO	1	3	0	0	0	0	0	2	0
M1- SY	1	2	1	1	0	0	0	1	0
M1-DI	2	0	1	0	0	0	0	0	0
M1-LE	0	1	0	0	0	0	0	0	0
M1-TR	0	0	0	0	0	0	0	0	0

	<i>Apis</i>	<i>Bombus</i>	Syrphid	Diptera	Alt 1	Alt2	Alt3	Mantaray	Chicon
M1- AM	1	0	0	0	1	0	0	1	0
M1-BO	0	1	0	0	1	0	0	1	0
M1- SY	0	0	1	0	1	0	0	1	0

Research questions

1. Is there a difference in the foraging profiles between functional groups of flower visitors?

This was addressed using a CCA and selecting from the environmental data the first 11 dummy variables and deleting the remaining 14 coding for altitude and valleys. A permutation test randomly assigned recorded visitor profiles to different functional groups of flower visitors.

2. Are there significant differences between the foraging profiles of functional groups of flower visitors after the compositional variability of valleys has been explained?

This was addressed using a partial CCA where the dummy variables coding for flower visitor identity were used as environmental variables, whilst the dummy variables coding for valleys were used as covariables. The remaining dummy variables for altitude were deleted.

3. Is there a difference in foraging profiles between *Apis* and other functional groups of flower visitors?

This was addressed using a partial CCA selecting the dummy variables coding for *Apis* as the constraining variable and deleting the other 10 dummy variables coding for the other functional groups of flower visitors (effectively merging the other functional groups together).

Indirect gradient analyses- detrended correspondence analysis

Data were initially analysed using detrended correspondence analysis (DCA) to determine whether a linear or unimodal type of ordination method was appropriate. The gradient length measures beta diversity and how unimodal the species' responses are along an ordination axis, expressed in standard deviation units of species turnover (Ter Braak and Šmilauer 2002; Lepš and Šmilauer 2003). A gradient length greater than 4 SD signifies a strong unimodal response (Ter Braak and Šmilauer 2002). The DCA analysis of the species abundance data indicated that a unimodal method was appropriate as gradient lengths for all

four axes exceeded lengths well over 4 SD, showing that species composition data were not homogenous.

Direct gradient analyses canonical correspondence analyses

Relationships between plant species on the basis of visitor profiles were further explored using canonical correspondence analysis (CCA) using CANOCO 4.5 (Ter Braak and Šmilauer 2002). CCA has the advantage over DCA in that it detects associations between species composition and environment, and also allows for the calculation of unconstrained residual axes summarising the variation that remains once the effect of the environmental variables have been accounted for (Ter Braak and Prentice 1988). Since the species abundance data contained many zero values and displayed a highly skewed distribution, data were log transformed [$y = \log_{10}(y + 1)$] in each of the CCAs to prevent a few high values unduly influencing the ordination results (Ter Braak and Šmilauer 2002). Hill's scaling focusing on inter-sample distances option was selected for all models since it equalises the average niche breadth for all axes and is therefore most suited to long gradients, and the distance rule is more informative when data displays a strong unimodal response, as indicated by large eigenvalues (>0.4) (Ter Braak and Verdonschot 1995; Ter Braak and Šmilauer 2002).

Testing the significance of individual constrained axes using a partial CCA

The second, third and fourth axes were tested for their significance by calculating a partial CCA with environmental variables identical to those of the first CCA. In partial CCA, species variation associated with explanatory variables that are not of primary interest (covariables) are partialled out in order to explore the explanatory variables of interest. For this analysis, the variability explained by the original first axis is partialled out by specifying the first CCA axis as a covariable in the new analysis, thus the original second axis becomes the first one, the third becomes the second one, and the fourth becomes the third axis (Ter Braak and Šmilauer 2002; Lepš and Šmilauer 2003). To convert the higher axes into covariables, the original solution file containing the sample scores (*SamE* scores) calculated from the species scores, were modified manually (Ter Braak and Šmilauer 2002; Lepš and Šmilauer 2003). Monte Carlo permutations analyses using 499 permutations from the partial

CCA were then performed. For all the analysis performed, restricted permutations selecting the time series or line transect option were used.

From the CCA analysis, a number of hypothetical associations were proposed. To test the prediction that there was no difference in foraging profiles between functional groups of flower visitors, a CCA was performed selecting from the environmental data only flower visitors, and deleting the remaining dummy variables coding for altitude and valley. The contribution of each environmental variable to the explained variation was determined by the inertia from marginal and conditional effects by using a forward selection. Marginal effects list the individual environmental variables ranked in order of the variance they explain singly. Conditional effects display the environmental variables ranked in order of their inclusion in the model and shows the amount of additional variation each variable contributes when it is added to the model ($\lambda - A$) (Ter Braak and Šmilauer 2002).

Flower visitor preferences were further examined by predicting that there was no difference in visitation profiles between functional groups, once the compositional variability explained by valleys was removed. Both altitude and valley indicators could not be used as covariables because there were not sufficient replications for each combination of valley and altitude (Šmilauer, personal communication). A CCA was performed selecting flower visitors as the only environmental variable and deleting valleys and altitude, and then by specifying valleys as the only covariables and deleting the remaining 16 dummy variables. A forward selection was used to determine which variables contributed significantly to the model.

In order to determine whether the foraging profile of *Apis* was different to other functional groups of flower visitors, the dummy variables coding for *Apis* were used as the only constraining variables, deleting the remaining ten dummy variables coding for other functional groups of flower visitors. A Monte Carlo global permutation was performed to test the significance of *Apis* using 499 permutations.

The results of ordinations were displayed as biplots where species points and environmental arrows approximate to the weighted averages of each of the species with regard to each of the environmental variables (Ter Braak and Verdonschot 1995). Qualitative environmental variables are usually displayed as centroids for individual categories (Lepš

and Šmilauer 2003). However, nominal environmental variables can also be plotted as arrows (see Ter Braak 1986). Arrow lengths and position of the arrowheads point from the centre of the diagram, the coordinates of which indicate the correlations between the explanatory variables and the CCA axis (Ter Braak and Verdonschot 1995). Environmental variables with long arrows are more strongly correlated with the ordination axis than those of shorter arrows, and smaller angles between arrows indicate stronger correlations between variables (Ter Braak and Verdonschot 1995; Lepš and Šmilauer 2003). For clarity, only those species with a fit >1% were displayed.

Terminology in CANOCO

Total inertia

The overall inertia is defined as the total variance in the species data as measured by the chi-square of the sample by species table divided by the table's total (Ter Braak and Šmilauer 2002).

Eigenvalue

Importance measure of the ordination axis, expressed as the amount of variability in the primary data (species data) explained by the corresponding axis (Lepš and Šmilauer 2003). Eigenvalues also represent the variance in sample scores (Palmer 1993).

Species-environment correlations

The species-environment correlation measures the strength of the association between species and environment for a given axis. Canoco calculates correlations between the sample scores for a given axis obtained from the species data and the sample scores that are linear combinations of the environmental variables (Ter Braak and Šmilauer 2002).

Cumulative percentage variance of the species-environment relation

Each axis explains a part of the variance. In Canoco, this information is given cumulatively as percentage variance of the species-environment relation. In CCA for unimodal models

this is a weighted regression for each species on the environmental variables (Ter Braak and Šmilauer 2002).

The sum of all canonical eigenvalues

The sum of all canonical eigenvalues relates to the sum of all eigenvalues in the corresponding canonical analysis. For example, how much of the variance could be explained by the environmental variables if they were used in similar, but constrained, analysis. Thus, the percentage variance of the species-environment relationship values represents percentages of this value (Lepš and Šmilauer 2003).

Supplementary environmental variables

Environmental variables that are not used during calculations carried out by the ordination method. Instead, the variables are added post-hoc to the ordination by projecting them passively into ordination space. Therefore, these variables do not contribute to the meaning of the ordination, but can still be judged from the ordination diagram using those results (Ter Braak and Šmilauer 2002; Lepš and Šmilauer 2003).

Results

Detrended correspondence analysis (DCA)

The detrended correspondence ordination used the whole data set of plants and retrospective projection of all environmental variables, based on the first two axes of the DCA (Figure 3.1A and 3.1B). The ordination diagram (Figure 3.1A) displayed the major variation in flower visitor composition across the Sacred Valley, with environmental variables presented in a separate plot for clarity (Figure 3.1B). The plant species were scattered across the plot. Inspection of the general plant distribution suggested that there was continuous variation in species composition. Many Asteraceae were concentrated towards the centre of the plot. In contrast, *Aegiphyla mertonii*, *Barnadesia horrida*, *Brachyotum nutans*, *Gynoxys longifolia*, *Siphocampulus actinothrix*, *Weinmannia pentaphyla*, Asteraceae sp.5. and Asteraceae sp. 8 were plant species clustered on the periphery of the ordination diagram and identified in the proceeding analyses to display strong associations with some of the flower visitors. Since the length of axis 1 was more than 8 SD, it is predicted that those plant species positioned at the opposite end of axis 1 are found at sites that have hardly any plant species in common (Jongman *et al.* 1987). For example, Asteraceae sp. 8 was only visited at Yanacocha and *Aegiphyla mertonii* was only visited at Huaran.

Evaluation of the heterogeneity among valleys and among altitudes by reviewing the spread of symbols indicated that valleys were more heterogeneous than altitude (Figure 3.1B). In contrast, the variables for altitude were not arranged across the plot in order of altitude value, but instead were arranged closer to the centre of the plot. Consequently, valley indicators were selected as covariables in CCA partial analysis (Table 3.9 and Table 3.10). The positions of the centroids for functional groups of flower visitors suggested that Trochilidae, Diptera, Syrphidae and *Apis* were important predictors of the variation in visitor profiles.

The length of the first gradient was the longest, explaining 4.8% of the total species variability (Table 3.2). For ecological data with strong gradients, the percentage explained inertia is typically low (<10%) (Ter Braak and Verdonschot 1995). The second and higher axes were also long with similar lengths (6 SD) suggesting that these axes also contribute

strongly to explaining the total species variability. The first eigenvalue was high implying that axis 1 represented a strong gradient. However, all the higher axes displayed strong gradients. Axis 1 showed the highest correlation with the environmental data ($r = 0.87$), whereas the correlations for the higher axes, although lower, were also well correlated. The results suggest that the whole data set was governed by more than one dominant gradient.

Table 3.2. Eigenvalues, gradient length for all four axes of ordinations by detrended correspondence analysis (DCA). Axis scores calculated for DCA is restricted by default to two (Lepš and Šmilauer 2003)

Type of analysis	Axis 1	Axis 2	Axis 3	Axis 4	Total inertia
DCA					
Eigenvalues	0.91	0.82	0.75	0.58	19.02
Length of gradient	8.11	6.32	6.64	6.70	
Species-environment correlations	0.87	0.51	0.66	0.50	
Cumulative variance of species data (%)	4.80	9.10	13.10	16.20	
Cumulative variance of species-environment relation (%)	14.80	18.40	0.00	0.00	
Sum of all eigenvalues					19.02
Sum of all canonical eigenvalues					4.84

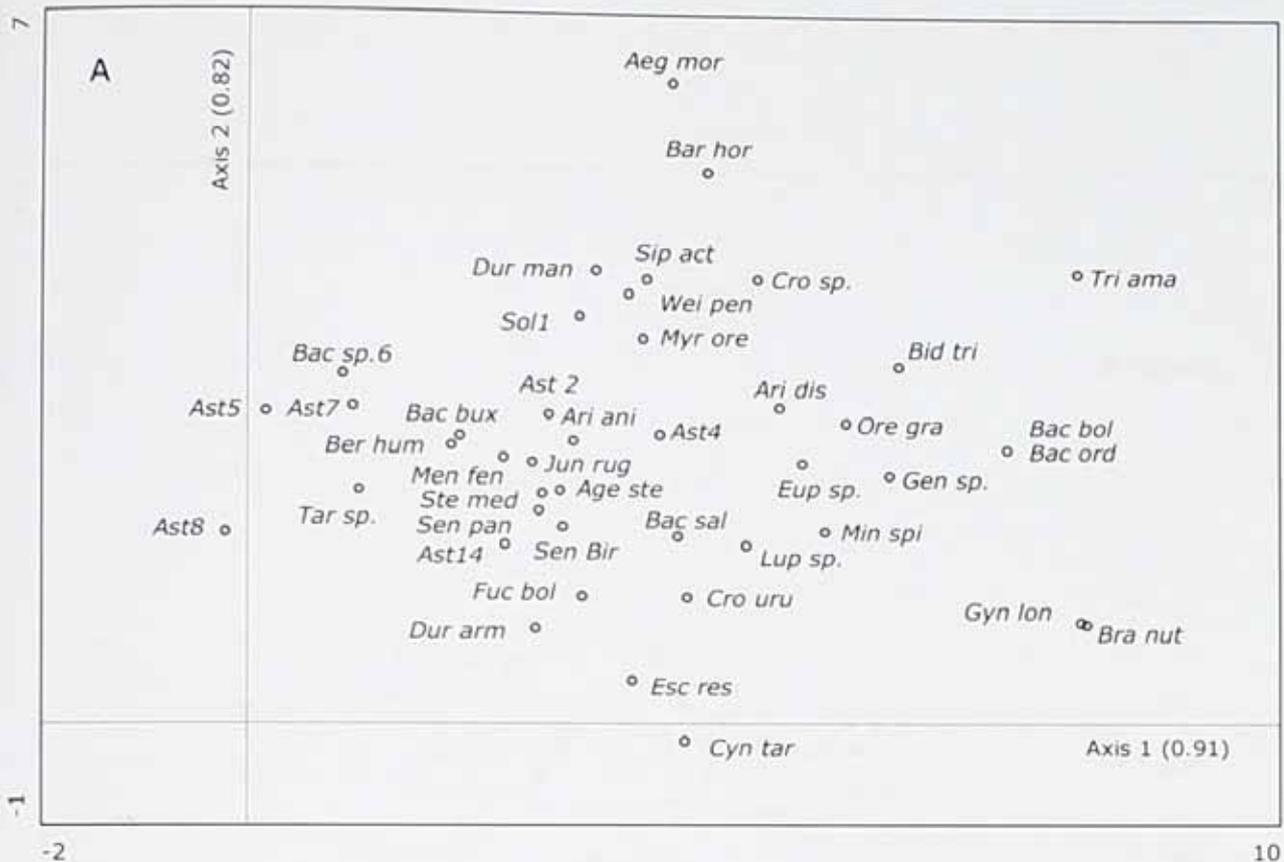


Figure 3.1A Scatter plot based on detrended correspondence analysis (DCA) of plant species according to their flower visitor profiles displaying the major variation in species composition in the Sacred Valley. The plot shows the DCA scores (O) of 43 plant species. Only those plants that received at least three visits were included in the analysis. Environmental variables are shown in a separate plot (Figure 3.1 B) for clarity. The first two ordination axes had eigenvalues of 0.91 and 0.82 respectively. They cumulatively explained 18.4% of the species-environment relationship, but only 9.10% of the variance in the species data itself. Plant species abbreviations are as follows: A. mor, *Aegiphya mortonii*; Ari ani, *Aristeguietia anisodonton*; Ari dis, *Aristeguietia discolor*; Ari ste, *Ageratina sternbergiana*; Ast 2, Asteraceae sp.2; Ast 4, Asteraceae sp. 4; Ast 5, Asteraceae sp. 5; Ast 7, Asteraceae sp. 7; Ast 8, Asteraceae sp. 8; Ast 14, Asteraceae sp.14; Bac sal, *Baccharis salicifolia*; Bac bol, *Baccharis boliviana*; Bac odo, *Baccharis odorata*; Bac bux, *Baccharis buxifolia*; Bac sp.6, *Baccharis* sp. Bar hor, *Barnadesia horrida*; Ber hum, *Berberis humbertiana*; Bid tri, *Bidens triplinervia*; Bra nut, *Brachyotum nutans*; Cro sp., *Cronquistianthus* sp.; Cro uru, *Cronquistianthus urubambensis*; Cyn tar, *Cynanchum tarmense*; Dur arm, *Duranta armata*; Dur. man, *Duranta mandonii*; Esc res, *Escallonia resinosa*; Eup sp., *Eupatorium* sp.; Fuc bol, *Fuchsia boliviana*; Gen sp., *Gentianella* sp.; Gyn lon, *Gynoxys longifolia*; Jun rug, *Jungia rugosa*; Lup sp., *Lupinus* sp 2.; Men fen, *Mentzelia fendleriana*; Min spi, *Minthostachys spicata*; Myr ore, *Myrcianthes oreophila*; Ore gra, *Oreocallis grandiflora*; Sen pan, *Senecio panticallensis*; Sen bir, *Senna birostris*; Sol sp., *Solanum* sp.; Sip act, *Siphocampulus actinothrix*; Ste mar, *Stellaria media*; Tar sp., *Tarraxacum* sp., Tri ama, *Trifolium amabile*; Wei pen, *Weinmannia pentaphyla*

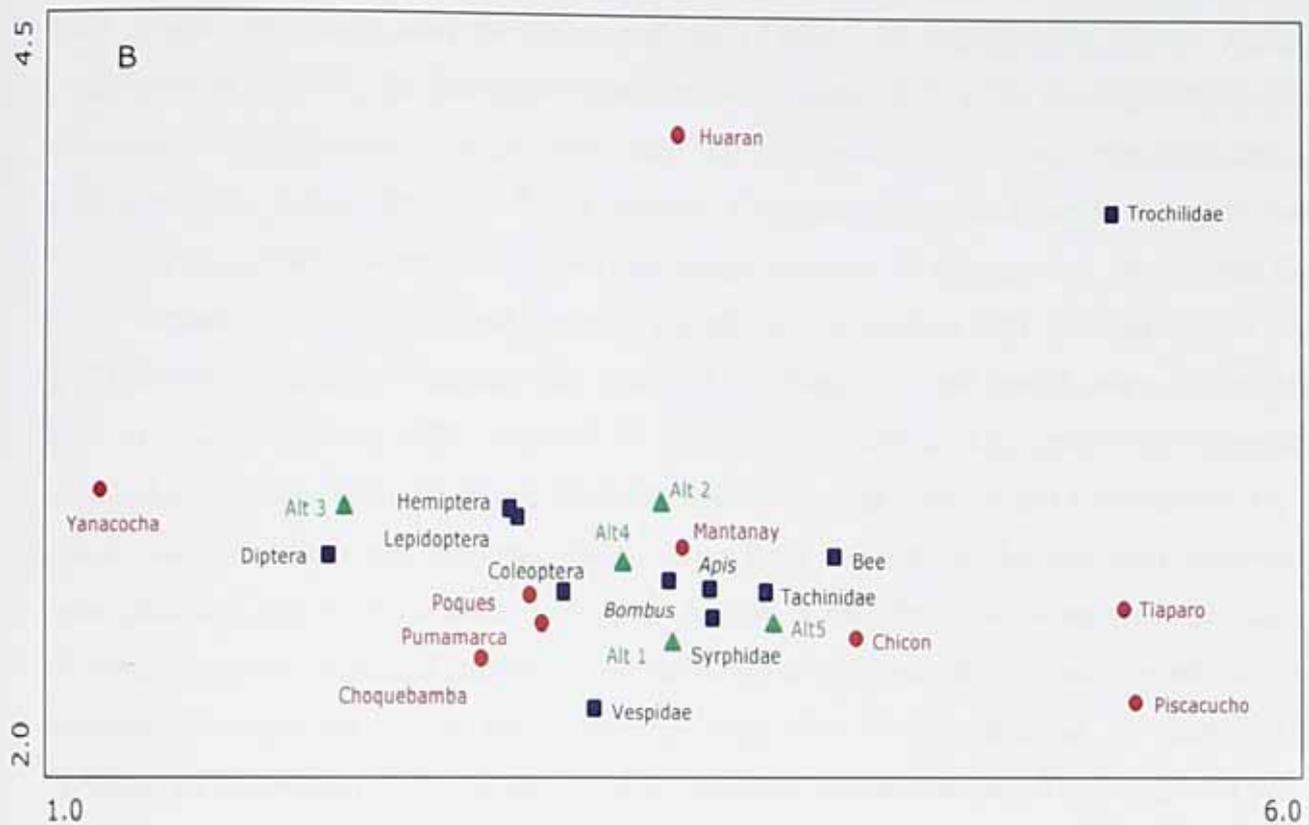


Figure 3.1B. DCA scatter plot of all the environmental variables. Qualitative variables altitude and valleys were transformed into binary dummy variables and were plotted as centroids into the ordination diagram. Abbreviations are as follows Alt1 = altitude 1; Alt2 = altitude 2; Alt3 = altitude 3; Alt4 = altitude 4; Alt5 = altitude 5.

CCA Analyses

Eigenvalues from the constrained ordination canonical correspondence analysis (CCA) were moderately lower than for DCA analysis (Table 3.3). Eigenvalues gave a similar description as in DCA; the first eigenvalue was also highest (0.71), but the higher axes also represented strong gradients. Axis 1 was also well correlated with the environmental data ($r = 0.93$) and was higher than in the DCA analysis. Correlations for the other three higher axes were only marginally lower. Cumulative percentage variance of species data showed that the axis 1 explained 4.1% of the total variation (inertia) in the species data; a similar result was also obtained in the DCA analysis. The results showed that the total inertia was considerably high (17.40) indicating high variance in the sample scores. The species-environment correlation indicated the strength of the relationship between species and environment for a given axis (Ter Braak and Šmilauer 2002). The results showed that all four axes were well correlated with the environmental data, also suggesting that the whole data set is governed by more than one dominant gradient. The cumulative variation of the species-environment relationship explained by all four canonical axes was 53.4%, whereas the cumulative variation in the species data was only 12.3%. The first and all canonical axes together were highly significant under the Monte Carlo permutation test ($P \leq 0.05$) demonstrating that there was a strong relationship between the species and environment in the plant flower visitor data set. The cumulative percentages of variance of the species data explained by the axes showed that the values were low, which is not unusual for species data and suggests a high level of noise (Ter Braak and Šmilauer 2002). The first axis did not explain more of the cumulative percentage variance of species data than the higher axes together, suggesting that all four axes determined the relationship between the species and environment (plant and flower visitor profiles).

Table 3.3. Summary of results of a constrained ordination by canonical correspondence analysis (CCA) of the plant flower visitor data. Significance level ($P \leq 0.05$) obtained with Monte Carlo permutation tests on the first axis and all axes (499 permutations under reduced model)

Type of analysis	Axis 1	Axis 2	Axis 3	Axis 4	Total inertia
CCA					
Eigenvalues	0.71	0.54	0.48	0.40	17.40
Species- environment correlations	0.93	0.85	0.83	0.79	
Cumulative variance of species data (%)	4.10	7.20	10.00	12.30	
Cumulative variance of species-environment relation (%)	17.70	31.30	43.40	53.40	
Sum of all eigenvalues					17.40
Sum of all canonical eigenvalues					4.01
	Eigenvalue	F-ratio	P-value		
Significance of first canonical axis	0.71	8.63	0.002		
	Trace	F-ratio	P-value		
Significance of all canonical axes (499 permutations under reduced model)	4.01	2.64	0.002		

Partial CCA analysis to test the significance of individual constrained ordination axes

Since the initial CCA analysis revealed strong gradients for all four axes (see Table 3.3), the remaining higher axes were tested for their significance by calculating a partial CCA with environmental variables identical to those of the first CCA. As shown in Table 3.3, the test for the axis 1 was significant ($F = 8.63$, $P = 0.002$) and indicated that despite being dominant, axis 1 alone was not sufficient to explain the species-environment relationship in the plant-flower visitor data set. To test the significance of the three remaining higher axes, the variability explained by the original first axis was partialled out by specifying the first CCA axis as a covariable in the new analysis, thus the original second axis becomes the first one, the third becomes the second one, and the fourth becomes the third axis (Ter Braak and Šmilauer 2002; Lepš and Šmilauer 2003). Monte Carlo permutation analyses using 499 permutations from the partial CCA showed that the second, third and fourth axes of the original analysis were all highly significant (Tables 3.4, 3.5 and 3.6).

The second axis showed the highest P value ($F = 7.57$; $P = 0.016$) (Table 3.4). The cumulative variation of the species-environment relationship explained by all four canonical axes was 53.4%, whereas the cumulative variation in the species data was 12%, indicating very similar results to the original analysis (see Table 3.3).

The test for axis 3 was significant ($F = 7.46$; $P = 0.024$). The cumulative variation of the species-environment relationship explained by all four canonical axes was 52.7%, whereas the cumulative variation in the species data was 10.7% (Table 3.5).

The test for axis 4 was also significant ($F = 6.49$; $P = 0.032$). The cumulative variation of the species-environment relationship explained by all four canonical axes was 50.8%, whereas the cumulative variation in the species data was 9.1%, the lowest of all axes tested (Table 3.6).

Table 3.4 Partial CCA to test the significance of the second ordination axis. The sum of all eigenvalues is after fitting covariables. Percentages are taken with respect to residual variances (i.e. variances after fitting covariables)

	Axis 1	Axis 2	Axis 3	Axis 4	Total inertia
Eigenvalues	0.66	0.62	0.51	0.41	19.02
Species-environment correlations	0.89	0.88	0.84	0.75	
Cumulative variance of species data (%)	3.60	7.00	9.80	12.10	
Cumulative variance of species-environment relation (%)	15.90	31.00	43.40	53.40	
Sum of all eigenvalues					18.14
Sum of all canonical eigenvalues					4.11
	Eigen value	<i>F</i> -ratio	<i>P</i> -value		
Significance of first canonical axis (499 permutations under reduced model)	0.655	7.575	0.0160		

Table 3.5. Partial CCA to test the significance of the third ordination axis. The sum of all eigenvalues is after fitting covariables. Percentages are taken with respect to residual variances (e.g. variances after fitting covariables)

	Axis 1	Axis 2	Axis 3	Axis 4	Total inertia
Eigenvalues	0.62	0.51	0.41	0.31	19.02
Species-environment correlations	0.88	0.84	0.75	0.75	
Cumulative variance of species data (%)	3.60	6.50	8.90	10.70	
Cumulative variance of species-environment relation (%)	17.60	32.10	43.90	52.70	
Sum of all eigenvalues					17.30
Sum of all canonical eigenvalues					3.52
	Eigenvalue	<i>F</i> -ratio	<i>P</i> -value		
Significance of first canonical axis (499 permutations under reduced model)	0.62	7.468	0.024		

Table 3.6. Partial CCA to test the significance of the fourth ordination axis. The sum of all eigenvalues is after fitting covariables. Percentages are taken with respect to residual variances (e.g. variances after fitting covariables)

	Axis 1	Axis 2	Axis 3	Axis 4	Total inertia
Eigenvalues	0.52	0.41	0.31	0.26	19.02
Species-environment correlations	0.85	0.75	0.75	0.65	
Cumulative variance of species data (%)	3.10	5.70	7.50	9.10	
Cumulative variance of species-environment relation (%)	17.50	31.50	41.90	50.80	
Sum of all eigenvalues					16.49
Sum of all canonical eigenvalues					2.96
	Eigenvalue	F-ratio	P-value		
Significance of first canonical axis (499 permutations under reduced model)	0.51	6.49	0.032		

Visitation profiles of different functional groups of flower visitors

Variation between plant species in terms of their flower visitor profiles and the relative importance of the measured environmental variables is shown in Figure 3.2. Both axis 1 and all canonical axes together were highly statistically significant under the Monte Carlo permutation test (Table 3.7). The cumulative variation of the species-environment relationship explained by all four canonical axes was 73.9%, whereas the cumulative variation in the species data was only 5.2% (Table 3.7). The individual environmental variables in order of the variance they explain singly (marginal effects) are presented in Table 3.8. Conditional effects show the environmental variables in order of their inclusion in the model, together with the additional variance each functional group of flower visitors explains at the time it was included (λ -A)(Ter Braak and Šmilauer 2002). The model resulting from a forward selection of explanatory variables identified four significant functional groups of flower visitors; the results showed that for marginal effects, Trochilidae was the most important variable in determining variation in flower visitor profiles, followed by *Apis*, Diptera and Coleoptera. Likewise, for the conditional effects, Trochilidae was the most important variable and contributed significantly to the model of already included variables ($P = 0.004$) followed by *Apis*, Diptera and Coleoptera, which were also significant ($P \leq 0.05$) predictors.

The ordination revealed that the vector described by the foraging profile of Trochilidae was positively associated with axis 1 of the plot, highlighting the strong association with

Duranta mandonii, *Gynoxis longifolia*; *Barnadesia horrida*; *Brachyotum nutans*; and *Siphocampulus actinothrix*. The distributions of plant species with the strongest affinity with hummingbirds lie in the top right hand corner, in the valleys of Huaran and Chicon. In contrast, the vector described by the foraging profile of *Apis* was negatively associated with axis 2 showing the strongest association with *Minthostachys spicata*, *Myrcianthes oreophila*, *Weinmannia pentaphyla* and *Oreocallis grandiflora*. It should be noted that *O. grandiflora* was not visited legitimately by honeybees, instead it was nectar robbed. These associations were strongest between the valleys of Mantanay and Tiaparo at altitudes 1 and 2. The vector described by the foraging profile of Diptera was positively associated with axis 2, showing the strongest association with *Ageratina sternbergiana*, *Jungia rugosa* and Asteraceae sp. 5. The distribution of plant species with the strongest affinity with Diptera lie in the top left hand corner, in the valleys of Pumamarca, Choquebamba and Poques at the three highest altitudes. Coleoptera had the strongest affinity with Asteraceae sp. 2, Asteraceae sp. 5 in the valley of Pumamarca. Syrphidae had the strongest associated with *Baccharis buxifolia* in the valleys of Yanacocha and Piscacucho between altitudes 1, 3 and 4. Vespidae, *Bombus*, Bee and Lepidoptera were represented by the shortest arrows and did not contribute significantly to explain the association between plant species and flower visitor profiles ($P > 0.05$). Foraging profiles of Coleoptera, Diptera and Hemiptera were similar, but opposite to *Apis*. The vector describing *Apis* was very similar to Tachinidae, but opposite to that of Trochilidae.

Table 3.7. Summary of Canonical correspondence analyses selecting functional groups of flower visitors as the only environmental variables

Type of analysis	Axis	Axis	Axis	Axis	Total
CCA	1	2	3	4	inertia
Eigenvalues	0.47	0.19	0.13	0.09	17.40
Species- environment correlations	0.79	0.58	0.51	0.45	
Cumulative % variance of species data	2.80	3.90	4.70	5.20	
Cumulative % of species-environment	39.10	54.90	66.00	73.90	
Sum of all eigenvalues					17.40
Sum of all canonical eigenvalues					4.01
	Eigen value	F-ratio	P-value		
Significance of first canonical axis (499 permutations under reduced model)	0.49	6.28	0.002		
	Trace	F-ratio	P-value		
Significance of all canonical axes (499 permutations under reduced model)	1.23	1.64	0.002		

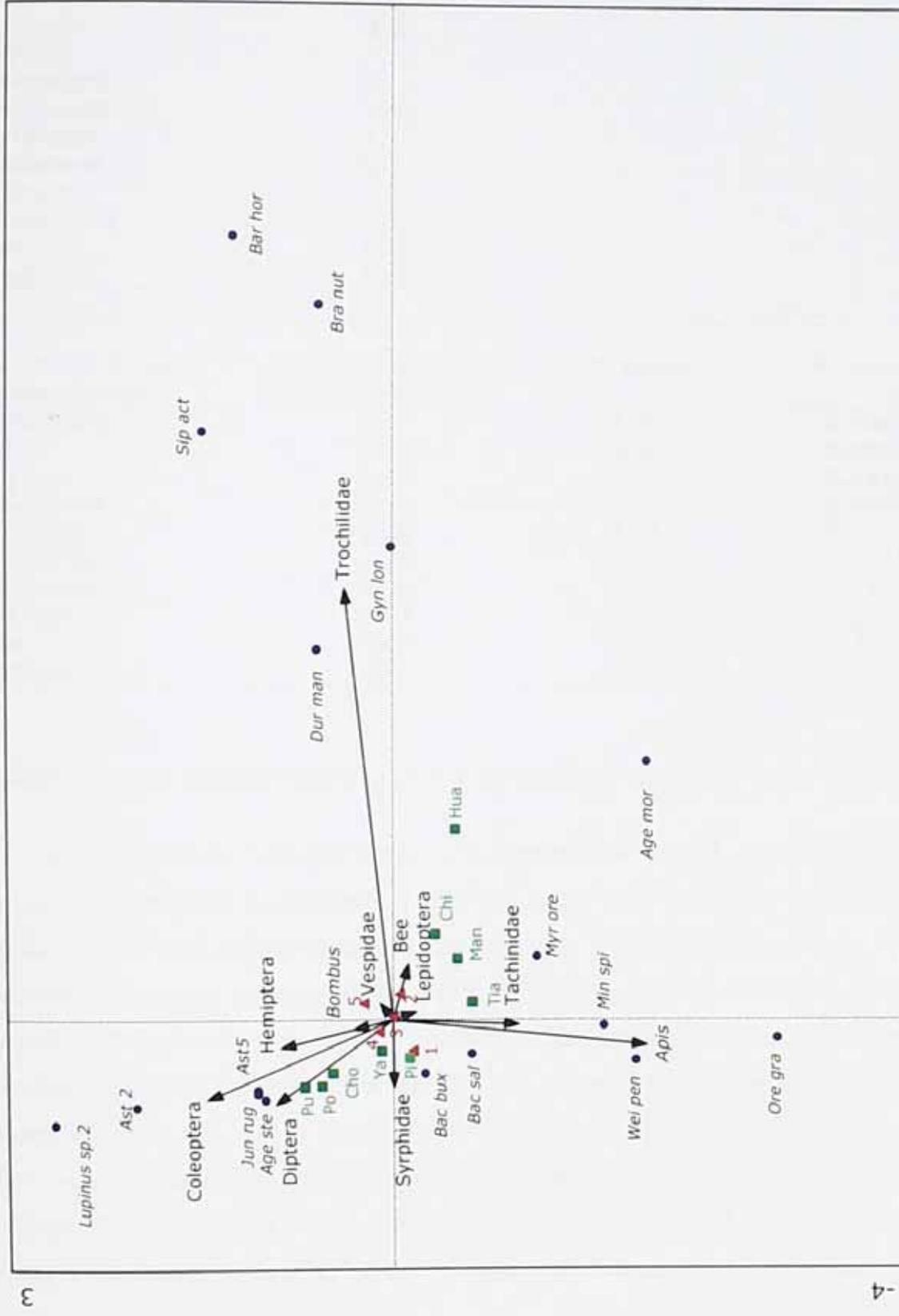


Figure 3.2. Species-environment biplot diagram of plants and functional groups of flower visitors based on the first 2 axes of canonical correspondence analysis (CCA). For clarity, only plant species with fit values > 1 in the ordination are displayed. The first two axes have eigenvalues of 0.47 and 0.19 respectively, explaining cumulatively 73.9% of the species environment relationship, but only 5.2% of the variance in the species data itself. See Figure 3.1A for plant species abbreviations.

Table 3.8. Canonical correspondence analyses selecting functional groups of flower visitors as the only environmental variables. Marginal and conditional effects obtained from a forward selection. Variables ranked in order of their importance by their marginal and conditional effects. A Monte Carlo permutation test was performed using 499 random permutations. The relative contribution to the model of already included variables is significant if ($P \leq 0.05$)

Functional group of flower visitors	Inertia marginal effects ($\lambda \sim 1$)		
Trochilidae	0.46		
<i>Apis</i>	0.15		
Diptera	0.13		
Coleoptera	0.12		
Tachinidae	0.09		
Syrphidae	0.08		
Hemiptera	0.08		
<i>Bombus</i>	0.08		
Lepidoptera	0.07		
Bee	0.06		
Vespidae	0.03		

Functional group of flower visitors	Inertia conditional effects ($\lambda \sim A$)	F - Value	P - value
Trochilidae	0.46	6.10	0.004
<i>Apis</i>	0.15	1.96	0.006
Diptera	0.10	1.42	0.016
Coleoptera	0.10	1.35	0.078
Tachinidae	0.09	1.15	0.194
Syrphidae	0.08	1.11	0.136
Hemiptera	0.08	1.01	0.370
<i>Bombus</i>	0.07	0.95	0.678
Bee	0.62	0.82	0.820
Lepidoptera	0.04	0.96	0.964

Species-environmental biplot with the second and third CCA axis

Apis, Diptera and Coleoptera were important predictors of the variation between plant species in their flower visitor profiles when the scores from the species and environmental variables were used to plot the second and third axes (Figure 3.3). In contrast, Trochilidae was not an important predictor of the variation for the species-environmental biplot for the second and third axes. The vector described by the foraging profile of Coleoptera was positively associated with axis 3 of the plot, still maintaining a strong association between Asteraceae sp. 2 and *Jungia rugosa*. Other taxa with strong positive associations with the third ordination axis were Hemiptera with Asteraceae sp. 2 and *Bombus* with *J. rugosa*. In contrast, Tachinidae and Lepidoptera were negatively associated with axis 3 and more strongly associated with *Escallonia resinosa* and *Weinmannia pentaphyla*. *Apis* still

maintained a strong association with *Oreocallis grandiflora*, *Minthostachys spicata* and *Myrcianthes oreophila*, and was positively correlated with axis 2. The association with *Aegiphyla mertonii*, was stronger than in the first ordination diagram (Figure 3.2). Noteworthy is the additional inclusion of *C. tarmense* and the strong association with *Apis*, whose fit to the diagram was >1%.

Species-environmental biplot with the third and fourth CCA axis

The spread of plant species were more scattered across the periphery of the plot and the arrows for the functional groups of flower visitors were shorter than for the axis 2 and axis 3, indicating weaker associations overall (Figure 3.4). Axis 3 and axis 4 were largely defined by Diptera, Syrphidae, Coleoptera, *Apis* and *Bombus*. Syrphidae had a strong positive association with axis 4 and Asteraceae sp. 4, *Aristeguietia sternbergiana*, *Gynoxis longifolia*, and *Stellaria media* which was not evident from the two previous species-environmental biplots (Figures 3.2 and 3.3). Bee and Syrphidae shared similar profiles, also not obvious from the previous two ordination diagrams. *Bombus* and *Apis* showed a strong affinity with *M. oreophila* and were negatively associated with axis 4, whereas Diptera was negatively associated with axis 3 and showed additionally affinities with Asteraceae sp. 5 and *Berberis humbertiana* whose fit to the diagram was >1% and not included in the previous two ordinations.

In summary, Axis 1 was strongly correlated with Trochilidae, axis 2 and 3 were more strongly correlated with *Apis*, Diptera and Coleoptera, and axis 3 and 4 were largely correlated with Diptera, Syrphidae, Coleoptera, *Apis* and *Bombus*. The overall results indicated that there was a significant difference in foraging profiles between functional groups of flower visitors.

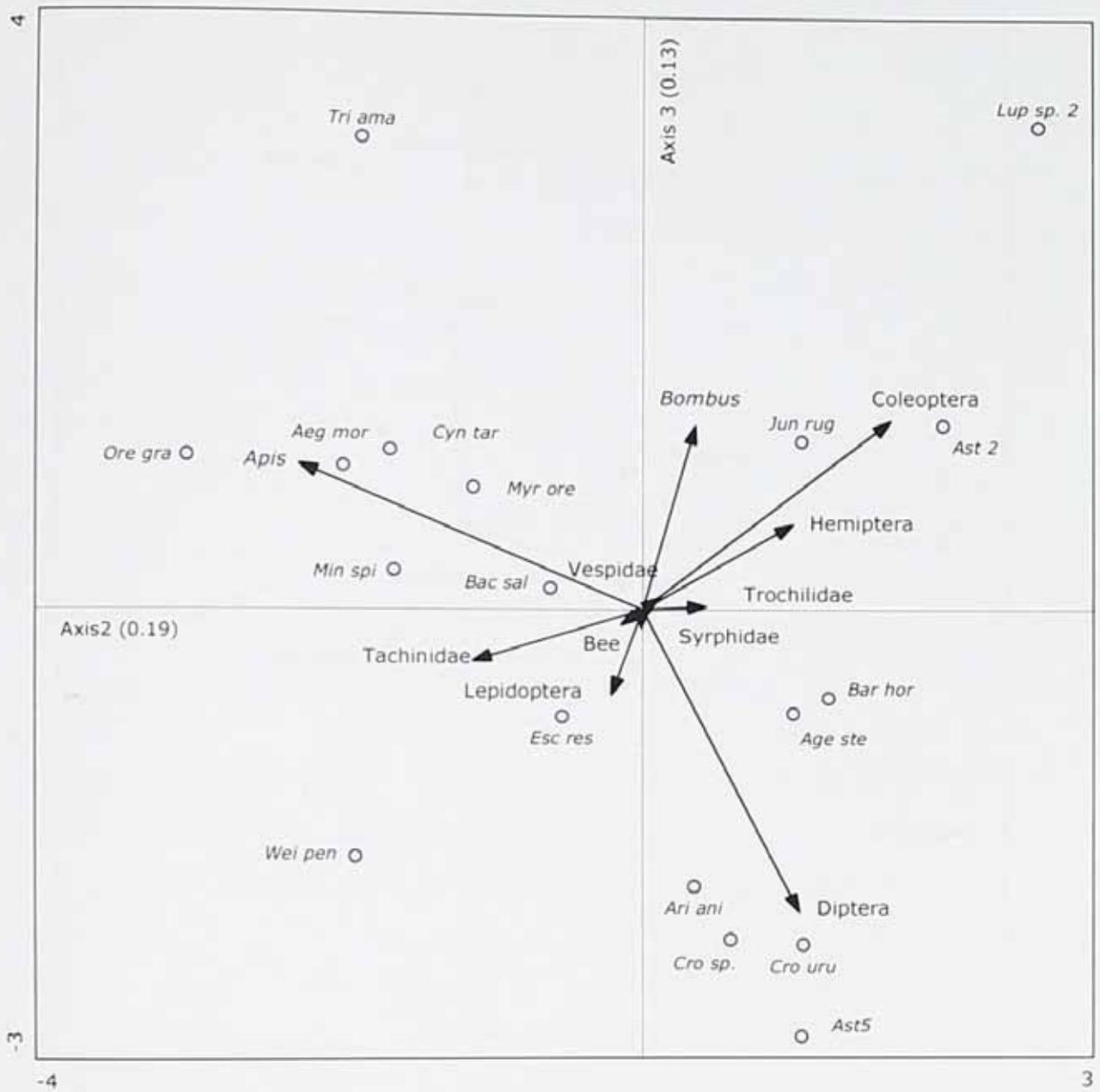


Figure 3.3. Species-environment biplot diagram of plants and functional groups of flower visitors based on axes 2 and 3 of canonical correspondence analysis (CCA). For clarity, only plant species with fit values >1% in the ordination are displayed. See Figure 3.1 A for plant species abbreviations.

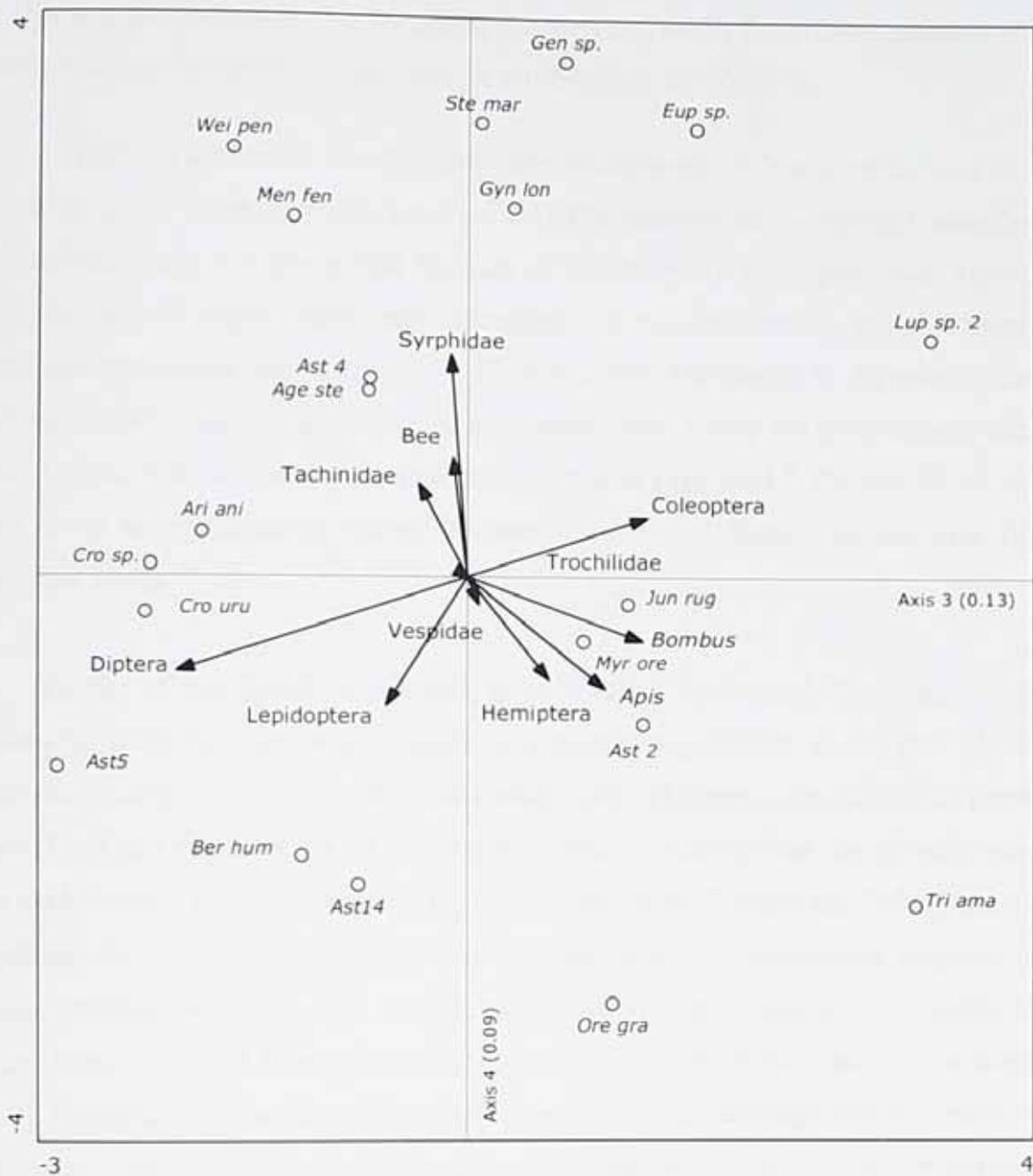


Figure 3.4. Species-environment biplot diagram of plants and functional groups of flower visitors based on axis 3 and 4 of canonical correspondence analysis (CCA). For clarity, only plant species with fit values >1 in the ordination are displayed. Axis 3 and 4 have eigenvalues of 0.13 and 0.09 respectively. See Figure 3.1A for plant species abbreviations. Length of the vectors indicate the strength of the correlation between functional groups of flower visitors.

Is there a difference in the foraging profiles between functional groups of flower visitors when the differences due to valleys are removed?

In order to determine the effects of functional groups of flower visitors, taking account of the effects of valleys, an additional partial CCA analysis was performed, using valleys as covariables. Table 3.9 shows that the sum of all unconstrained eigenvalues was no longer equal to the total inertia, since the covariables (valleys) had already explained some of the inertia in the species data (i.e. $17.34 - 15.04 = 2.39$). The inertia in the species data (plant species visited by functional groups of pollinators) after fitting the covariables (valleys) was 1.03. Of this residual inertia, the first ordination axis explained 2.7% and 39.3% of what in total could be explained by the environmental variables (flower visitors) (Ter Braak and Šmilauer 2002).

Results of the global permutation tests (Table 3.9) showed that both axis 1 and all canonical axes together were highly statistically significant under the Monte Carlo permutation test ($P = 0.0020$), the lowest achievable value given the number of permutations used (Lepš and Šmilauer 2003). In the full analysis resulting from the forward selection of the explanatory variables (Table 3.10), Trochilidae, *Apis*, Diptera and Coleoptera accounted significantly to both the marginal and conditional effects, in determining variation in flower visitor profiles. However, once the effects of valleys were accounted for (partial analysis), Trochilidae, *Apis* and Diptera were still the most important explanatory variables (Table 3.10). Hemiptera became more important than Coleoptera, although neither functional group of flower visitor contributed significantly to the variation explained in the model, evident from the change in closeness in angles of the arrows between Diptera and Coleoptera compared to Coleoptera and Hemiptera in the ordination diagram (Figure 3.5). In conclusion, there still remained systematic differences between functional groups of flower visitors in their foraging profiles after accounting for the effects of the nine valleys in which plants and flower visitors occurred.

Table 3.9. Summary of canonical correspondence analyses selecting functional groups of flower visitors as the only environmental variable and valleys as the covariable

Type of analysis	Axis	Axis	Axis	Axis	Total
CCA	1	2	3	4	inertia
Eigenvalues	0.40	0.13	0.11	0.08	17.43
Species- environment correlations	0.77	0.53	0.54	0.45	
Cumulative % variance of species data	2.70	3.60	4.40	4.90	
Cumulative % of species-environment	39.30	52.30	63.80	72.00	
Sum of all eigenvalues					15.04
Sum of all canonical eigenvalues					1.03

	Eigen value	F-ratio	P-value
Significance of first canonical axis (499 permutations under reduced model)	0.46	5.76	0.002
	Trace	F-ratio	P-value
Significance of all canonical axes (499 permutations under reduced model)	1.03	1.53	0.002

Table 3.10. Canonical correspondence analyses selecting functional groups of flower visitors as the only environmental variables and valleys as the covariables. Marginal and conditional effects obtained from a forward selection. Variables ranked in order of their importance by their marginal and conditional effects. A Monte Carlo permutation test was performed using 499 random permutations. The relative contribution to the model of already included variables is significant if ($P \leq 0.05$)

Functional group of flower visitors	Inertia marginal effects (λ^2)		
Trochilidae	0.39		
<i>Apis</i>	0.13		
Diptera	0.10		
Coleoptera	0.09		
Hemiptera	0.09		
Tachinidae	0.08		
Syrphidae	0.07		
Bombus	0.06		
Lepidoptera	0.06		
Bee	0.06		
Vespidae	0.03		

Functional group of flower visitors	Inertia marginal effects (λ^2)	F - Value	P - value
Trochilidae	0.39	5.78	0.004
<i>Apis</i>	0.12	1.71	0.008
Diptera	0.08	1.30	0.052
Hemiptera	0.09	1.28	0.254
Coleoptera	0.08	1.24	0.150
Tachinidae	0.07	1.03	0.300
Syrphidae	0.07	1.01	0.268
Bee	0.40	0.95	0.454
Bombus	0.04	0.62	0.938
Lepidoptera	0.03	0.44	0.954

Is there a difference in foraging profiles between *Apis* and other functional groups of flower visitors?

In order to determine if the foraging profile of *Apis* was different to other functional groups of flower visitors, the dummy variables coding for *Apis* were used as the only constraining variables, deleting the remaining 10 dummy variables coding for other functional groups of flower visitors. The canonical axis (axis 1) explained 14.7% of the total variability in the species data, while the remaining three unconstrained axes explained individually less variability than the canonical axis (8.6% for axes 2, 8.4% for axis 3 and 8.1% for axis 4). The results showed that the explanatory variable *Apis* was significant,

(Monte Carlo Permutation test, $P = 0.002$) (Table 3.11). Therefore the visitation profile of *Apis* was significantly different to the visitation profiles of the other functional groups.

Table 3.11. Summary of Canonical correspondence analyses selecting *Apis* as the only environmental variable

Type of analysis CCA	Axis 1	Axis 2	Axis 3	Axis 4	Total inertia
Eigenvalues	0.147	0.863	0.847	0.817	17.38
Species- environment correlations	0.53	5.80	10.6	15.3	
Cumulative % variance of species data	0.80	3.90	4.70	5.20	
Cumulative % of species-environment	100.00	0.00	0.00	0.00	
Sum of all eigenvalues					17.43
Sum of all canonical eigenvalues					0.147
	Trace	<i>F</i> -ratio	<i>P</i> -value		
Significance of all canonical axes (499 permutations under reduced model)	0.14	1.9 1	0.002		

Discussion

This study further assessed plant-flower visitor assemblages within a broader framework using canonical correspondence analysis (CCA) to obtain a representative summary of visited plant species across the different valleys using ordination diagrams. Several studies have used correspondence analysis which have included explanatory variables to detect pattern in assemblages of plant-pollinator interactions (i.e. Hingston and McQuillan 2000; Dicks *et al.* 2002; Potts *et al.* 2003; Morales and Aizen 2006). To my knowledge, this is one of the few studies that has used both nestedness (Chapter 4) and several multivariate methods to probe further community-level plant-flower visitor interaction data for patterns. In multivariate analysis, the use of quantitative data as opposed to binary data (e.g. Hingston and McQuillan 2002) has the advantage that it gives a better assessment of how species fall into groups according to the strengths of their interactions (Dicks *et al.* 2002).

Visitation profiles of different functional groups of flower visitors

The canonical correspondence analysis resulted in a well resolved pattern of reciprocal relationships between functional groups of flower visitors and plants species (Figure 3.2). Four visitation profiles differed significantly between the functional groups: Trochilidae, *Apis*, Diptera and Coleoptera. The amount of variability explained by the individual axes did not decrease gradually, instead the CCA analysis revealed strong gradients for all four ordination axes (Table 3.2). By adding post-hoc the supplementary environmental variables altitude and valleys to the ordination and projecting them passively into ordination space, their relation to plant species and flower visitors could be interpreted. The ordination diagram (Figure 3.2) revealed that hummingbirds exhibited the strongest affinities with plant species distributed in the valleys of Huaran, Chicon and Mantamay and occupied higher altitude areas. *Apis* showed the strongest association with those plant species distributed closest to Machu Picchu, between in the valleys of Tiaparo and Piscacucho that tended to be distributed at low altitudes. Diptera and Coleoptera showed the strongest affinities with those plant species located in the valleys of Pumamarca, Choquebamba and Poques that occupied the highest altitudes.

The strong reciprocal patterns shown in this present study were not in accordance with the visitation profiles described by Hingston and McQuillan (1998), where birds, bees and bumblebees, along with muscoid flies, were also similar in their visitation profiles, while wasps and all beetles were opposite. The dissimilarities between visitation profiles in Tasmania may be due to different flowering phenologies, increases in population sizes of *Bombus terrestris* during in early summer, the restriction of foraging by the majority of flower visitors to summer and autumn, and the dependence of the bee sub-family Colletidae and bumblebees on flowers of the family Myrtaceae (Hingston and McQuillan 1998).

One fundamental difference between many alpine areas such as Argentina, Chile, New Zealand, USA and Norway, compared to the tropical high Andes of Perú, is the short length of season, low winter temperatures and snow accumulation which restrict vegetative activity to spring and autumn (Arroyo *et al.* 1981; Totland 1993). Consequently, flowering must be completed early enough to permit pollination, seed maturation and seed dispersal (Arroyo *et al.* 1981). Early flowering is characteristic of many studies conducted in short-season environments (Totland 1993 and references therein). Primack (1983) found that in the subalpine areas of Cass in New Zealand, for the majority of plant species, flowering times were concentrated over a period of five months. Similarly, in the alpine plant communities of Norway, Totland (1993) found that flowering was concentrated at the beginning of the season, five insect-pollinated plant species flowered simultaneously, and that most species pairs overlapped considerably in flower visitor species. In contrast, snow accumulation in the Sacred Valley is usually restricted to much higher altitudes (>5000m) (SW personal observation) than many other alpine regions, and instead the seasons are clearly divided into wet and dry seasons, therefore flowering is not restricted to just one short season and can continue throughout the year.

Once the effects of valleys were accounted for, Trochilidae, *Apis* and Diptera still maintained significantly different visitation profiles (Figure 3.5, Table 3.10). The remaining functional groups did not differ markedly in visitation profiles, but still retained similar patterns to that of the original analysis with flower visitors as the only environmental variables (Figure 3.2). For example, Coleoptera and Hemiptera mainly foraged within a few plant genera such *Aristeguietia* and *Jungia* and were closest in visitor profile to Diptera. The close association between beetles and Asteraceae sp. 2 and *Jungia rugosa* was also evident on axes 2, 3, and 4, though their overlap with flies no longer remained. The overlap in

visitation profiles between flies and beetles was also illustrated by Hingston and McQuillan (1998), where beetles strongly favoured flowers of Asteraceae.

Syrphidae and Tachinidae

Syrphids and tachinids were similar in foraging profiles and this was more evident once the compositional variability explained by valleys was removed (Figure 3.5). Flowers visited by these functional groups are typically radially symmetric with open access to the reward such as *Baccharis* and the flowers of *Escallonia resinosa*. This finding is in agreement with Hingston and McQuillan (1998) who also showed that Tachinidae and Syrphidae overlapped in visitor profile, with some preference to flowers of the Asteraceae. *Bombus*, Lepidoptera, Vespidae and Bee showed no particular affinity with any of the plant species once the effect of valleys was already accounted for. This was evident from the short length of the arrows in the ordination diagrams, on the first two axes (Figures 3.2 and 3.5). This result was not surprising for Bee and Vespidae since these groups recorded the lowest number of visits and only plant species with more than three visits were included in the analysis. In contrast, the species-environment biplots based on the remaining higher axes, resulted in slightly more defined foraging profiles for *Bombus*, Lepidoptera and Syrphidae, indicated by the longer arrows. For *Bombus*, the only strong preference, although not significant, was between bumblebees and *M. oreophila* and *J. rugosa*. Hingston and McQuillan (1998) also showed that bumblebees strongly favoured flowers of the Myrtaceae more than any other plant family. Likewise, in the Sacred Valley, although the association was not evident from the ordination based on axis 1 and 2, the biplot based on axis 3 and 4 did reveal a preference to the brush-like flowers of *M. oreophila* (Myrtaceae) (Figures 3.4). Differences among visitation profiles across geographical regions are not surprising given that some plant communities are highly zonal in character such as the Andean vegetation of central Chile (Arroyo *et al.* 1982) or contain many different life zones, each one characterised by a distinct vegetation type such as the Vilcanota Highlands (Holdridge 1967; Tuypayachi 2005) (see Table 1.4 Chapter 1). The dominant plant families most frequently visited in Tasmania were Myrtaceae, Fabaceae and Epacridaceae (Hingston and McQuillan 1998), whereas in this present study, Asteraceae, Lamiaceae and Myrtaceae were the most heavily visited families.

Hummingbirds

The CCA analyses demonstrated that hummingbirds showed the most distinct foraging profile and were a dominant component of the canonical community ordination. Once the effect of valleys was removed, hummingbirds still maintained the same strong association with all five plant species shown in the previous ordination (Figure 3.2). Hummingbirds predominately foraged on tubular flowers *Barnadesia horrida* (Asteraceae) appeared to have a functionally specialised pollination system; the flowers possess long pink corolla tubes, which are actually a head of small closely fitting tubular flowers surrounded by involucre bracts (Gentry 1996) and produce copious nectar (SW personal observation). However, *B. horrida* was visited by five species of hummingbirds of varying sizes and bill morphologies, so in that sense, are to some degree ecological generalists (Ollerton *et al.* 2007b). Furthermore, this plant was not visited exclusively by hummingbirds, but also hosted syrphid flies, small dipteras, bumblebees and native bees. However, a combination of foraging behaviour and low visitation rates suggested that these insects were not effective pollinators.

The flowers of *Siphocampylus actinothrix* are gullet shaped with a cleft lowermost lobe and exposed anthers and stigma. The flowers are drab yellow to greenish with no odour and were visited exclusively by five hummingbird species. This drab colouration is in contrast to the majority (90%) of *Siphocampylus* species which have vivid red, red and yellow, or orange flowers (Sazima *et al.* 1994). During hovering the head and bill of *Aglaeactis castelnauldii* made contact with the reproductive organs and the close fit between bird head and flower corolla suggested that this plant was hummingbird pollinated. Although data on the pollination biology are scarce, *Siphocampylus* is regarded as mainly an ornithophilous genus (Sazima *et al.* 1994).

The remaining plant species visited by hummingbirds possessed flowers with varying morphological traits. For example, *D. mandonii* has short, white corollas, and were not effectively pollinated by hummingbirds (Chapter 5) and *B. nutans* possess long dark purple whorl shaped flowers which were visited by an array of insect robbers, while the flowers of *Gynoxys longiflora* (Asteraceae) possess open access, small yellow flowers which produce small quantities of nectar. The strong affinity with *G. longiflora* is interesting since this plant has small flowers, allowing easy access to pollen and small amounts of nectar which

conformed more towards entomophily. This finding is in accordance with other studies showing the utilisation and profitability of small flowers used by birds. For example, in New Zealand, Castro and Robertson (1997) surveyed plants visited by three species of native honeyeaters and suggested that the nectar produced by entomophilous flowers provided sufficient energy to sustain the energetic requirements of birds. The results also suggest that small flowers may be valuable to hummingbirds as complements to larger so called ornithophilous flowers such as *B. horrida* and *Passiflora* sp. This finding is also in agreement with McDade and Weeks (2004) who found that in Costa Rica and Panama, hummingbirds visited flowers with a wide range of reward types.

Diptera

Diptera comprised of a combination of small flies (Sciaridae, Muscoidea and Anthomyiidae) and a variety of larger unidentified fly species with a diverse range of proboscis lengths. Diptera showed well resolved patterns of reciprocal relationships on all of the axes, indicating strong affinities with Asteraceae and particularly members of the genera *Aristeguetia* and *Cronquistianthus*. Of the functional groups, flies were the most important vectors with a significant influence on the differences between functional groups on axes 2 and 3, and 3 and 4 (illustrated by long length of arrows in Figures 3.3 and 3.4). The strong association was also retained after the partial CCA analysis was performed, highlighting the same visitor profile (e.g. *A. anisodonton*, *Cronquistianthus* sp., *C. urubambensis* and Asteraceae sp. 5.). These findings showed that flies were non-randomly distributed among this suite of plant species. The overall results were also in agreement with those of Kearns (1992) who found that most fly species visited flowers of several plant species and that in the case of muscoid flies, Asteraceae was an important pollen source.

Diptera are often considered opportunistic, ineffective flower visitors, unlikely to transfer pollen between conspecific plants but their importance as pollinators is often underappreciated (Kearns 1992; Kearns and Inouye 1994; Ollerton 1999). For example, small primitive Diptera such as flies from the families Sciaridae and Mycetophilidae are generally considered ineffective pollinators in comparison to larger flies such as bombyliids and syrphids (Mesler *et al.* 1980). However, Muscoidea have been listed as important pollinators in high altitude systems (Primack 1983; Kearns 1992; Kearns and Inouye 1994), whilst fungus gnats (Sciaridae and Mycetophilidae) have been reported as principal pollinators of

Listera cordata (Orchidaceae) and *Scoliopus bigelovii* (Liliaceae) in coastal redwoods of California (Mesler *et al.* 1980) and *Ceropegia* spp. (Apocynaceae) are pollinated by small flies from a range of families, Ollerton, personal communication.). The floral specificity of Diptera to generalist open access flowers, coupled with the foraging activities and sheer numbers observed on some plants, and their strong presence at all altitudes, suggested that they may provide good pollination services, although this would require further study.

If functional groups as defined by Fenster *et al.* (2004) are important for driving floral evolution and community interaction structure, this suggest that there should be differences in the visitation profiles of these functional groups within the communities. Fenster *et al.* (2004) defined plant species as being specialised in pollination and to occupy pollination niches if they were successfully pollinated by a small subset of functionally grouped potential pollinators. Although the canonical correspondence analyses did show significant differences in the visitation profiles among functional groups of flower visitors, their contribution to pollination was not measured. Therefore conclusions regarding the potential selective pressures of hummingbirds, flies and honeybees may have exerted through plant reproductive success could not be made. Since functional groups may vary considerably in their effectiveness as pollinators to plants over space and time, the selective pressures they may exert are likely to be different (Fenster *et al.* 2004). Furthermore, spatiotemporal variation in pollinator availability, community population dynamics and the phylogenetic identity of the participants are likely to have some bearing on the evolutionary potential of plant-pollinator interactions (Minkley and Roulston 2006; Ollerton *et al.* 2007b).

In the CCA analyses quantitative data was used (i.e. abundance measures) in order to give a better assessment of the strength of interaction between plant species and each of the functional groups. The CCA analyses suggested that the suite of plant species with the strongest associations did not exhibit specialisation onto functional groups. None of the plant species were visited exclusively by honeybees, hummingbirds and flies. Diptera showed strong affinities with phenotypically generalist plant species of the Asteraceae and comprised of a variety of species of flies with varying morphologies and a diverse range of probosces. Honeybees showed the strongest associations with plant species which were both phenotypically generalised (e.g. Asteraceae sp. 8) and to *Oreocallis grandiflora*, which they nectar robbed. Although *O. grandiflora* appeared to have a functionally specialised pollination system, it is probably pollinated by a variety of hummingbird species of varying

sizes and bill morphologies (SW personal observation). Similarly, *Barnadesia horrida* also appeared to have a functionally specialised pollination system, but was visited by five species of hummingbirds and an array of other insect taxa. Thus, those hummingbird pollinator species are to some degree ecological generalists (Ollerton *et al.* 2007b). Additionally it should be noted that despite the strong affinity between hummingbirds and *Duranta mandonii*, they were not pollinators (see Chapter 5). As Fenster *et al.* (2004) pointed out, sometimes the most frequent flower visitors are poor pollinators, thus for this particular plant species hummingbirds are probably not important for driving floral evolution.

Apis mellifera

Considering all canonical axes, honeybees were most strongly associated with *O. grandiflora*, *M. spicata*, *W. pentaphyla*, *M. oreophila*, *Cynanchum tarmense* and *A. mertonii*. Honeybees showed the strongest affinity with *O. grandiflora* (Figures 3.2 and 3.5) but were prevented legitimate access to the nectar of the long narrow pink corolla tubes, and therefore behaved as nectar robbers. *Minthostachys spicata*, and *A. mertonii* have small tubular flowers, *W. pentaphyla* has small white tubular flowers clustered in narrow terminal racemes (Gentry 1996), *C. tarmense* possess open access white flowers and *M. oreophila* has large white open access brush-like flowers. Once the influence of valleys were accounted for, the affinity with *O. grandiflora* and *M. spicata* still persisted, but Asteraceae sp. 8, also became important. In contrast, those plants species such as *M. oreophila*, *W. pentaphyla* and *C. tarmense* which were associated with particular valleys, were no longer plant species with fit values >1% in the ordination diagram.

The strong affinity between honeybees and *O. grandiflora* is noteworthy since this Andean shrub is associated with elfin forest and was only found at sites close to the Sanctuary of Machu Picchu (Piscacucho and Tiaparo). Despite its distributional limits, honeybees still showed a strong affinity with this plant. Likewise, *M. spicata* had a limited distribution, visited by an array of different insects, but still maintained this strong association with honeybees.

The importance of *O. grandiflora* for honeybees and the reproductive success of the plant are difficult to assess since additional observations (some 80 person hours) showed that

this plant is visited by as many as 18 flower visitors, but with the exception of hummingbirds all visitors behaved as nectar robbers. Foraging behaviour of hummingbirds and the morphology of the flowers suggest that they were the legitimate pollinators. However, as Maloof and Inouye (2000) pointed out, it should not be necessarily assumed that nectar robbers are detrimental and do not pollinate the flowers they visit. Maloof and Inouye (2000) reviewed the effect of nectar robbers on seed set on eighteen plant species and showed that the occurrence of negative, neutral and positive effects were equal and depended in part on the identity of the legitimate pollinator and robber, the amount of reward removed by the robber, the growth form of the plant species and the other resources available in the environment. The importance of hummingbirds and honeybees to *O. grandiflora* would need to be evaluated through careful field experiments, as has been done for *Duranta mandonii* (Chapter 5).

The possible impacts from *Apis mellifera* on native flora and fauna

By further assessing the plant-flower visitor assemblages within a broader framework using CCA, the results suggested that honeybees had a distinct visitor profile which only overlapped with Tachinidae on the axis 1 and 2 (Figure 3.2) and *Bombus* on axes 3 and 4 (Figure 3.4). After accounting for the effects of valleys, the overlap between *Apis* and Tachinidae was not so prominent (Figure 3.5). Similarly, the overlap between bumblebees and honeybees was negligible, illustrated by the short length of the arrow for *Bombus*. The CCA analyses revealed that the foraging profiles of honeybees did not overlap with native bees, bumblebees, hummingbirds, flies and syrphid flies. Many studies on mutualistic networks have shown that most interactions are weak and, when considering the mutual effects on plants and pollinators, strongly asymmetric (Jordano 1987; Olesen and Jordano 2002; Bascompte *et al.* 2003). Therefore, even though honeybees collected resources from multiple plant species (see Chapter 2) many of these interactions were weak dependencies. By using multivariate analysis, the CCA revealed which particular plant species honeybees formed the strongest associations with. Of the 114 plant species surveyed throughout the entire Sacred Valley, only two plant species were exclusively visited by honeybees. This finding contrasts with the conclusion reached by some authors (i.e. Donovan 1980; Menezes Pedro and Camargo 1991; Memmott and Waser 2002; Dupont *et al.* 2003; Kato and Kawakita 2004; Kwak and Bekker 2006) that honeybees overlap substantially with native

bees and other native fauna in terms of the flowers they visit. For example, such studies have reported that the proportion of total flora utilised by honeybees can range from 33% up to as much as 88%). In Tasmania, where the bumblebee *Bombus terrestris* has recently been introduced, Hingston and McQuillan (1998) found that this bee overlapped with that of all insect families, all bee subgenera, and all species of birds and proposed that *B. terrestris* had the potential to have a major impact on Tasmanian ecosystems. However, in the Sacred Valley, honeybees visited 13% of the total flora surveyed. Furthermore, in Perú, *Apis mellifera* has been established for some five hundred years (Javier Llaxacondor, personal. Communication, 2001) and based on historical records from Incan bee keepers, the native bees and wasps are still the same species used for honey today (Roubik 2000). This suggests that because of their long coexistence with other native bee species they may have already reached a state of equilibrium with the native fauna. Predicting the outcome of honeybee introductions is highly problematic and potentially challenging (Goulson 2003). The impact of honeybees would need to be evaluated through careful field experiments using direct multiple methods with sufficient sampling replicability over multiple seasons and years. This could prove difficult since honeybees are wide ranging and ubiquitous at all altitudes in this region, thus control sites would be difficult to establish.

Visitation profiles and Sampling intensity

It should be recognised that since the sampling intensity differed among plant species given that their abundance along transects varied, this may have affected the outcome of the results for visitation profiles presented here. It has been shown that differences in sampling effort among species can influence the variation in pollinator assemblages to a given plant species (Ollerton and Cranmer 2002; Herrera 2005; Nielsen and Bascombe 2007). In addition to differences in sampling intensity, a number of other factors were likely to influence visitation profiles within the Sacred Valley. These include habitat fragmentation (Kremen *et al.* 2002; Steffan-Dewenter *et al.* 2002), both seasonal and annual dynamics within and between plant populations (Herrera 1998), temporal and spatial variation in the visitor community (Ollerton and Cranmer 2002; Price *et al.* 2005), population size and relative density of floral displays which could facilitate pollination (Ghazoul 2006). An important characteristic of such generalist flowers described above is that the identity of the main pollinators is likely to be influenced by inter-annual changes in their abundance (Ollerton *et al.* 2007b). Therefore changes in visitation profiles within the Sacred Valley

would be expected not only between the wet season (when peak flowering occurs) and during the dry season, but also between subsequent years.

Summary

The canonical correspondence analysis established which functional groups were the most important according to the strengths of their interactions and in which valleys. Trochilidae, *Apis*, Diptera and Coleoptera were the functional groups of flower visitors with significantly different visitation profiles. Once the effects of valleys were accounted for, hummingbirds, honeybees and flies still maintained significantly different visitation profiles. The CCA analyses demonstrated that overall hummingbirds exhibited the most distinct foraging profile, which was maintained once the effect of valleys was removed, followed by *Apis* and Diptera. Honeybees did not overlap with native bees, bumblebees, hummingbirds, flies and syrphid flies. Of the 114 plant species surveyed throughout the entire Sacred Valley, only two plant species were exclusively visited by honeybees. The CCA analyses also suggested that the suite of plant species with the strongest associations did not exhibit specialisation onto functional groups. None of the plant species were visited exclusively by honeybees, hummingbirds and flies. Although the multivariate analysis identified how species fell into groups based on the strengths of their interactions, those visitation profiles which differed significantly were mainly functional groups of flower visitors that were ecologically generalised. These findings suggested that moderate to substantial ecological and functional generalisation occurs in the Sacred Valley, in agreement with Waser *et al.* (1996).

CHAPTER FOUR

Structure of a Plant-Flower Network in the Sacred Valley, Perú

Introduction

Over recent decades, the ecology and evolution of generalisation has attracted a great deal of interest among pollination ecologists (Waser *et al.* 1996 and references therein; Armbruster *et al.* 2000; Johnson and Steiner 2000; Fenster *et al.* 2004; Herrera 2005; Waser Ollerton *et al.* 2007b). Many mutualistic networks involving plants and their pollinators, animal-seed dispersers, and ant-plants have recently been shown to display several important structural properties (Vázquez *et al.* 2005). The architecture of most networks follow regular patterns; they are typically sparse and reveal high heterogeneity; the majority of species have few interactions, but a few species are much more connected than expected by chance alone (Fortuna and Bascompte 2006). One of the most repeatedly used parameters within these interaction webs is “Connectance” (C), which is defined as the proportion of all possible interactions within a network which are actually realised (Jordano 1987; Olesen and Jordano 2002; Petanidou and Potts 2006). Parameters such as “linkage” have also been used as a descriptor for the mean number of number of interactions across animal species (L_m) (Olesen and Jordano 2002; Dupont *et al.* 2003), as an index for the number of species of flower visitors per plant species (Ollerton and Cranmer 2002), or as specialisation indexes for the number of insect species visiting a plant species (phily) and the number of plant species visited by an insect (tropy) (Petanidou and Potts 2006).

Many mutualistic data sets fit a power-law relationship where the number of interactions increases with network size, while the percentage connectivity decreases with network size and with increasing species richness, and larger communities tend to be more loosely connected than smaller communities (Jordano 1987; Fonseca and Leighton 1996; Dunne *et al.* 2002; Olesen and Jordano 2002; Bascompte *et al.* 2003). Most plant-flower visitor and other mutualistic webs display a nested structure and are highly asymmetric (Olesen and Jordano 2002; Bascompte *et al.* 2003; Jordano *et al.* 2006; Ollerton *et al.* 2007a). One of the most pervasive features arising from the application of network analysis is the degree of generalisation found in most pollination systems (Bascompte *et al.* 2003; Vázquez and Aizen

2004; Jordano *et al.* 2006). In nested assemblages, specialists interact with a subset of the species that the more generalised species interact with Bascompte *et al.* (2003). Nested patterns have implications for understanding community assembly, co-evolution and the conservation of pollination ecosystem services and biodiversity maintenance (Memmott *et al.* 2004; Bascompte *et al.* 2006; Jordano *et al.* 2006). Asymmetrical interactions and the presence of a core of taxa with high density of interactions not only affects the robustness of the mutualistic network, but may also provide pathways for the persistence of rare species (Jordano 1987; Bascompte *et al.* 2003; Jordano *et al.* 2006). For example Bascompte *et al.* (2003) showed that specialised species frequently depended on a core of generalist species and suggested that this core of taxa may drive the evolution of the entire community.

Nestedness theory was originally developed in the context of island biogeography to characterise the distribution of species on island archipelagos or within fragmented landscapes (Patterson 1987; Cutler 1991; Atmar and Patterson 1993). Within sets of islands, biotas show a nested subset pattern if the species present on depauperate islands are a proper subset of those on richer islands (Patterson 1987). The flower visitor species are considered to be equivalent to islands and the plant species to those that inhabit the islands (Bascompte *et al.* 2003). The data are presented in presence and absence matrices, where realised interactions correspond to 1 or shaded squares and absent interactions to 0 or white squares (Figure 4.1). In the pollination matrix, rows represent flower visitor species and columns plant species. In order to measure nestedness, rows and columns of the matrix are packed to a state of maximal nestedness, so that plant and animals species are ranked in order of linkage level. The interaction matrix is ranked from the most generalised species with the highest number of interactions represented in the first rows and columns of the matrix, to the most specialised species with the lowest number of interactions (represented at the bottom left and top right corners of the matrix).

In a perfectly nested matrix the data will fill the matrix so that all interactions will be packed into the upper left corner, ordered in a way to minimise unexpected species absences and presences (Figure 4.1C). Presences of species in a given column will be a proper subset the more specialised species tend to be nested within the interactions already observed among shape and fill. A perfectly ordered matrix (e.g. 50% fills or less) forms a smooth isocline in the upper left hand corner of the matrix (Atmar and Patterson 1993) (Figure 4.1C).

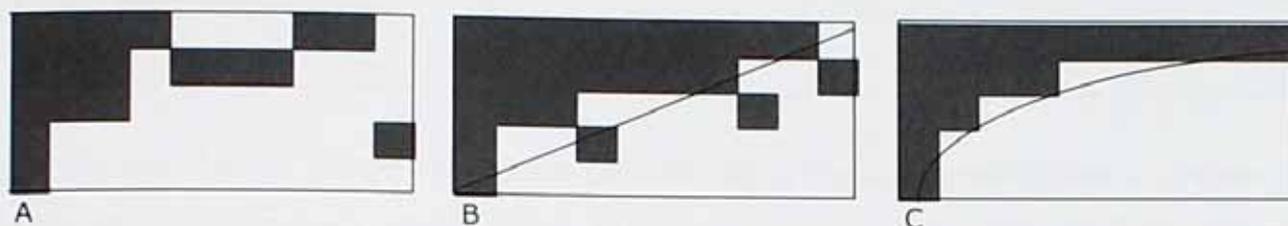


Figure 4.1. Presence /absence matrices showing (A) low, (B) high and (C) a maximally packed matrix of a perfectly nested system. Presences indicated by black squares. Adapted from Guimãraes and Guimãraes (2006).

More recently advances in evaluating the various measures of nestedness have been developed using multivariate statistical methods, including randomisation procedures and the application of appropriate null models (Wright and Reeves 1992; Atmar and Patterson, 1993; Bascompte *et al.* 2003; Memmott *et al.* 2004; Vázquez and Aizen 2004; Vázquez *et al.* 2005; Fortuna and Bascompte 2006; Lewinsohn *et al.* 2006; Stang *et al.* 2006; Nielsen and Bascompte 2007; Santamaria and Rodriguez-Gironés 2007). These modelling techniques have been used to address a wide range of questions relating to the ecology and evolution of generalised and specialised plant-pollinator interactions, landscape fragmentation and habitat degradation, biodiversity loss and the tolerance of mutualistic networks to species extinctions.

Another pervasive feature of some plant-pollinator interaction networks is that those species of flower visitors at the core of the interactions are also the species in greatest abundance, implying that the networks are abundance structured (Dupont *et al.* 2003; Ollerton *et al.* 2003; Stang *et al.* 2006). For example, Dupont *et al.* (2003) found that specialised locally rare plants tended to be visited by generalised locally abundant visitors, and specialised locally rare visitors tended to forage on generalised, locally abundant plants. Generalisation level of a species was significantly correlated with local abundance for flower visitors, thus species interacting with many other species tended to be the most abundant in the community. The factors promoting asymmetry were examined by Stang *et al.* (2006) focusing on the impact of morphological size constraints (nectar holder depth and width) and species abundances in a Mediterranean plant-flower interaction web. Although abundance produced asymmetry, species-species specific predictions about the degree of generalisation of the interaction partners could not be made. The study revealed that size threshold was a good predictor for the level of ecological generalisation within the community as a whole, and emphasised the importance of including morphological traits when characterising generalist and specialist species (Stang *et al.* 2006).

Aims and objectives

In this chapter, plant-flower visitor networks using the same transect data in Chapter 2 were analysed separately constituting a temporal window ranging from April to October 2002. Cumulative webs from pooled valleys and pooled altitudes were then calculated for the entire sampling period. This investigation is one of the few that specifically addresses questions on where *Apis* fits into the plant-flower visitor networks and whether honeybees are likely to affect network composition in the Sacred Valley. In particular, the study addressed the following questions:

1. Which structural features characterise the plant-pollinator networks of the nine valleys? For example, are the matrices compartmented, do they display gradients, are they nested or are the interaction matrices a combinations of compartmented and nested structures?
2. Which species and functional groups of plants and flower visitors dominate the network core, and are their positions constant across the nine valleys?
3. Are the core flower visiting species the most abundant species in the network, as suggested by previous studies (Dupont *et al.* 2003; Ollerton *et al.* 2003; Jordano *et al.* 2006) and is there a correlation between relative abundance and of a given species and its degree of generalisation within each network?
4. Do the species of plants at the core of interactions possess a particular morphology, e.g. an open flower shape as found by Stang *et al.* (2006)?
5. Are the species of flower visitors at the core of interactions defined by possessing relatively long mouth parts as suggested by Stang *et al.* (2006)?
6. Do plants with open access flowers (phenotypically generalised, sensu Ollerton *et al.* 2007b) receive significantly more visits and a higher number of visitor species than plants with tubular flowers and hidden nectar (phenotypically specialised)?
7. Where does *Apis* fit into this network of interactions and is this position constant across the nine valleys?
8. How do these results compare with those previously published for alpine and sub-alpine communities?

Methods

Quantitative measures on the plant-flower visitor networks

The same visitation data from the 90 transects described in Chapter 2 were subjected to nestedness analyses. All data were tested for normality using the Kolmogorov-Smirnov test. Since data were not normally distributed, Mann-Whitney U tests were performed to test for differences between median number of flower visits and flower visitor species to open access flowers compared to tubular, bell, flag and open-tubular flowers. Approaches used by Dupont *et al.* (2003) were used for calculations for network characteristics and linkage levels using the following definitions:

- P = number of plant species visited
- A = number of flower visitor species
- I = number of interactions in the network
- M = system size ($A \times P$)

Linkage level denotes the number of interactions per species:

- L_m of a species of flower visitor (m) = the number of plant species visited by m
- L_n of a plant species (n) = the number of flower visitor species visiting n .

Comparisons of linkage level distributions were estimated for plants and flower visitor species. Linkage levels were standardised as:

- $l_m = L_m / P$ = relative linkage of flower visitor species
- $l_n = L_n / A$ = relative linkage of plant species

To test for a possible relationship between linkage level and abundance, Spearman rank-correlations for both plants and flower visitor species were used. Core plant species and core species of flower visitors were ranked according to 10% of the relative linkage (l_m) scores. Statistical analysis was performed using SPSS 11.5 for Windows (2006, SPSS Inc, Chicago, IL, USA). Means are presented as \pm 1SD.

Network statistics

In the present analysis, the same procedure adopted by Bascompte *et al.* (2003) was followed. The degree of nestedness is defined as N , as $N = (100-T/100)$, in which T is the matrix temperature. Matrix temperature (T) is a simple thermodynamic measure, an analogy with physical disorder (Atmar and Patterson 1993; Bascompte *et al.* 2003). A matrix temperature of 100° denotes a completely random pattern and 0° indicates a perfectly nested pattern. The significance of nestedness of the resultant matrices was assessed using Monte Carlo simulations. Nine binary matrices of plant-flower visitor interactions and all pooled valley matrices were subjected to nestedness analysis using the ANINHADO software (<http://www.guimaraes.bio.br/software.html>; Guimãraes and Guimãraes 2006). ANINHADO is based on the Nested Temperature Calculator (NTC), which was originally developed by AICS Research Inc. (Atmar and Patterson 1993). This software has the advantage that it allows for rapid and independent calculation of T and incorporates more realistic pre-determined null models than the previous software (Guimãraes and Guimãraes 2006). The same method used by Nielsen and Bascompte (2007) and Ollerton *et al.* (2007a) was followed by using the CE null model based on the probabilities in each cell being the mean of the connection probabilities (e.g. generalisation of each plant and flower visitor species). Additionally, the CE model is also considered the most realistic when compared with the other three null models included in the ANINHADO software package because it gives the most conservative inference regarding the significance of nestedness (Bascompte *et al.* 2003).

Results

Network structure of individual valleys

Altogether, 144 species and morphospecies of flower visitors were observed visiting 114 species of plants in the Sacred Valley, forming a total of 620 interactions (Table 4.1). All networks, except for one, were highly significantly nested: generalist flower visitors interacted with generalist and specialist plant species; specialist flower visitors interacted with generalist plant species; and generalist plants interacted with generalist and specialist flower visitors (Figures 4.2-4.10). In all valleys system temperatures T of all matrices (except Huaran) were highly significantly nested (Table 4.2). The plant-flower visitor interaction matrices fitted a power-law relationship; the number of interactions significantly increased with the network size (Pearson $r = 0.98$, $P = <<0.0001$, $N = 9$) and the percentage connectivity (fill) significantly decreased with network size (Pearson $r = -0.83$, $P = 0.006$, $N = 9$). Yanacocha, Poques, Choquebamba and Piscacucho contained the highest number of interactions respectively, were the most species rich and were some of the least connected valleys. Flower visitors out-numbered plant species in all networks, and between 7% and 20% of plant species in each valley were specialists, and were limited to a single visitor species (Figures 4.2-4.10). In all valleys with the exception of Huaran, only a small fraction (mean 10.1 ± 3.9) of the total possible interactions was recorded (Table 4.1). *Apis* was the most generalised species of flower visitor and *Baccharis sacilifolia* was the most generalised plant species. Honeybees interacted with up to 11 species of plants and *B. sacilifolia* was associated with a maximum of 31 species of flower visitors (Figure 4.3).

Network structure across altitudinal zones

Across all five altitudes, system temperatures T of all matrices were highly significantly nested (Table 4.3). System temperature T and percentage connectivity was lower than for individual valleys, with the exception of Yanacocha ($T = 3.3^{\circ}$). Percentage Connectance (C) was similar across all altitudes, whereas system temperature was lowest at altitude 1 and reached a maximum at altitude 3. The highest number of interactions was recorded at altitude 2 and the lowest was recorded at altitude 1. Species richness of flower visitors decreased with altitude, whereas the number plants visited increased with altitude.

Network structure for the single cumulative web

The system temperature T of the single cumulative web was the lowest of the analysed matrices (1.64° , $P = \ll 0.0001$). System temperature T and percentage connectivity decreased to 3.5 % with network size and increasing species richness, resulting in a total of 107 interactions being duplicated across all valleys (Table 4.1). Thus, out of a total of 727 realised interactions, 620 of those interactions were unique and only observed in single valleys, a pattern which was not evident from each matrix analysed separately. Additionally some core species previously identified in individual valleys were not core species in the cumulative web.

Table 4.1. Comparison of network characteristics for the nine plant-flower visitor communities of the Sacred Valley

Valley	Species richness	Number of flower visitors	Number of plant species	Matrix size: total number of potential interactions	Number of interactions recorded	% realised interactions
Huaran	24	16	8	128	26	20.3
Yanacochoa	73	51	22	1122	96	8.5
Chicon	50	32	18	576	63	10.9
Mantanay	58	34	24	816	76	9.1
Pumamarca	62	36	26	936	84	8.9
Choquebamba	72	43	29	1247	100	7.9
Poques	79	47	32	1504	119	8.0
Tiaparo	57	32	25	800	67	8.3
Piscacucho	65	38	27	1026	96	9.7
All valleys pooled	258	144	114	17556	620	3.5

Table 4.2. Quantitative measures of the nine plant-flower visitor communities of the Sacred Valley

Valley	System T	Fill (%) C	P	L_m (mean \pm SD)	Relative Linkage (l_m)	L_n (mean \pm SD)	Relative Linkage (l_n)	Spearman (L_m) r_s P N	Spearman (L_n) r_s P N
Huaran	30.4	20.3	0.258	1.62 \pm 0.88	0.12-0.37	3.25 \pm 2.37	0.06-0.50	0.85 0.00 16	0.59 0.01 8
Yanacocho	3.3	8.5	<<0.0001	1.88 \pm 1.81	0.04-0.52	4.40 \pm 7.52	0.01-0.60	0.92 0.00 22	0.76 0.00 51
Chicon	15.2	10.9	<<0.0001	1.96 \pm 1.28	0.01-0.60	3.50 \pm 3.83	0.03-0.53	0.92 0.00 32	0.90 0.00 18
Mantanay	10.2	9.1	<<0.0001	2.23 \pm 2.17	0.04-0.41	3.16 \pm 3.52	0.02-0.48	0.90 0.00 34	0.90 0.00 24
Pumamarca	11.0	8.9	<<0.0001	2.33 \pm 1.56	0.03-0.26	3.23 \pm 4.42	0.02-0.41	0.94 0.00 32	0.90 0.00 25
Choquebamba	11.7	7.9	<<0.0001	2.32 \pm 1.91	0.03-0.27	3.41 \pm 3.55	0.02-0.34	0.94 0.00 43	1.00 0.01 29
Poques	7.9	8.0	<<0.0001	2.53 \pm 2.10	0.02-0.28	3.71 \pm 4.33	0.02-0.48	0.98 0.00 47	1.00 0.01 32
Tiaparo	9.2	8.3	<<0.0001	2.09 \pm 1.63	0.04-0.32	2.68 \pm 2.79	0.03-0.43	0.94 0.00 32	0.90 0.00 25
Piscacucho	9.3	9.7	<<0.0001	2.56 \pm 1.82	0.03-0.29	3.55 \pm 3.81	0.02-0.42	0.93 0.00 38	1.00 0.01 27

Notes to table: Fill (connectance) is equal to the number of realised links/possible links. P values relate to the null model analysis calculated using the ANINHADO software (Guimarães and Guimarães 2006). L_m = linkage level for flower visitors. L_n = linkage level of plant species. Spearman rank correlations are between abundance and generalisation level (linkage) of species of plants and flower visitors.

Table 4.3. Comparison of network characteristics for the nine plant-flower visitor communities using pooled data across five altitudinal zones

	System T	Fill (%) C	P	Species richness	Number of species of flower visitors	Number of visited plant species	Matrix size: total number of potential interactions	Number of interactions recorded
Altitude 1	4.32	4.7	<<0.0001	105	67	38	2546	118
Altitude 2	5.76	4.9	<<0.0001	110	66	44	2904	143
Altitude 3	7.76	4.4	<<0.0001	94	52	42	2184	130
Altitude 4	7.62	4.4	<<0.0001	110	62	48	2976	122
Altitude 5	6.40	4.6	<<0.0001	107	55	52	2860	125

Notes to table: Fill (connectance) is equal to the number of realised links/possible links. P values relate to the null model analysis calculated using the ANINHADO software (Guimarães and Guimarães 2006).

Network structure of the nine valleys

Huaran

The system temperature T for Huaran, i.e. departure from perfect nestedness, was the highest of all the valleys analysed (30.46°) and was not significantly different to either T in 1000 Monte Carlo simulations, or the CE null model ($P = 0.258$) (Table 4.2). Therefore, the network was the only one characterised by a non-nested structure, which was not more ordered than if flower visitors were randomly visiting plant species. In total 16 species of animals were observed visiting eight plant species, forming only 25 interactions. Compared to the rest of the valleys surveyed, very little insect activity was observed. This was attributed to the poor weather conditions on the day of surveying in comparison with the rest of the valleys surveyed. *Apis*, *Metallura tyrianthina* and *Aglaeactis cupripennis* were the most generalised flower visitors, all forming associations with four plant species (Figure 4.2). The most specialised flower visitor was the endemic hummingbird *Aglaeactis castelnauldii*. Apart from *Apis*, collectively hummingbirds dominated the network core and were the second most abundant group of species. *Baccharis buxifolia* was the most generalised plant species, forming the highest number of interactions, followed by *Aegiphyla mertonii* and *Barnadesia horrida*. *Fuchsia apetala* and *Passiflora* sp. were strict specialist plant species and only interacted with *Aglaeactis castelnauldii*.

	<i>Baccharis salicifolia</i>	<i>Aegiphyla mortonii</i>	<i>Barnadesia horrida</i>	<i>Baccharis buxifolia</i>	<i>Minthostachys spicata</i>	<i>Duranta mandonii</i>	<i>Fuchsia apetala</i>	<i>Passiflora</i> sp.	
<i>Apis mellifera</i>	20	15			5				3
<i>Metallura tyrianthina</i>		6	1			1			3
<i>Aglæactis cupripennis</i>		1	8			2			3
<i>Bombus funebris</i>		1	1	1					3
<i>Muscina</i> sp. 1	2			1					2
<i>Aglæactis castelnauldii</i>							1	2	2
<i>Toxomerus</i> sp. 3	2								1
Syrphidae sp. 2	2								1
Syrphidae sp. 7	1								1
<i>Copestylum</i> sp.1					1				1
Syrphidae sp. 1	1								1
<i>Oreonimpha nobilis</i>			3						1
<i>Eristalis</i> sp.1				1					1
<i>Colibri coruscans</i>		1							1
Tachindae sp. 3	1								1
Diptera sp.15	1								1
	8	5	4	3	2	2	1	1	

Figure 4.2. The maximally packed plant-flower matrix for Huaran. Realised interactions are shown shaded and numbers within squares indicate number of visits. Colour coding is as follows: green=core visitor species to core plant species identified in Tables 4.4 and 4.5, pink= core visitor species to ≥ 2 plant species, blue= specialist visitor species to a single generalist plant species, yellow= specialist visitor species to a single specialist plant species.

Yanacocha

The system temperature T for Yanacocha was the lowest of all the valleys analysed (3.32° , $P = \ll 0.0001$) and thus the closest to perfect nestedness (Table 4.2). The flower visitor data matrix yielded 51 species visiting 21 plant species, forming a total of 96 interactions. *Apis* was the most generalised species and formed associations with 11 plant species. Of those plant species, *Platymiscium* sp. 1, Fabaceae sp.1 and *Berberis humbertiana* were strict specialist plants and interacted exclusively with honeybees (Figure 4.3). Apart from honeybees, those insect species which clustered in the upper left of the matrix were dominated by the butterfly *Metardaris cosinga* and other Lepidoptera spp., muscoid and syrphid flies. These functional groups also tended to be both generalists (e.g. visiting *Baccharis buxifolia*, Asteraceae sp. 5, Asteraceae sp. 6 and Asteraceae sp. 2) and specialists (Asclepiadaceae sp., *Monnina salicifolia*, *Brachyotum nutans* and *Escallonia resinosa*). The most specialised flower visitors were the hummingbirds *Metallura tyrianthina* and *Aglaeactis cupripennis* which only interacted with *Barnadesia horrida*, *Fuchsia apetala* and *Passiflora trifoliata* respectively. Over 30% of the species of flower visitors were specialists, interacting with the most generalised plant species (i.e. those insect species which clustered at the bottom left hand half of the matrix). *Baccharis sacilifolia* was the most generalised plant species and was associated with 31 species of flower visitors. Asteraceae sp. 5 was ranked second and formed interactions with 18 species of flower visitors.

	<i>Baccharis salicifolia</i>	Asteraceae sp. 5	Asteraceae sp. 6	Asteraceae sp. 2	<i>Duranta armata</i>	<i>Coletea spinosa</i>	Asteraceae sp. 8	Asteraceae sp. 7	Asteraceae sp. 4	<i>Passiflora trifoliata</i>	<i>Duranta mandonii</i>	<i>Tarraxacum</i> sp.1	<i>Barnadesia horrida</i>	<i>Fuchsia apetala</i>	Asclepiadaceae sp. 1	<i>Momina salicifolia</i>	<i>Berberis humbertiana</i>	Fabaceae sp.	Asclepiadaceae sp. 2	<i>Brachyotum nutans</i>	<i>Platymiscum</i> sp.	<i>Escallonia resinosa</i>	
<i>Apis mellifera</i>	46	5	8	1		5		8	1		1						2	1			1		11
<i>Metardaris cosinga</i>	10	11	3		5						1					1					1		8
Muscidae sp. 5	3	2	1																	1			4
<i>Muscina</i> sp.1	9	9					1	2															4
Syrphidae sp. 2	9			2					1														4
Lepidoptera sp. 2		1			1						1												3
Muscidae sp.6	4							1							1								3
Diptera sp. 13		1	1				1									1							3
Muscidae sp. 1	5	2				1																	3
<i>Platychierus</i> sp.2	1	1	1																				3
Anthomyiidae sp. 2	4									1													2
<i>Platychierus</i> sp.3		1					1																2
Diptera sp. 3		1						1															2
Diptera sp. 1		1				1																	2
Sphaeroceridae sp.1			1			1																	2
Lepidoptera sp.1	1			1																			2
Tachinidae sp. 10	1	1																					2
<i>Copestylum</i> sp.1	1	1																					2
<i>Metalura tyrianthina</i>													2	1									2
Tachinidae sp. 15	1																						1
<i>Sciara</i> sp. 4	15																						1
<i>Lasioglossum</i> sp.	1																						1
<i>Agealcis culpripennis</i>										1													1
Lepidoptera sp. 5	1																						1
Muscidae sp. 3	1																						1
Diptera sp. 12	1																						1
Tachinidae sp. 8	1																						1
Diptera sp. 4	1																						1
Coleoptera sp. 12	2																						1
Coleoptera sp. 4					1																		1
Diptera sp. 2						1																	1
Syrphidae sp. 1	1																						1
Diptera sp. 9					1																		1
Diptera sp. 16					1																		1
Tachinidae sp. 13		5																					1
Diptera sp. 17		4																					1
Diptera sp. 6					1																		1
Vespididae sp. 5	1																						1
Lepidoptera sp. 3					1																		1
<i>Eristalis</i> sp.1		1																					1
<i>Sciara</i> sp. 3		15																					1
Diptera sp. 8	2																						1
Coleoptera sp. 8		1																					1
Diptera sp. 11		1																					1
Vespididae sp.2	1																						1
Tachinidae sp. 4	1																						1
Diptera sp. 14	9																						1
Diptera sp. 10	2																						1
Syrphidae sp. 4	1																						1
<i>Copestylum</i> sp. 2	1																						1
<i>Toxomerus</i> sp. 2	1																						1
	31	18	7	6	4	4	4	3	3	3	2	2	1	1	1	1	1	1	1	1	1	1	1

Figure 4.3. The maximally packed plant-flower matrix for the valley of Yanacocha. Numbers along the borders of the matrix indicate linkage levels of the species. Realised interactions are shown shaded and numbers within squares indicate number of visits. Colour coding is as follows: green=core visitor species to core plant species identified in Tables 4.4 and 4.5, pink= core visitor species to ≥ 2 plant species, blue= specialist visitor species to a single generalist plant species, yellow= specialist visitor species to a single specialist plant species.

Chicon

The system temperature T for Chicon was the second highest (15.2°) and was highly significantly nested ($P = \ll 0.0001$) (Table 4.2). In total, 32 species of animals were observed visiting 18 plant species, forming 63 interactions (Figure 4.4). *Apis* was the most generalised species and formed associations with five plant species. Apart from honeybees, *Bombus funebris*, syrphid flies, hummingbirds, dipterans, Coleopterans and Lepidoptera sp. 6 were the most generalised functional groups of flower visitors. The most specialist plant species were *Jungia rugosa*, *Melilotus alba*, *Bidens* sp.1, Scrophulariaceae sp.1. and *Baccharis odorata*. The dipteran fly Muscidae sp. 6 was the only strict specialist to the specialist plant *Baccharis odorata* observed at Chicon. The matrix structure was similar to Yanacocha: more than half the species of flower visitors were specialists interacting with the most generalised plant species. *Baccharis sacilifolia* was the most generalised plant species and interacted with 17 species of flower visitors. *Myrcianthes oreophila* was ranked second and formed interactions with eight species of flower visitors. Chicon recorded the least number of plants (6%) forming interactions with only one visitor species. As at Huaran and Yanacocha, abundance values of flower visitors also showed that those insect and bird species which visited the most plant species were also the species in greatest abundance (Figure 4.4).

	<i>Baccharis salicifolia</i>	<i>Myrcianthes oreophila</i>	<i>Baccharis buxifolia</i>	<i>Gynoxys longiflora</i>	Asteraceae sp.2	<i>Brachyotum nutans</i>	Asteraceae sp.4	<i>Escallonia resinosa</i>	<i>Ageratina sternbergiana</i>	<i>Puya ferruginea</i>	<i>Aristeguieta anisodonton</i>	<i>Barnadesia horrida</i>	<i>Mimthostachys spicata</i>	<i>Baccharis odorata.</i>	<i>Jungia rugosa</i>	Scrophulariaceae sp.1	<i>Bidens pilosa</i>	<i>Melilotus alba</i>	
<i>Apis mellifera</i>	45	36	8					6					9						5
Chrysomelidae sp. 2	4		1						1							1			4
<i>Bombus funebris</i>	1	2	1	1															4
Syrphidae sp. 2	7				3			1					2						4
<i>Aglaeactis cupripennis</i>	2			3		12						4							4
Syrphidae sp. 3				1	2						1							1	4
Lepidoptera sp. 6	1				1													1	3
Muscidae sp. 1	6						1										1		3
<i>Pterophanes cyanopterus</i>		1				4				1									3
Diptera sp.15		2	1					2											3
Diptera sp. 11			1										1						2
<i>Sciaria</i> sp. 4.	3										1								2
<i>Sciaria</i> sp. 2	1								1										2
Coleoptera sp. 1	1				1														2
Muscidae sp. 6													1						1
<i>Sciaria</i> sp. 5	1																		1
Diptera sp. 2	1																		1
<i>Oreotrochilus estella</i>										1									1
Coleoptera sp. 3	1																		1
Diptera sp. 6							1												1
<i>Oreonimpha nobilis</i>						1													1
Diptera sp. 1		1																	1
<i>Toxomerus</i> sp. 2							1												1
Hymenoptera sp. 5				1															1
Syrphidae sp. 6	1																		1
<i>Platychierus</i> sp. 2		1																	1
Lepidoptera sp. 10			1																1
<i>Aglaeactis castelnauldii</i>				2															1
<i>Colibri coruscans</i>		2																	1
Tachindae sp. 1	1																		1
Tachinidae sp. 12	1																		1
Tachinidae sp. 17	2																		1
	17	8	5	5	4	3	3	3	2	2	2	2	2	2	1	1	1	1	1

Figure 4.4. The maximally packed plant-flower matrix for the valley of Chicon. Numbers along the borders of the matrix indicate linkage levels of the species. Realised interactions are shown shaded and numbers within squares indicate number of visits. Colour coding is as follows: green=core visitor species to core plant species identified in Tables 4.4 and 4.5, pink= core visitor species to ≥ 2 plant species, blue= specialist visitor species to a single generalist plant species, yellow= specialist visitor species to a single specialist plant species. Red = specialist visitor species to a specialist plant species.

Mantanay

The system temperature T for Mantanay was (10.2°) and was highly significantly nested ($P = \ll 0.0001$) (Table 4.2). The flower visitor data matrix yielded 35 species visiting twenty four plant species, forming a total of 76 interactions (Figure 4.5). *Apis* was the most generalised species and formed associations with 10 plant species. Syrphidae sp. 2 and *Bombus funebris* were ranked second and third most generalist species of flower visitors respectively. Apart from honeybees, syrphid flies, tachinid flies and hummingbirds were the most generalised functional groups. Coleoptera sp. 3 was the only strict specialist flower visitor to the most specialist plant *Lupinus mutabilis*. Nearly a third of the species of flower visitors were specialists interacting with the most generalised plant species.

Myrcianthes oreophila was the most generalised plant species and formed associations with 15 species of flower visitors, closely followed by *Baccharis buxifolia*. Compared to Chicon, the rankings of plant species were reversed at Mantanay. Of those plant species, *Prunus serotina* was a strict specialist plant, being one of the only plant species in the whole of the Sacred Valley visited exclusively by honeybees. Although at Mantanay *Jungia rugosa* could be considered a specialist plant species, at Pumamarca, this plant is an extreme generalist. Other specialist plants were *Oenothera rosea*, *Duranta armata* and *Passiflora* sp.1.

	<i>Myrcianthes oreophila</i>	<i>Baccharis salicifolia</i>	<i>Escallonia resinosa</i>	<i>Weinmannia pentaphylla</i>	<i>Cynanchum tarmense</i>	Asteraceae sp.2	<i>Aristeguietia anisodonton</i>	Asteraceae sp.4	<i>Passiflora tripartita</i>	<i>Barnadesia horrida</i>	<i>Tecoma sambucifolia</i>	<i>Baccharis buxifolia</i>	<i>Mimthostachys spicata</i>	<i>Aegiphyla mortonii</i>	Solanaceae sp. 1	<i>Duranta mandonii</i>	<i>Lupinus mutabilis</i>	<i>Jungia rugosa</i>	<i>Siphocampulus actinotrix</i>	<i>Duranta armata</i>	<i>Gynoxys longifolia</i>	<i>Prunus serotina</i>	<i>Oenothera rosea</i>	<i>Passiflora tripartita</i>		
<i>Apis mellifera</i>	13	12	1		10	2	2							2	1			2				2			10	
Syrphidae sp. 2		3	2	2				3		1			1		1									1		8
<i>Bombus funebris</i>	7	2	3			2			1		1														1	7
Syrphidae sp. 3	1		2		1		1					10														6
Lepidoptera sp. 6	1		1																							3
<i>Aglæactis cupripennis</i>	3								1	5																3
<i>Aglæactis castelnauldii</i>										2						3			2							3
Tachinidae sp. 6	2			5										1												3
Tachinidae sp. 2		2	1	1																						3
Diptera sp.15	1												1													2
Tachinidae sp. 8	2																					1				2
Hymenoptera sp. 3		1						1																		2
Diptera sp. 5	1				2																					2
<i>Bombus melaleucus</i>			2		1																					2
<i>Metalura tyrianthina</i>									1								1									2
Muscidae sp. 1	3																									1
Coleoptera sp. 3																		2								1
Diptera sp. 16	1																									1
<i>Colibri coruscans</i>	1																									1
<i>Sciara</i> sp. 4.													1													1
Tachinidae sp. 1											1															1
<i>Chrysomelidae</i> sp. 2		2																								1
Tachinidae sp. 9									1																	1
<i>Eristalis</i> sp. 2							1																			1
Diptera sp. 11						1																				1
Tachinidae sp. 10	1																									1
<i>Copestylum</i> sp.1			1																							1
<i>Toxomerus</i> sp. 2		1																								1
Diptera sp. 3				1																						1
Tachinidae sp. 14	2																									1
Coleoptera sp. 2		1																								1
Tachinidae sp. 5	1																									1
Lepidoptera sp. 11	1																									1
Lepidoptera sp. 2	1																									1
	17	8	8	4	4	3	3	3	3	3	2	2	2	2	2	2	2	1	1	1	1	1	1	1	1	

Figure 4.5. The maximally packed plant-flower matrix for the valley of Mantaray. Numbers along the borders of the matrix indicate linkage levels of the species. Realised interactions are shown shaded and numbers within squares indicate number of visits. Colour coding is as follows: green=core visitor species to core plant species identified in Tables 4.4 and 4.5, pink= core visitor species to ≥ 2 plant species, blue= specialist visitor species to a single generalist plant species, yellow= specialist visitor species to a single specialist plant species. Red = specialist visitor species to a specialist plant species.

Pumamarca

The system temperature T for Pumamarca was (11.1°) and was highly significantly nested ($P = \ll 0.0001$) (Table 4.2). Thirty six species were observed visiting 26 plant species, forming a total of 82 interactions (Figure 4.6). *Bombus funebris* formed associations with the highest number of plant species, compared to both bumblebee species, but honeybees were more abundant (Figure 4.6). The assemblage of flower visitors also showed that Dipterans and Coleopterans were more generalised flower visitors than in previous valleys and tended to replace syrphid and tachinid flies, and hummingbirds. *B. buxifolia* and Asteraceae sp. 2, followed closely by *Jungia rugosa* were the most generalised species of plants, forming associations with up to 15 species of flower visitors. Fifteen percent of plant species were specialists, interacting with only one species of flower visitor. Species 24 and 55 were strict specialists and were visited exclusively by beetles and wasps. An important characteristic of Pumamarca, Choquebamba and Poques was the apparent level of endemism and the high number of plant and flower visitors shared in common. Also noteworthy was the overall high numbers of specialist interactions recorded in these three valleys (see Figure 4.11).

Choquebamba

The system temperature T for Choquebamba was (11.7°) and was highly significantly nested ($P = \ll 0.0001$) (Table 4.2). Forty three species of flower visitors were observed visiting 29 plant species, forming a total of 99 interactions (Table 4.1). At Choquebamba, both bumblebees, and some flies were the most generalised flower visitors and all formed associations with eight plant species each (Figure 4.7). Compared to most valleys, honeybees were a much less dominant component of the network, forming associations with only three generalist plant species. Coleoptera sp. 3, *Eriopsis* sp. 1 and *Aglaeactis cupripennis* were strict specialist flower visitors, whereas in Huaran, the hummingbird *A. cupripennis* was an extreme generalist. The matrix structure was similar to Pumamarca; more than a third of the species of flower visitors were specialists and interacted with the most generalised plant species. *Jungia rugosa*, *Ageratina sternbergiana* and *Baccharis buxifolia* were the most generalised plant species in this valley, forming associations with up to 15 species of flower visitors and only 9% of plant species were limited to one visitor species.

	<i>Baccharis salicifolia</i>	Asteraceae sp.2	<i>Jungia rugosa</i>	<i>Ageratina sternbergiana</i>	Species 61	<i>Eupatorium</i> sp.1	<i>Baccharis buxifolia</i>	<i>Stellaria media</i>	<i>Lobelia tenera</i>	<i>Oxalis urubambensis</i>	Species 24	Species 55	Species 22	<i>Trifolium amabile</i>	Species 63	<i>Oxalis lotoides</i>	Species 57	<i>Lupinus</i> sp.3	<i>Ranunculus praemorsus</i>	<i>Baccharis</i> sp.6	<i>Croquistanthus</i> sp.	Species 62	Species 54	Species 35	Species 30	<i>Escallonia resinosa</i>	
<i>Bombus funebris</i>	2	2	8		1				1								1					1					7
<i>Apis mellifera</i>	7	1	11				3	1												1							6
<i>Sciana</i> sp. 4.	5	1		1	1	14		1																			6
<i>Bombus melaleucus</i>	2		1											1		1										1	5
Diptera sp. 1				1			1															1			1	1	4
Muscidae sp. 1	6		2																					1			3
<i>Sciara</i> sp.5	1	1																							1		3
<i>Lasiglossum</i> sp.	1							1		1																	3
Muscidae sp. 5		1	1			1																					3
Muscidae sp. 4	3						1												1								3
<i>Asylus</i> sp.3	4	3	2																	1							3
<i>Toxomerus</i> sp.2	8	4	3																								3
<i>Astylus</i> sp.2		2												1													2
<i>Eriopsis</i> sp. 1		2																	1								2
Sphaeroceridae sp.2			1						1																		2
<i>Eriopsis</i> sp.2				2	2																						2
Curculionidae sp.1		2			1																						2
Coleoptera sp. 5		1	1																								2
Coleoptera sp. 7	3		2																								2
Curculionidae sp.4	1	1																									2
Sphaeroceridae sp. 1		1	1																								2
Syrphidae sp. 8									2					1													2
Coleoptera sp. 1		1	1																								2
<i>Eristalis</i> sp. 2														1													1
Vespidae sp. 7													1														1
Coleoptera sp. 3				1																							1
<i>Eristalis</i> sp.1				1																							1
Muscidae sp.6						1																					1
Lepidoptera sp.13				1																							1
Lepidoptera sp. 14				1																							1
<i>Toxomerus</i> sp. 1	1																										1
Chrysomelidae sp. 2		2																									1
Curculionidae sp. 3	2																										1
Coleoptera sp. 14			1																								1
Coleoptera sp. 4			1																								1
Curculionidae sp. 2	2																										1
	15	15	14	7	4	3	3	3	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Figure 4.6. The maximally packed plant-flower matrix for the valley of Pumamarca. Numbers along the borders of the matrix indicate linkage levels of the species. Realised interactions are shown shaded and numbers within squares indicate number of visits. Colour coding is as follows: green=core visitor species to core plant species identified in Tables 4.4 and 4.5, pink= core visitor species to ≥ 2 plant species, blue= specialist visitor species to a single generalist plant species, yellow= specialist visitor species to a single specialist plant species. Red = specialist visitor species to a specialist plant species.

	<i>Junglia rugosa</i>	<i>Ageratina stenbergiana</i>	<i>Baccharis salicifolia</i>	<i>Escallonia resinosa</i>	<i>Senecio paniculatus</i>	<i>Senna birostris</i>	Asteraceae sp. 2	<i>Oxalis urubambensis</i>	<i>Salvia oppositiflora</i>	<i>Berberis humbertiana</i>	<i>Myrcianthes oreophila</i>	<i>Larrium amplexicaule</i>	<i>Fuchsia boliviana</i>	Asteraceae sp. 14	<i>Aristeguietia anisodonta</i>	Asteraceae sp. 37	<i>Stellaria maris</i>	Asteraceae sp. 52	<i>Taraxacum</i> sp.	Melastromaceae sp.	Asteraceae sp. 5	Species 46	<i>Oenothera versicolor</i>	Species 31	<i>Melilotus alba</i>	<i>Oxalis lotoides</i>	Species 29	species 48	Umbelliferaceae sp.	
<i>Bombus funebris</i>	1	5	3	2				1				1													1			1	8	
<i>Sciara</i> sp. 4		5	24	6	3							1		1										1		1			1	8
Muscidae sp. 5	1	2	1							1	1	1														1			1	8
Diptera sp. 1	3	2				1	2																	1						6
<i>Toxomerus</i> sp. 2	4		3				1								1	1														5
<i>Platychierus</i> sp. 2		3	1														1										1			4
Muscidae sp. 1	2		2	2											1															4
<i>Lygaeus albomatus</i>							10							2						1										3
Coleoptera sp. 2	1		1					1																						3
Diptera sp. 11		1								1																				3
Syrphidae sp. 3	1	1		1							1																			3
<i>Apis mellifera</i>	5		1	1																										3
<i>Astylus</i> sp. 2	1																						1							2
<i>Platychierus</i> sp. 3	1									4																				2
<i>Sciara</i> sp. 5		1													1															2
Syrphidae sp. 2			1														1													2
<i>Bombus</i> sp.	1									1																				2
<i>Bombus melaleucus</i>								1	1																					2
Curculionidae sp. 1							1	1	1																					2
Curculionidae sp. 2	1							1																						2
Coleoptera sp. 7	1						1																							2
<i>Sciara</i> sp. 3										1				1																2
Diptera sp. 15														1																2
Vespidae sp. 4	1		1																											2
<i>Agleactis cupripennis</i>																														1
Coleoptera sp. 3																														1
<i>Eropis</i> sp. 1																				1										1
Tachinidae sp. 13														1																1
Muscidae sp. 7											1																			1
Hymenoptera sp. 1										1																				1
Anthomyiidae sp. 1						2																								1
Coleoptera sp. 1										1																				1
Curculionidae sp. 3																														1
Muscidae sp. 4			2																											1
Sphaeroceridae sp. 1																														1
Tachinidae sp. 19																														1
Muscidae sp. 6			1																											1
Tachinidae sp. 10																														1
Chironomidae sp.																														1
<i>Sciara</i> sp. 2			1																											1
<i>Toxomerus</i> sp. 1																														1
Anthomyiidae sp. 2			1																											1
Vespidae sp. 6																														1
	15	11	11	7	5	5	5	5	4	3	3	3	3	3	2	2	2	2	2	2	1	1	1	1	1	1	1	1	1	1

Figure 4.7. The maximally packed plant-flower matrix for the valley of Choquebamba. Numbers along the borders of the matrix indicate linkage levels of the species. Realised interactions are shown shaded and numbers within squares indicate number of visits. Colour coding is as follows: green=core visitor species to core plant species identified in Tables 4.4 and 4.5, pink= core visitor species to ≥ 2 plant species, blue= specialist visitor species to a single generalist plant species, yellow= specialist visitor species to a single specialist plant species. Red = specialist visitor species to a specialist plant species.

Poques

The system temperature T for Poques was the third lowest (7.9°) and was highly significantly nested ($P = \ll 0.0001$) (Table 4.2). The flower visitor data matrix for Poques yielded 45 species visiting 32 plant species, forming the highest number of interactions (114) of all valleys (Table 4.1). *Apis* was a dominant component and interacted with nine plant species; the majority of interactions were with generalised plant species (Figure 4.8). In contrast to some of the other valleys, Poques did not have any specialist plant species. At Poques *Trifolium amabile* was visited by honeybees and Vespidae sp.3. However, in Pumamarca, *Bombus melaleceus* was the sole flower visitor to this plant species. The assemblage of flower visitors also showed that the most generalised functional groups were Diptera and Syrphidae. As in the majority of valleys, rare specialist flower visitors tended to interact with the most generalised plant species. *Jungia rugosa*, Asteraceae sp. 2 and *Baccharis buxifolia* were the most generalised plant species in this valley, forming associations with up to 22 species of flower visitors. Over 31% of plant species formed associations with two or more species of flower visitor.

Tiaparo

The system temperature T for Tiaparo was (9.2°) and was highly significantly nested ($P = \ll 0.0001$) (Table 4.2). Thirty two species of flower visitors were observed visiting 25 plant species, forming a total of 66 interactions (Table 4.1). Although the degree of linkage with plants was greatest for Syrphidae and Diptera, many of those species visited by these functional groups were to specialist plants (Figure 4.9). In contrast, *Apis* tended to form interactions with more generalised plants. Muscidae sp. 2 and *Muscina* sp.1 were the least linked species of flower visitors, interacting with rare specialist plants species. Most specialist flower visitor species however, interacted with generalised plants. *Minthostachys spicata* was the most linked plant species, forming interactions with 14 species of flower visitors. The degree of linkage with flower visitors for the plant species ranked second most generalised was noticeably less linked at Tiaparo than in the rest of the valleys and 18% of plants were limited to a single visitor species.

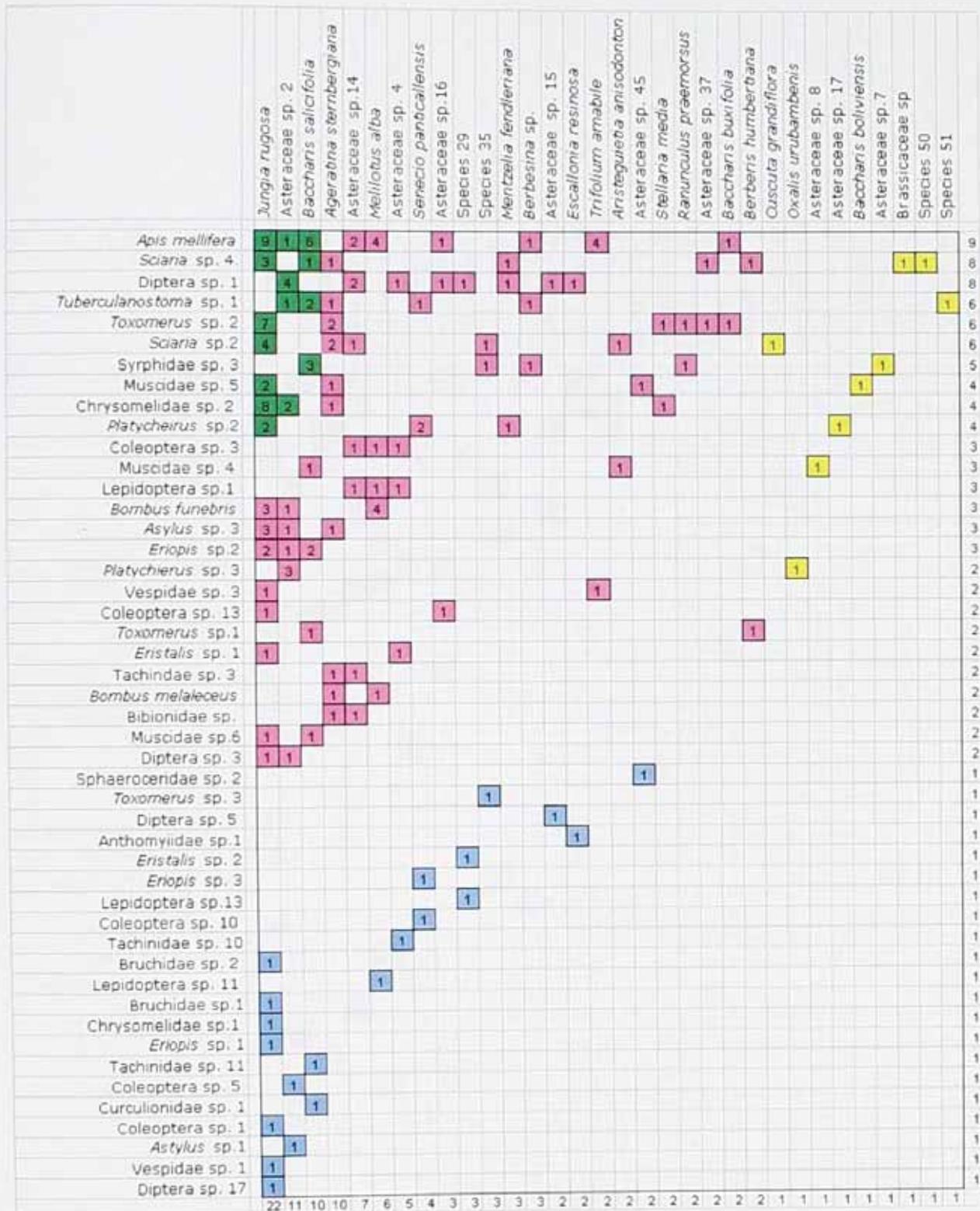


Figure 4.8. The maximally packed plant-flower matrix for the valley of Poques. Numbers along the borders of the matrix indicate linkage levels of the species. Realised interactions are shown shaded and numbers within squares indicate number of visits. Colour coding is as follows: green=core visitor species to core plant species identified in Tables 4.4 and 4.5, pink= core visitor species to ≥ 2 plant species, blue= specialist visitor species to a single generalist plant species, yellow= specialist visitor species to a single specialist plant species.

	<i>Mimthachys spicata</i>	<i>Ageratina sternbergiana</i>	<i>Cronquistianthus urubambensis</i>	<i>Aristeguietia discolor</i>	<i>Lupinus</i> sp.1	<i>Oreocallis grandiflora</i>	<i>Barnadesia horrida</i>	<i>Bidens triplinervia</i>	<i>Aristeguietia anisodonton</i>	<i>Drimaria</i> sp.	<i>Baccharis salicifolia</i>	<i>Gentianaceae</i> sp. 1	<i>Bidens andicola</i>	Species 22	<i>Asteraceae</i> sp. 17	<i>Solanum ochrophylum</i>	<i>Fragaria</i> sp.	<i>Agalinis</i> sp.	<i>Salvia opositiflora</i>	<i>Aristeguietia andicolor</i>	<i>Cronquistianthus</i> sp.	<i>Brachyotum nutans</i>	<i>Eupatorium</i> sp.	<i>Jungia rugosa</i>	<i>Escallonia resinosa</i>		
<i>Sciaria</i> sp. 4.		13		4			1	3		1		2										2				3	8
Syrphidae sp. 3				2								1	1										1	2	1		6
Syrphidae sp. 2	7		1								1		1														4
<i>Apis mellifera</i>	57		5			11		1																			4
Diptera sp. 17		1							1										1								3
<i>Sciaria</i> sp. 2				1							1										1						3
Coleoptera sp. 11	1				1				1																		3
<i>Colibri coruscans</i>						1	1											1									3
<i>Copestylum</i> sp. 2				1	1			1																			3
Diptera sp.15	1	1	1																								3
<i>Eristalis</i> sp. 2	9																	1									2
Hymenoptera sp. 2	1						1																				2
Tachinidae sp. 10	2				1																						2
<i>Platychierus</i> sp. 2	1		1																								2
Diptera sp. 5						1																					2
Muscidae sp. 2																											1
<i>Muscina</i> sp.1														1													1
Hymenoptera sp. 4	1																										1
<i>Copestylum</i> sp.1	1																										1
Lepidoptera sp. 11									1																		1
Diptera sp. 14										1																	1
Lepidoptera sp.8	1																										1
Coleoptera sp. 3						1																					1
<i>Agleactis cupripennis</i>							1																				1
<i>Astylus</i> sp.1					1																						1
Tachinidae sp. 2		1																									1
Coleoptera sp. 6			1																								1
Coleoptera sp. 9						1																					1
<i>Helicobia</i> sp. 1			2																								1
<i>Ophion</i> sp.	1																										1
Diptera sp. 11	1																										1
Tachinidae sp. 8	1																										1
	14	5	5	5	5	4	3	3	3	2	2	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1

Figure 4.9. The maximally packed plant-flower matrix for the valley of Tiaparo. Numbers along the borders of the matrix indicate linkage levels of the species. Realised interactions are shown shaded and numbers within squares indicate number of visits. Colour coding is as follows: green=core visitor species to core plant species identified in Tables 4.4 and 4.5, pink= core visitor species to ≥ 2 plant species, blue= specialist visitor species to a single generalist plant species, yellow= specialist visitor species to a single specialist plant species.

Piscacucho

The system temperature T for Piscacucho was close to that of Tiaparo (9.3°) and was highly significantly nested ($P = \ll 0.0001$) (Table 4.2). Although the matrix yielded similar numbers of plants and animal species to Tiaparo, the total number of interactions was much higher in Piscacucho (66 compared to 95, respectively) (Table 4.1). *Apis* was the most generalised species and formed associations with eight species of plants. The beetle Chrysomelidae sp. 2 was ranked second and tended to interact with the most generalised plant species (those clustered in the left hand side of the matrix). Syrphid flies were an important functional group of visitors, tending to interact with both generalised and specialised plants. Flower visitors at Piscacucho recorded the highest degree of linkage with plant species in all of the valleys; 65% of all the flower visitors formed associations with at least two plant species (Figure 4.10).

Minthostachys spicata was also the most linked plant, forming associations with sixteen flower visitors. *Gentianella* sp.1 was also an important plant species, (though only recorded being visited in this valley). Compared to the rest of the valleys, those plant species which were ranked the most generalised tended to be common species found in most valleys. In Choquebamba and Poques *Senecio panticallensis* was one of the most generalised plant species but in Piscacucho was a specialist plant.

	<i>Mintostachys spicata</i>	<i>Gentianella</i> sp.1	<i>Aristeguietia discolor</i>	<i>Baccharis salicifolia</i>	<i>Lupinus</i> sp.2	Asteraceae sp.4	Species 22	<i>Ageratina</i> sp.1	<i>Baccharis boliviana</i>	<i>Baccharis odorata</i>	<i>Geranium</i> sp.	<i>Brachyotum rutans</i>	<i>Eupatorium</i> sp.	<i>Desmodium rotundus</i>	Asteraceae sp.2	<i>Siphocampylus</i> sp.	Melastromaceae sp.	<i>Solanum</i> sp.	<i>Ranunculus praemorsus</i>	<i>Saracha</i> sp.	<i>Gentianella</i> sp.2	<i>Arenaria lanuginosa</i>	<i>Senecio pamtcallensis</i>	<i>Croquistanthus</i> sp.	<i>Lobelia tenera</i>	<i>Bidens trip linervia</i>	<i>Gaidendrum punctatum</i>	
<i>Apis mellifera</i>	17			1	1	1	1	1				1													1	1	8	
Chrysomelidae sp. 2		3		1	1	1			1	1			1														1	7
<i>Platychierus</i> sp. 1	5	4		3	1								1													1	6	
<i>Copestylum</i> sp. 2	2		1						1			1															1	6
<i>Sciara</i> sp. 4.				4			2	2							1					1		1		1			1	6
Syrphidae sp. 2		2	3	1						1							1											5
<i>Eristalis</i> sp. 2					1	1		1																				3
<i>Toxomerus</i> sp.1	1					1			1																			3
Syrphidae sp. 5		2				1										1												3
Tachinidae sp. 8	5					2	1																					3
Lepidoptera sp. 6				1			1		1																			3
Syrphidae sp. 3	6			7	1																							3
<i>Bombus funebris</i>	1																			2								2
Syrphidae sp. 7		1																				1						2
Sphaeroceridae sp. 2								1	1		1																	2
Diptera sp.15				1						1																		2
Coleoptera sp. 10					1																1							2
Coleoptera sp. 2		1			1																							2
Lepidoptera sp. 7							1							1														2
<i>Astylus</i> sp.1		1			1																							2
Lepidoptera sp. 4	2			1																								2
Lepidoptera sp. 11	1			2																								2
<i>Sciara</i> sp. 3	1			1																								2
<i>Platycheirus</i> sp.1	1	1																										2
<i>Tuberculanostoma</i> sp. 1		1	1																									2
Diptera sp. 5	2		1																									2
Lepidoptera sp. 9											1																	1
Lepidoptera sp. 12											1																	1
Diptera sp. 2											1																	1
Tachinidae sp. 16								1																				1
Bruchidae sp. 3						1																						1
<i>Bombus melaleucus</i>						1																						1
<i>Copestylum</i> sp.1	1																											1
Tachinidae sp. 7	1																											1
Tachinidae sp. 18	2																											1
Diptera sp. 7				1																								1
Lepidoptera sp.12			1																									1
Tachinidae sp. 1	2																											1
	16	10	9	8	8	7	5	5	4	3	3	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Figure 4.10. The maximally packed plant-flower matrix for the valley of Piscacucho. Numbers along the borders of the matrix indicate linkage levels of the species. Realised interactions are shown shaded and numbers within squares indicate number of visits. Colour coding is as follows: green=core visitor species to core plant species identified in Tables 4.4 and 4.5, pink= core visitor species to ≥ 2 plant species, blue= specialist visitor species to a single generalist plant species, yellow= specialist visitor species to a single specialist plant species.

Abundance and linkage level

Mean linkage levels for flower visitors (L_m) ranged from 1.62 in Huaran to 2.56 in Piscacucho and mean linkage levels for plant species (L_n) ranged from 2.68 in Tiaparo and 4.40 in Yanacocha (Table 4.2). Flower visitors at Yanacocha were the most species rich, but recorded the lowest mean linkage level for species of flower visitors ($L_m = 1.88 \pm 1.81$) (excluding Huaran), and the highest linkage levels for plant species ($L_n = 4.40$) (Figure 4.11). Mean linkage level for plants was higher than for flower visitors. Plants were visited by a mean of 3.43 ± 1.47 (mean \pm SD) insect and bird species. On average, each insect or bird species visited 2.17 ± 0.41 (mean \pm SD) plant species. Overall, the range of relative linkage values for both plants and flower visitors were similar across valleys. Distributions of linkage levels for all of the valleys, with the exception of Huaran, were highly right-skewed, with a large spread around the mean. When abundance values of flower visitors were overlain on each nestedness matrix, strong significant positive correlations were found between abundance and generalisation level (linkage) of a species for both plants and flower visitors in all of the valleys (Table 4.2). Thus, those species of flower visitors at the core of the interactions were also the species in greatest abundance (Figures 4.2-4.10)

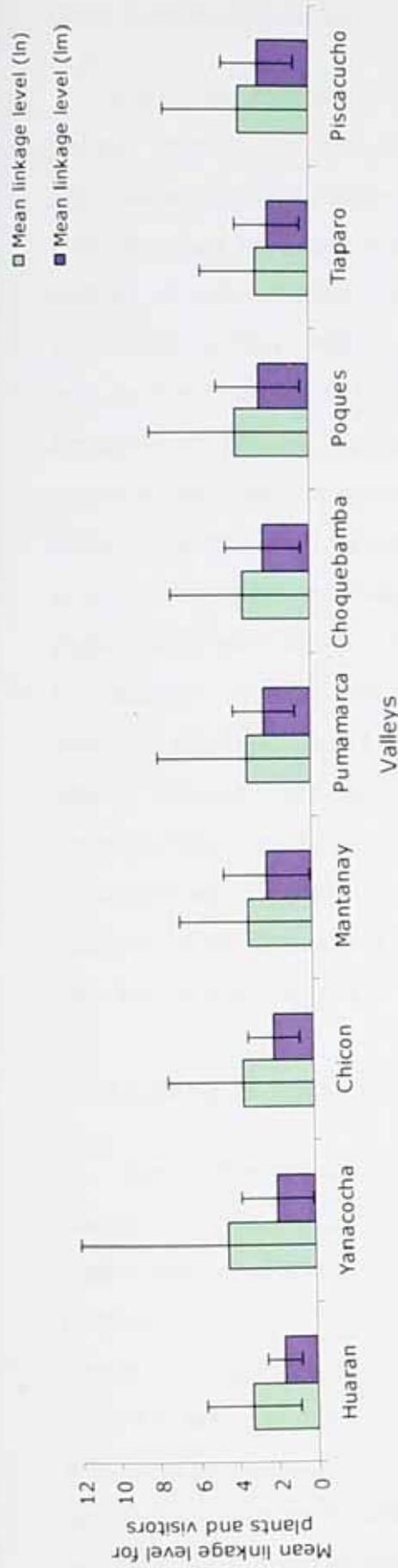


Figure 4.11. Mean \pm SD linkage level scores for plant species (l_n) and flower visitors (l_m) for each valley.

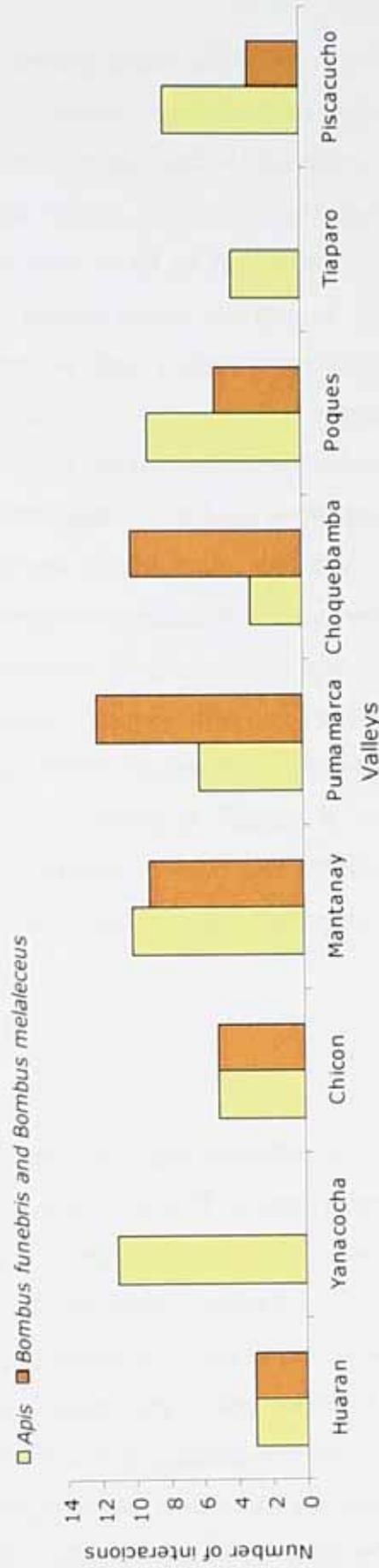


Figure 4.12. Number of plant interactions recorded in each valley for *Apis* and *Bombus funebris* and *Bombus melaleuceus* combined.

Core species of plants

Core plant species were identified by ranking plants according to 10% of their relative linkage levels (L_n). *Baccharis sacilifolia*, *Jungia rugosa*, *Minthostachys spicata* and *Myrcianthes oreophila* formed the central core of plant species. *Baccharis sacilifolia* was the dominant plant species in four out of the nine valleys in terms of both linkage levels and the number of visits received, and formed the core in all of the valleys except Tiaparo and Piscacucho. In those valleys geographically located closer to Pisac, *B. sacilifolia* interacted with the most species of flower visitors, whereas those valleys located closer to the Historic Sanctuary of Machu Picchu, *Jungia rugosa* and *Minthostachys spicata* were the most generalist plant species and recorded the highest number of visits (Table 4.4, Figures 4.7 - 4.10). Consistent with flower visitors, the rankings of the core plant species were constant across some valleys but changed order in others. Additionally, the status of some species of plants and flower visitors reversed from specialist to generalist or vice versa between valleys. For example, at Mantanay, Chicon and Tiaparo *Jungia rugosa* was considered to be a specialist plant species, whereas at Pumamarca, Choquebamba and Poques this plant species was an extreme generalist. At Pumamarca, *Trifolium amabile* was considered a specialist plant species visited only *Bombus melaleceus*, whilst at Poques it was visited by both honeybees and Vespidae sp. 3. Similarly, at Huaran, Chicon and Mantanay, hummingbirds were one of the generalised functional groups of flower visitors, whereas at Yanacocha they were the most specialised.

Core species of flower visitors

Core visitor species were identified by ranking plants according to 10% of their relative linkage levels (L_m). The networks centred around a core of generalist species recording the highest number of interactions, surrounded by many species with only one or two interactions (Table 4.5, Figures 4.2 - 4.10). The fraction of core species in each valley varied according to species richness of plants and visitors. Hymenoptera dominated the network core in all of the valleys in terms of linkage levels, except at Tiaparo, where dipterans and syrphids interacted with more plant species (Figures 4.2 - 4.10). *Apis* was a dominant flower visitor in six of the nine valleys in terms of abundance and linkage levels, whilst *Bombus funebris* replaced *Apis* in Pumamarca and Choquebamba (Figure 4.12). When abundances of bumblebees were

higher or roughly equal to honeybee numbers (i.e. at Pumamarca and Choquebamba), *B. funebris* was a more generalist visitor than *Apis*.

The position of *Apis* was remarkably constant across all the valleys except for Tiaparo. Even though *Apis* was not included in the core at Tiaparo, honeybees were still more than twice as abundant as *Sciaria* sp. 4. (Figure 4.9). Rankings of the remaining core species of flower visitors did change position across valleys; however, the core comprised of the same species (e.g. *Apis*, *Bombus funebris*; Syrphidae sp. 2; *Sciaria* sp. 4; Chrysomelidae sp. 2; Muscidae sp. 1; and Diptera sp. 1) across the majority of the valleys. Only at Huaran, and Yanacocha and Chicon were hummingbirds present in the network core and only at Yanacocha were Lepidoptera prominent and abundant (Figures 4.2, 4.3 and 4.4).

Table 4.4. Rankings of core species of plants according to 10% of the relative linkage (l_n) scores

Huaran	Yanacocha	Chicon	Mantanay	Pumamarca	Choquebamba	Poques	Tiaparo	Piscacucho
<i>B. sacillifolia</i>	<i>B. sacillifolia</i> Asteraceae sp. 5	<i>B. sacillifolia</i> <i>M. orephila</i>	<i>M. orephila</i> <i>B. sacillifolia</i>	<i>B. sacillifolia</i> <i>J. rugosa</i> Asteraceae sp. 2	<i>J. rugosa</i> <i>A. sternbergiana</i> <i>B. sacillifolia</i>	<i>J. rugosa</i> Asteraceae sp. 2 <i>B. sacillifolia</i>	<i>M. spicata</i> <i>A. sternbergiana</i> <i>C. urubambensis</i>	<i>M. spicata</i> <i>Gentianella</i> sp.1 <i>A. dicolor</i>

Morphology of plants: *B. salicifolia*, open brush; *M. orephila*, open brush; *J. rugosa*, open; *M. spicata*, short tube; Asteraceae sp. 2, open; *A. sternbergiana*, open brush; *Gentianella* sp.1, tube; *C. urubambensis*, open; *A. dicolor*, open brush; Asteraceae sp. 5.

Table 4.5. Rankings of core species of flower visitors according to 10% of the relative linkage (I_m) scores

Huaran	Yanacocha	Chicon	Mantanay	Pumamarca	Choquebamba	Poques	Tiaparo	Piscacucho
<i>A. mellifera</i>	<i>A. mellifera</i>	<i>A. mellifera</i>	<i>A. mellifera</i>	<i>B. funebris</i>	<i>B. funebris</i>	<i>A. mellifera</i>	<i>Sciaria</i> sp. 4	<i>A. mellifera</i>
<i>M. tyrianthina</i>	<i>M. cosinga</i>	Chrysomelidae sp. 2	Syrphidae sp. 2	<i>A. mellifera</i>	<i>Sciaria</i> sp. 4	<i>Sciaria</i> sp. 4	Syrphidae sp. 3	Chrysomelidae sp. 2
<i>A. cupripennis</i>	Muscidae sp. 5	<i>B. funebris</i>	<i>B. funebris</i>	<i>Sciaria</i> sp. 4	Muscidae sp. 5	Diptera sp.1	Syrphidae sp. 2	<i>Platychieirus</i> sp. 1
<i>B. funebris</i>	<i>Muscina</i> sp.1	Syrphidae sp.2		<i>B. melaleucus</i>	Diptera sp. 1	<i>Tuberculanostoma</i> sp. 1		Copestylum sp. 2
<i>Muscina</i> sp.1	Syrphidae sp. 2	<i>A. cupripennis</i>		Diptera sp. 1	<i>Toxomerus</i> sp. 2	<i>Platychieirus</i> 2		<i>Sciaria</i> sp. 4
<i>A. castelnauldii</i>	Lepidoptera sp. 2	Syrphidae sp. 3			<i>Platychieirus</i> sp. 2	<i>Sciaria</i> sp. 2		Syrphidae sp. 2
	Muscidae sp. 6	Lepidoptera sp. 6			Muscidae sp. 1	Syrphidae sp. 3		Syrphidae sp. 2
	Diptera sp. 13	Muscidae sp. 1			<i>L. albornatus</i>	Muscidae sp. 5		
	Muscidae sp. 1	<i>P. cyanopterus</i>			Coleoptera sp. 2	Chrysomelidae sp. 2		
	<i>Platychieirus</i> sp. 2	Diptera sp.15			Diptera sp. 11	<i>Platychieirus</i> sp.1		
	Anthomyiidae sp. 2				Syrphidae sp. 3			
	<i>Platychieirus</i> sp.3				<i>A. mellifera</i>			
	Diptera sp. 3							
	Diptera sp. 1							
	Diptera sp. 13							
	Lepidoptera sp.1							
	Tachinidae sp. 10							
	Copestylum sp.1							
	<i>M. tyrianthina</i>							

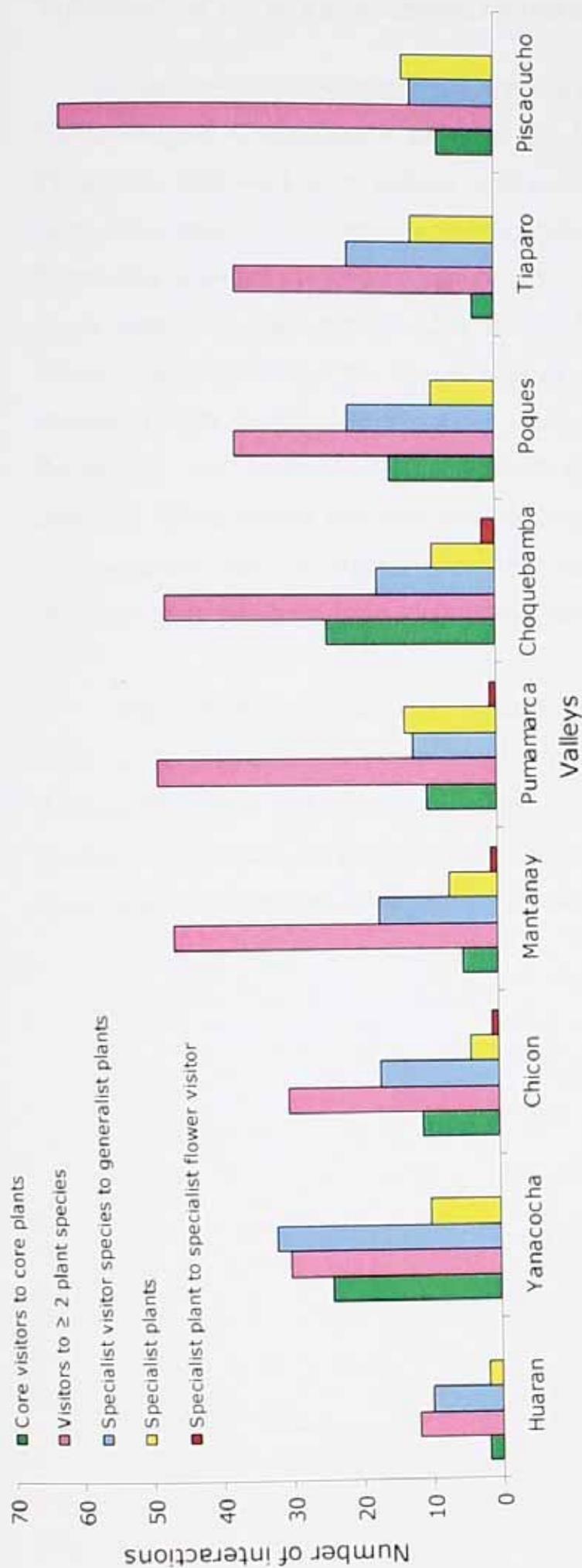


Figure 4.13 Network characteristics for each valley summarising the number of **1**: core generalist interactions by visitor species to core plant species according to 10% of the relative linkage (l_m) and (l_n) scores, **2**: the number of interactions from core visitor species to ≥ 2 plant species, **3**: the number of interactions from specialist visitor species to a single generalist plant species, **4**: the number of interactions to specialist plant species by one visitor species, **5**: the number of interactions by a specialist flower visitors to specialist plant species.

Summary of network structure of the nine valleys

A summary of the network characteristics of the nine valleys is presented in Figure 4.13. Yanacocha and Choquebamba recorded the highest number core generalist interactions. Piscacucho comprised of the highest number of generalist interactions (62) involving two or more plant species, followed by Pumamarca, Choquebamba and Mantanay respectively. Yanacocha recorded the greatest number of interactions from specialist visitor species to a single generalist plant species (32) followed by Tiaparo and Poques. Piscacucho and Pumamarca both recorded the highest number of interactions (13) involving specialist plants associated with single visitor species, followed by Tiaparo. Only Chicon, Mantanay, Pumamarca and Choquebamba comprised of specialist specialist interactions where a specialist flower visitor was observed visiting a specialist plant species. Of these valleys, Choquebamba had the highest specialist interactions which included the hummingbird *Aglaeactis cupripennis* visiting *Melastromaceae* sp. and *Coleoptera* sp, 3 to *Tarraxcum* sp.

From a conservation perspective, those valleys which recorded the highest mean linkage level (l_n) for plants and a greater number of insect and bird species such as Yanacocha, Poques, Piscacucho and Chicon are valleys that may be less vulnerable to perturbations. In contrast, valleys such as Tiaparo and Mantanay could be more vulnerable, especially since these valleys were ranked as the lowest in terms of core species.

Core species of visited plants across altitudes

Largely in accordance with rankings for individual valleys, *Baccharis salicifolia*, *Jungia rugosa*, *Minthostachys spicata* and *Myrcianthes oreophila* also formed the central core of plant species across altitudes (Table 4.6). *Baccharis salicifolia* was the dominant plant species across all altitudes in terms of both linkage levels and the number of visits received. In contrast to individual valleys, this ranking did not change across altitudes. Although in some valleys *Jungia rugosa* was considered to be a specialist plant species, across altitudinal bands it was confirmed as a core plant species. Other important core plant species included *Brachyotum nutans*, *Ageratina sternbergiana*, Asteraceae sp. 4 and Asteraceae sp. 5 (altitudes 4 and 5).

Table 4.6. Rankings of core species of plants according to 10% of the relative linkage (l_n) scores. Data pooled data across five altitudinal zones. Numbers indicate rankings

Altitude 1	Altitude 2	Altitude 3	Altitude 4	Altitude 5
1. <i>B. salicifolia</i>	1. <i>B. salicifolia</i>	1. <i>B. salicifolia</i>	1. <i>B. salicifolia</i>	1. <i>B. salicifolia</i>
2. <i>J. rugosa</i>	2. Asteraceae sp. 2	2. <i>M. spicata</i>	2. <i>M. oreophila</i>	2. <i>J. rugosa</i>
3. <i>M. oreophila</i>	3. <i>E. resinosa</i>	3. <i>J. rugosa</i>	3. <i>J. rugosa</i>	3. <i>A. sternbergiana</i>
4. <i>M. spicata</i>	4. <i>B. horrida</i>	4. Asteraceae sp. 6	4. Asteraceae sp. 4	4. <i>B. nutans</i>
			5. <i>A. sternbergiana</i>	5. Asteraceae sp.5

Core species of flower visitors across altitudes

Rankings of flower visitors were similar across altitudes to those in valleys (Table 4.7). *Apis* dominated the core at all altitudes in terms of abundance and linkage levels. *Bombus funebris* formed the core at every altitude except altitude 2. The Hemiptera *Lygaeus albornatus* was a core species at altitudes 1 and 4. The Coleoptera *Astylus* sp. 2 featured as a core species at altitudes 4 and 5. The hummingbird *Aglaeactis cupripennis* formed the central core at altitudes 2, 4 and 5, whereas *Aglaeactis castelnaudii* was a core species at altitude 3 and ranked second at altitude 5. The results also showed that across altitudes some of species identified as specialist flower visitors in each of the valleys transferred from being specialists to generalist core flower visitors. These species included *Toxomerus* sp. 2., *Eristalis* sp.2., Tachinidae sp. 10, Coleoptera sp. 3, *Colibri coruscans* (see Figures 4.2, 4.4 and 4.5).

Table 4.7. Rankings of core species of flower visitors according to 10% of the relative linkage (l_m) scores. Data pooled data across five altitudinal zones. Numbers indicate rankings

Altitude 1	Altitude 2	Altitude 3	Altitude 4	Altitude 5
1. <i>A. mellifera</i> 2. Diptera sp. 1 3. <i>B. funebris</i> 3. <i>Muscina</i> sp.1 4. <i>Lasioglossum</i> sp. 4. Syrphidae sp.2 4. <i>L. albornatus</i> 4. Tachinidae sp.1 4. Diptera sp.11 4. Diptera sp.5 5. Muscidae sp.5 5. Syrphidae sp.3	1. <i>A. mellifera</i> 2. <i>B. funebris</i> 3. <i>Sciaria</i> sp. 4 4. <i>M. cosinga</i> 5. Diptera sp.1 5. Syrphidae sp.2 5. <i>A. cupripennis</i> 5. Muscidae sp. 6. Syrphidae sp.3 6. Anthomyiidae sp.3 6. Syrphidae sp.2	1. <i>Sciaria</i> sp. 4 2. <i>A. mellifera</i> 2. Syrphidae sp.2 3. <i>Sciaria</i> sp.2 3. Muscidae sp.5 4. <i>B. funebris</i> 4. Muscidae sp.4 4. <i>C. coruscans</i> 5. Tachinidae sp.10 5. <i>Eristalis</i> sp.2 5. Syrphidae sp.2 5. Muscidae sp.1 5. <i>Platycheirus</i> sp.2 5. Diptera sp.5 5. <i>Astyplus</i> sp. 3 5. Syrphidae sp.3 5. <i>A. castelinaudii</i> 5. Lepidoptera sp.6	1. <i>A. mellifera</i> 2. <i>B. funebris</i> 3. <i>Platycheirus</i> sp.2 3. Syrphidae sp.3 4. <i>A. cupripennis</i> 4. Muscidae sp.5 4. <i>B. melaleucus</i> 5. Chrysomelidae sp. 2 6. Coleoptera sp. 3 6. <i>Sciaria</i> sp.2 6. Tachinidae sp.2 6. <i>L. albornatus</i> 6. <i>Toxomerus</i> sp.2 6. Muscidae sp.1 6. Curculionidae sp.1 6. <i>M. cosinga</i> 6. Syrphidae sp.1 6. <i>Astyplus</i> sp.2	1. <i>A. mellifera</i> 2. <i>Toxomerus</i> sp.2 2. <i>Astyplus</i> sp.2 2. <i>B. funebris</i> 2. Syrphidae sp.2 2. <i>A. castelinaudii</i> 3. Chrysomelidae sp. 2 3. Syrphidae sp.3 3. <i>Sciaria</i> sp.4 4. <i>Sciaria</i> sp.5 4. <i>A. cupripennis</i> 4. <i>Copestylum</i> sp.2 5. <i>B. melaleucus</i> 5. <i>Platycheirus</i> sp.2 5. Tachinidae sp.8 5. Diptera sp. 17 5. Muscidae sp.1

Flower visitor traits

Morphological data on mouth part lengths and bill lengths (taken in the field) of flower visitors was limited to a few species with small sample sizes. Nevertheless, the dominant flower visitors at the core of interactions can be broadly divided into functional groups of large bees with medium to long proboscis (*Bombus funebris*, *Bombus melaleucus* and *Apis*), large tachinid flies and syrphid flies with long probosces, small syrphid flies and small flies and beetles with shorter mouthparts, and hummingbirds (*Aglaeactis cupripennis* and *Metallura tyrianthinia*) with relatively medium bill lengths (mean 14.5 ± 2.4 mm, $N = 8$) when compared to the larger *Pterophanes cyanopterus* (30mm) and *Colibri coruscans* (25mm) (Figure 4.14).



Figure 4.14. Core hummingbird species (A) *Metallura tyrianthinia* and (B) *Aglaeactis cupripennis* with medium bill lengths caught with mist nets. Photos by the author.

Plant species traits

The majority of the plant species at the core of interactions had similar morphological traits. The most generalist plant species *Baccharis salicifolia* is characterised by numerous small open, white brush heads with short corollas (approx 5mm) (Figure 4.15). The dioecious flowers allow easy access to both pollen and nectar for flower visitors with a wide range of proboscis or bill lengths. All the remaining Asteraceae species present in the central core species possessed similar traits; the flower heads are generally small, mostly white or yellow, abundant, and open or brush like. Similarly, the large white, brush flowers of the tree

Myrcianthes oreophila also possess openly accessible nectar and pollen. *Minthostachys spicata* have numerous, small white tubular flowers which permit access to a wide variety of flower visitors, whilst *Gentianella* sp. has longer bell shaped corollas. More than half the plant species visited in all of the valleys (except Huaran) possessed an open morphology, and the majority were Asteraceae.



Figure 4.15. Morphology of core species *Baccharis salicifolia*. Permission to reproduce photo from © 2003 BonTerra Consulting.

Associations between floral morphology and visitor numbers

Results of the Mann Whitney U test of pairwise comparisons between floral morphology and number of visits revealed that only two sample medians were significantly different from one another (Figure 4.16). Plants species with open access flowers did not receive more visits than plants with tubular flowers and hidden nectar. The median number of visits made by visitors to flowers with open morphology was significantly more than to flag shaped flowers (Mann Whitney U test, $P = 0.002$). Plants possessing open and bell and tubular and bell shaped morphology did not differ significantly. Plant species with flag shaped morphology received significantly less visits than flowers with open tube morphology (Mann Whitney U test, $P = <0.02$). The visitation data were highly variable for open access flowers and open tube flowers, with many visits limited to one visit. The results indicated that open access flowers received the greatest number of visits (mean = 9.4 ± 17.6 and median = 3), whereas the mean number of visits received to open tubular flowers was the highest (mean = 13.5 ± 26.4 , median = 1.5).

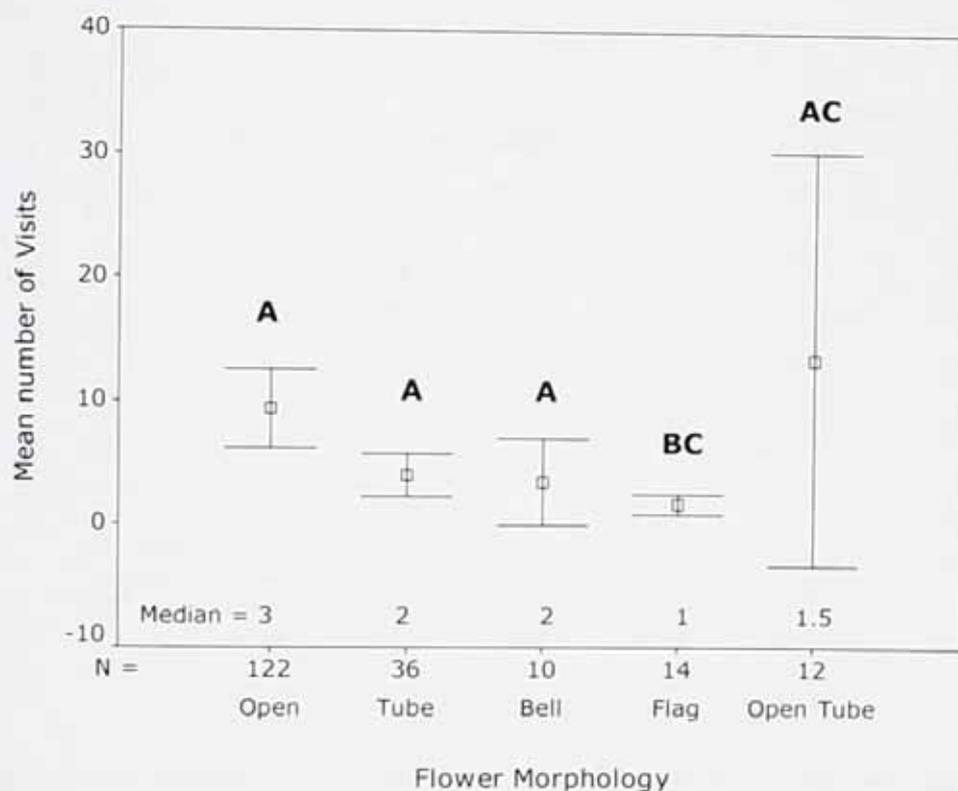


Figure 4.16. Average number of visits to plant species with open access, tubular, bell, flag and open tube morphology. Medians represented by numbers. Columns with the same letter did not differ significantly (Mann Whitney U test, $P \geq 0.05$). Sample sizes are indicated by N on the x axis.

Associations between floral morphology and the number of visitor species

A Mann-Whitney U test of pairwise comparisons between floral morphology and the number of visitor species showed significantly stronger differences among morphological traits compared to visitor numbers. Three sample medians were significantly different from one another (Figure 4.17). Plants with open access flowers were visited by a significantly larger number of visitor species than flowers with tubular flowers (Mann Whitney U test, $P = <0.05$). Flowers with flag shaped corollas were visited by a significantly smaller number of visitor species than open access flowers (Mann Whitney U test, $P = 0.002$) and tubular flowers were visited by a significantly higher number of visitor species than flowers with flag shaped corollas (Mann Whitney U test, $P = <0.05$). In contrast, flowers with tubular, bell shaped and open tubular flowers did not differ significantly in the median number of visitor species they hosted. The visitation data were also highly variable for open access flowers and open tube flowers. The results (Figure 4.17) showed that open access flowers received the greatest number of visitor species (mean = 4.20 ± 4.93 and median = 2).

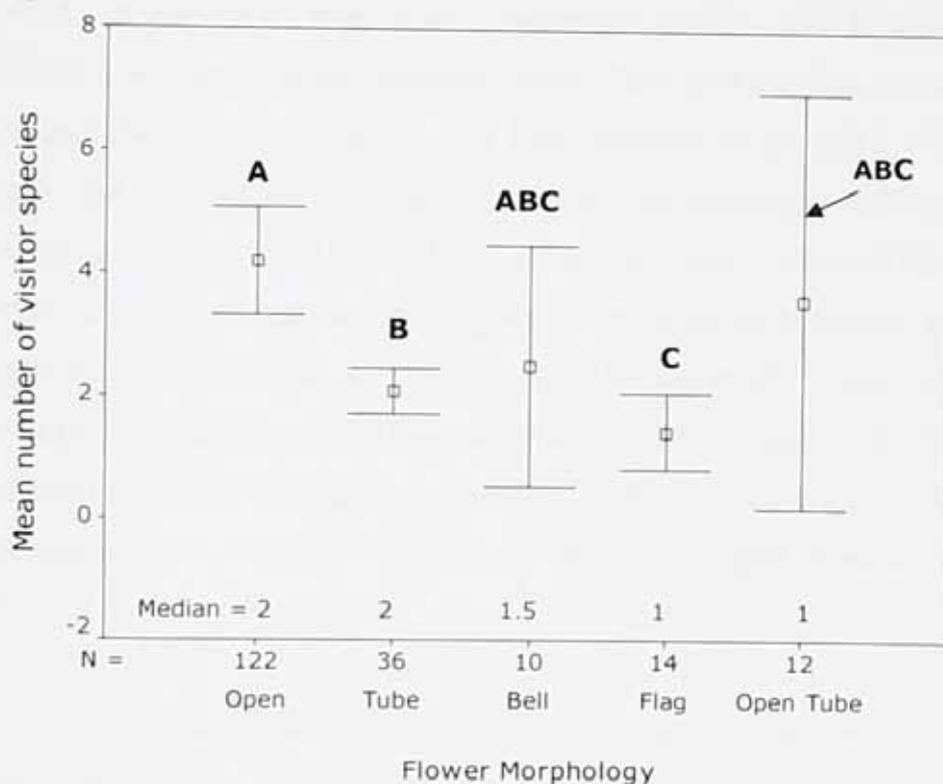


Figure 4.17. Average number of visitor species to plant species with open access, tubular, bell, flag and open tube morphology. Medians are represented by numbers. Columns with the same letter did not differ significantly (Mann Whitney U test, $P \geq 0.05$). Sample sizes are indicated by N on the x axis.

Discussion

Structure of pollination networks

The plant-flower visitor networks in the Sacred Valley were all highly significantly nested, with the exception of Huaran, and this was attributed to sampling in poor weather conditions. In this valley, the network was dominated by hummingbird species, which unlike many insect species are able to forage in all weather conditions (Cruden 1972). The analyses for individual valleys, pooled valleys and pooled altitudinal bands, produced strongly organised and significantly nested matrices. Therefore, if a plant species depends strongly on a pollinator, the pollinator depends weakly on the plant, and vice versa (Vázquez and Aizen 2004; Bascompte *et al.* 2006). These patterns of interactions were evident from limited sampling effort; each valley was only surveyed once. This fits with a study of plant-pollinator networks in Norway by Nielsen and Bascompte (2007) which focused on the extent to which nestedness was sensitive on sampling effort. They manipulated sampling effort in space, and time and found that nestedness was less sensitive to sampling effort than the number of species and links within the networks. Nielsen and Bascompte (2007) suggested that sampling more plots or days would probably not have significantly affected the estimate of the network pattern. Altogether these findings support the hypothesis that most, or possibly all mutualistic relationships, independent of network size, latitude or the nature of the interaction, display strong patterns of nestedness (Bascompte *et al.* 2003; Dupont *et al.* 2003; Ollerton *et al.* 2003; Memmott *et al.* 2004; Vázquez and Aizen 2004; Guimarães *et al.* 2006; Jordano *et al.* 2006; Lewinsohn *et al.* 2006; Stang *et al.* 2006; Nielson and Bascompte 2007; Ollerton *et al.* 2007a).

The networks of the Sacred Valley were characterised by a core of strong interactions with a few highly abundant species, whilst the majority of other flower visitors were comparatively rare and formed weak interactions. Strong significant positive correlations were found between abundance and generalisation level (linkage) of a species for both plants and flower visitors in all of the valleys. Thus, those species of flower visitors at the core of the interactions were also the species in greatest abundance, implying that the networks are abundance structured (Dupont *et al.* 2003). Similar results were also reported by Ollerton *et*

al. (2003) and Dupont *et al.* (2003), suggesting that in terms of the number of plant species visited, insect and bird population size within the Sacred Valley may determine the degree of ecological generalisation of flower visiting animals. Furthermore, the most cited reason to explain the existence of generalisation within plant-pollinator networks is the spatiotemporal variability in the composition and abundance of flower visitor assemblages (Waser *et al.* 1996; Gómez *et al.* 2007 and references therein).

The plant-flower visitor interaction matrices for each of the valleys were sparse; only 10% (mean 10.1 ± 3.8) of the potential interactions were recorded and the majority of species had few interactions. The relatively small percentage of all possible interactions (C) established across valleys and altitudes was similar to other sub-alpine and alpine pollination networks (Table 4.8). The datasets for individual valleys and pooled valleys fitted the predicted power-law relationship, the number of interactions per valley significantly increased with species richness, while the percentage connectivity significantly decreased with network size, as demonstrated by Jordano (1987), Fronseca and John (1996), Olesen and Jordano (2002), Bascompte *et al.* (2003) and Petanidou and Potts (2006) (Tables 4.2). However, when data was combined across altitudes, connectance was similar across all altitudes, but species richness of visited plant species was greatest at altitude 5 (Table 4.3).

A number of factors may have influenced network properties along the elevational gradient: differences in spatial sampling effort, anthropogenic disturbance at lower altitudes and facilitative effects among plants via shared pollinators at higher elevations (Moeller 2004; Ghazoul 2006). For example, fragmentation and the loss of native habitats within agricultural landscapes may lead to the disruption of plant-pollinator interactions (Memmott and Waser 2002; Klein *et al.* 2003). Several studies have found that diversity and abundance of some taxa declined significantly with increasing distance from forest edge (Klein *et al.* 2003; Ricketts 2003; Chacoff and Aizen 2006). Given these findings, it is expected that flower visitors in the Sacred Valley may be sensitive to the quality of the matrix, particularly to agricultural pastures and *Eucalyptus* plantations that surround natural habitats. Above 3500m the life zones are largely pristine with less anthropogenic disturbance. In addition, multiple-species floral displays have been proposed to facilitate pollination by attracting a greater diversity or abundance of pollinators (Ghazoul 2006), thus the diverse species-rich floral mixtures found at the higher elevations may potentially facilitate pollination and influence the diversity and abundance of flower visitors.

Table 4.8. Summary statistics of published sub-alpine and alpine pollination networks

Location	Latitude	Altitude (m)	S	A	P	M	K	% Fill	T	P	Source
Tenerife, Canary Islands	28° N	2050	49	38	11	418	108	25.8	33.2	<0.05	Dupont <i>et al.</i> (2003)
Cordón del Cepo, Chile	33° S	2200-2600	107	64	43	2752	196	7.1	3.9	<0.0005	Arroyo <i>et al.</i> (1982)
Cordón del Cepo, Chile	33° S	2700-3100	61	25	36	900	81	9.0	9.0	<0.0005	Arroyo <i>et al.</i> (1982)
Cordón del Cepo, Chile	33° S	3200-3600	185	101	83	8484	361	4.3	7.5	<0.0005	Arroyo <i>et al.</i> (1982)
Snowy mountains, Australia	36° S	1860-2040	117	81	36	2916	253	8.6	9.57	<0.0005	Inouye and Pyke (1988)
Arthur's Pass New Zealand	43° S	900	78	60	18	1080	120	11.1	26.46	>0.05	Primack (1983)
Cass New Zealand	43° S	600-800	180	139	41	5699	374	6.6	7.47	<0.0005	Primack (1983)
Craigieburn, New Zealand	43° S	1600-1800	167	118	49	4782	346	6.0	5.98	<0.0005	Primack (1983)
This study	13° S	2900-4050	258	144	114	17556	620	3.5	1.64	<0.0001	

Notes to table: (S) species richness; (A) number of pollinator species; (P) number of plant species; (M) matrix size (total number of potential interactions); (K) number of interactions recorded; (% fill) connectance or density of network; (T) matrix temperature; (P) P values relate to the null model analysis. Adapted from Dupont *et al.* (2003) and Jordano *et al.* (2006).

Linkage levels and abundance

Between 7% and 20% of plant species in each valley were specialists, and were limited to a single visitor species, in accordance with patterns previously reported from other network studies (Figures 4.2-4.10, Table 4.2). For example, Memmott *et al.* (2004) reanalysed the data generated from important studies by Clements and Long (1923) and Robertson (1929) and found that pollinators outnumbered plants by 3:1 and in both networks, only between 11% and 18% of plant species were limited to a single visitor species and three pollinators per plant was usual. In this present study, mean linkage level for plants was higher than for flower visitors. On average, plants were visited by a mean of 3.43 insect and bird species whereas each insect or bird species visited 2.17 plant species, suggesting that the generalisation level for flower visitors was lower than for plants. Similarly, in Argentina, Basilio *et al.* (2006) also found that linkage level was higher for plants than for flower visitors, where plants were visited on average by 4.1 insect species.

In this study, approaches used by Dupont *et al.* (2003) were followed to calculate network characteristics and linkage levels. Linkage level and abundance was then tested statistically by using Spearman's correlations. However, data on link strength was not included because such information on the relative frequency of visits was lacking. More recent advances regarding how specialisation varies across ecosystems have since been developed. For example, previous studies on the structure of mutualistic networks has been criticised because they were based on qualitative data where all links were largely considered equal, without considering the importance of interaction strength for community dynamics (Vázquez *et al.* 2005; Bascompte *et al.* 2006; Blüthgen *et al.* 2006).

Vázquez *et al.* (2005) have shown that the frequency of interaction between two species in ecological networks serves as surrogate for the total effect of animal mutualists on plant reproduction and seed dispersal. Later, Blüthgen *et al.* (2006) developed a scale independent quantitative index to define the overall degree of specialisation in 51 mutualistic plant-animal networks. They found that across networks, the degree of specialisation was independent of network size, largely insensitive to sampling effort, and the average specialisation of plants and animals was correlated. This pattern was constrained by the topology of the ratio of animal to plant species in the interaction web (typically 3.6:1), therefore if animal species are more numerous than plants, the animal guild was more generalised than the plant guild. These

authors suggested that the correlation between pollinator frequency and specialisation showed that resource partitioning was particularly pronounced among the most active species, whereas rarely interacting species were more opportunistic in resource use.

In the Sacred Valley, the ratio of animal to plant species in the interaction webs were not typical of the 3:1 reported by Blüthgen *et al.* (2006). Instead, the ratio of flower visitors to plant species across valleys and altitudes was $\leq 2:1$. The reason for this may be related to a number of factors. It should be recognised that there were several limitations to this study. Firstly, there was no independent measure of the abundance of plants; instead an abundance measure was based on selecting plants species at random and recording the number of visits. Given that the abundances of plant species along transects are likely to vary substantially (Herrera 1988), the relative differences in abundances of plants may not be consistent across valleys. Secondly, species richness of insects was estimated using a morphospecies approach. It should also be acknowledged that the use of higher-taxon levels as surrogates for the number of real species could potentially bias estimates of total flower visitor richness in this present study (see the discussion in Chapter 2). Therefore, the degree of specialisation or generalisation reported across valleys may not necessarily hold true if species richness of insects was either over or under-estimated. Given the limited sampling effort, the potential consequences for the results are likely to be an increase in generalisation levels for plants and flower visitors.

Forbidden interactions

The low proportion of the possible interactions observed in mutualistic networks is a major component of sparse interaction matrices which Jordano *et al.* (2003, 2006) refer to as forbidden interactions. These authors proposed that connectivity decreases because interactions are prevented by a mismatch of biological or phenological attributes of species. However, not all zero interactions are forbidden interactions. For example, Ollerton *et al.* (2007a) analysed the mutualistic interaction between anemonefish and their hosts and suggested three quarters of the zero interactions they recorded were possibly mediated by local factors such as competition, habitat preferences and host-fish biochemical signalling.

A small proportion of the zero interactions between hummingbirds and plants were due to habitat uncoupling between the plant species and flower visitor, causing the interaction not to occur. For example, the Andean Hillstar (*Oreotrochilus estella*) observed at Chicon, is adapted to harsh climatic conditions with a range restricted to puna grasslands and *Puya* stands (Fjelds  and Krabbe 1990) and therefore does not visit some of the plant species visited by other hummingbird species. Similarly, at Chicon and Mantanay, where the Shining Sunbeam (*Aglaeactis cupripennis*) and the White-tufted Sunbeam (*Aglaeactis castelnauldii*) are sympatric, *A. castelnauldii* has a higher distributional range, and visits plant species typically associated with lower strata open shrub and *Polylepis* stands (e.g. *Gynoxis longiflora*). In contrast, *A. cupripennis* seasonally migrates to *Polylepis* woodlands (Fjelds  and Krabbe 1990), and also forages in the sub-canopy (SW personal observation). The Sword Billed hummingbird (*Ensifera ensifera*) was never recorded in the transects because this species forages by traplining in the canopy and moving up and down the valley, even though it was present in some valleys (SW personal observation).

A small fraction (7.6%) of plant species were visited by insects and birds which had flowers with longer corolla tubes than their proboscis length or bill length (Table 4.9). In the majority of cases, these visitors acted as nectar robbers or pollen thieves. Phenological non-coincidence between plant and pollinator has also been suggested to prevent interactions occurring between species (Jordano *et al.* 2006). In the Sacred Valley many of core plants species were long lived species with wide distributional ranges, which flowered for the whole duration of the dry season (i.e. *Baccharis sacilifolia*, *Jungia rugosa*, *Minthostachys spicata*), although *Myrcianthes oreophila* did not simultaneously flower in all of the valleys. Arroyo *et*

al. (2006 and references therein) proposed the “increased pollination probability hypothesis” as compensation through larger display size and longer flower longevity to attract scarce pollinator visits and maintain outcrossing under unfavourable conditions such as high altitudes. The causes of the asymmetric specialisation observed in the data sets are not thought to be due to pollinator availability at high altitudes, given that with the exception of honeybees, abundances of functional groups of flower visitors do not vary with altitude (Chapter 2). However, detailed quantitative data between traits of pair-wise interactions were not available to test whether phenological mismatches between core plant species and flower visitor are possible sources of forbidden interactions.

Table 4.9. Plant species visited by floral larcenists

<i>Barnadesia horrida</i>	<i>Passiflora</i> sp.	<i>Oreocallis grandiflora</i>	<i>Brachyotum nutans</i>	<i>Fuchsia boliviana</i>	<i>Syphocampilus</i> sp.	<i>Passiflora tripartita</i>	<i>Oenothera versicolor</i>	<i>Salvia apetala</i>
Syrphidae sp. 2	<i>Apis mellifera</i>	<i>Apis mellifera</i> (SNR)	Syrphidae sp. 3 (NR)	<i>Sciara</i> sp. 3 (PR)	Syrphidae sp. 5 (PR)	<i>Aglaeactis cupripennis</i>	Diptera sp. 1 (PR)	Diptera sp. 17
<i>Sciara</i> sp. 4. (PR)	Diptera sp. 5 (SNR)	<i>Apis mellifera</i> (SNR)	<i>Apis mellifera</i> (SNR)	Diptera sp.15 (PR)		<i>Metallura tyrianthinia</i> (NR)		
Hymenoptera sp.		<i>Copestylum</i> sp. 2 (NR)		Tachinidae sp. 13 (PR)				
Diptera sp.11								

Note: letters in parenthesis are as follows: (PR) pollen robber; (NR) nectar robber; (SNR) secondary nectar robber. *A. mellifera* is considered as a secondary robber (personal communication David Roubik). Hummingbirds also behave as nectar robbers.

Core plant species

Of the plants, *B. sacilifolia*, *J. rugosa*, *M. spicata* and *M. oreophila* formed the central core of plant species in the network. *B. sacilifolia* was by far the most dominant plant species in the Sacred Valley and formed associations with up to 31 species of flower visitors. It was ranked highest in four of the nine valleys both for linkage levels and the number of visits received, and formed the core in all of the valleys except Tiaparo and Piscacucho. As mentioned above, the networks consisted of a cohesive core to which the rest of the community was attached (Bascompte *et al.* 2003). The results showed that the networks were highly asymmetric, so that a strong dependence on a given interaction in one direction was often followed by a weak dependence in the other direction. Thus, *B. sacilifolia* might depend heavily on *Apis* for plant reproduction but *Apis* depends weakly on this plant species because it collects resources from multiple plant species. In contrast, the majority of interactions consisted of only one flower species interacting with one plant species. The results showed that rare specialised species only interacted with the most generalist plant species. Hence, the resources provided by these core plant species also served the rare flower visitors in the networks and may provide pathways for their persistence (Jordano 1987; Bascompte *et al.* 2003; Jordano *et al.* 2006).

Consistent with flower visitors, the cores also shared a similar structure; the positions among species were remarkably constant and these patterns were repeated across the network. One possible explanation for the observed patterns is the degree of temporal matching between sets of mutualists, whereby the most generalist plant species tended to be those which exhibited extended phenologies such as *B. salicifolia*, or insects such as honeybees which were active throughout the entire year (SW personal observation).

It is noteworthy that despite these regular patterns, the status of some plant species and the identity of flower visitors changed along a continuum from extreme specialists to extreme generalists or vice versa among valleys. For example, *T. amabile* was considered a specialist plant at Pumamarca, visited exclusively by *B. melaleceus*, whereas at Poques it was visited by *Apis* and Vespidae sp. 3. Additional observations at Choquebamba revealed that *T. amabile* is visited in roughly equal proportions by *Apis*, *B. melaleceus* and *B. funebris*. Therefore, *T. amabile* can be considered to be functionally specialised on three species of bees from the same family and two from the same genus. The flowers of *J. rugosa* are phenotypically

generalised, open access flowers which produce small amounts of nectar. At Mantamay this plant seemed to be ecologically specialised on its sole visitor *Apis*, whereas at Pumamarca this species appeared to be more ecologically generalised, and was visited by a taxonomically wide range of insects such as butterflies, bumblebees, honeybees, beetles, flies and syrphids. In fact, this plant was a core species in Choquebamba and Poques. Similarly, the flowers of *S. panticallensis* are also phenotypically generalised with open access to rewards. Despite the open access nature of these flowers, at Piscacucho this plant seemed to specialise on small flies, whereas at Choquebamba and Poques it appeared more ecologically generalised on many different functional groups of insects. These results suggest that the degree of ecological generalisation is influenced by the setting in which the plants exist (Ollerton *et al.* 2007b). Local abundance of plant species and their particular flowering phenologies is also likely to play an important role in determining core species. For example, in some of the same valleys studied by Servat *et al.* (2002), *B. odorata*, *A. sternbergiana*, *F. apetala* and *O. lotoides* flower in both the rainy and dry season in some valleys but in others were restricted to just one season.

Similar changes from ecological specialists to generalists were also evident for hummingbird assemblages. Many of the plant species probably pollinated by hummingbirds appeared to be functionally specialised (possessed long tubular red or pink flowers, producing copious amounts of nectar) but were visited by several hummingbird species. Hummingbirds were only central to the network core at Huaran and Chicon and this could be attributed to habitat uncoupling and an artefact of the sampling method. Hummingbirds were not evenly distributed throughout habitats; *Ensifera ensifera* foraged by traplining and avoided the lower strata. *M. tyrianthinia* was highly territorial and tended to remain in the same area of its host plant (*Duranta mandonii*), only visiting additional plant species which grew on the host plant (*Passiflora* sp.) or nearby. Different foraging heights and behaviour among species are a common characteristic of hummingbird assemblages and have been shown to result in spatial partition of flowers that occur in open habitats (Snow and Snow, 1972; Stiles 1975; Feinsinger and Colwell 1978, SW unpublished data). It has also been suggested that traplining hummingbirds track temporal changes (Gill 1998; Henderson *et al.* 2006). As Olesen and Jordano (2002) pointed out, pollination networks are difficult to define spatially. For example, many hummingbird species are altitudinal migrants and visit flowers over several altitudinal zones (Snow and Snow 1972; Colwell *et al.* 1974; Feinsinger and Colwell 1978) and this can

be further exacerbated where an entire valley with many different habitats is treated as one unit.

The observed reversal from specialist to generalist or vice versa among valleys suggest that these patterns are influenced by the setting in which the plants exist and that flower visitors and plants can only be defined as ecological generalists or specialists relative to other visitor and plant species (Olesen and Jordano 2002; Ollerton *et al.* 2007b). For a given plant species, the realised generalisation will be determined by the number of effective pollinator species which interact with it and affect its fitness, within the setting of geographical and temporal variability in the community context (Ollerton *et al.* 2007b). In some valleys, local abundances and fluctuations in population sizes of plants and flower visitors, variable visitation rates between populations (i.e. highly variable visitation rates between populations of *Duranta mandonii*, Chapter 5), natural or anthropogenic habitat disturbance (widespread in the study area), and the presence and absence of other species such as honeybees may disrupt or modify the interaction between a plant species and its visitors. Interestingly, when honeybees were scarce in some valleys, bumblebees replaced *Apis* as the dominant core species. Since bee assemblages vary in their composition depending on the time of year and some oligolectic solitary bees are short lived and therefore only interact with a small fraction of available resources (Cruden 1972; Heinrich 1975; Ginsberg 1983; Jordano 1987; Torchio 1987; Hingston 1988), the observed patterns suggest that network structure in the Sacred Valley is context dependent. Insufficient sampling effort is also likely to account for the observed patterns because longer observations (not included in this study) at patches of *T. amabile* and *O. grandiflora* recorded many more species of flower visitors. This is in agreement with the sampling biases reported by Ollerton and Cranmer (2002), suggesting that studies carried out over longer periods show a higher level of generalisation than those studies conducted over shorter time spans.

Core species of flower visitors

The networks in most valleys centred around a core of strong interactions with a few highly abundant species, whilst the majority of other flower visitors were comparatively rare. The core was dominated by Hymenoptera (*Apis* and *Bombus funebris*) followed by flies (mainly syrphids, muscids and *Sciaria* spp.); beetles, butterflies and hummingbirds only featured in a few valleys and core species varied according to species richness and

abundances. Memmott *et al.* (2004) simulated removal of pollinators using two large networks of plants and flower visitors and also found that in both networks, Hymenoptera (honeybees and bumblebees) and Lepidoptera were the core insect orders. In this study, Lepidoptera (*Metardaris cosinga*) only featured in the core at Yanacocha which was probably related to the coincidence of the emergence of adult butterflies. At high altitude sites in Chile, similar network cores, dominated mainly by *Bombus* spp. and Syrphid flies have also been documented (Arroyo *et al.* 1982). A dominance of Hymenoptera was also demonstrated in the plant-flower visitor network of the sub-alpine desert of Tenerife, Canary Islands (Dupont *et al.* 2003).

The structural patterns of the network core and the identities and positions of some core plant and flower visitor species remained relatively constant among some valleys but fluctuated across others. Honeybees were the most abundant and generalised flower visitors and dominated the core in six of the nine valleys. Even in those valleys where honeybees were not ranked highest, they still featured in the core of species interactions. Interestingly, at Pumamarca and Choquebamba, when honeybee numbers were lower or about equal to bumblebees, *Bombus funebris* replaced *Apis* as the dominant core species. These findings suggest that the identities and positions of the species comprising of the core change spatially and temporally according to natural variation in population sizes of plants and animals. Indeed, species diversity, evenness and abundances for some taxa did vary significantly between valleys and altitudes (Chapter 2). In Norway, Nielsen and Totland (in review) found a high degree of temporal and spatial variation in species composition and diversity in the plant-pollinator networks of fragmented boreal forests. Even on a relatively small spatial scale within a single season, they found that interaction evenness of the overall most abundant species varied significantly. Spatio-temporal variation in abundance of many functional groups of pollinators may be significant over seasons and between consecutive years (Heinrich 1976; Ackerman 1983; Herrera 1988; Eckhart 1992; Roubik 2001). Lewinsohn *et al.* (2006) predicted that in studies conducted across sites or time periods, species will shift their positions within a nested structure far more often than they should shift compartments. Changes in the positions and abundances of the core taxa in the Sacred Valley would be expected when peak flowering occurs during the wet season. Throughout December, January, February and March, heavy rains influence visitation rates and abundances of insects, thus hummingbirds which forage in all weather conditions, are likely to play a more dominant role as core species. Moreover, peak flowering of certain plant species permits the onset of

breeding for some hummingbird species (Fjeldså and Krabbe 1990; Baltosser and Scott 1996, SW personal observation).

Core species of flower visitors and plant species across altitudes

Combining the data across valleys for altitudinal zones further reinforced the strong dominance of core species identified in individual valleys. For example, *Baccharis sacilifolia*, *Jungia rugosa*, *Minthostachys spicata* and *Myrcianthes oreophila* also formed the central core of plant species across altitudes (Table 4.6). *Baccharis sacilifolia* was ranked the highest core plant species across all altitudinal bands in terms of both linkage levels and the number of visits received, further endorsing the importance of this plant species in the community for the persistence of rare specialist species (Jordano 1987; Bascompte *et al.* 2003; Jordano *et al.* 2006). In contrast to individual valleys, the observed reversal from specialist to generalist plant species or vice versa (i.e. *Jungia rugosa*) was not apparent. Instead, *Jungia rugosa* was confirmed as a core plant species across four out of the five altitudes. As discussed above, the limitations to this study should be recognised. Hence, the network properties are likely to be affected by the abundance measure used to estimate plants and by the differences in sampling intensity within and across plant species. Nevertheless, despite these caveats, using pooled data still provided a valid summary of network structure and how network properties changed across altitudes.

As reported for valleys, *Apis* was a key core species across all altitudes, followed by bumblebees and a similar suite of dipterans to those found in each of the valleys. While the analyses from individual valleys identified which plants and flower visitors were core species, by combining data across altitudes, information was provided regarding their importance along the altitudinal gradient. Examples include species typically associated with high elevations such as *Brachyotum nutans*, a páramo plant species (Gentry 1996), the hummingbirds *Aglaeactis castelnaudii* and *Aglaeactis cupripennis* and *Bombus melaleceus*. Additionally, the analyses also showed that across altitudes some of species identified as specialist flower visitors in each of the valleys changed from being specialists to generalist core flower visitors. These species included *Eristalis* sp.2, Tachinidae sp. 10, Coleoptera sp. 3, *Colibri coruscans* and *Toxomerus* sp.2. This result suggests that limited sampling of individual valleys may have masked the degree of specialisation and generalisation of some flower visitor species.

Phenotypic traits of plants at the core of interactions

In most valleys, the network core was dominated by phenotypically generalised plant species. For example, the dioecious flowers of *B. salicifolia* are small open, white brush heads with short corollas. The flowers allow easy access to resources for flower visitors with a wide range of proboscis lengths. All the remaining Asteraceae core species possessed similar traits; open access, small white or yellow flowers which produce small amount of nectar. Similarly, the flowers of *Myrcianthes oreophila* are also phenotypically generalised, open brush white flowers, but produce large amounts of nectar. In contrast, flowers of *Minthostachys spicata* are relatively more phenotypically specialised in the sense that they have small tubular flowers which produce small amounts of nectar, but the short corolla tubes are proportionally wider at the mouth, permitting easy access to a wide variety of flower visitors (see Chapter 1 Figure 1.4G.). Similarly, *Gentianella* sp. 1 has longer bell shaped corollas allowing access mostly to syrphid flies and beetles. An important characteristic of such open access flowers present in the network cores is that most of the visitors are likely to provide equally good pollination services, and the identity of the main pollinators will be largely determined by their abundance in one year, and this may fluctuate greatly (Ollerton *et al.* 2007b).

Morphological traits of flower visitors at the core of interactions

The open access nature of the core flowers, permitted access to a wide variety of core species of insects with an array of different lengths of probosces. Those core flower species ranked highest (*Apis* and *B. fumebris*) had medium to long probosces, in agreement with Stang *et al.* (2006) who demonstrated that ecologically morphologically generalist visitor species mainly had long probosces with a high number of individuals and high number of interaction partners. They also found that visitors with short probosces rarely exploited nectar from flowers with deeply hidden nectar, although in this present study, in some cases, visitors with either short probosces or bill lengths were not prevented access to long corolla tubes since they acted as floral larcenists (Table 4.9). Moreover, these authors were only concerned with nectar holder depth and width and did not include pollen foraging visitors. In contrast, many of the core plant taxa had easily accessible pollen, thus allowing visitation from small *Sciarida* and Chrysomelidae species with relatively shorter mouth parts.

Morphological traits of plants and patterns of visitation

Phenotypically generalised plants with open access flowers received significantly more visitor species, whereas phenotypically specialised plants received significantly fewer visits and fewer visitor species, in accordance with a study of a Mediterranean flower visitation web reported by Stang *et al.* (2006). However, the number of visits to tubular flowers with hidden nectar was not significantly different to open flowers with freely accessible nectar. An important characteristic of the visitation data was the highly skewed non-normal distributions and large standard deviations. Thus, the lack of difference may be due in part to the uneven sample sizes between categories, and the low visitation rates from visitors to both flowers with open and tubular morphology (Figures 4.16 and 4.17). This finding suggested floral traits were not restricting visitation, and in some cases species with either short probosces or bill lengths gained access to long corolla tubes by nectar robbing (Table 4.6). In addition, some of the smaller insects were easily able to gain entrance to flowers with open tubular morphology.

The structure of interactions webs and network stability

Human landscape modification and destruction have been documented as one of the greatest threats to biodiversity (Allen-Wardell *et al.* 1998; Kremen and Ricketts 2000; Paton 2000; Roubik 2000). Given that the Sacred Valley has a long history of anthropogenic pressures (Chepstow-Lusty and Winfield 2000) it might be expected that habitat degradation will have a detrimental effect on the plant-flower visitor networks. Despite this, it has been argued that the asymmetric nature of the networks and the presence of a core of generalist species may have important implications for the persistence of plant-pollinator communities. These two properties of nestedness will not only affect the robustness of the mutualistic network, but may also provide pathways for the persistence of rare species and could drive the evolution of the entire community (Jordano 1987; Bascompte *et al.* 2003; Jordano *et al.* 2006). Memmott *et al.* (2004) explored potential extinction patterns in two large plant-pollinator networks by simulating the removal of pollinators at random from the most generalised to the most specialised. They found that if plants and pollinators were pooled and species were removed from both networks according only to their linkage levels, both networks would collapse upon the removal of the top 21% or so of the most linked species.

However, it should be noted that Memmott *et al.* (2004) did admit to several caveats because their method of simulating the removal of flower visitors using different algorithms assumed that all the visitors were equally effective as pollinators, meaning that there would be detrimental on population growth only if all the visitors were lost, and may have underestimated the severity of losing flower visitors.

It has been proposed that networks with broad-scale distributions of connectivities are more robust to the loss of highly connected nodes (species) than scale-free networks, demonstrated by Jordano *et al.* (2006). They simulated the loss of an increasing fraction of either plant or pollinator species by removing species according to decreasing order of their number of interactions, starting with the most generalised. A scale free network (Ashu, Kyoto Japan, Kato *et al.* 1990, cited in Jordano *et al.* 2006) and a broad-scale network (Cordón del Cepo, Chile, Arroyo *et al.* 1982) were chosen from the networks they analysed to highlight the most general trends. For each node removed, connectance was estimated as a fraction of the original connectance. Both networks were found to more robust to the loss of pollinator species and more susceptible to the loss of plant species. The scale-free network was less robust to the loss of plant species; the network collapsed when only a small fraction of the most connected nodes were removed (20%), whereas for the broad-scale network, a collapse was predicted after the removal of half of the most connected species, illustrating that broad-scale networks may be more robust, particularly to the loss of plant species.

The Cordón del Cepo plant-pollinator network (Arroyo *et al.* 1982) was most similar in terms of system characteristics to this present study, suggesting that the plant-pollinator networks of the Sacred Valley could be fairly resilient to the loss of plant species and their associated flower visitors (Table 4.8). This prediction is further supported because the network core is dominated by locally abundant, wide-ranging generalist plant species. Although pollinator effectiveness was not measured, the open access nature of the core flowers and the crawling activities of the foraging core insect species, coupled with high abundances, suggest that all insects that visit the core plant species have the potential to be pollinators (Ollerton *et al.* 2007b). From the plant's perspective, if shifts occur in the identity of the core species of flower visitors, as shown in Pumamarca and Choquebamba, then pollination is still likely to occur because the core plant species have replacement pollinators. This prediction is supported by Pauw (2007), who studied a portion of a pollination web concerning a generalist oil-collecting bee (*Rediviva peringueyi*) pollinator and a guild of oil-

secreting plants, in the Cape Flora Region of South Africa. He found that when the bee was absent from small conservation areas, seed set did not occur in six specialist plant species which were only pollinated by *R. peringueyi*, whereas for the pollination generalist *Hemimeris racemosa* (Scrophulariaceae), declines in seed set were not found because it had replacement pollinators, suggesting that generalisation buffers this species against the effects of pollinator loss.

The integration of honeybees into interaction webs and the possible implications

Although honeybees have integrated into the webs potentially contributing to the long-term persistence of community structure, it should be recognised that they may have the potential to alter the structural attributes of the networks. In the following section the possible consequences will be discussed. The results from Chapter 2 showed that honeybees were a dominant component of the flower visiting assemblages; they visited a relatively large proportion of the total flora surveyed and were the most abundant flower visitor at the two lowest altitudes. In all networks, except Tiaparo, honeybees were a prominent core species. Dupont *et al.* (2003) suggested that the massive introduction of honeybees was likely to have affected network composition and could be a strong competitor of native pollinators. It is widely accepted by scientists and conservationists that in many parts of the world, invasive exotic species cause changes in ecological ecosystems and threaten biodiversity and ecosystem functioning. (Memmott and Waser 2002; Morales and Aizen 2002; Gurevitch and Padilla 2004; Traveset and Richardson 2006). The global loss of natural habitats caused by anthropogenic disturbance promotes the invasion of alien species of plants which may facilitate the invasion of some exotic flower visitors (Kearns and Inouye 1997; Morales and Aizen 2002; Aizen and Feinsinger 2004). Recent advances on invasive mutualisms has provided novel insights into how alien flower visitors and plants integrate and how they spread into native pollination webs (Memmott and Waser 2002; Morales and Aizen 2006; Lopezaraiza-Mikel *et al.* 2007; Aizen *et al.* 2008). These studies have shown that if alien species integrate into mutualistic webs, they could potentially alter plant-pollinator network structure, with consequences for the long-term persistence of species.

To test the effects of alien species on the structure of pollination networks, Aizen *et al.* (2008) analysed webs in Argentina with differing degrees in the incidence of alien species. They measured interaction strength and connectivity of ten paired plant-pollinator webs, eight

from forests of the southern Andes and two from oceanic islands. Mutualism strength for all pairs of interacting species was estimated using the average frequency of interactions between plants and flower visitors. Frequency of interaction between two species is a measure that relates to plant reproduction and seed dispersal, and for a given flower visitor may predict pollinator importance (Vázquez *et al.* 2005; Morales and Aizen 2006).

For all five pairs of webs analysed by Aizen *et al.* (2008), webs with the highest number of visits to alien plants also had the highest incidence of visits from alien pollinators. On average, highly invaded webs exhibited weaker mutualistic interactions than those in less invaded webs, potentially increasing network stability. Therefore, some exotic species engage disproportionately in the most asymmetric interactions as the invasion progresses. Although alien integration did not alter the architecture of the pollination networks in terms of their overall connectivity, during invasion links were transferred from generalist native species to “super-generalist” invaders. Consequently, connectivity among native species declined in highly invaded webs. Their results revealed that interaction strength attained by some of these generalist invaders was stronger than for any of the native species, suggesting that those species that were ecologically specialised interacted solely with at least some of the exotic species. Thus, if these super-generalist exotic species become central nodes within these networks due to their dominance they could modify these structural properties by increasing the degree of nestedness, with implications for the persistence of many native species. Aizen *et al.* (2008) suggested that ultimately, the shift of interactions from native to alien generalists could cause a positive feedback loop, facilitating invasiveness and exposing many native species to novel ecological and evolutionary dynamics.

As Vázquez *et al.* 2005; Bascompte *et al.* 2006; Blüthgen *et al.* 2006; Aizen *et al.* 2008) have highlighted, interaction strength and high asymmetry are essential ingredients for the stability of interaction webs. These recent findings may have important implications for the long-term persistence of the networks in the Sacred Valley. For example, if nestedness, asymmetry, a low number of strong reciprocal dependencies and high heterogeneity in species strength promote web stability (Bascompte *et al.* 2006) this suggests that alien dominated webs may resist the restoration of interactions among native species to their previous non-invaded state (Tylianakis 2008).

From the networks analysed in the Sacred Valley one alien flower visitor and three exotic plant species were identified; *Apis mellifera* and the plants, *Melilotus alba*, *Lamium amplexicaule* and *Prunus serotina*. *Melilotus alba* was the only alien species visited by both honeybees and native bees, whilst native bees were exclusive visitors to the exotic species *Lamium amplexicaule*, and honeybees were exclusive visitors to the introduced tree *Prunus serotina* (Figures 4.4, 4.5, 4.7, and 4.8). Furthermore, neither honeybees nor bumblebees formed strong associations with these species. This is in contrast with the findings of Morales and Aizen (2002) in Argentina, where honeybees were most closely associated with alien plants. However, it should be noted that further changes caused by anthropogenic habitat disturbance such as overgrazing, agricultural intensification, and forest clearing could potentially disrupt interactions between native plants and pollinators (Morales and Aizen 2002; Steffan-Dewenter *et al.* 2002; Klein *et al.* 2003; Morales and Aizen 2006; Traveset and Richardson 2006). For example, Morales and Aizen (2002) assessed whether the invasion of exotic plants mediated by habitat disturbance facilitated the invasion of exotic flower visitors in southern the temperate forests of the southern Andes, Argentina. They measured the frequencies of insect visits (including *Apis*) to flowers of 15 native and 15 exotic plant species. They found that habitat disturbance did not affect the frequency of visits, but plants in disturbed areas received a greater proportion of visits by exotic insects. Therefore it is proposed that the strong association between honeybees and some exotic biennials and perennial herbs could facilitate their establishment in disturbed habitats and increase the abundance and expansion of exotic plants visited by honeybees (Morales and Aizen 2002).

In the Sacred Valley, anthropogenic pressures generally occur at lower elevations and include the fragmentation and destruction of natural habitats through, agricultural intensification, overgrazing, soil erosion and widespread planting of *Eucalyptus*. Exotic species introduced in the nearby Historic Sanctuary of Machu Picchu, include some 40 species of plants which have modified the natural landscape, particularly in the case of *Eucalyptus* plantations, pastures and land cleared for agricultural purposes (ParksWatch 2004). In the Vilcanota highlands the vast majority of plant species are native, with a high concentration of endemic species (Tupayachi, 2005). Although the networks studied only included three alien plant species, it seems likely that similar plant introductions may also have spread into the Sacred Valley, particularly in the case of introduced crop species. Therefore, perhaps the greatest threat posed to plant-flower visitor communities in this region is from habitat disturbance and the recent introduction of exotic plant species. Given that

honeybees were ubiquitous at all altitudes, if they were to form strong associations with exotic plants, this could potentially facilitate their establishment in these disturbed habitats and increase abundance and expansion of invasive plant populations via seed set (Morales and Aizen 2002; Morales and Aizen 2006; Lopezaraiza-Mikel *et al.* 2007).

Although recent studies have demonstrated that alien species may alter network structure through a number of direct and indirect pathways (e.g. Memmott and Waser 2002; Morales and Aizen 2002; Morales and Aizen 2006; Lopezaraiza-Mikel *et al.* 2007), it is difficult to assess to what extent honeybees will affect these interactions, particularly since *Apis* has a long coevolutionary history with the native flora and fauna in the region (Javier Llaxacondor, personal communication, 2001). It could be argued that the present day nested patterns observed in the Sacred Valley may have been generated by a process of natural "ecological filtering" that have largely removed most specialist-specialist interactions from communities (Ollerton *et al.* 2003). Ultimately, honeybees are likely to negatively impact the reproductive success of native plant populations if they affect the quality and quantity of the pollen transferred among plants, resulting in reduced seed set (Traveset and Richardson 2006).

In order to achieve a better understanding of these complex networks and the potential large-scale impacts of perturbations, future work should include sufficient replications and quantify the strength of interactions. Furthermore, Olesen *et al.* (2007) suggest that modules (subgroups of frequently acting species) dictate the basic building blocks of networks and may play a critical role in both their stability and in the potential for coevolution of plants and pollinators. Therefore, since all larger pollination networks are modular and modularity is complementary to nestedness, testing for this should be the next step towards a more profound understanding of network complexity in the Sacred Valley (Olesen *et al.* 2007).

Summary

Reinforcing many of the findings of previous research, the Sacred Valley networks were characterised by many generalised interactions and highly significantly nested structures. Separate network analyses of individual valleys and cumulative valleys fitted the predicted power-law relationship, the number of interactions per valley significantly increased with

species richness, while percentage connectivity significantly decreased with network size. The networks consisted of core groups of generalists in which specialised plants interacted mainly with generalised flower visitors and specialised flower visitors mainly interacted with generalised plants. Only a small proportion of plant species in each valley were specialists and limited to a single visitor species. Mean linkage level for plants was higher than for flower visitors, indicating that the generalisation level for flower visitors was lower than for plants. Generalisation level was positively correlated with species abundance, implying that the networks were abundance structured.

Combining the data across valleys for altitudinal zones further reinforced the strong dominance of core species such as *Baccharis sacilifolia*, highlighting the importance of this plant species in the community for the persistence of rare specialist species (Jordano 1987; Bascompte *et al.* 2003; Jordano *et al.* 2006). In contrast to individual valleys, the observed reversal from specialist to generalist plant species or vice versa (i.e. *Jungia rugosa*) was not apparent. Similarly, the dominance of *Apis* was also confirmed across all altitudes, followed by bumblebees and a similar suite of dipterans to those found in each of the valleys. While the analyses from individual valleys identified which plants and flower visitors were core species, by combining data across altitudes, information was provided regarding their distribution along the altitudinal gradient. Additionally, some of species identified as specialist flower visitors in each of the valleys changed from being specialists to generalist core flower visitors. This is not surprising given that networks which include many different plant species and flower visitors are highly dynamic both spatially and temporally (Herrera 1988). Furthermore, a high spatial variation in the distribution of pollinator species may promote generalisation (Gómez and Zamora 1999). However, the results should be taken cautiously since species richness of insects may have been either over or under-estimated and no independent measure was taken to estimate plant species richness. Given the limitations of this study, the potential consequences for the results are likely to be an increase in generalisation for some plants and flower visitors. Nevertheless, combining data across altitudes still provided a valid summary of network structure and how network properties changed across altitudes.

Core plant species were mostly composed of phenotypically generalised species, of which *Baccharis sacilifolia* was the most generalised. Although generalised plants with open access flowers received significantly more visitor species than to flowers with tubular and flag morphologies, visitation to tubular flowers with hidden nectar was not significantly

different to open flowers with freely accessible nectar, suggesting that floral traits were not restricting visitation. The dominant core flower visitors were honeybees and bumblebees followed by flies, beetles and butterflies, whereas hummingbirds only featured as core species in a few valleys. Additionally, the identities and positions of some core plant and flower visitor species remained relatively constant among some valleys but fluctuated across others.

Implications for conservation

The asymmetric nature of the networks and the presence of a core of generalist species could have implications for the persistence of the communities of the Sacred Valley. It has been argued that this dense core of generalist species is very robust to habitat loss (Fortuna and Bascompte 2005). Since networks with broad-scale distributions of connectivities could be more robust to the loss of highly connected nodes, it was suggested that networks of the Sacred Valley might be fairly resilient to the loss of plant species and their associated flower visitors. Furthermore, because specialised rare species are frequently dependent on a core of generalist taxa (Bascompte *et al.* 2003; Vázquez and Aizen 2006) honeybees may play an important role for the possibilities of rare species to persist. However, despite these findings it was also recognised a future threat posed to plant-flower visitor communities in this region is from habitat disturbance and the recent introduction of exotic plant species. This could potentially facilitate their establishment in disturbed habitats and modify the structure of the pollination webs. Therefore, conservation efforts should focus not only on the rare species but also on the generalist core pollinator groups. Future goals should include exploration of facilitative interactions between honeybees and alien plant species, and removal campaigns should be promoted to eradicate *Eucalyptus* and invasive herbs (ParksWatch 2004). Future studies should also target those valleys identified with the highest proportions of specialist interactions to confirm whether those interactions were rare or influenced by the setting in which the plants exist, or an artefact of sampling effort.

CHAPTER FIVE

Pollinator effectiveness of native and non-native flower visitors to an Andean shrub, *Duranta mandonii* (Verbenaceae)

Introduction

Foraging insects and other animals that visit a given plant species may fluctuate extensively within a spectrum that ranges from floral larcenists, low-efficiency pollinators, secondary pollinators, non-pollinators, to major pollinators (McDade and Kinsman 1980; Arizmendi *et al.* 1996; Butz Huryn 1997; Maloof and Inouye 2000; Gross 2001; Irwin *et al.* 2001; Navarro 2001; Castellanos *et al.* 2003). The majority of flowering plants are pollinated by a wide taxonomic range of pollinators, each of which will provide a different quality of pollinator service in most cases (reviewed by Waser *et al.* 1996; Ollerton *et al.* 2007b). These differences have been attributed to factors such as spatio-temporal variation in pollinator abundance (Herrera 1988; Ollerton and Cranmer 2002; Price *et al.* 2005), climatic conditions (Cruden 1972; Kessler and Krömer 2000) and the presence and relative abundance of other species of flower visitors (Gross and Mackay 1988; Paton 1993; Hansen *et al.* 2002).

Previous studies of pollinator efficiency have focused on female plant fitness components (number of pollen grains deposited on stigmas and the number of seeds set, e.g. Primack and Silander 1975; Herrera 1987; Waser and Price 1990; see also a comprehensive review by Inouye *et al.* 1994). Less attention, however, has been paid to pollinator efficiency via male fitness components (such as pollen removal from anthers (Spears 1983; Snow and Roubik 1987; Wilson and Thompson 1991; Inouye *et al.* 1994; Freitas and Paxton 1998; Castellanos *et al.* 2003). There is now growing consensus that one must consider both male and female components of reproductive success in a given plant species when evaluating pollinator efficiency, because some visitors may be effective at one component but ineffective at another (e.g. Roubik and Snow 1987; Freitas and Paxton 1998; Young and Stanton 1990; Wilson and Thompson 1991; Fishbein and Venable 1996).

In this chapter data are presented on observations and experiments in which the pollination efficiency of honeybees was compared with those of hummingbirds, native bees, and moths on both the male and female components of fitness of the Andean shrub *Duranta mandonii* (Verbenaceae). This plant was selected because initial observations indicated that it was visited by an array of different flower visitors including honeybees. The Vilcanota Highlands of south-eastern Perú contain a unique flora and fauna with high levels of diversity and endemism (Wege and Long 1995; Stattersfield *et al.* 1998; Servat *et al.* 2002). Before the introduction of the European honeybee some five hundred years ago, the only source of honey came from stingless bees, *Melipona* and *Trigona* spp. exploited by the Incas (Javier Llaxacondor personal communication 2001). There has been no previous work on what role honeybees play as pollinators in the Sacred Valley and little work in Perú as a whole. Since *D. mandonii* was locally abundant and was visited by an array of different flower visitors including honeybees, this presented an excellent opportunity to compare pollinator efficiency among native and non-native flower visitors and look for evidence of interference or resource competition by honeybees.

This study addresses the following questions:

1. Which native flower visitors of *Duranta mandonii* are the most efficient pollinators in terms of visitation rate, pollen deposition and pollen removal?
2. Are honeybees as efficient as the native pollinators of *D. mandonii*?
3. Is there evidence that honeybees compete with native flower visitors for floral resources, to the detriment of the native fauna?
4. Does the interaction between *D. mandonii* and the introduced *A. mellifera* have a negative effect on the reproductive success of the plant?

Materials and Methods

Study Species

The study species was provisionally identified as *Duranta mandonii* Moldenke (Verbenaceae) with the help of staff from the Herbario Vargas (UNSSAC), however, there has been some confusion with the identification of species within this genus (see Moldenke 1941; Sanders 1984). *Duranta mandonii* is a multi-stemmed large shrub (to > 5m), mostly associated with disturbed cloud forest and dry Andean shrub forest. This species is characterised by axillary spines and small entire leaves (Gentry 1996). The fruits are usually round and fleshy and turn orange when mature and contain eight seeds. Peak blooming of *Duranta mandonii* occurs in the rainy season between December and mid April. Flowers are hermaphroditic with a white zygomorphic corolla tube (mean length = 10.7 ± 2.1 mm; mean width = 3.4 ± 0.5 ; $n = 51$) and are unscented. Individual plants surveyed often produce more than 500 open blossoms at a time. Flowers have four anthers which are slightly exerted beyond the stigma. Thus, visitors contact the anthers first and then the stigma before reaching the nectar. The nectar is located at the base of the corolla tube and access to nectar is only possible from the opening of the corolla. The calyx of this species is very thick and thus prevents nectar robbers from piercing the corolla at the base (no pierced flowers were observed on this species).

Observations and experiments were conducted in the forests of the Vilcanota Highlands at Mantabay (Province of Urubamba, Perú) ($13^{\circ}12'S$; $72^{\circ}5'W$), between 9 January and 15 March 2002. The study sites contained a mixture of Arid Montane Scrub, Semi-Humid Scrub, Montane Forest and Riparian Thickets (bmh-PM *sensu* Holdridge, 1967; Fjeldsá and Krabbe 1990).

Nine patches of *D. mandonii* were selected for study at a range of altitudes (3200-3850m) and locations, based partly on accessibility to the flowers of this spiny shrub. Direct observations of avian and insect visitors were made in each of the nine patches, mostly between 0600h and 1830h. Surveys later at night revealed no flower visitors and therefore was regarded as being largely diurnally pollinated. In total, 263 person-hours of observations were carried out. Some of the smaller patches could be easily observed at a

distance of 2-4 m, whereas the larger shrubs were observed from distances of between 5 and 10 m using binoculars. For each visit the time, species of flower visitor and the number and duration of flowers probed was recorded. For bee visits, focal flowers were selected and watched for 5 minute periods. Identification of bees to the genus level was not possible without collection; therefore, "native bees" in the text refers collectively to all species of native bees, and *Bombus* spp. are referred to pollen removal experiments.

Nectar production

To investigate nectar production over time 25 flowers in late stage buds on 5 plants were bagged. The following day nectar volume was measured in each flower from 0600h to 1800h. Flowers were re-bagged after each sampling session to exclude visitors. Samples were initially taken at hourly intervals, but since the flowers did not re-secrete any nectar during this time period, it was decided to sample the flowers every two hours. Twenty flowers were also bagged with fine mesh cloth to determine nectar volume and concentration after 24 hour accumulation. Sugar concentration (as sucrose equivalents) was measured using Bellingham and Stanley sugar pocket refractometers and volume was determined using microcapillary pipettes (see Dafni 1992; Kearns and Inouye 1993 for details of these techniques).

Breeding System

Levels of autogamy and self compatibility were examined using flowers from 16 labelled branches from 4 individual plants. A total of 52 flowers were tagged and bagged on separate randomly selected branches. Flowers were treated as follows: (1) open (unbagged) control: no treatment ($n = 9$), (2) emasculated treatment: flowers were carefully emasculated to remove anthers and left unbagged throughout the duration of the observation period ($n = 8$), (3) autogamy: bagged unmanipulated flowers were left covered with exclusion bags until the end of the observation period ($n = 12$), (4) self – incompatibility: bagged flowers were carefully emasculated and hand pollinated with pollen from other flowers on the same individual ($n = 12$ flowers), (5) xenogamy: bagged flowers were carefully emasculated and hand pollinated with pollen from flowers from other individuals ($n = 11$). After the autogamy and xenogamy experiments flowers were re-covered. Note that on all the experiments some flowers were lost when pollination bags

were removed. It was not possible to have a larger sample because of the lack of available late stage buds per branch.

Pollen removal

Pollen removal by *Bombus* spp., honeybees and hummingbirds was examined using single flower visits to a sample of virgin flowers from six different plants. Moths, hawk-moths and flies were infrequent visitors (see Results), so it was not possible to measure pollen removal for these taxa. Each flower was bagged in late bud stage with a fine mesh exclusion bag and left for 24 hours prior to collection. Open flowers were then collected using fine forceps and mounted in holes on a c.3m long pole. The treatment groups and sample sizes were as follows: (1) control: virgin flowers mounted on the pole but not visited ($n = 9$ flowers, 36 anthers), (2) open pollinated (unbagged) control: ($n = 4$ flowers, 16 anthers), (3) bagged control ($n = 6$ flowers, 23 anthers), (4) experimental virgin flowers ($n = 17$ flowers, 70 anthers). Once again, a lack of suitable late-stage buds prevented larger sample sizes. The pole was placed at different locations within the periphery of the plant and I then waited for visitors to approach the target flowers. After single flower visits, the pole was removed from the plant and all four anthers were separated and placed in Ependorff tubes containing 70% ethanol. Due to the morphology of *Duranta mandonii* it was decided not to separate anthers from stigmas in the field because of possible contamination of the stigma from pollen in the anthers. In the laboratory the anthers were removed from the flowers, individually crushed and placed in a sonic bath for fifteen minutes. The number of pollen grains remaining per anther was then counted using an electronic Coulter Counter.

Fruit set

The efficiency of hummingbirds, native bees, honeybees and moths at fruit and seed production was evaluated using a total of 234 late stage buds from 69 branches at nine different sites. The experiment was conducted between 11 and 16 March 2002 at an elevation of 3200m. Flowers were randomly selected over a six day period and bagged using fine mesh. When the flowers opened they were coded with coloured string to denote each experimental day. Of the 234 flowers bagged, 61 flowers fell when the pollination bags were removed from the branches. Each day was divided into three time periods based

on prior flower visitation field data at one site. From prior observations, it was assumed that the most likely flower visitors in each time period were: hummingbirds 06:00-10:30, bees 12:00-16:00 and hummingbirds/moths 17:00-21:00. In addition, some flowers were assigned to open pollinated control and bagged control treatments (06:00-21:00). At the start of each time period the treatment bags were removed to allow visitation and when the time period was concluded the flowers were re-bagged. The flowers were subsequently monitored and when the petals had dropped the bags were removed. Fruits from experimental flowers were collected and the number of seeds per fruit counted.

To determine the importance of each flower visitor as a pollinator, mean percentage visitation data were multiplied by percentage of pollen grains removed per anther. Pollinator efficiency rankings across the different components of fitness (pollen removal and fruit set) measured for each taxon were then compiled to compare how consistent the different flower visitors were as pollen removers and depositors.

Competition with native flowers visitors

To ascertain if honeybees have the potential to compete with native species by overlapping with them in their foraging activity, correlations of mean percentage visitation rates for each taxon against mean percentage visitation rates for *Apis mellifera* at each time period were calculated. To determine if honeybees restrict foraging to times of greatest nectar availability and thus potentially deplete flowers of nectar for other flower visitors, Pearson or Spearman Rank correlations of mean percentage visitation rates for each taxon against nectar production data were calculated. To ascertain if the presence of *A. mellifera* resulted in active or passive displacement of native fauna, the frequencies of encounters were calculated from visitation data recorded at each site. Visitation records were standardized to only include encounters where *Apis* and other taxa occurred together within time periods of up to five minutes.

Data analysis

All data were tested for normality using the Kolmogorov-Smirnov test. A one-way ANOVA was used to test for significant differences in the mean number of pollen grains removed from flowers in each treatment group. Post-hoc Tamhane T2 tests were used to determine which pairs of means were statistically significantly different. This test was chosen because it does not assume homogeneity of variances. Visitation rates were originally calculated on a per site basis and as visits per hour. However, due to very large standard deviations of means caused by large between site variation in visitation rates, data were pooled and calculated as visits per minute and percentage of visits per taxon per site. Flower visitation rates and the number of seeds set were not normally distributed, therefore Mann-Whitney *U* tests were performed to test for differences between sample medians. Pearson correlations were used when data were normally distributed; Spearman rank correlations were used when data were non-normally distributed. All statistical analyses were performed using SPSS version 11.5 for Windows (2003, SPSS Inc. Chicago, IL, USA). All means are presented ± 1 SD.

Results

Nectar production

The mean volume of nectar produced by a flower in 24 hours was $3.0 \pm 4.8 \mu\text{l}$ ($n = 20$) and mean sugar concentration was $20.8 \% \pm 2.6$. Mean nectar volume was low during the morning, followed by a gradual increase, peaking in mid to late afternoon, whilst nectar concentration was relatively constant over time (Figure 5.1). Sugar concentration was relatively constant over time. Total sugar content of nectar peaked at 08:00 and approximately tracked volume levels (Figure 5.1).

Flower Visitors

Duranta mandonii was visited by three species of hummingbirds; *Metallura tyrianthina*, and *Aglaeactis cupripennis* (Trochilidae), and the flower-piercer *Diglossopsis cyanea* (Coerebidae). Insect visitors were Hymenoptera: Apidae (*Apis mellifera*, *Bombus* spp.), Lepidoptera, Diptera: Syrphidae and Tachinidae. Visitation rates for Syrphidae and Tachinidae were very low and therefore were not included in analysis of flower visitation rates by taxa. All insect taxa observed made legitimate visits to flowers, in the sense that they did not pierce the corollas. The insects and the hummingbirds foraged only on nectar, no pollen collecting by bees was observed. The flower-piercer *Diglossopsis cyanea* foraged for nectar by moving slowly along the branches and entering flowers legitimately.

Four distinct patterns of flower visitor activity were recognised between different taxa (Figure 5.2): bimodal with peaks in the morning and afternoon/early afternoon (*Aglaeactis cupripennis*, hawkmoths and moths), unimodal with a sustained peak during the middle of the day (native bees, *Apis mellifera* and butterflies) and unimodal with a peak in the early morning, though with some activity during the rest of the day (*D. cyanea*). Visitation by *Metallura tyrianthina* was relatively constant throughout the day. Native bees foraged earlier and later in the day than honeybees (Figure 5.2).

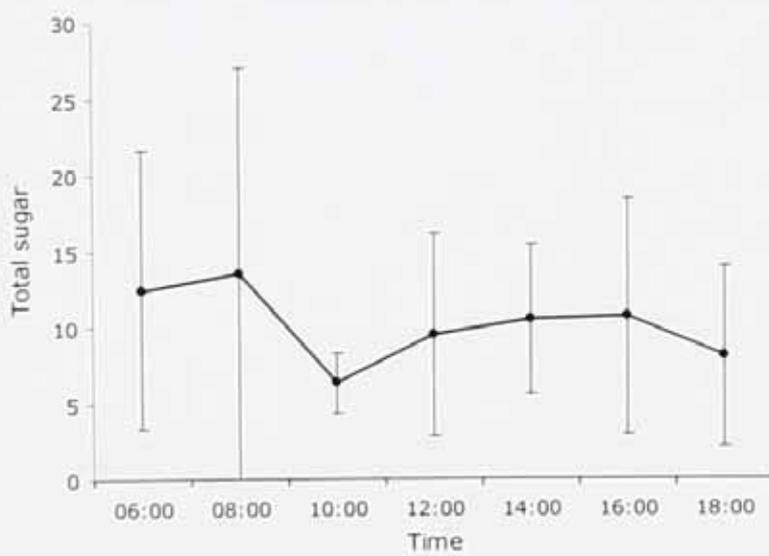
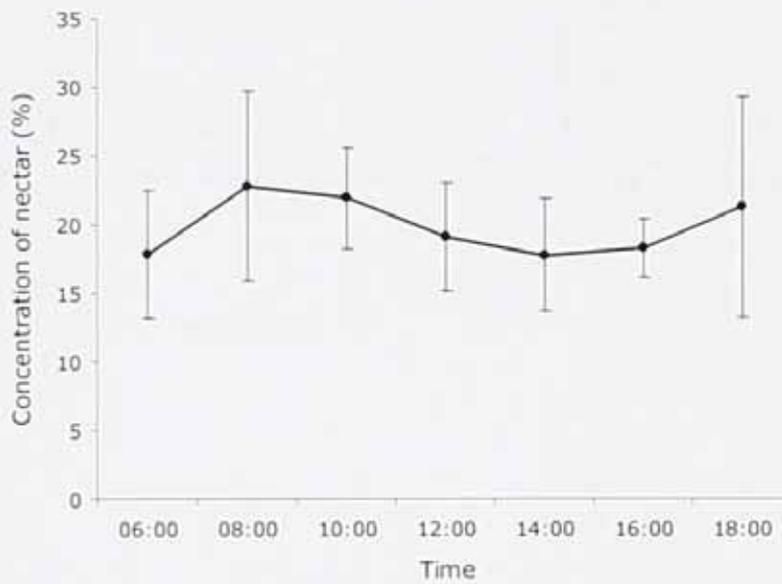
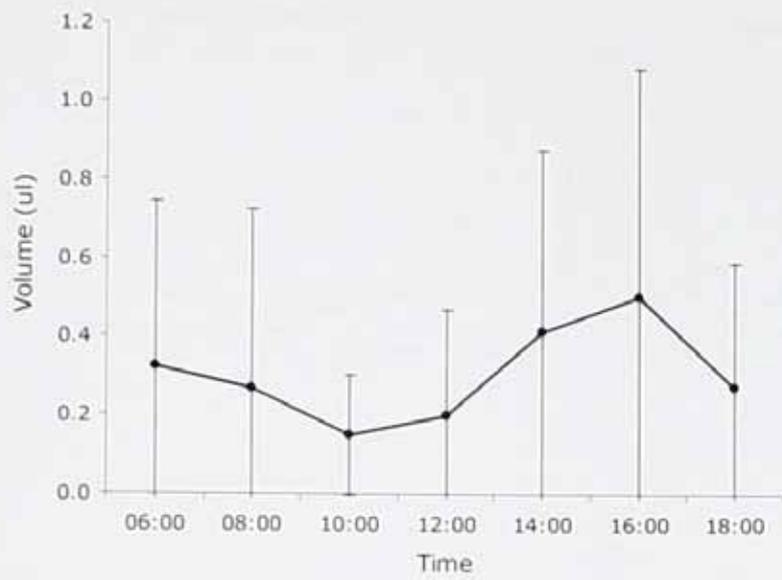


Figure 5.1 Nectar volume, concentration and sugar content for flowers of *Duranta mandonii*. Data are means ± 1 SD ($n = 16$). Error bars (\pm SE).

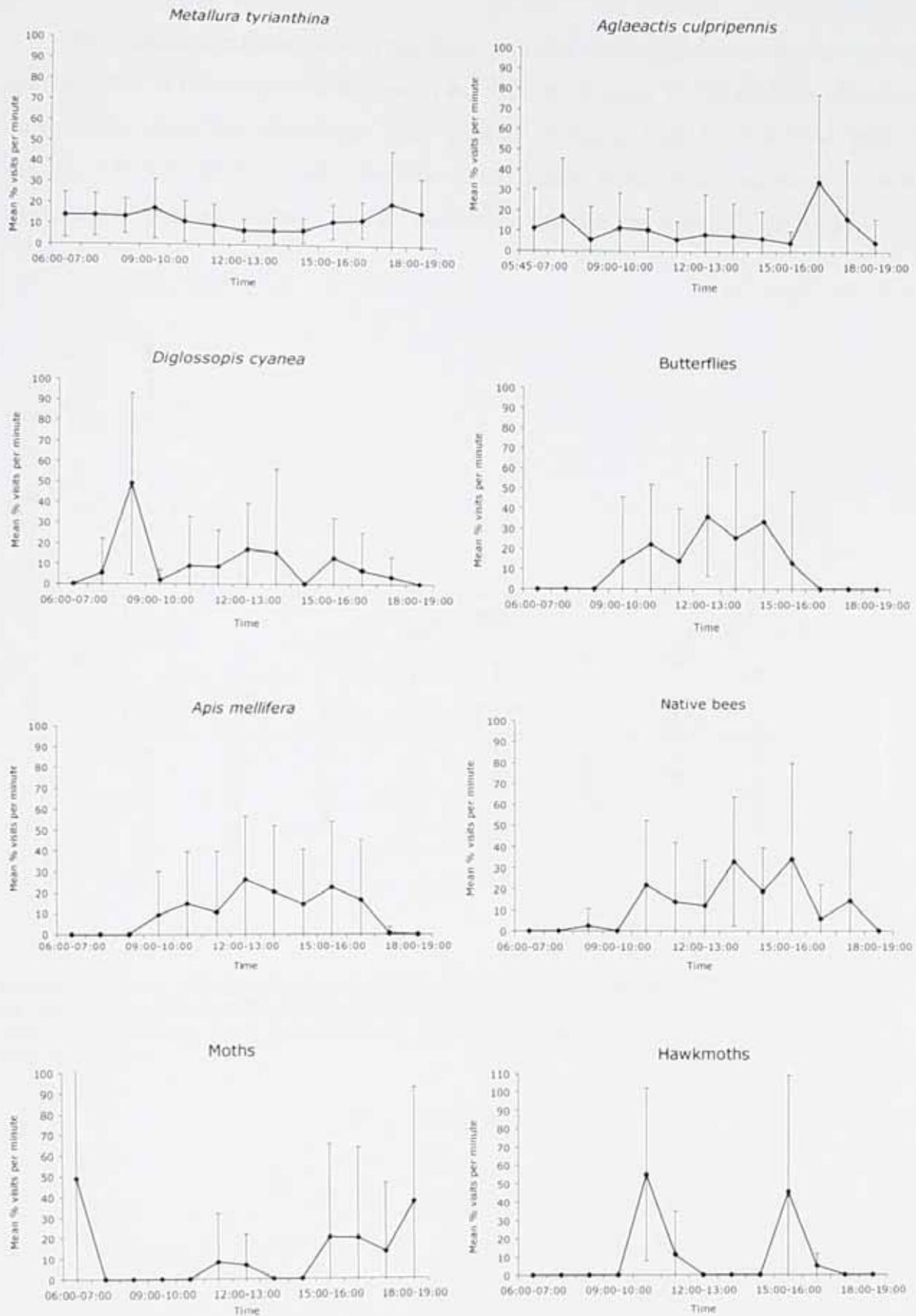


Figure 5.2 Flower visitation rate expressed as mean percentage of visits per minute for 13 census periods. Error bars (\pm SD).

The hummingbird *Metallura tyrianthina* contributed the highest number of visits per minute (68%) in comparison to the rest of the visitors. Kruskal Wallis analysis of variance showed that there was significant heterogeneity among groups for visitation rates ($\chi^2 = 142.0$, d.f. = 8, $P \ll 0.001$). Results of the Mann Whitney U test revealed strong significant differences amongst taxa for percentage of visits per minute (Figure 5.3).

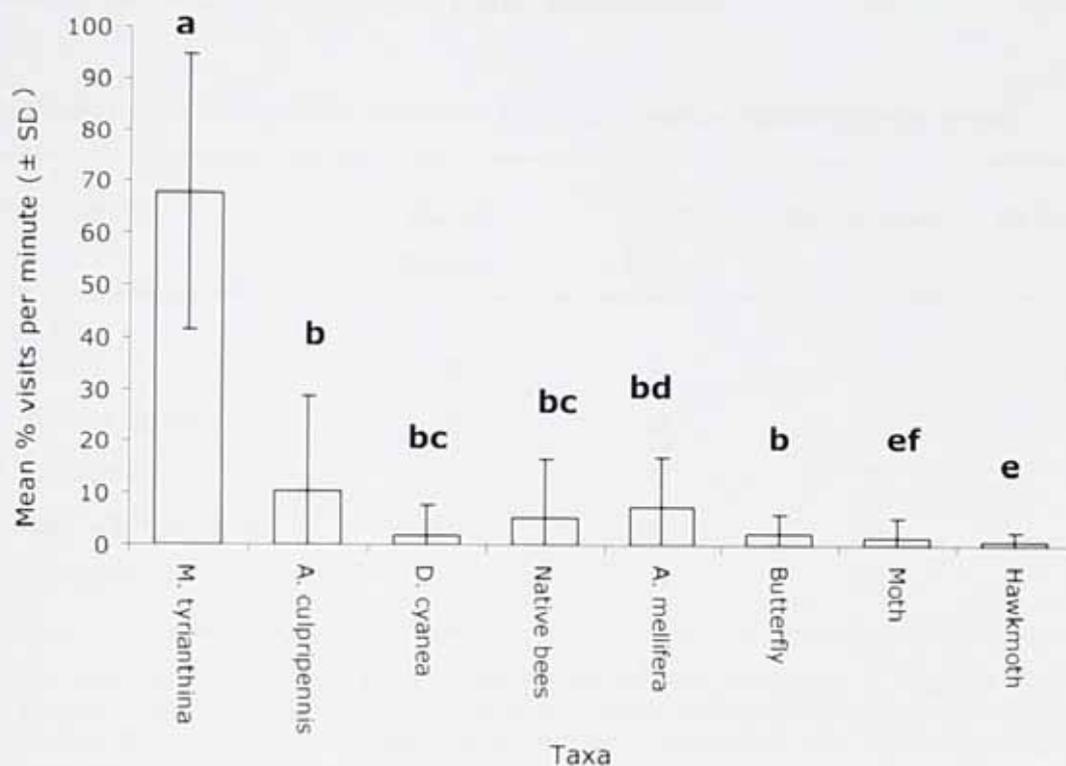


Figure 5.3 Mean percentage of visits (\pm SD) per minute for each taxa. Columns with the same letter do not differ significantly (Mann-Whitney U - test, $P > 0.05$). Median values were: *M. tyrianthina*, 77.3, *A. cupripennis*, 1.34, *A. mellifera*, 2.14; values for all other taxa were zero.

Determination of breeding system

The results from the breeding system experiments are summarised in Table 5.1. Of the 52 flowers tagged only cross pollinated treatments and control plants (no treatment) resulted in fruit set. The narrow tubed flower morphology and fragile pedicels of *Duranta mandonii* made it difficult to cross-pollinate, resulting in low sample sizes that precluded statistical analysis. However, it was concluded from the lack of fruit set in the bagged flowers and the observations of floral morphology, that flowers of *D. mandonii* are not autogamous and require animal vectors as pollinators.

Table 5.1 Fruit set levels in flowers of *Duranta mandonii* following treatments.

Treatment	No. of flowers	No. of fruits	No. of seeds	% fruit set
1. Open	9	4	32	44
2. Emasculated	8	0	0	0
3. Autogamy	12	0	0	0
4. Self-compatibility	12	0	0	0
5. Xenogamy	11	4	23	36

1. No treatment. **2.** Flowers were emasculated and left unbagged. **3.** Bagged unmanipulated **4.** Bagged flowers emasculated and hand pollinated with pollen from other flowers on the same individual. **5.** Bagged flowers emasculated and hand pollinated with pollen from flowers of other individuals.

Pollen removal efficiency of flower visitors

Flower visitors varied considerably in their abilities to remove pollen in a single visit (Figure 5.4). The one-way ANOVA indicated that the mean number of pollen grains removed from each of the treatments varied significantly among groups ($F = 6.70$, d.f. = 4, $P \ll 0.001$). Post-hoc tests of multiple comparisons show that six sample means were highly significantly different from one another. Open pollinated and native bee treatments removed the most number of pollen grains and *Metallura tyrianthina* removed the least (statistically indistinguishable from bagged virgin flowers). Note no differences in the mean number of pollen grains remaining per anther by *Apis mellifera* and *Bombus* spp. were found (Post-hoc Tamhane tests, $P = 0.988$). Similarly, *Bombus* and open pollinated and

Apis and open pollinated treatments were statistically indistinguishable. Rates of pollen removal from single visits for both native and honeybees were high (51% and 39% of the pollen per anther respectively).

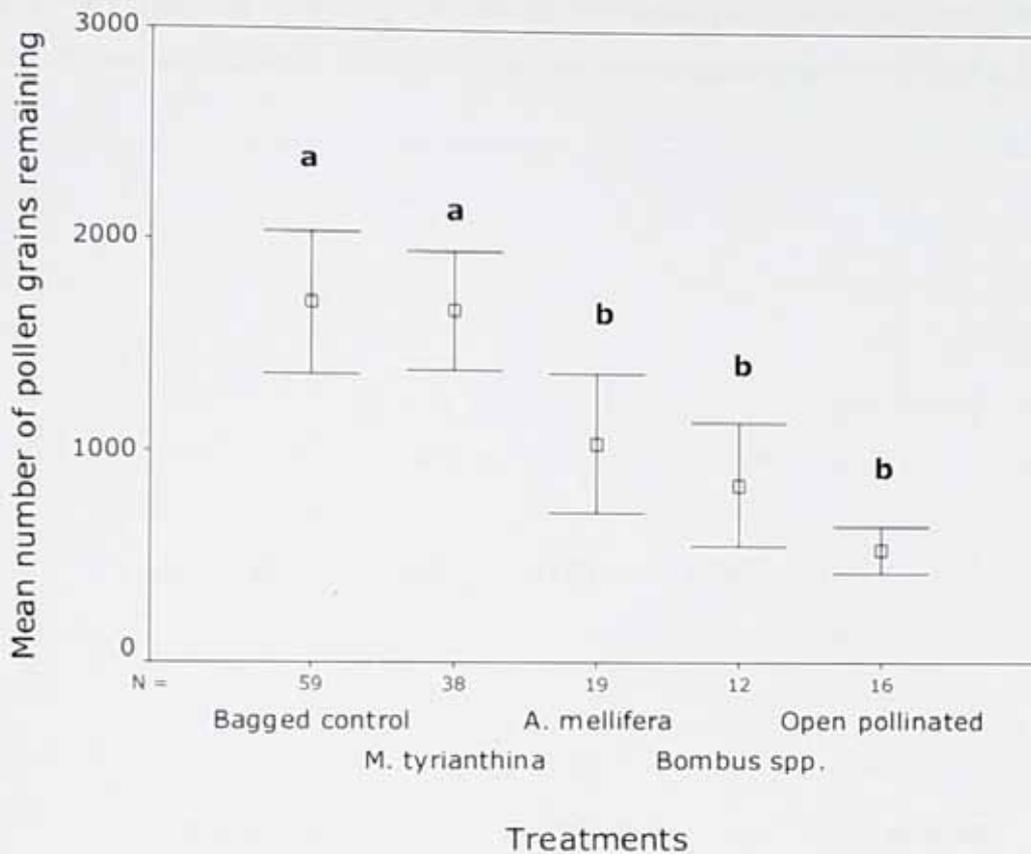


Figure 5.4 Mean (\pm SD) number of pollen grains remaining from *Duranta mandonii* anthers following single visits to flowers. Columns with the same letter did not differ significantly (Post-hoc Tamhane test, $P \geq 0.05$). Sample sizes (number of anthers) are indicated by N on the X axis.

Fruit and seed set efficiency of flower visitors

Kruskal Wallis analysis of variance showed that there was significant heterogeneity among groups for median number of seeds set ($\chi^2 = 25.03$, d.f. = 4, $P \ll 0.001$, Figure 5.5). Mann Whitney *U* tests of multiple pairwise comparisons showed that four sample medians were highly significantly different from one another and two treatments were marginally significantly different (Figure 5.5). Seed set between bee and open pollinated treatments did not differ significantly (Mann Whitney *U* test, $P = 0.34$). The data were highly variable for bee and open pollinated treatments, with many outliers and high standard deviations. The results indicated that open pollinated and bee treatments produced the greatest number of seeds (mean = 1.85 and mean = 1.23 respectively). Hummingbird and bagged control treatments did not result in seed set. It should be noted that maximum seed number per fruit

collected for this experiment was eight. Because seed numbers were low, all treatments recorded a median value of zero. Likewise, percentage of fruits set was also low, ranging from 0-30% (Table 5.1). Naturally pollinated flowers yielded fewer percentage fruit set than those pollinated by hand (30% versus 44% open pollinated treatments and 36% versus 18.2% for cross pollinated treatments and bee treatments) (Tables 5.1 and 5.2).

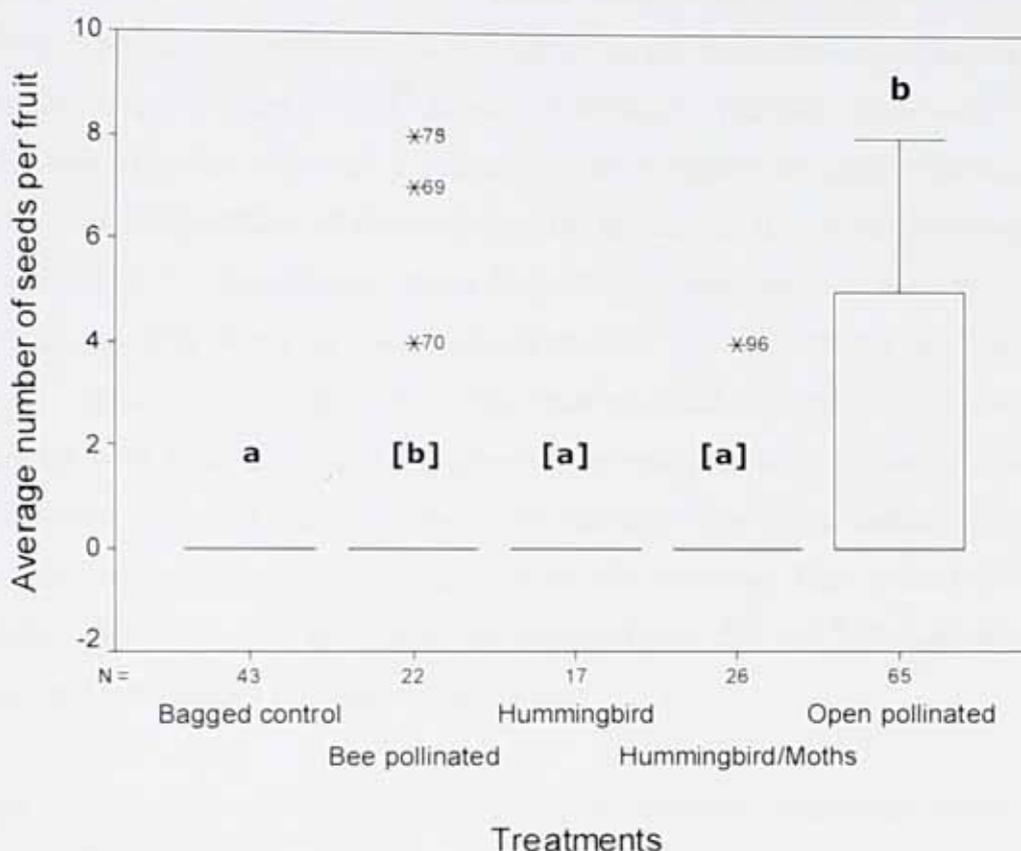


Figure 5.5 Average number of seeds set in flowers of *Duranta mandonii*. Medians are represented by horizontal lines and stars represent outliers. Columns with the same letter did not differ significantly (Mann-Whitney U test, $P \geq 0.05$ [$P \geq 0.10$]). Sample sizes are indicated by N on the X axis.

Table 5.2 Number of fruits set for each treatment in flowers of *D. mandonii*.

Treatment	Number of flowers	Number of fruits	% fruit set
Bagged control	43	0	0.0
Bee	22	4	18.2
Hummingbird	17	0	0.0
Hummingbird/ moth	26	1	3.8
Open Control	64	19	30.0

Pollinator efficiency rankings

Comparisons of the different plant fitness components measured (i.e. total pollen removal, percentage of pollen removed, visitation rate and fruit set) revealed that flower visitors were not consistent in their rankings (Table 5.3). Hummingbirds were ranked highest for visitation; however, for every other component they were ranked lowest. *Bombus* spp. and honeybees did not differ in the average proportion of pollen grains removed from anthers. When mean percentage visitation data were multiplied by percentage of pollen removed to create pollinator importance index values, honeybees and native bees were ranked as about as equally important. It was not possible to distinguish from the fruit set experiments which bee species contributed to seed production, so both *Bombus* spp. and honeybees were jointly ranked first. Honeybees and *Bombus* spp., had similar visitation rates (Figure 5.3) and therefore seed set was likely to be approximately equal for both taxa. Since hummingbirds removed almost no pollen, any fruit set here is most likely to be by small moths; consequently they were ranked second after bees. Visitation rates for *D. cyanea* peaked within the treatment time period for hummingbirds (0600h-1030h); however no fruit set occurred (Figures 5.2 and 5.3) suggesting that this bird is also not an effective pollinator of this plant.

Table 5.3 Pollinator efficiency rankings across the different plant fitness components measured for each taxon visiting *D. mandonii*

Ranking	Observations of flower visitors	Pollen removal	Fruit/seed set **	Pollinator importance (% pollen removed x mean % visitation)
1	Hummingbirds	<i>Bombus</i> spp.† <i>Apis mellifera</i> †	Native bees/ <i>Apis mellifera</i>	<i>Apis mellifera</i> 288
2	<i>Apis mellifera</i>		Moths	Native bees 260
3	Native bees	Hummingbirds*	Hummingbirds	Hummingbirds*136
4	Butterflies			
5	Moths			
6	Flies			

Pollinator importance = percentage of pollen grains removed multiplied by mean visitation rate. Native bees = all bee species. Hummingbirds = *M. tyrianthina* and *A. cupripennis*. Hummingbird* = *M. tyrianthina* only. **Rankings of seed/fruit are based on assumption that either native bees and/or honeybees contributed most toward female reproductive success. † Not significantly different.

Competition between honeybees and native flower visitors

Correlations of visitation rates between *Apis mellifera* and the other flower visiting taxa are presented in Table 5.4. There was a strong statistically significant correlation between *Apis mellifera* and butterflies, and *Apis mellifera* and native bees visiting *D. mandonii*. Thus, honeybees do overlap their foraging activity with both native bees and butterflies and visit flowers at similar rates (Figure 5.2). In contrast, there was a strong negative relationship between *Apis mellifera* and *Metallura tyrianthina*; hummingbirds did not overlap foraging activity with honeybees and visited flowers less frequently during the middle part of the day when bees were more active.

For all flower visitors no statistically significant relationships were found between flower visitation rates and nectar volume and sugar content. *D. mandonii* flower visitors did not coincide their foraging activity to periods of greatest nectar availability.

Table 5.4 Pearson and Spearman Rank* correlations of visitation rate between *Apis mellifera* and other taxon. Statistically significant correlations are in bold.

<i>Apis mellifera</i> versus	<i>R</i>	<i>N</i>	<i>P</i>
Butterflies	0.770	13	0.002
<i>Diglossopsis cyanea</i>	0.032	13	0.916
Moths	-0.302	13	0.317
Native bees	0.691	13	0.009
<i>Metallura tyrianthina</i>	-0.721	13	0.005
<i>Aglaeactis cupripennis</i>	-0.037	13	0.905
Hawkmoths	0.449	13	0.124*

Overall, very few observations were recorded where *Apis mellifera* and other taxa foraged in very close proximity of each other. Of a total of 56 observations, no encounters were recorded where *Apis* displaced the native fauna (Table 5.5). Most of the foraging activity resulted in neutral encounters between *Apis* and other taxa in which there was no displacement from flowers, and in no cases were honeybees the aggressor. In contrast, interference aggression did occur between hummingbirds and *Apis*, resulting in honeybees being displaced from flowers.

Table 5.5 Observations and outcomes of encounters between *Apis mellifera* and other flower visiting taxa.

<i>Apis</i> with	No. of encounters	No. of neutral encounters	No. of displacements <i>Apis</i> loses	No. of displacements <i>Apis</i> wins
Hummingbirds*	34	29	5	0
Native bees	11	11	0	0
Other taxa**	11	11	0	0

*Both species of hummingbirds. **Butterflies moths and flies.

Discussion

Fruit and seed set efficiency of flower visitors

Seed set experiments indicated that bees were the most efficient pollinators, although the experimental design made it impossible to pinpoint which bee species (native or honeybees) were the most efficient (Figure 5.5 and Table 5.2). Similarly, the same could be argued for hummingbird and moth treatment groups. However, because hummingbird treatments did not produce fruits and they removed almost no pollen, fruit set during this time period was most likely to be by small moths. Despite the higher number of seeds sired by multiple visits (open pollinated treatments) there was no statistical difference between the median number of seeds produced by bee and open pollinated treatments. This suggests that few visits are required to initiate seed set. However, it should be pointed out that the results from these experiments may be biased by the bagging effect. Since the bags were left on flowers over a period of days, physiological effects in the general environment may have affected the quality of pollen and the quantity of seeds produced.

Pollen removal efficiency of bees

Despite the higher proportion of pollen grains removed from anthers by *Bombus* spp., there was no statistically significant difference in sample means between the two bee taxa. Therefore, in this respect, both taxa could be considered equally efficient at removing pollen from *D. mandonii*. Freitas and Paxton (1998) reported similar pollen removal efficiencies between the native bee *Centris tarsara* and *Apis mellifera* visiting wild cashew in Brazil. The results also showed that *Bombus* and open pollinated treatments (unrestricted visitation) were statistically indistinguishable, once again reinforcing the conclusion that bees are the main pollinators of *D. mandonii*.

Pollen removal efficiency of hummingbirds

One of the most striking findings was that although *Metallura tyrianthina* was the most abundant visitor (68% of all visits) it effectively removed zero pollen and was

statistically indistinguishable from virgin and bagged controls (Figures 5.3 and 5.4). *Metallura tyrianthina* was not a pollinator, probably due to a mismatch of morphology between flower and hummingbird. Mean length of corolla tubes was $10.7 \text{ mm} \pm 2.1$, while the mean length of the exposed culmen (tip of bill to nares) was $14 \text{ mm} \pm 1.0$. During visits by both species of hummingbirds, no part of their feathered bodies contacted either anthers or stigma. Instead pollen could only be deposited on bills, but the results from pollen removal experiments suggest that the bills of these hummingbirds are too narrow to remove pollen from anthers. Therefore, both *M. tyrianthina* and *Aglaeactis cupripennis* must be considered as nectar thieves. These results are comparable to observations made in Mexico, where the efficiency of hummingbird species as pollinators of *Salvia mexicana* was related to bill length (Arizmendi *et al.* 1996).

Pollinators of *Duranta mandonii*

The combination of field observations and experiments demonstrated variation in several components of pollinator efficiency among the different taxa, consistent with the findings of a number of other studies (e.g. Young and Stanton 1990; Ivey *et al.* 2003). For example, frequency of visits ranked hummingbirds highest, but for all remaining components, they were consistently ranked last because of zero pollen removal (Table 5.3). Similarly, when pollen removal was considered by itself, *Bombus* spp., and honeybees were ranked highest. Based on pollinator importance (pollen removal multiplied by visitation rates), *Apis mellifera* was the most efficient pollinator of *D. mandonii*. Thus, if pollen removal increases with number of visits (e.g. Young and Stanton 1990) honeybees should be the most efficient pollinators. Based on the assumption that *Bombus* species removed a constant amount of pollen in each visit to flowers (51% of pollen available in all four anthers), only two visits would be needed to deplete all the pollen available. For honeybees three visits would be sufficient. Pooled visitation data across nine sites showed that between 12:00-16:00 (bee treatments) native bees made an average of 6 visits per flower and honeybees 6.8 per flower. Therefore, this is easily enough to ensure maximum pollination. However, the low fruit production after hand pollinations (xenogamy = 36%) and fruit set experiments (open pollinated = 30%) suggests that *D. mandonii* is resource limited, rather than limited by pollinator services (Table 5.1).

Competition with native flowers visitors

Field observations showed that competition between native flower visitors and *Apis mellifera* was negligible, possibly because of the low population densities at which honeybees occurred. Although honeybees did overlap in their foraging activity with native bees and butterflies, they did not outnumber native species (Figure 5.2). As Goulson (2003) has pointed out, niche overlap does not demonstrate that competition is occurring and it is difficult to prove. The results are not in accordance with many other studies (e.g. Roubik 1978; Schaffer *et al.* 1979; Ginsberg 1983; Schaffer *et al.* 1983; Paton 1993) that reported lower abundances of native flower visitors in the presence of honeybees. The impact of honeybees depleting nectar resources is probably minimal for *D. mandonii*, not only because honeybees occurred in relatively low numbers, but foraging patterns did not match peak nectar availability, and both bee species visited flowers at similar rates and removed equal amounts of pollen. In addition, the impact of floral larceny by native hummingbirds, which were easily the commonest flower visitors, was likely to have a far greater impact on the nectar availability than the presence of non-native honeybees. The results suggest that in fact *D. mandonii* has probably evolved under a regime of repeated nectar depletion by non-pollinating hummingbirds, and therefore may be to some extent buffered from the negative effects of nectar removal by honeybees. Note however, that floral larceny may not always have a negative impact on plant fitness (Maloof and Inouye 2000).

The presence of *Apis mellifera* did not result in either active or passive displacement of native fauna by honeybees. Interference aggression did occur infrequently between hummingbirds and honeybees, but in all cases honeybees were displaced from flowers (Table 5.5). This result contrasts with some previous work; for example Schaffer *et al.* (1983) found that the presence of honeybees deterred hummingbirds from foraging. However, displacement in Schaffer *et al.*'s study occurred at artificial feeders which represented an almost inexhaustible resource. Similarly, 91% of interactions between honeybees and native bees in Queensland resulted in disruption of native bee foraging (Gross and Mackay 1998). The observations are in agreement with the view that honeybees are not aggressive (e.g. Schaffer *et al.* 1979; Ginsberg 1983; Roubik 1991; Butz Huryn 1997 and references therein), and for some pollinators and plant species do not represent a conservation threat. However, to determine decisively whether honeybees do impact on

native pollinator communities, population-level experimental studies over many seasons are necessary (Goulson 2003).

Conclusions

In conclusion, the results showed significant variation among flower visitors both in pollen removal ability and contribution to fruit set. This variation was not always correlated, such that taxa which regularly visited flowers did not remove the most pollen or contribute to fruit set. Despite the taxonomic diversity of visitors, the main natural pollinators of this shrub are large native bees such as *Bombus* spp. In the terminology of Fenster *et al.* (2004), Ollerton *et al.* (2007b) and Vázquez and Aizen (2006), *D. mandonii* has high apparent generalisation, but low realised generalisation and can be considered a moderate to generalist (a number of species of large bees provide pollination services) but a functional specialist as the majority of pollinators belong to a single functional group (Fenster *et al.* 2004, though see discussion by Ollerton *et al.* 2007b). This study has highlighted the importance of measuring efficiency components when documenting plant-pollinator interactions, and has also demonstrated that visitation rates give little insight into the relative importance of flower visitors. No real evidence of interference or resource competition was apparent between honeybees and the native pollinators. For *D. mandonii* and its guild of flower visitors at least, honeybees are not an obvious threat to the stability of this interaction.

Conclusions

In this thesis the main objectives were to examine flower visitor abundance, diversity and composition and identify the main components which structured the plant-flower visitor communities in the Sacred Valley. To my knowledge, no other community study has examined plant-flower visitor interactions along an altitudinal gradient in the tropical high Andes of Perú. This concluding chapter will consolidate and summarise the findings from the entire investigation and discuss the limitations of the study. Additionally, the chapter considers the conservation implications and current threats to the biodiversity of the region, making recommendations for sustaining or restoring these plant-flower visitor communities. Finally suggestions are made for opportunities for future work.

Chapter 2 examined the generality of the prediction that the altitudinal distribution patterns among functional groups of flower visitors in the Sacred Valley were typical of pollinator distributions in high altitude environments. The results from Chapter 2 confirmed that the nine tributary valleys contained high level of habitat heterogeneity, from Subtropical thorn steppe found at the valley floor to Subtropical pluvial Sub-andino tundra at the highest elevations surveyed. Five altitudinal bands were surveyed passing through five life zones of the Holdridge Life Zone system (Holdridge 1967). It was not feasible to compare life zones because the elevation at which some vegetation occurred varied from one valley to another. Species richness and diversity of both flower visitors and visited plant species was high and field surveys yielded a total of 114 plant species visited by 144 flower visitor species. Many of the insects recorded, particularly Tachinidae, were new species to the region, and for Perú as a whole. Nevertheless, there were several limitations in this present study regarding estimations of species richness. Firstly, it should be acknowledged that the use of higher-taxon levels as surrogates for the number of real species could have potentially biased estimates of total flower visitor richness. Although it has been suggested that for some environmental monitoring, invertebrate morphospecies can be used as surrogates for real species, the accuracy of morphospecies separation has been shown to vary greatly among different invertebrate groups (Oliver and Beattie 1995; Derraiik *et al.* 2002). Although specimens were identified by experts, the estimation of 137

morphospecies was probably conservative and may not be representative of species diversity for the whole valley. However, the flora of this region is taxonomically well known and the majority of plant specimens were identified as named species by the Herbarium. Secondly, it should be also be recognised that there was no independent measure for the abundance of plants; instead abundance was estimated based on the number of visits. Given that the abundance of plants was not likely to be consistent along transects and among valleys, the results may be biased by variation in sampling intensity. Ideally, mean floral abundance per transect for each plant species should have been measured.

When calculating diversity indices it is important to assess the significance of the observed differences in community structure, otherwise no formal inferences can be made (Seaby and Henderson 2006). Therefore, a randomisation approach was used to test for significances between diversity indices. A comparison of species richness and evenness for visited plant species and flower visitors revealed high heterogeneity amongst some of the valleys and remarkable homogeneity amongst others. An important finding was that those valleys located closest to each other, such as Pumamarca, Choquebamba were the most diverse and shared many plants and flower visitor species in common. Pumamarca, Choquebamba and Poques are part of the Patacancha Valley, which is a tributary of the Sacred Valley. These valleys contained more plant species associated with Subtropical montane dry forest than other valleys located further east towards Calca. Similarly, Tiaparo and Piscacucho are located at the opposite end of the Sacred Valley close to Machu Picchu where species richness of plants and flower visitors was found to be high; diversity and evenness of visited plants was equal, with some endemic and geographically restricted species of plants and hummingbirds. Similarly, Mantamay was also a valley which had high species richness and species diversity of visited plants. Species richness and abundances of flower visitors was the highest at Yanacocha but this was not the most diverse valley, demonstrating that community structure was less uniform than in other valleys and was dominated by Diptera.

The results from the species diversity investigation generated a number of important questions such as: do the valleys that share many of the same species of plants and visitors display higher levels of endemism than other valleys? Which species of plants and flower visitors are able to disperse across valleys and which species have range restricted distributions? To what extent were the observed differences in species diversity among

valleys a result of anthropogenic modifications of the landscape? Is the rarity of the butterflies and small solitary bees due to sampling biases and weather conditions?

Since the assemblages studied were taxonomically very different in life histories, nesting preferences and behaviour, the transect census method undertaken may not have been appropriate to adequately characterise some of the taxa. For example, hummingbirds may have been under-represented in different samples because the composition and the relative abundance of hummingbird species is likely to be affected by their morphological-behavioural attributes, available resources and the distributional limits of a particular bird species (Terborgh 1971; Feinsinger and Colwell 1978; Borgella *et al.* 2001). Furthermore hummingbirds were easily disturbed from foraging by observers and did not tend to visit plants within the sampling area, but instead either remained on the periphery or in the canopy. Flower visiting beetles can be inactive, infrequent visitors, whereas some small solitary bees are short-lived, have short flight ranges and are not easily detected (Heinrich 1975; Torchio 1987; Gathmann and Tschardt 2002).

Due to the logistical time constraints and problems faced when undertaking field work in such remote, rugged locations at high altitudes, it was not feasible to sample transects in each valley more than once. It was estimated that the most frequently the nine valleys could be sampled using one team of assistants would be twice per month. Although this investigation suffered from lack of sampling replication and was restricted to one dry season, this present study is one of the few that has incorporated a number of different elevations and locations. Despite sampling limitations, nevertheless this study revealed some valid and consistent trends and has provided novel information about the plant-flower visitor assemblages of the Sacred Valley.

Confirming some of the findings of previous research in alpine areas, Chapter 2 showed that the animal assemblages in the Sacred Valley were similar in abundances to those in the Andes of Mendoza, Argentina (Medan *et al.* 2002) (Appendix IV). One prominent difference between the pollinator assemblages at higher latitudes in South America and those in the Sacred Valley was the high hummingbird diversity. Although the results suggested that the animal communities were dominated by Diptera, followed by Hymenoptera, mean abundance of most functional groups of flower visitors did not vary statistically with altitude, whereas species diversity did. The trend towards a decrease in

diversity and abundance of Hymenoptera with increasing altitude, and an increasing contribution of Diptera and Lepidoptera alluded to in some studies was not evident in the Sacred Valley. Contrary to other temperate montane areas, Hymenoptera were more diverse at the highest elevations. Diptera followed by Hymenoptera, Coleoptera, Trochilidae and Lepidoptera were the most abundant functional groups. Flies did not significantly increase in abundance with altitude and dominate the flower visitor fauna at the highest elevations as predicted. Instead Diptera were equally prominent at all altitudes and only honeybee abundance varied statistically with altitude. This is perhaps not surprising since the Tropical Peruvian Andes are situated closer to the equator, where thermoregulation and climate may not be as significant in explaining species abundance and diversity as in other temperate alpine regions, and these patterns may only emerge at the highest elevations. Nevertheless, given that habitat disturbance, habitat loss and fragmentation have been cited by many authors as a major contributory factors leading to the disruption of pollination systems (Kremen and Ricketts 2000); it is likely that the anthropogenic disturbance observed throughout the Sacred will influence the diversity and abundance of some taxa.

An important characteristic of the visitation data in this thesis was the highly skewed non-normal distributions and the large spread around the means and medians. This was particularly evident in Chapter 5, where visitation rates to nine populations of *Duranta mandonii* were highly erratic and in Chapter 4 with comparisons between floral morphology and visitor numbers and number of visitor species. Similarly, this was also evident in Chapter 3, where the initial DCA analysis for species abundance data indicated that a unimodal method was appropriate as gradient lengths for all four axes exceeded lengths well over 4 SD, showing that species composition data were not homogenous. This finding was not unusual given that spatio-temporal variation in pollinator abundance and identity of the participants may be significant over seasons and between consecutive years (Herrera 1988; Petanidou and Potts 2006).

The predicted trend of diminishing species richness among visited plants species and families with elevation was not evident. Instead Chapter 2 demonstrated that species richness for visited plants reached a maximum at the highest altitudes. It has also been predicted that increased plant diversity will result in an increase in flower visitor diversity (Heithaus 1974). The results suggested that visited plant species diversity could be

correlated with flower visitor diversity. Other contributory factors for the observed patterns might be the presence mass-flowering plant species which may facilitate pollination, habitat heterogeneity, flower longevity and the general decrease from anthropogenic pressures with increasing elevation. Furthermore, most plants surveyed were not exposed to the harsh environmental and climatic conditions experienced in other alpine ecosystems where the timber-line commences at lower elevations, the growing season is much shorter and pollen limitation is a factor (e.g. Arroyo *et al.* 1982).

To my knowledge only one other study (Arroyo *et al.* 1982) has compared altitudinal trends in the proportions of plant species utilised by different taxa. Chapter 2 demonstrated that differences were found between honeybees and flies, syrphids, hummingbirds and beetles in terms of the proportions of plant species being visited with elevation, although no significant differences were found between small native bees, bumblebees and honeybees suggesting some overlap in visitor profiles. The altitudinal patterns observed in this thesis did not support the findings of the plant-pollinator system in the temperate Andes of Chile, where bees pollinated a diminishing percentage of the flora along a gradient of increasing altitude and flies and butterflies pollinated an increasing proportion along the same gradient. Instead, Dipterans (all other flies and Syrphidae), butterflies, honeybees and beetles all visited the highest proportion of plants at low to mid elevations, Syrphidae visited more plant species at altitudes 2 and 4 and only hummingbirds utilised an increasing percentage as elevation increased.

No previous work has quantified differences that may exist in abundances and flower use between honeybees and native fauna in the Sacred Valley. In Chapter 2 one of the objectives was to collect data on how honeybees fitted within these communities. Although it was beyond the scope of the thesis to use direct measurements to detect potential competitive effects from honeybees, nevertheless by using indirect measurements the study has been a valuable first step in evaluating the potential for competition. The results from Chapter 2 were relatively consistent for the various components measured and showed that honeybees were a dominant part of the flower visitor assemblages and occurred in relatively high densities throughout the Sacred Valley. However, the correlative data showed only one negative correlation between abundances of honeybees and bumblebees at altitude 5 and between honeybees and beetles from pooled altitudes. Thus, on the whole, native flower visitor abundances did not decline in the presence of honeybees. Although

Apis visited a relatively large proportion of the total flora surveyed in comparison to the rest of the taxa, honeybees only intensively utilised a small proportion of available plant species, consistent with previous studies (Butz Huryn 1997 and references therein). Furthermore, resource overlap by honeybees and native bees was low, and no evidence was found to suggest that honeybees used interference competition and displaced other species when foraging. In Chapter 2 it was concluded that since only limited data were collected over one season and observations were based on indirect measures, little could be inferred on native bee survival, fecundity or population density in relation to honeybees. However, it was acknowledged that anthropogenic disturbance was likely to influence the diversity and abundance of some taxa, particularly if honeybees were to form strong associations with exotic plants, potentially facilitating their establishment and expansion via seed set.

In Chapter 3 the structural patterns of plant-pollinator interactions were further probed by using multivariate analysis as an exploratory tool. Correspondence analysis was chosen because it is designed precisely to draw out reciprocal associations and dissimilarities between sets of species (Lewinsohn *et al.* 2006). Chapter 4 further examined the interaction structure using nestedness analysis. To my knowledge, this is one of the few studies that used both nestedness (Chapter 4) and several multivariate methods (Chapter 3) to probe community-level plant-flower visitor interaction data for patterns. The canonical correspondence analysis established which functional groups were the most important according to the strengths of their interactions, some of which were not revealed from the nestedness analysis because species rather than functional groups were used. One particular advantage multivariate analysis has over nestedness analyses is that by including quantitative data rather than binary data, a better assessment can be made of how species fall into groups according to the strengths of their interactions (Dicks *et al.* 2002). Results from ordination diagrams showed that honeybees had a distinct visitor profile which did not overlap with native bees, bumblebees, hummingbirds, flies or syrphid flies. Although the nestedness analyses showed that honeybees visited many of the same resources utilised by native visitors, their relative dependence on plants was generally weak. In contrast to the nested analyses, the CCA summarised which particular plant species honeybees formed the strongest associations with across the whole of the Sacred Valley. On the other hand, by dividing species into functional taxonomic groups of pollinators (*sensu* Fenster *et al.* 2004), the identities of individual species within some functional groups could not be established

without separating these groups into separate species and then undertaking further rigorous analysis. Furthermore, since sampling was limited to surveying transects once, the data lacked any true replications of each combination of valley and altitude and thus both the valley and altitude indicators could not be used as covariables.

In Chapter 3 it was shown that by using quantitative data sets some of the reciprocal relationships between honeybees and Diptera and core plant species were further identified. Hummingbirds, honeybees, flies and beetles were identified as the major functional groups of flower visitors with significantly different visitation profiles. Once the effects of valleys were accounted for, hummingbirds, honeybees and flies still maintained significantly different visitation profiles. It was demonstrated in Chapter 4 that the nestedness approach had an advantage over the CCA analyses since it established which core species were important in which valleys. In contrast, by utilising the same data set and performing CCA analysis by pooling valleys, some important plant-flower visitor interactions such as bumblebees were overlooked. The nestedness analysis identified butterflies as core species at Yanacocha which was also complemented in the CCA analysis. The CCA analyses demonstrated that overall hummingbirds exhibited the most distinct foraging profile, which was maintained once the effect of valleys was removed. This finding was in contrast to the nestedness analysis, where hummingbirds were identified as only central to the network core at Huaran and Chicon. These results emphasised the importance of assessing the assemblage structure by using several comparative methods (Lewinsohn *et al.* 2006). By plotting the higher axes, the ordination diagrams showed that flies were the most important vectors on axes 2 and 3, and axes 3 and 4. This complemented the nestedness analysis, by demonstrating that this functional group of flower visitors as non-randomly distributed among this suite of plant species.

In Chapter 4 the network structure of flower visitor webs from nine valleys were described separately using nestedness analysis and then compared to a cumulative web by pooling data sets. The nested analysis was then repeated by combining data across altitudinal bands. All networks, except Huaran, were highly significantly nested and displayed network structures in accordance with many other studies (see Jordano *et al.* 2006). The networks consisted of core groups of generalists in which specialised plants interacted mainly with generalised flower visitors and specialised flower visitors mainly interacted with generalised plants.

In Chapter 4 it was shown that by pooling data from all valleys the degree of interaction strength asymmetry increased, the nestedness (N) of the network increased with network size, while the percentage connectivity decreased with network size, as predicted by Santamaria and Gironés (2007). A key finding was that some core species previously identified in individual valleys did not feature as core species in the cumulative web. Therefore important information regarding core species may be overlooked by pooling data. Furthermore, the disadvantage of pooling data into accumulative matrix is that those plant and visitor species which exhibited extended phenologies such as *Baccharis salicifolia* and *Apis* may have accounted for more interactions than they actually had at a particular point in time, potentially resulting in an over estimation of network size and connectance and the degree of generalisation within the system as a whole (Basilio *et al.* 2006). On the other hand, producing a single cumulative web had an advantage over individual webs since it revealed that only 107 interactions were common to all valleys and that 85% of the interactions were unique and only observed in single valleys, a pattern which did not emerge from summarising valleys separately. Altogether, these results suggested that the plant-flower visitor networks of the Sacred Valley may be context specific. The results from the single cumulative web posed an intriguing question: Why were the majority of interactions unique to some valleys and why were so few interactions common to all? This is likely to depend on many extrinsic and intrinsic factors such as the setting in which the plant finds itself, spatiotemporal variation in pollinator availability, presence of co-flowering plant species and plant absolute and relative abundance (Waser *et al.* 1996; Gómez and Zamora 1999; Gómez *et al.* 2007; Ollerton *et al.* 2007b).

Combining the data across valleys for altitudinal zones further reinforced the strong dominance of core species such as *Baccharis salicifolia*, highlighting the importance of this plant species in the community for the persistence of rare specialist species (Jordano 1987; Bascompte *et al.* 2003; Jordano *et al.* 2006). In contrast to individual valleys, the observed reversal from specialist to generalist plant species or vice versa (i.e. *Jungia rugosa*) was not apparent. Similarly, the dominance of *Apis* was also confirmed across all altitudes, followed by bumblebees and a similar suite of dipterans to those found in each of the valleys. While the analyses from individual valleys identified which plants and flower visitors were core species, by combining data across altitudes, information was provided regarding their distribution along the altitudinal gradient. Additionally, some of species identified as specialist flower visitors in each of the valleys changed from being specialists

to generalist core flower visitors. This is not surprising given that networks which include many different plant species and flower visitors are highly dynamic both spatially and temporally (Herrera 1988). Furthermore, a high spatial variation in the distribution of pollinator species may promote generalisation (Gómez and Zamora 1999). However, the results should be taken cautiously since species richness of insects may have been either over or under-estimated and no independent measure was taken to estimate plant species richness. Given the limitations of this study, the potential consequences for the results are likely to be an increase in generalisation for some plants and flower visitors. Despite these caveats, combining data across altitudes still provided a valid summary of network structure and how network properties changed across altitudes.

The results reported in Chapter 4 supported the notion that the plant-flower visitor networks follow a power-law regime as demonstrated by Jordano (1987), Olesen and Jordano (2002), Bascompte *et al.* (2003). All analyses, except for Huaran, produced strongly organised and significantly nested matrices. The networks were characterised by sparse matrices with only a small percentage of all possible interactions realised. Between 7% and 20% of plant species in each valley were specialists, and were limited to a single visitor species, in accordance with patterns previously reported from other network studies (e.g. Memmott *et al.* 2004; Basilio *et al.* 2006). On average relative linkage for plants was higher than for flower visitors, suggesting that the generalisation level for flower visitors was lower than for plants. It was shown that the ratio of animal to plant species in the interaction webs were not typical of the 3:1 reported by Blüthgen *et al.* (2006), but instead was closer to 2:1. As stated previously, because there were limitations regarding sampling protocols, it was also accepted that the degree of specialisation or generalisation for plants and flower visitors was likely to change.

Reinforcing some of the findings of previous research (e.g. Dupont *et al.* 2003; Ollerton *et al.* 2003; Memmott *et al.* 2004), Chapter 4 revealed strong correlations between abundance and the apparent degrees of generalisation for both plant and flower visitor species in all valleys. Those species of flower visitors at the core of the interactions were also the species in greatest abundance, implying that the networks were abundance structured. The strong correlation between relative abundance and the apparent degree of generalisation was not likely to be explained by sampling effort alone where rare species have few interactions and therefore appear more specialised. Instead the status of some

plant species and the identity of flower visitors changed along a continuum from extreme specialists to extreme generalists or vice versa among valleys, suggesting they were context specific. Given that the composition, diversity and abundance of pollinators may vary between and within plant populations (Herrera 1988), in terms of the number of plant species visited, insect and bird population sizes within the Sacred Valley may determine the degree of ecological generalisation of flower visiting animals. Future work should investigate interaction frequency since it has been shown to be a surrogate for the total effect on animal mutualists on plants (Vázquez *et al.* 2005).

The Sacred Valley core plants comprised of phenotypically generalised species, the majority belonging to the Andean genus *Baccharis*, *Ageratina* and *Aristeguietia*. *Baccharis sacilifolia* was the most prominent, forming associations with up to 31 species of flower visitors. The core flower visitors were dominated by honeybees and bumblebees followed by flies (mainly Muscidae, *Sciaria* and Syrphidae), beetles and butterflies, whereas hummingbirds only featured as core species in a few valleys. However, in Chapter 3 by using canonical correspondence analyses, it was shown that hummingbirds were a very important component of the plant-flower visitor assemblages. Honeybees were the most generalised flower visitors and dominated the core in six of the nine valleys. At Pumamarca and Choquebamba, when honeybee numbers were lower or about equal to bumblebees, *Bombus funebris* replaced *Apis* as the dominant core species. These findings suggested that the identities and positions of the species comprising the core change spatially and temporally according to natural variation in population sizes of plants and animals. Indeed, species diversity, evenness and abundances for some taxa did vary significantly between valleys and altitudes as demonstrated in Chapter 2. The results support the work of Nielsen and Totland (in review) who found that even on a relatively small spatial scale within a single season, that interaction evenness of the overall most abundant species varied significantly.

The positions of some core species were constant among valleys but varied in others; the status of some plant species and the identity of flower visitors changed along a continuum from extreme specialists to extreme generalists or vice versa among valleys. It was suggested that the observed reversal from specialist to generalist or vice versa among some valleys was influenced by the setting in which the plants exist and these patterns may be context specific. For a given plant species, the realised generalisation will be determined

by the number of effective pollinator species which interact with it and affect its fitness, within the setting of geographical and temporal variability in the community context (Ollerton *et al.* 2007b). It was argued that local abundance of plant species and their particular flowering phenologies was likely to play an important role in determining core species, particularly since some plant species flowered in both the rainy and dry season in some valleys but in others were restricted to just one season or in some valleys did not flower at all.

Chapter 4 also examined the species at the core of interactions to determine whether they defined a distinct morphological type. The results showed that the open access nature of the core flowers, permitted access to a wide variety of core species of insects with an array of different lengths of proboscis. Those core flower visitor species which recorded the highest linkage levels, such as *Apis* and *Bombus funebris* had medium to long probosces, or relatively medium length bills. Although confirming some of the findings of Stang *et al.* (2006) who showed that ecologically generalist visitor species mainly had long probosces with a high number of individuals and high number of interaction partners, these authors however did not include pollen visitors. In contrast, many of the core plant taxa in this present study possessed easily accessible pollen, thus allowing visitation from an array of pollen collectors such as small *Sciaria* and Chrysomelidae species with relatively shorter mouth parts. One of the most important findings was that although it appeared that phenotypically generalised plants with open access flowers received significantly more visitor species than flowers with tubular and flag morphologies, plant species with open access did not receive more visits than tubular flowers with hidden nectar. This suggested that floral traits were not restricting species visitation, and in some cases species with either short probosces or bill lengths gained access to long corolla tubes by nectar robbing. An important characteristic of flowers with such open access to rewards is that most of the visitors were likely to provide equally good pollination services, and the identity of the main pollinators will be largely determined by their abundance in space and time (Ollerton *et al.* 2007b).

In Chapter 4 it was suggested that the asymmetric nature of the networks and the presence of a core of generalist species had important implications for the persistence of the communities of the Sacred Valley. For example, because specialised rare species are frequently dependent on a core of generalist taxa (Bascompte *et al.* 2003; Vázquez and

Aizen 2006) it was proposed that honeybees may play an important role for the possibilities of rare species to persist. It was anticipated that networks with broad-scale distributions of connectivities could be more robust to the loss of highly connected nodes and thus the networks of the Sacred Valley might be fairly resilient to the loss of plant species and their associated flower visitors. This proposal was further endorsed since the network cores were dominated by locally abundant, wide-ranging generalist plant species and from the plant's perspective, if shifts occurred in the identity of the core species of flower visitors, then pollination was still likely to occur because the core plant species have replacement pollinators. Moreover, generalisation within these networks could buffer some plant species against the effects of pollinator loss (Pauw 2007). This poses an important question: what ecological and evolutionary processes produced these patterns of generalisation and specialisation in the Sacred Valley networks?

In addition to analysing network structure, Chapter 4 also addressed questions on how *Apis* fitted into the plant-flower visitor networks and whether honeybees were likely to affect network composition in the Sacred Valley. Although it was argued that honeybees may play an important role for the possibilities of rare species to persist. It was also recognised that honeybees also had the potential to alter network composition. For example, the global loss of natural habitats caused by anthropogenic disturbance has been shown to promote the invasion of alien species of plants which may facilitate the invasion of some exotic flower visitors (Aizen and Feinsinger 1994; Kearns and Inouye 1997; Morales and Aizen 2002; Morales *et al.* 2006; Lopezaraiza-Mikel *et al.* 2007; Aizen *et al.* 2008). Although only three non-naïve plant species were identified and no strong associations were formed between the native fauna or honeybees, it was acknowledged that this integration may affect native plant-pollinator interactions. In the study area, anthropogenic pressures generally occur at lower elevations and include the fragmentation and destruction of natural habitats through livestock farming, agricultural intensification, overgrazing, burning, soil erosion and widespread planting of *Eucalyptus*. Although the vast majority of plant species in the Vilcanota highlands are native (Tupayachi, 2005), some 40 exotic species have been introduced into the nearby Historic Sanctuary of Machu Picchu which have modified the landscape considerably (ParksWatch 2004). It was suggested that similar plant introductions may also have spread into the Sacred Valley, particularly in the case of introduced crop species. Therefore if honeybees were to form strong associations with exotic plants, this could potentially facilitate their establishment in

these disturbed habitats and increase the abundance and expansion via seed set (Morales and Aizen 2002; Morales and Aizen 2006; Lopezaraiza-Mikel *et al.* 2007).

Chapter 4 also pointed out the difficulty in assessing to what extent the integration of honeybees into these webs will affect these interactions, particularly since *Apis* has a long coevolutionary history with the native flora and fauna in the region and the networks were not considered to be highly invaded. Moreover, with the exception of the study of *Duranta mandonii* in Chapter 5, pollinator effectiveness and seed set were not measured in this study. Ultimately, honeybees are likely to negatively impact the reproductive success of native plant populations if they affect the quality and quantity of the pollen transferred among plants, resulting in reduced seed set (Traveset and Richardson 2006).

It was concluded that in order to achieve a better understanding of these complex networks and the potential large-scale impacts of perturbations, future work should include sufficient replications and quantify the strength of interactions. Furthermore, Olesen *et al.* (2007) suggest that modules (subgroups of frequently acting species) dictate the basic building blocks of networks and may play a critical role in both their stability and in the potential for coevolution of plants and pollinators. Therefore, since all larger pollination networks are modular and modularity is complementary to nestedness, testing for modularity should be the next step towards a more profound understanding of network complexity in the Sacred Valley (Olesen *et al.* 2007).

In Chapters 3 and 4, by following a more comprehensive approach and using several multivariate methods it was possible to detect some of the more complex relationships among associated plants and flower visitors which were not highlighted by testing the assemblages using solely one approach. However, used on its own the CCA analyses were a limited tool since pair-wise interactions alone are not sufficient for understanding the ecological and evolutionary processes shaping these interactions (Bascompte *et al.* 2003). Moreover, since the CCA analyses pooled data, it gave no indication of the identity of core taxa for each valley and which species of plants and flower visitors were potentially more vulnerable or robust to environmental perturbation.

The CCA analyses also suggested that the suite of plant species with the strongest associations did not exhibit specialisation on to functional groups. None of the plant species was visited exclusively by honeybees, hummingbirds or flies. Although the multivariate analyses identified how species fell into groups based on the strengths of their interactions, those visitation profiles which differed significantly were mainly functional groups of flower visitors that were ecologically generalised. These findings complemented the nestedness analyses and suggested that moderate to substantial generalisation occurs in the Sacred Valley, in agreement with Waser *et al.* (1996).

Chapter 5 focused on a single species and compared pollinator efficiency of hummingbirds, native bees and moths on both male and female components of fitness of the Andean shrub, *Duranta mandonii* (Verbenaceae) during the wet season. The plant was visited by a wide range of insects and birds. The results demonstrated significant variation among flower visitors in rates of visitation, pollen removal ability and contribution to fruit set. This variation was not correlated, such as taxa which regularly visited flowers such as hummingbirds did not remove the most pollen or contribute to fruit set. However, it was pointed out that the results from these experiments may have been biased by the bagging effect. Since the bags were left on flowers over a period of days, physiological effects in the general environment may have affected the quality of pollen and the quantity of seeds produced.

Chapter 5 demonstrated that despite the taxonomic diversity of flower visitors, the main pollinators of this shrub were large native bees such as *Bombus funebris*, *Bombus melaleceus* and *Apis*. Despite the higher proportion of pollen grains removed from anthers by *Bombus* spp., there was no statistically significant difference in sample means between the two bee taxa. Thus, in this respect, both bumblebees and honeybees were considered equally efficient at removing pollen.

It was concluded that *D. mandonii* had a high apparent generalisation, but low realised generalisation and can be considered a moderate ecological generalist (a number of species of large bees provide pollination services) but a functional specialist (the majority of the pollinators belonged to a single functional group). Chapter 5 highlighted the importance of measuring efficiency components when documenting plant-pollinator interactions, and also demonstrated that visitation rates may give little insight into the

relative importance of flower visitors. No real evidence of interference or resource competition was apparent between honeybees and native pollinators, possibly because *D. mandonii* has evolved under a regime of regular nectar depletion from hummingbirds which do not pollinate the flowers. Consistent previous chapters, it was concluded that for *D. mandonii* and its guild of flower visitors at least, honeybees were not an obvious threat to the stability of its interactions with pollinators.

Conservation implications

In light of the present scarcity of information on the pollination ecology of the species assemblages in the Sacred Valley, this thesis has provided valuable insights regarding the structural properties of plant-flower visitor networks. The nestedness analysis proved a useful approach for making an initial assessment of the vulnerability of interactions to species decline from anthropogenic pressures, by identifying reciprocally specialist and generalist associations (Ollerton *et al.* 2007a). From a conservation perspective, future landscape restoration projects in the Sacred Valley should focus attention on dominant species identified in the plant-flower visitor communities which interact with large numbers of flower visitors (Traveset and Richardson 2006). Particular attention should be paid to honeybees because specialised rare species were shown to frequently depend on a core of generalist taxa suggesting that honeybees and bumblebees may play an important role for allowing species to persist in the Sacred Valley. However, since the Sacred Valley is threatened by large-scale anthropogenic disturbance, future goals should also include exploration of facilitative interactions between honeybees and alien plant species, and removal campaigns should be promoted to eradicate *Eucalyptus* and invasive herbs (ParksWatch 2004).

Target species for future conservation

Candidates for future conservation efforts within the Sacred Valley should include those species of plants and animals which have already been identified as IUCN Red List species (see Table 1.5, Chapter 1 and Appendix III). Most noteworthy was the verification of the strong link between hummingbirds and endangered *Polylepis-Gynoxys* woodlands. Three hummingbird species *Aglaeactis castelnaudii*, *Oreonympha nobilis* and *Lesbia victoriae* showed strong preferences in some valleys to plant species associated with

Polylepis forests such as *Fuchsia apetala*, *Barnadesia horrida*, *Passiflora* spp. and *Gynoxys longiflora*. The IUCN Red List categorises all the hummingbird species observed in this present study as having a status of *Least Concern* (taxa which do not qualify for Near Threatened or Conservation Dependent status but are still interesting because of their restricted ranges (Stattersfield *et al.* 1998). An important finding from additional observations was the Green and White hummingbird (*Leucippus viridicauda*), previously placed in the genus *Amazilia*. This Peruvian endemic is common along the Urubamba River at Machu Picchu (Walker 2005) but was observed at low elevations at Piscacucho, along with *Eriocnemis luciani* and *Coeligena violifer*, also unique to this valley (SW unpublished data).

Also significant from a conservation perspective were the occasional observations of various species of hummingbirds and bumblebees to *Myrcianthes oreophila* (*Vulnerable*) and the strong association for honeybees. *Myrcianthes oreophila* was identified as one of the core plant species forming associations with up to 17 visitor species. Other important endangered plant species included the specialist plant *Fuchsia apetala* (*Threatened*) (which was visited solely by *Aglaeactis castelnaudii*) (*Least Concern*), and *Duranta armata* (*Near Threatened*). This thesis has also provided novel information on the ecology and distributional limits of two species of bumblebees; *Bombus funebris* and *Bombus melaleucus*. A total of 14 species of *Bombus* bumblebees have been listed for Perú (Rasmussen 2003) of which three out of the eight species distributed for the Department of Cusco were observed in this present study. Future conservation effort should be also focus on bumblebees since they are already established as effective pollinators of potatoes (*Solanaceae*) and many other crops and fruits in Perú (Rasmussen 2003).

Current conservation efforts and threats to biodiversity

The Vilcanota Highlands are currently the focus of a long-term conservation project, initiated by the Asociación Ecosistemas Andinos (ECOAN). *Polylepis* woodlands are important habitats for *Polylepis*-adapted birds and also provide ecosystem services that are vital to Andean people (Stattersfield *et al.* 1998; Fjeldså 2002b; Jameson and Ramsey 2007). The long term-objectives of the ECOAN project is to create and consolidate a system of *Polylepis* reserves controlled and led by local rural communities, with real alternatives conducive to the use and sustainable development of high Andean forests, and

which will allow the recovery of bird populations most at risk. The communities which are presently benefiting in the Vilcanota Highlands are Mantabay, Cancha Cancha (Huaran) and Abra Malaga (ECOAN 2004). So far 23 patches of *Polylepis* woodlands have been evaluated, totalling 485.4 hectares (ECOAN 2004). The project has reported that the majority of forests are threatened by over-exploitation and land management practices. Also in the Vilcanota Highlands, Jameson and Ramsey (2007) examined changes in *Polylepis* forest cover and quality over a 50 year period by using a combination of satellite imagery, photography and interviews. These authors found that of the three valleys they examined Mantabay (part of this present study) had the greatest cover and in general the locations of forest patches has not changed significantly from 1956 to 2005. They reported that 10% of *Polylepis* forest in the three valleys had degraded, with only 1% of forest area having disappeared by 2005. However, in Mantabay they found changes in the forest quality and a decline in the area of woodland with medium to high canopy densities which was caused by selective timber extraction of tall straight trees. Jameson and Ramsey (2007) also found that the majority of people interviewed from the local communities utilised the forest for wood extraction for fuel, construction, grazing and cultivation and accepted that these activities were a continuing threat to these forests. Some of the most serious anthropogenic threats to the biodiversity of the Sacred Valley are presented in Table 6.1.

If these activities continue in the Sacred Valley, this could potentially trigger a cascade of declines among the specialist plant species to which they are linked (Pauw 2007). Potentially at risk will be some endemic hummingbird species and insects which rely on the assemblages of plants associated with *Polylepis* woodlands. Also of particular concern are the climbing *Passiflora* species associated with tall trees and their dependence on the traplining hummingbird *Ensifera ensifera*. At lower altitudes, another considered threat is the cutting of *Duranta armata* and *Duranta mandonii* (and *Passiflora* which grow on them) because their thorny branches are used to prevent livestock from passing over farm walls (SW personal observation). A similar threat could be the loss of many endemic orchid species found in *Eucalyptus* plantations which are destroyed when trees are felled (SW personal observation).

Fjeldså (2002a) assessed biodiversity loss in the Andean highlands and found a positive correlation between endemism, ecoclimatic stability and human settlement. Because of this association, he recommended that initiatives to minimise extinctions of

plants and animals should include support for sustainable land use, provide local communities with economic incentives and better information for making their own decisions. In Perú, the resistance to nature conservation in populated areas means some of

Table 6.1 Some of the most serious threats to plant-flower visitor communities and biodiversity in the Sacred Valley

Threats to Biodiversity	
Solid waste	Communities of the Sacred Valley dispose of waste water and garbage on the banks of the Urubamba River (ParksWatch Perú 2004).
Burns and forest fires	Use of fire to stimulate re-growth of grasses (Fjeldså 2002a) which includes burning <i>Puya</i> spp. and accidental burning of <i>Polylepis</i> (SW personal observation).
Felling of <i>Polylepis</i> forests for firewood	Wood is mainly collected by rural communities as a source of fuel wood or for construction. For native people living close to patches of <i>Polylepis</i> forest, this timber constitutes their only source of firewood (ECOAN 2004)
Agriculture and grazing	Many communities are dependent on livestock farming and agriculture. <i>Polylepis</i> woodland provides grazing for livestock. In some cases crops are introduced species (ECOAN 2004)
Landslide activity	Certain anthropogenic activities such as agriculture, grazing, felling of <i>Eucalyptus</i> trees and intentional fires and burns cause landslides. Landslides are also attributed to loss of soil, erosion and sedimentation
Plantations	The Peruvian government initiated large-scale planting of <i>Eucalyptus</i> and pine, often in <i>Polylepis</i> zones. <i>Eucalyptus globus</i> saplings are planted to stop erosion. This is not an appropriate solution. Water consumption of <i>Eucalyptus</i> trees is high which is not advantageous in a seasonal environment. The planting of monocultures also increases pest damage. <i>Eucalyptus</i> is also a valuable cash crop (Chepstow-Lusty and Winfield 2000).
Illegal hunting	Piscacucho, Chillca and Ollantaytambo are zones vulnerable to illegal hunting of Andean deer (<i>Hippocamelus antisensis</i>) particularly near Piscacucho and Ollantaytambo (ParksWatch Perú 2004)
Legal land tenure conflicts	Territorial conflicts have occurred between local residents and Municipal leaders over the plan to increase the Buffer Zone of the Historic Sanctuary of Machu Picchu by increasing it in 2008 by 18,000 hectares (ParksWatch Perú 2004)

the places with the highest concentrations of endemic species are still not protected (Fjeldså 2002b). Indeed many of the endemic bird areas (EBAs) in the Vilcanota highlands (3 in this present study) remain unprotected (Wege and Long 1995). From a conservation perspective there has been some progress in the Vilcanota Highlands; over the last five years it is estimated that approximately 160,000 trees have been planted at a cost of \$US112,000 (Jameson and Ramsey 2007). However, some of these trees include *Eucalyptus* spp. whose water consumption is extremely high and the leaves contain toxic compounds that once shed prevent germination of other plants (Chepstow-Lusty and Winfield 2000; ECOAN 2004). As Jameson and Ramsey (2007) point out, it is the change in forest quality that is most apparent rather than the loss of entire *Polylepis* stands, therefore some of the conservation efforts should be refocused on enhancing and protecting habitat quality of existing patches. Conservation effort should also be extended to include studies which focus on plant-pollinator networks which are entirely lacking in *Polylepis* zones. Perhaps the greatest challenge for conservationists in the Sacred Valley will be how to maintain biological diversity in areas adjacent to dense rural populations (Walker 2005).

Other potential threats come from the deliberate introduction of the hybridised European African-derived honeybee into the Sacred Valley. In Perú, beekeepers first experienced the effects of the hybrid in 1977 in the Departments of Pasco, Junin and Carjamarca. In these areas beekeepers reported frequent swarms of feral honeybees (Kent 1989). It was proposed that although the hybrid established itself throughout many regions below 1500m on the eastern Andes of Perú, the hybrid had not been successfully established above this elevation. Some beekeepers in other areas of Perú have practised migratory beekeeping between the Amazonian lowlands to the highlands (Kent 1989). If the same practice was adopted in the Sacred Valley, this could potentially threaten the plant-pollinator communities of the region. More recently, samples of worker honeybees from 7 altitudinal regions of Perú where beekeeping is practised with minimal management showed evidence of an extensive process of hybridisation (Quezada-Euán *et al.* 2003). Surprisingly, in areas sampled between 2500 and 3500m they found that the numbers of “pure” European derived honeybees increased but were not dominant, and also found evidence of European–African hybrids. Quezada-Euán *et al.* (2003) proposed that this was either the result of beekeepers moving colonies to higher altitudes from the Africanised-saturated tropical lowlands or that colonisation of Africanised honeybees was not restricted by temperature alone.

No information was available from the beekeepers maintaining colonies in the study area regarding possible honeybee hybridisation. Since beekeeping in the Sacred Valley is strictly regulated by the Asociación de Apicultura de Urubamba and permission is required to keep hives, migratory beekeeping and increased apiculture from lowland Quillabamba seems unlikely, especially since the access route into the Sacred Valley is across high mountain passes. Furthermore the majority of professional beekeeping is practised along the coastal plains of Perú, whereas in the Sacred Valley most are part-time and hobby beekeepers.

Opportunities for future work

Considering the inherent difficulties with observational studies using indirect measures, future work assessing competitive effects from honeybees should include density manipulation experiments of colonies by removing hives from some valleys and augmenting hives in others. Of course this could only be achieved providing the Asociación de Apicultura de Urubamba was willing to participate and by offering financial incentives. Given that honeybees are ubiquitous at all elevations in most valleys, it would be difficult to find sites completely devoid of honeybees for control sites, especially since nothing is known about feral populations. If however this was achievable, abundance manipulations should be followed up over many seasons and years with careful experiments using direct measures to assess pollinator efficiency and reproductive success of the plants identified as core species.

This thesis has shown that to fully understand the asymmetric structure of the visitation webs in the Sacred Valley further studies are needed. Future work would profit from measuring the relative effectiveness of all flower visitors identified in the plant-flower networks, since as Memmott *et al.* (2004) highlighted, not all plants require pollination to reproduce and not all flower visitors are pollinators. However this presents a considerable challenge with such large networks as in the Sacred Valley. Most importantly pollinator efficiency of core species should be measured, coupled with experiments where honeybees are excluded from visitation to core plant species. This approach may answer questions on how network structure changes in the absence of honeybees and which species would be most tolerant to extinction. Networks should also be compared between dry and wet season and would also benefit from including visitation frequency data. Since connectance has

been shown to decline for entire year-long systems, (e.g. Basilio *et al.* 2006), future work should calculate average values of connectance from the dry and wet season to take into account species which overlap in phenology. A useful future analysis would be to include quantitative information on interaction strength by calculating resource use and evenness indices for the entire year to explore whether the observed specialisation and generalisation in the Sacred Valley is context specific. Finally, since the Sacred Valley is the most important area in Perú for many crops, future work should include agricultural pollination webs in the context of the habitat matrix.

This thesis has emphasised the importance of assessing the assemblage structure by using several comparative methods as some species identified as core species in the nestedness analyses were not highlighted in the CCA analyses or vice versa. Therefore future work would benefit by including both approaches to complement one another and to probe for more complex relationships. Finally, careful experiments should be undertaken to explore whether morphological thresholds such as nectar-holder depth and width of flower corollas restrict visitation.

For future work a number of alternative sampling designs might be incorporated in conjunction with the transect method to eliminate some of the potential biases, one of which could have included fixed observation plots. Compared to the transect method which is prone to sampling biases such as data aggregation, uneven sampling and observer bias, the fixed plot method is especially suited for the study of bees as it is easier to detect the movement of small insects (Stang *et al.* 2006; Sjödin 2007). By using equal observation times for each plant species in addition to walking transects, flower visitor behaviour and mobility can be measured. Additionally, mean floral abundance per transect for each plant species should have been measured.

Insects should be identified to species level by the same experts. To overcome taxonomic differences in life histories, nesting preferences and behaviour of some flower visitors, collection of small solitary bees from pithy stems of Asteraceae may provide a more realistic estimate of species diversity and abundance. Additionally, by collecting butterflies from baited traps, bees from ground malaise traps and aerial malaise traps and beetles from pan pitfall traps, pollen samples from these insects could be obtained. Similarly, pollen samples from hummingbird species can be taken by capturing birds using

mist nets, although this is a very labour intensive technique. Mist-netting was undertaken at Chicon, Tiaparo and Piscacucho; a total of 40 net/hours only yielded 22 individual birds, of which 11 were hummingbirds (SW unpublished data).

Future work ideally should include the use of a variety of diversity indices such as SHE analysis (Seaby and Henderson 2006) which is useful for identifying ecotones and changes in diversity with increasing sampling effort. Similarly, beta diversity indices measure the increase in species diversity along transects which would be especially appropriate to study changes in species diversity along the altitudinal gradient if sufficient sampling replication was undertaken.

In conclusion this thesis has used a number of different approaches to answer a range of questions relating to trends in species diversity and abundance with altitude and structure of plant-flower visitor interaction webs in the Sacred Valley, Perú. This study has given valuable insights into the ecology of vulnerable and endangered plant and animal species of the region. The results contribute knowledge to the way in which plant-flower visitor networks are structured and the role that honeybees play in these communities. The findings have implications for conservation management. The nestedness analyses proved a useful approach as an initial assessment of the vulnerability or robustness of some species, although the results strongly suggest that the plant-flower visitor networks in the Sacred Valley are context specific. Botanical collections have yielded 871 plant species for the Vilcanota Highlands, suggesting that there is still much work needed to catalogue the full diversity of pollination interactions. Clearly much more is yet to be discovered about the ecology of the plant-flower visitor communities of the Vilcanota Highlands of Perú. The evidence presented in this thesis suggests that perhaps some of the greatest threats to biodiversity and the survival and persistence of the plant-flower visitor communities in the Sacred Valley are from the destruction and fragmentation of habitats and from facilitative interactions between native and alien plants, mediated through visitation from honeybees. The greatest challenge for conservationists will be how to maintain biodiversity in these areas so close to indigenous populations. Ultimately, extinction risks can only be eliminated if there is political support for more sustainable land management.

REFERENCES

- Ackerman, J.D. (1983) Diversity and seasonality of male Euglossine bees (Hymenoptera: Apidae) in Central Panama. *Ecology* **64**: 274-283
- Aizen, M.A. and Feinsinger, P. (1994) Forest fragmentation, pollination, and plant reproduction in a Chaco dry forest. *Ecology* **75**:330-351
- Aizen, M.A., Morales, C.L. and Morales, J.M. (2008) Invasive mutualists erode native pollination webs. *PLoS Biology* **6**: e31.doi:10.1371/journal.pbio.0060031
- Allen-Wardell, G., Bernhardt, P., Bitner, R., Burquez, A., Buchmann, S., Cane, J., Cox, P.A., Dalton, V., Feinsinger, P., Ingram, M., Inouye, D., Jones, E.C., Kennedy, K., Kevan, P., Koopowitz, H., Medellín, R., Medellín-Morales, S., Nabhan, G.P., Pavlik, B., Tepedino, V., Torchio, P. and Walker, S. (1998) The potential consequences of pollinator declines and stability of food crop yields. *Conservation Biology* **12**: 8-17
- Alonso, L.E., Alonso, A., Schulenberg, T. S. and Dallmeier, F. (2001) Biological and social assessment of the Cordillera de Vilcabamba, Perú. Conservation International/ Smithsonian Institute. *RAP Working Papers SI/MAB Series 6*
- Arizmendi, M.C., Domínguez, C.A. and Dirzo, R. (1996) The role of an avian nectar robber and of hummingbird pollinators in the reproduction of two plant species. *Functional Ecology* **10**: 119-127
- Armbruster, W.S., Fenster, C.B. and Dudash, M.R. (2000) Pollination "principles" revisited: specialization, pollination syndromes, and the evolution of flowers. *Norske Videnskaps-Akademi. I, Mat. Naturv. Klasse, Ny Serie* **39**: 179-200
- Arroyo, M.T.K., Armesto, J.J. and Villagran, C. (1981) Plant phenological patterns in the high Andean Cordillera of central Chile. *Journal of Ecology* **69**:205-223
- Arroyo, M.T.K., Primack, R. and Armesto, J. (1982) Community studies in pollination ecology in the temperate Andes in Central Chile. I. pollination mechanisms and altitudinal variation. *American Journal of Botany* **69**: 82-97
- Arroyo, M.T.K., Muñoz, M.S., Henríquez, C., Till-bottraud, I. and Pérez, F. (2006) Erratic pollination, high selfing levels and their correlates and consequences in an altitudinally widespread above-tree-line in the high Andes of Chile. *Acta Oecologia* **30**: 248-257
- Asociación Ecosistemas Andinos (ECOAN) (2004) *Communal Polylepis forest reserves in the Vilcanota mountains*. <http://ecoanperu.org/sfenglish/index4-1.htm>
- Atmar, W. and Patterson, B.D. (1993) The measure of order and disorder in the distribution of species in a fragmented habitat. *Oecologia* **96**:373-382
- Atwal, A.S. and Sharma, O.P. (1971) The dominance of *Apis mellifera* over *Apis indica*. *American Bee Journal* **111**: 343

- Baltosser, W.H. and Scott, P.E. (1996) Costa's Hummingbird (*Calypte costae*). In: *The Birds of North America*, No. 251 (Poole, A. and Gill, F. eds.) The Academy of Natural Sciences, Philadelphia, PA, and The American Ornithologist's Union, Washington, DC
- Bascompte, J., Jordano, P., Melián, C.J. and Olesen, J.M. (2003) The nested assembly of plant-animal mutualistic networks. *Proceedings of the National Academy of Sciences of the United States of America* **100**: 9383-9387
- Bascompte, J., Jordano, P. and Olesen, J.M. (2006) Asymmetric coevolutionary networks facilitate biodiversity maintenance. *Science* **312**: 431-433
- Basilio, A. M., Medan, D., Torretta, J.P. and Bartoloni, N.J. (2006) A year-long plant network. *Austral Ecology* **31**: 975-983
- Beekman, M. and Ratnieks, F.L.W. (2000) Long-range foraging by the honeybee, *Apis mellifera* L. *Functional Ecology* **14**: 490-496
- Begon, M., Harper, J.L. and Townsend, C.R. (1990) *Ecology: individuals, populations and Communities*. Second Edition. Cambridge (MA): Blackwell Science
- Berry, P.E. and Calvo, R.N. (1989) Wind pollination, self-incompatibility, and altitudinal shifts in pollination systems in the high Andean genus *Espeltia* (Asteraceae). *American Journal of Botany* **76**: 1602-1614
- Bingham, H. (1922) Inca land explorations in the highlands of Perú. <http://Gutenberg.org/files/10772/10772-h/10772-h.htm>
- Blüthgen, N., Menzel, F., Hovestadt, T., Fiala, B. and Blüthgen, N. (2006) Specialization, constraints, and conflicting interests in mutualistic networks. *Current Biology* **17**:341-346
- Borgella, R. Jr., Snow, A.A. and Gavin, T.A. (2001) Species richness and pollen loads of hummingbirds using forest fragments in southern Costa Rica. *Biotropica* **33**: 90-109
- Burns, J.M., Janzen, D. H., Hajibabaei, Hallwachs, W. and Herbert, P.D.N. (2008) DNA barcodes and cryptic species of skipper butterflies in the genus *Perichares* in Area de Conservación Guanacaste, Costa Rica. *Proceedings of the National Academy of Sciences of the USA* **105**:6350-6355
- Butz Huryn, V.M. (1997) Ecological impacts of introduced honeybees. *The Quarterly Review of Biology* **72**: 275-295
- Cahill, J.R. and Matthysen, E. (2007) Habitat use by two specialist birds in high-Andean *Polylepis* forests. *Biological Conservation* **140**: 62-69
- Campbell, D.R. (1987) Interpopulational variation in fruit production: the role of pollination-limitation in the Olympic Mountains. *American Journal of Botany* **74** : 269-273
- Cane, J.H. (2001) Habitat fragmentation and native bees: a premature verdict? *Conservation Ecology* **5** (1):3.[online] URL: <http://www.consecol.org/vol5/iss1/art3/>

- Cane, J.H. and Sipes, S. (2006) Characterising floral specialization by bees: analytical methods and a revised lexicon for oligolecty. *Plant-Pollinator Interactions: from Specialization to Generalization* (eds N.M. Waser and J. Ollerton), pp. 99-122. The University of Chicago Press, Chicago
- Casagli, N., Fanti, R., Nocentini, M. and Righini, G. (2006) Assessing the capabilities of VHR satellite data for debris flow mapping in the Machu Picchu area (C101-1). Proceedings of the ICL Symposium, Cusco.
- Castellanos, M.C., Wilson, P. and Thomson, J.D. (2003) Pollen transfer by hummingbirds and bumblebees, and the divergence of pollination modes in *Penstemon*. *Evolution* **57**: 2742-2752
- Castro, I. and Robertson, A.W. (1997) Honeyeaters and the New Zealand forest flora: The utilisation and profitability of small flowers. *New Zealand Journal of Ecology* **21**:169-179.
- Celebrezze, T. and Paton, D.C. (2004) Do introduced honeybees (*Apis mellifera*, Hymenoptera) provide full pollination service to bird-adapted Australian plants with small flowers? An experimental study of *Brachyloma ericoides* (Epacridaceae). *Austral Ecology* **29**: 129-136
- Chacoff, N.P. and Aizen, M.A. (2006) Edge effects on the flower-visitor insects in the grapefruit plantations bordering premontane subtropical forest. *Journal of Applied Ecology* **43**: 18-27
- Chapman, F.M. (1921) The distribution of bird life in the Urubamba Valley of Perú. *Bulletin 117*, United States National Museum.
- Chepstow-Lusty, A., Bennett, K.D., Switsur, V.R. and Kendall, A. (1996) 4000 years of human impact and vegetation change in the central Peruvian Andes- with events paralleling the Maya record? *Antiquity* **70**: 824-833
- Chepstow-Lusty, A. and Winfield, M. (2000) Inca agroforestry: lessons from the past. *Ambio* **29**:322-328
- Chepstow-Lusty, A., Frogley, M.R., Bauer, B.S., Bush, M.B. and Tuypachi, H. A. (2003) A late Holocene record of arid events from the Cuzco region, Perú. *Journal of Quaternary Science* **18**: 491-502
- Collins, P.D., Harper, K.T. and Pendleton, B.K. (1983) Comparative life history and floral characteristics of desert mountain in Utah. *Great Basin Naturalist* **43**: 385-393
- Colwell, R.K., Betts, B.J., Bunnell, P., Carpenter, F.L. and Feinsinger, P. (1974) Competition for the nectar of *Centropogon valerii* by the hummingbird *Colibri thalassinus* and the flower-piercer *Diglossa plumbea* and its evolutionary implications. *The Condor* **76**: 447-452
- Constanza, R., d'Arge, R., de Groot, R., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., O'Neill, R., Parneto, J., Raskin, R., Sutton, P. and van den Belt, M. (1997) The value of the world's ecosystem services and natural capital. *Nature* **387**:253-260

- Cox, P.A. and Elmqvist, T. (2000) Pollinator extinction in the Pacific Islands. *Conservation Biology* **14**: 1237-1239
- Cruden, R.W. (1972) Pollinators in high-elevation ecosystems: relative effectiveness of birds and bees. *Science* **176**:1439-1440
- Cutler, A. (1991) Nested fauna and extinction in fragmented habitats. *Conservation Biology* **5**: 496-505
- Dafni, A. (1992) Pollination ecology – a practical approach. Oxford, IRL Press
- Derraik, J.G. B., Closs, G.P., Dickerson, K.J.M., Sirvid, P., Barratt, B.I.P. and Patrick, B.H. (2002) Arthropod morphospecies versus taxonomic species: a case study with Araneae, Coleoptera and Lepidoptera. *Conservation Biology* **16**: 1015-1023
- Descimon, H. (1986) Origins of Lepidopteran faunas in the high tropical Andes. In Vuillemier, F. and Monastrio (eds) pp. 500-532. *High Altitude Tropical Biogeography*. New York
- Diamond, J. (1988) Factors controlling species diversity: overview and synthesis. *Annals Missouri Botanical Gardens* **75**: 117-129
- Dicks, L.V., Corbet, S.A. and Pywell, R.F. (2002) Compartmentalization in plant-insect flower visitor webs. *Journal of Animal Ecology* **71**: 32-43
- Donovan, B.J. (1980) Interactions between native and introduced bees in New Zealand. *New Zealand Journal of Ecology* **3**: 104-116
- Dunne, J.A., Williams, R.J. and Martinez, N.D. (2002) Network structure and biodiversity loss in food webs: robustness increases with connectance. *Ecology Letters* **5**:558-567
- Dupont, Y.L. Hansen, D.M. and Olesen, J. M. (2003) Structure of a plant-flower visitor network in the high altitude sub-alpine desert of Tenerife, Canary Islands. *Ecography* **26**: 301-310
- Dupont, Y.L. Hansen, D.M., Valido, A., and Olesen, J. M. (2004) Impact of introduced honey bees on native pollination interactions of the endemic *Echium wildpretii* (Boraginaceae) on Tenerife, Canary Islands. *Biological Conservation* **118**: 301-311
- Eckhart, V.M. (1992) Spatio-temporal variation in abundance and variation in foraging behaviour of the pollinators of gynodioecious *Phacelia linearis* (Hydrophyllaceae). *Oikos* **64**: 573-586
- Fabbro, T. and Körner, C. (2004) Altitudinal differences in flower traits and reproductive allocation. *Flora* **199**:70-81
- Feinsinger, P. and Colwell, R. (1978) Community organization among neotropical nectar-feeding birds. *American Zoologist* **18**: 779-795

- Feinsinger, P. (1983) Variable nectar secretion in a *Heliconia* species pollinated by hermit hummingbirds. *Biotropica* **15**: 48-52
- Feinsinger, P., Beach, J.H., Linhart, Y.B., Busby, W.H. and Murray, K.G. (1987) Disturbance, pollinator predictability and pollination success among Costa Rican cloud forest plants. *Ecology* **68**: 1294-1305
- Fenster, C.B., Armbruster, W.S., Wilson, P., Dudash, M.R. and Thomson J.D. (2004) Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution and Systematics*. **35**: 375-403
- Fishbein, M. and Venable, D.L. (1996) Diversity and temporal change in the effective pollinators of *Asclepias tuberosa*. *Ecology* **77**: 1061-1073
- Fjeldså, J. and Krabbe, N. (1990) *Birds of the High Andes*. Apollo Books, Denmark
- Fjeldså, J. (2002a) *Polylepis* forests-vestiges of vanishing ecosystem in the Andes. *Ecotropica* **8**: 111-123
- Fjeldså, J. (2002b) Key areas for conserving the avifauna of *Polylepis* forests. *Ecotropica* **8**: 125-131
- Fonseca, C.R. and Leighton, J. J. (1996) Connectance: a role for community allometry. *Oikos* **77**: 353-358
- Fortuna, M.A. and Bascompte, J. (2006) Habitat loss and structure of plant-animal mutualistic networks. *Ecology Letters* **9**: 281-286
- Forup, M.L. and Memmott, J. (2005) The relationship between abundances of bumblebees and honeybees in native habitat. *Ecological Entomology* **30**: 47-57
- Freitas, B.M. and Paxton, R.J. (1998) A comparison of two pollinators: the introduced honeybee *Apis mellifera* and an indigenous bee *Centris tarsata* on cashew *Anacardium occidentale* in its range of NE Brazil. *Journal of Applied Ecology* **35**: 109-121
- Galiano, W. (2000) Situación ecológico-ambiental del Santuario Histórico de Machu Picchu: Una aproximación. *Programa Machu Picchu Cusco*
- Gathmann, A. and Tschardt, T. (2002) Foraging ranges of solitary bees. *Journal of Animal Ecology* **71**: 757-764
- Gentry, A.H. (1988) Changes in plant community diversity and floristic composition on environmental and geographical gradients. *Annals of the Missouri Botanical Garden* **75**: 1-34
- Gentry, A.H. (1996) *A field guide to the families and genera of woody plants Northwest South America (Colombia, Ecuador, Perú) with supplementary notes on herbaceous taxa, 2nd ed.* University of Chicago Press Chicago and London

- Ghazoul, J. (2005) Buzziness as usual? Questioning the global pollination crisis. *Trends in Ecology and Evolution* **20**: 367-373
- Ghazoul, J. (2006) Floral diversity and the facilitation of pollination. *Journal of Ecology* **94**: 295-304
- Gill, F.B. (1988) Trapline foraging by hermit hummingbirds: competition for an undefended renewable resource. *Ecology* **69**:1933-1942
- Ginsberg, H. (1983) Foraging ecology of bees in an old field. *Ecology* **64**: 165-175
- Gómez, J.M. and Zamora, R. (1999) Generalization vs. specialization in the pollination system of *Hormathopylla spinosa* (Cruciferae). *Ecology* **80**: 796-805
- Gómez, J.M. and Zamora, R. (2006) Ecological factors that promote the evolution of generalization in pollination systems. *Plant-Pollinator Interactions: from Specialization to Generalization* (eds N.M. Waser and J. Ollerton), pp. 145-166. The University of Chicago Press, Chicago
- Gómez, J.M., Bosch, J., Perfectti, F., Fernández, J. and Abdelaziz, M. (2007) Pollinator diversity affects plant reproduction and recruitment: the tradeoffs of generalisation. *Oecologia* **153**: 597-605.
- Gonzalez, V.H. and Engel, M.S. (2004) The tropical Andean bee fauna (Insecta: Hymenoptera: Apoidea), with examples from Colombia. *Entomologische Abhandlungen* **62**: 65-75
- Gotelli, N.J. and Colwell, R.K. (2001) Quantifying biodiversity: procedures and pitfalls in the measurements and comparisons of species richness. *Ecology Letters* **4**: 379-391
- Goulson, D. (2003) Effects of introduced bees on native ecosystems. *Annual Review of Ecology and Systematics* **34**: 1-26
- Grados, J. (2002) Arctiidae y Sphingidae (Lepidoptera: Heterocera) del Santuario Histórico de Machu Picchu, Cusco, Perú: Estudio preliminar. *Revista Peruana Biología*. **19**: 16-22
- Gross, C.L and Mackay, D. (1998) Honeybees reduce the fitness in the pioneer shrub *Melastoma affine* (Melastomataceae) *Biological Conservation* **86**: 169-178
- Gross, C.L. (2001) The effect of introduced honeybees on native bee visitation and fruit-set in *Dillwynia juniperina* (Fabaceae) in a fragmented ecosystem. *Biological Conservation* **102**: 89-95
- Guadalupe, E.G., Zea, M.A., Villafuerte, I.S. and Flores, D.O. (2002) Estudio geológica-geotécnico para el relleno sanitario de Machu Picchu y pueblos aledaños. Rev. Inst. Investig. Fac. minas metal cienc. geogr. ([online].jul/ago. 2002, **5**:25:23
- Guimarães, P. R. and Guimarães, P. (2006) Improving the analyses of nestedness for large sets of matrices. *Environmental Modelling and Software* **21**: 1512-1513.

- Guimarães, P. R., Sazima, C., Furtado dos Reis, S. and Sazima, I. (2006) The nested structure of a marine cleaning symbiosis: is it like flowers and bees? *Biology Letters* **3**: 51-54.
- Gurevitch, J. and Padilla, D.K. (2004) Are invasive species a major cause of extinctions? *Trends in Ecology and Evolution* **19**: 470-474
- Hansen, D.M., Olesen, J. M. and Jones, C.G. (2002) Trees, birds and bees in Mauritius: exploitative competition between introduced honey bees and endemic nectarivorous birds? *Journal of Biogeography* **29**: 721-73
- Heinrich, B. and Raven, P.H. (1972) Energetics and pollination ecology. *Science* **176**:597-603
- Heinrich, B. (1975) Energetics of pollination. *Annual Review of Ecology and Systematics* **6**: 139-170
- Heinrich, B. (1976) Resource partitioning among some eusocial insects: bumblebees. *Ecology* **57**: 597-602
- Heithaus, E.R. (1974) The role of plant-pollinator interactions in determining community structure. *Annals of the Missouri Botanical Gardens* **61**: 675-691
- Hellmich, R.L. and Rinderer, T.E. (1991) Beekeeping in Venezuela. In: Spivak, M., D.J. C. Fletcher, and M. D. Breed, editors. The "African" honey bee. Westview Studies in Insect Biology. Westview Press, Inc. Boulder, Colorado. Pages 399-411.
- Henderson, J., Hurly, T.A., Bateson, M. and Healey, S.D. (2006) Timing in free-living rufous hummingbirds *Selasphorus rufus*. *Current Biology* **61**: 512-515
- Herrera, C.M. (1987) Components of pollinator: 'quality' comparative analysis of a diverse insect assemblage. *Oikos* **50**: 79-90
- Herrera, C.M. (1988) Variation in mutualisms: the spatio-temporal mosaic of a pollinator assemblage. *Biological Journal of the Linnean Society* **35**: 95-125
- Herrera, C.M. (2005) Plant generalization on pollinators: species property or local phenomenon? *American Journal of Botany* **92**: 13-20
- Hingston, A.B. (1998) Temporal and spatial variation in abundances of native bee species on an altitudinal gradient in southern Tasmania. *Australian Journal of Zoology* **46**: 497-507
- Hingston, A.B. and McQuillan, P. B. (1998) Does the recently introduced bumblebee *Bombus terrestris* (Apidae) threaten Australian ecosystems? *Australian Journal of Ecology* **23**: 539-549
- Hingston, A.B. and McQuillan, P. B (2000) Are pollination syndromes useful predictors of floral visitors in Tasmania? *Austral Ecology* **25**: 600-609
- Holdridge, L.R. (1967) *Life Zone Ecology*. Costa Rica Tropical Science Centre.

- Horskins, K. and Turner, V.B. (1999) Resource use and foraging patterns of honeybees, *Apis mellifera*, and native insects on flowers of *Eucalyptus costata*. *Australian Journal of Ecology* **24**: 221-227
- Hughes, C. and Eastwood, R. (2006) Island radiation on a continental scale: exceptional rates of plant diversification after uplift of the Andes. *Proceedings of the National Academy of Sciences of the United States of America*. **103**: 10334-10339
- Inouye, D.W., Gill, D.E., Dudash, M.R. and Fenster, C.B. (1994) A model and lexicon for pollen fate. *American Journal of Botany* **81**: 1517-1530
- Irwin, R.E., Brody, A.K. and Waser, N.M. (2001) The impact of floral larceny on individuals, populations, and communities. *Oecologia* **129**: 161-168
- Ivey, C.T., Martinez, P. and Wyatt, R. (2003) Variation in pollinator effectiveness in swamp milkweed, *Asclepias incarnata* (Apocynaceae) *American Journal of Botany* **90**: 214-225
- Jacquemyn, H., Micheneau, C., Roberts, D.L. and Paillet, T. (2005) Elevational gradients of species diversity, breeding system and floral traits of orchid species on Réunion Island. *Journal of Biogeography* **32**: 1751-1761.
- Jameson, J. and Ramsey, P.M. (2007) Changes in high altitude *Polylepis* forest cover and quality in the Cordillera de Vilcanota, Perú, 1956-2005. *Biological Conservation* **138**: 38-46
- Johnsgard, P.A. (1997) *The hummingbirds of North America*. Christopher Helm, A and C Black, London.
- Johnson, S.D. and Steiner, K.E. (2000) Generalization versus specialization in plant pollination systems. *Trends in Ecology and Evolution* **14**: 140-143
- Jongman, R. H.G., Ter Braak, C.J.F. and Van Tongeren, O. F. G. (1987) *Data analysis in community and landscape ecology*. Pudoc, Wageningen, The Netherlands. Reissued in 1995 by Cambridge University Press, Cambridge
- Jordano, P. (1987) Patterns of mutualistic interactions in pollination and seed dispersal: Connectance, dependence asymmetries, and coevolution. *The American Naturalist* **129**: 657-677
- Jordano, P., Bascompte, J. and Olesen, J. M. (2003) Invariant properties in coevolutionary networks of plant-animal interactions. *Ecology Letters* **6**: 69-81
- Jordano, P., Bascompte, J. and Olesen, J. M. (2006) The ecological consequences of complex topology and nested structure in pollination webs. *Plant-Pollinator Interactions: from Specialization to Generalization* (eds N.M. Waser and J. Ollerton), pp. 173-199. The University of Chicago Press, Chicago
- Kato, M. and Kawakita, A. (2004) Plant-pollinator interactions in New Caledonia influenced by introduced honey bees. *American Journal of Botany* **91**: 1814-1827

- Kearns, C.A. (1992) Anthophilous fly distribution across an elevational gradient. *The American Midland Naturalist* **127**: 172-182
- Kearns, C.A. and Inouye, D.W. (1993) *Techniques for pollination biologists*. Niwot, University Press of Colorado.
- Kearns, C.A. and Inouye, D.W. (1994) Fly pollination of *Linum lewisii* (Linaceae). *American Journal of Botany* **81** (9):1091-1095
- Kearns, C.A. and Inouye, D.W. (1997) Pollinators, flowering plants, and conservation biology. *BioScience* **47**: 297-307
- Keating, P.L. (1999) Changes in páramo vegetation along an elevational gradient in southern Ecuador. *Journal of the Torrey Botanical Society* **126**: 159-175
- Kent, R.B. (1989) The African honeybee in Perú: an insect invader and its impact on beekeeping. *Applied Geography* **9**: 237-257
- Kerr, J.T., Sugar, A. and Packer, L. (2000) Indicator taxa, rapid biodiversity assessment, and nestedness in endangered ecosystem. *Conservation Biology* **14**: 1726-1734
- Kessler, M. and Krömer, T. (2000) Patterns and ecological correlates of pollination modes among bromeliad communities of Andean forests in Bolivia. *Plant Biology* **2**: 659-669
- Klein, A. M., Steffan-Dewenter, I. and Tschardt, T. (2003) Pollination of *Coffea canephora* in relation to local and regional agroforestry management. *Journal of Applied Ecology* **40**: 837-845
- Krebs, C.J. (1994) *Ecology: The Experimental Analysis of Distribution and Abundance, Fourth Edition*. Harper Collins College Publishers
- Kremen, C. and Ricketts, T. (2000) Global perspectives on pollination disruptions. *Conservation Biology* **14**:1226-1228
- Kremen, C., Williams, N.M. and Thorpe, R.W. (2002) Crop pollination from native bees at risk from agricultural intensification. *Proceedings of the National Academy of Sciences of the United States of America* **99**: 16812-16816
- Kühn, I., Bierman, S.M., Durka, W. and Klotz, S. (2006) Relating geographical variation in pollination types to environmental and spatial factors using novel statistical methods. *New Phytologist* **172**: 127-139
- Kwak, M.M. and Bekker, R.M. (2006) Ecology of plant reproduction: extinction risks and restoration. *Plant-Pollinator Interactions: from Specialization to Generalization* (eds N.M. Waser and J. Ollerton), pp. 362-386. The University of Chicago Press, Chicago
- Lepš, J. and Šmilauer, P. (2003) *Multivariate analysis of ecological data using Canoco*. Cambridge University Press

- Lewinsohn, T.M., Prado, P. I., Jordano, P., Bascompte, J. and Olesen, J. M. (2006) Structures in plant-animal interaction assemblages. *Oikos* **113**: 174-184
- Lieberman, D., Lieberman, M., Peralta, R. and Hartshorn, G.S. (1996) Tropical forest structure and composition on a large-scale altitudinal gradient in Costa Rica. *Journal of Ecology* **84**:137-152
- Lopezaraiza-Mikel, M.E., Hayes, R.B., Whalley, M.R. and Memmott, J. (2007) The impact of an alien plant-pollinator network: an experimental approach. *Ecology Letters* **10**:539:550
- Malo, J.E. and Baonza, J. (2002) Are there predictable clines in plant-pollinator interactions along altitudinal gradients? The example of *Cytisus scoparius* (L.) link in the Sierra de Guadarrama (Central Spain). *Diversity and Distributions* **8**:365-371
- Maloof, J.E. and Inouye, D.W. (2000) Are nectar robbers cheaters or mutualists? *Ecology* **81**: 2651-2661
- Marlin, J.C. and LaBerge, W. E. (2001) The native bee fauna of Carlinville, Illinois, revisited after 75 years: a case for persistence. *Conservation Ecology* **5**: 9[online] URL: <http://www.consecol.org/vol5/iss1/art9>
- Martins, F.Q. and Batalha, M.A. (2006) Pollination systems and floral traits in cerrado woody species of the upper Taquari region (central Brazil). *Brazilian Journal of Ecology* **66**: 543-552
- McCall, C. and Primack, R.B. (1992) Influence of flower characteristics, weather, time of day, and season on insect visitation rates in three plant communities. *American Journal of Botany* **79**: 434-442
- McDade L.A., Kinsman S (1980) The impact of floral parasitism in two neotropical hummingbird-pollinated plant species. *Evolution* **3**:994-958
- McDade L.A. and Weeks, J.A. (2004) Nectar in hummingbird-pollinated neotropical plants II: Interactions with flower visitors. *Biotropica* **36**: 196-215
- Medan, D., Montaldo, N.H., Deveto, M., Mantese, A., Vasellati, V., Roitman, G.G. and Bartoloni, N.H. (2002) Plant-pollinator relationships at two altitudes in the Andes of Mendoza, Argentina. *Artic, Antarctic, and Alpine Research* **34**: 223-241
- Memmott, J. and Waser, N.M. (2002) Integration of alien plants into a native flower-pollination visitation webs. *Proceedings of the Royal Society of London B* **269**: 2395-2399
- Memmott, J. and Waser, N.M. and Price, M.V. (2004) Tolerance of pollination networks to species extinctions. *Proceedings of the Royal Society of London B* **271**: 2605-2611
- Menezes Pedro, S.R. and Camargo, J.M.F. (1991) Interactions on floral resources between the Africanized honey bee *Apis mellifera* L. and the native bee community (Hymenoptera: Apoidea) in a natural "cerrado" ecosystem in southeast Brazil. *Apidologie* **22**: 397-415

- Mesler, M.R., Ackerman, J.D. and Lu, K.L. (1980) The effectiveness of fungus gnats as pollinators. *American Journal of Botany* **67**:564-567
- Michener, C.D. (1979) Biogeography of the bees. *Annals of the Missouri Botanical Garden* **66**: 277-347
- Michener, C.D. (2002) The bee genus *Chilicola* in the tropical Andes, with observations on nesting biology and a phylogenetic analysis of the subgenera (Hymenoptera Colletidae, Xeromelissinae). *Scientific Papers, Natural History Papers, The University of Kansas*. **26**:1-47
- Minkley, R.L. and Roulston, T.H. (2006) Incidental mutualisms and pollen specialization among bees. *Plant-Pollinator Interactions: from Specialization to Generalization* (eds N.M. Waser and J. Ollerton), pp. 69-98. The University of Chicago Press, Chicago
- Moeller, D.A. (2004) Facilitative interactions among plants via shared pollinators. *Ecology* **85**: 3289-3301
- Moldenke, H.V. (1941) New species and varieties of Verbenaceae from Central and South America. *Bulletin of the Torrey Botanical Club* **68**: 498-506.
- Moldenke, A. R. (1975) Niche specialization and diversity along a Californian Transect. *Oecologia*. **21**: 219-242
- Morales, C.L. and Aizen, M.A. (2002) Does invasion of exotic plants promote invasion of exotic flower visitors? A case study from the temperate forests of the southern Andes. *Biological Invasions* **4**: 87-100
- Morales, C.L. and Aizen, M.A. (2006) Invasive mutualisms and the structure of plant-pollinator interactions in temperate forests of north-west Patagonia, Argentina. *Journal of Ecology* **94**: 171-180
- Morell, V. (1999) Wilderness headcount. *National Geographic* **195**: 32-41
- Murdoch, W.W., Evans, F.C. and Peterson, C.H. (1972) Diversity and pattern in plants and insects. *Ecology* **53**: 819-829
- Myers, N., Mittermeyer, R.A., Mittermeyer, C.G. and da Fonseca, G.A.B., and Kent, J. (2000) Biodiversity hotspots for conservation priorities. *Nature* **403**: 853-858
- Navarro, L. (2001) Reproductive biology and effect of nectar robbing on fruit production in *Maclenia bullata* (Ericaceae). *Plant Ecology* **152**: 59-65
- New, T.R. (1997) Significance of honey bees in the Australian environment: setting the scene. *The Victorian Naturalist* **114**: 4-7
- Nielsen, A. and Bascompte J. (2007) Ecological networks, nestedness and sampling effort. *Journal of Ecology* **95**: 1134-1141

- Nielsen, A. and Totland, Ø. (in review) The pollinator distribution in a boreal forest landscape responds to fragmentation. Submitted to *Ecological Entomology*.
- Olesen, J.M. and Jordano, P. (2002) Geographic patterns in plant-pollinator mutualistic networks. *Ecology* **83**: 2416-2424
- Olesen, J.M., Bascompte, J., Dupont, Y.L. and Jordano, P. (2007) The Modularity of pollination networks. *Proceedings of the National Academy of Sciences of the United States of America* **104**:19891-19896
- Oliver, I. and Beattie, A.J. (1995) Invertebrate morphospecies as surrogates for species: a case study. *Conservation Biology* **10**: 99-109
- Ollerton, J. (1996) Reconciling ecological processes with phylogenetic patterns: the apparent paradox of plant-pollinator systems. *Journal of Ecology* **84**: 767-769
- Ollerton, J. and Leide, S. (1997) Pollination systems in Asclepiadaceae: a survey and preliminary analysis. *Biological Journal of the Linnean Society* **62**: 593-610
- Ollerton, J. (1999) La evolución de las relaciones polinizador-planta en los Artropodos. *Boletín de la Sociedad Entomológica Aragonesa* **26**:741-758
- Ollerton, J. and Cranmer, L. (2002) Latitudinal trends in plant-pollinator interactions: are tropical plants more specialised? *Oikos* **98**: 340-350
- Ollerton, J., Johnson, S.D., Cranmer, L. and Kellie, S. (2003) The pollination ecology of an assemblage of grassland Asclepiads in South Africa. *Annals of Botany* **92**: 807-834
- Ollerton, J., Armbruster, S.W. and Vázquez, D.P. (2006a) The ecology and evolution of specialized and generalized pollination. *Plant-Pollinator Interactions: from Specialization to Generalization* (eds N.M. Waser and J. Ollerton), pp. 19-22. The University of Chicago Press, Chicago
- Ollerton, J., Johnson, S.D. and Hingston, A.B. (2006b) Geographical variation in diversity and specificity of pollination systems. *Plant-Pollinator Interactions: from Specialization to Generalization* (eds N.M. Waser and J. Ollerton), pp. 283-308. The University of Chicago Press, Chicago
- Ollerton, J., McCollin, D., Daphne, G., Fautin, G. and Allen, G.R. (2007a) Finding NEMO: nestedness engendered by mutualistic organisation in anemonefish and their hosts. *Proceedings of the Royal Society* **274**: 591-598
- Ollerton, J., Killick, A., Lamborn, E., Watts, S. and Whitston, M. (2007b) Multiple meanings and modes: on the many ways to be a generalist flower. *Taxon* **56**: 717-728
- Paini, D.R. (2004) Impact of the introduced honey bee (*Apis mellifera*) (Hymenoptera: Apidae) on native bees: A review. *Austral Ecology* **29**:399-407
- Palmer, M. W. (1993) Putting things in even better order: the advantages of canonical correspondence analysis. *Ecology* **74**: 2215-2230

ParksWatch Perú (2004) *Perfil de Área Protegida – Perú Santuario Histórico de Machu Picchu*. www.parkswatch.org

Paton, D.C. (1990) Budgets for the use of floral resources in Mallee heath. In *The Mallee Lands: A conservation perspective*, ed. J.C. Noble, P.J. Joss, G.K. Jones, pp.189-193. Melbourne: CSIRO

Paton, D.C. (1993) Honeybees in the Australian environment- does *Apis mellifera* disrupt or benefit the native biota? *Bioscience* **43**: 95-103

Paton, D.C. (1995) Impact of honeybees on the flora and fauna of *Banksia* heathlands in Ngarkat Conservation Park. *SASTA Journal*. **95**: 3-11

Paton, D.C. (2000) Disruption of bird-plant pollination systems in Southern Australia. *Conservation Biology* **14**: 1232-1234

Patterson, B.D. (1987) The principle of nested subsets and its implications for biological conservation. *Conservation Biology* **1**: 323-334

Patterson, B.D. and Brown, J.H. (1991) Regionally nested patterns of species composition in granivorous rodent assemblages. *Journal of Biogeography* **18**: 395-402

Pauw, A. (2007) Collapse of a pollination web in small conservation areas. *Ecology* **88**: 1759-1769

Percival, M. (1974) Floral ecology of coastal scrub in Southwest Jamaica. *Biotropica* **6**: 104-129

Petanidou, T. and Potts, S.G. (2006) Mutual use of resource in Mediterranean plant-pollinator communities: how specialized are pollination webs? *Plant-Pollinator Interactions: from Specialization to Generalization* (eds N.M. Waser and J. Ollerton), pp. 220-244. The University of Chicago Press, Chicago

Pleasants, J.M. (1980) Competition for bumblebee pollinators in Rocky Mountain plant communities. *Ecology* **61**: 1446-1459

Pojar, J. (1974) Reproductive dynamics of four plant communities in southwestern British Columbia. *Canadian Journal of Botany* **52**: 1819-1834

Potts, S.G., Vulliamy, B., Dafni, A., Ne'eman, G. and Willmer, P. (2003) Linking bees and flowers: how do floral communities structure pollinator communities? *Ecology* **84**: 2628-2642

Price, M.V., Waser, N.M., Irwin, R.E., Campbell, D.R. and Brody, A.K. (2005) Temporal and spatial variation in pollination of a montane herb: a seven year study. *Ecology* **86**: 2106-2116

Primack, R.B. and Silander, J.A. (1975) Measuring the relative importance of different pollinators to plants. *Nature* **277**: 143-144

- Primack, R.B. (1983) Insect pollination in the New Zealand mountain flora. *New Zealand Journal of Botany* **21**: 317-333
- Proctor, M., Yeo, P. and Lack, A. (1996) *The natural history of pollination*. Harper Collins, London
- Pyke, G. H. and Balzer, L. (1985) The effects of the introduced honey-bee on Australian native bees. *New South Wales National Parks Wildlife Service, Occasional Papers*, Number 7
- Quezada-Euán, J.J.G., Pérez-Castro, E.E. and May-Itzá, W. (2003) Hybridization between European and African-derived honeybee populations (*Apis mellifera*) at different altitudes in Perú. *Apidologie* **34**: 217-225
- Rasmussen, C. (2003) Clave de indentificación para las especies peruanas de *Bombus* Latreille, 1809 (Hymenoptera, Apidae), con notas sobre su biología y distribución. *Revista Peruana de Entomología* **43**: 31-45
- Revilla, N.S., Zambrano, D.M. and Huari, W.N. (2003) Problemática de la familia Orchidaceae en el Valle Sagrado de los Incas. *Lyonia* **4**: 19-24
- Richerson, P.J. and Lum, K. (1980) Patterns in plant species diversity in California: relation to weather and topography. *The American Naturalist* **116**: 504-536
- Ricketts, T.H. (2003) Tropical forest fragments enhance pollinator activity in nearby coffee crops. *Conservation Biology* **18**:1262-1271
- Roubik, D.W. (1978) Competitive interactions between neotropical pollinators and Africanized honeybees. *Science* **201**:1030-1032
- Roubik, D.W. (1980) Foraging behaviour of competing Africanized honeybees and stingless bees. *Ecology* **61**: 836-845
- Roubik, D.W. (1983) Experimental community studies: time-series tests of competition between African and neotropical bees. *Ecology* **64**:971-978
- Roubik, D.W. (1989) *Ecology and natural history of tropical bees*. Cambridge, UK: Cambridge University Press
- Roubik, D.W. (1991) *Aspects of Africanized honeybee ecology in tropical America*. In M Spivak *et al.*, eds. The "African" honeybee. Boulder Colorado: Westview Press pp 259-281
- Roubik, D.W. (2000) Pollination system stability in tropical America. *Conservation Biology* **14**: 1235-1236
- Roubik, D.W. (2001) Ups and downs in pollinator populations: when is there a decline? *Conservation Ecology* **5**(1): 2 [online] URL: <http://www.consecol.org/vol5/iss/art2>

- Rymer, P.D., Whelan, R.J., Ayre, D.J., Weston, P.H. and Russell, K.G. (2005) Reproductive success and pollinator effectiveness differ in common and rare *Persea* species (Proteaceae). *Biological Conservation* **123**: 521-532
- Sanders, R.G. (1984) Provisional synopsis of the species and natural hybrids in *Duranta* (Verbenaceae). *Sida* **10**: 308-318
- Santamaria, L. and Rodríguez-Gironés, M.A. (2007) Linkage rules for plant-pollinator networks: trait complementarity or exploitation barriers. *PLOS Biology* **5**: 354-362
- Sarmiento, G. (1986) Ecological features of climate in high tropical mountains. In Vuilleumier, F. and Monastrio (eds) pp. 11-45. *High Altitude Tropical Biogeography*. New York
- Sazima, M., Sazima, I. and Buzato, S. (1994) Nectar by night: *Siphocampylus sulfureus* (Lobeliaceae) pollinated by hummingbirds and bats. *Plant Systematics and Evolution* **191**: 237-246
- Schaffer, W.M., Jensen, D.B., Hobbs, D.E., Gurevitch J, Todd, J.R. and Schaffer, M.V. (1979) Competition, foraging energetics, and the cost of sociality in three species of bees. *Ecology* **60**: 976-987
- Schaffer, W.M., Zeh, D.W., Buchmann, S.L., Kleinhaus, M., Schaffer, M.V. and Antrim, J. (1983) Competition for nectar between introduced honeybees and native North American bees and ants. *Ecology* **64**: 564-577
- Seaby, R.M. and Henderson, P.A. (2006) *Species Diversity and Richness Version 4*. Pisces Conservation Ltd., Lymington, England
- Servat, G. P., Mendoza, W., Ochoa, J. A. (2002) Flora y fauna de cuatro bosques de *Polylepis* (Rosaceae) en la Cordillera del Vilcanota Cusco Perú. *Ecología Aplicada* **1**: 25-35
- Shapiro, A.M. (1992) Why are there so few butterflies in the high Andes? *Journal of Research on the Lepidoptera* **31**: 35-56
- Sjödin, E.N. (2007) Pollinator behavioural responses to grazing intensity. *Biodiversity and Conservation* **16**: 2103-2121
- Smith, A.P. (1975) Insect pollination and heliotropism in *Oritrophium limnophilum* (Compositae) of the Andean páramo. *Biotropica* **7**: 284-286
- Smith, A.P. and Young, T.P. (1987) Tropical alpine plant ecology. *Annual Review of Ecology and Systematics* **18**: 137-158
- Snow, B.K. and Snow, D.W. (1972) Feeding niches of hummingbirds in a Trinidad valley. *Journal of Animal Ecology* **41**: 471-485
- Snow, A.A. and Roubik, D.W. (1987) Pollen deposition and removal by bees visiting two tree species in Panama. *Biotropica* **19**: 57-63

- Snow, D.W. and Snow, B.K. (1980) Relationships between hummingbirds and flowers in the Andes of Colombia. *Bulletin of British Natural History (Zoology)* **38**: 105-139
- Solow, A.R. (1993) A simple test for change in community structure. *Journal of Animal Ecology* **62**: 191-193
- Spears, E.E. Jr. (1983) A direct measure of pollinator effectiveness. *Oecologia (Berlin)* **57**: 196-199
- Spivak, M. (1992) The relative success of Africanized and European honey-bees over a range of life-zones in Costa Rica. *Journal of Ecology* **29**: 150-162
- Stang, M., Klinkhamer, P.G.L. and van der Meijden, E. (2006) Asymmetric specialization and extinction risk in plant-flower visitor webs: a matter of morphology or abundance? *Oecologia* **151**: 442-453
- Stattersfield, J.A., Crosby, M.J., Long, A.J. and Wege, D.C. (1998) *Endemic bird areas of the world: priorities for biodiversity conservation*. Birdlife International United Kingdom
- Steffan-Dewenter, I. and Tschardtke, T. (2000) Resource overlap and possible competition between honeybees and wild bees in central Europe. *Oecologia* **122**: 288-296
- Steffan-Dewenter, I. (2002) Landscape context affects trap-nesting bees, wasps, and their natural enemies. *Ecological Entomology* **27**: 631-637
- Steffan-Dewenter, I., Münzenberg, U., Bürger, C., Thies, C. and Tschardtke, T. (2002) Scale-dependent effects of landscape context on three pollinator guilds. *Ecology* **83**:1421-1432
- Steffan-Dewenter, I. and Kühn, A. (2003) Honeybee foraging in differentially structured landscapes. *Proceedings of the Royal Society of London series B* **270**: 569-575
- Stevens, G.C. (1992) The elevational gradient in altitudinal range: an extension of Rapoport's latitudinal rule to altitude. *The American Naturalist* **140**: 893-911
- Stiles, F.G. (1975) Ecology, flowering phenology, and hummingbird pollination of some Costa Rican *Heliconia* species. *Ecology* **56**: 285-301
- Sugden, E.A., Thorp, R. W. and Buchmann, S.L. (1996) Honey bee-native bee competition: focal point for environmental change and apicultural response in Australia. *Bee World* **77**: 26-44
- Terborgh, J. (1971) Distribution of environmental gradients: theory and a preliminary interpretation of distributional patterns in the avifauna of the Cordillera Vilcabamba, Perú. *Ecology* **52**: 23-40
- Ter Braak, C.J.F (1986) Canonical Correspondence Analysis: A new eigenvector technique for multivariate direct gradient analysis. *Ecology* **67**:1167-1179

- Ter Braak, C.J.F. and Prentice, C. (1988) A theory gradient analysis. *Advances in Ecological Research* **18**: 271-317
- Ter Braak, C.J.F. and Verdonschot, P.F.M. (1995) Canonical correspondence analysis and related multivariate methods in aquatic ecology. *Aquatic Sciences* **57**: 255-289
- Ter Braak, C.J.F. and Šmilauer, P. (2002) *Canoco reference manual and CanoDraw for Windows User's guide: software for Canonical Community Ordination (version 4.5)*. Microcomputer Power (Ithaca, NY, USA)
- Thomson, D.M. (2006) Detecting the effects of introduced species: a case study of competition between *Apis* and *Bombus*. *Oikos* **114**: 407-418
- Torchio, P.F. (1987) Use of non-honey bee species as pollinators of crops. *Proceedings of the Entomological Society of Toronto* **118**: 111-124
- Totland, Ø. (1993) Pollination in alpine Norway: flowering phenology, insect visitors, and visitation rates in two plant communities. *Canadian Journal of Botany* **71**: 1072-1079
- Traveset, A. and Richardson, D.M. (2006) Biological invasions as disruptors of plant reproductive mutualisms. *Trends in Ecology and Evolution* **21**: 208-216
- Tupayachi, A.T. (2005) Flora de la Cordillera de Vilcanota. *Arnaldoa* **12**: 126-144
- Tylianakis, J.M. (2008) Understanding the web of life: the birds, the bees, and sex with aliens. *PLoS Biology* **6** (2): ed47.doi:10.1371/journal.pbio.0060047
- Van der Hammen, T. and Cleef, A.M. (1987) Development of the high Andean páramo flora and vegetation. In Vuilleumier, F. and Monastrio (eds) pp.153-201. *High Altitude Tropical Biogeography*. New York
- Vázquez, J.A. and Givnish, T.J. (1998) Altitudinal gradients in tropical forest composition, structure, and diversity in the Sierra de Manantlán. *Journal of Ecology* **86**: 999-1020
- Vázquez, D.P. and Aizen, M.A. (2003) Null model analysis of specialization in plant-pollinator interactions. *Ecology* **84**: 2493-2501
- Vázquez, D.P. and Aizen, M.A. (2004) Asymmetric specialization: a pervasive feature of plant-pollinator interactions. *Ecology* **85**: 1251-1257
- Vázquez, D.P., Morris, W.F. and Jordano, P. (2005) Interaction frequency as a surrogate for the total effect of animal mutualists on plants. *Ecology Letters* **8**: 1088-1094
- Vázquez, D.P. and Aizen, M.A. (2006) Community-wide patterns of specialisation in plant-pollinator interactions revealed by null models. *Plant-Pollinator Interactions: from Specialization to Generalization* (eds N.M. Waser and J. Ollerton), pp. 200-219. The University of Chicago Press, Chicago

- Villanueva-G, R.G. and Roubik, D.W. (2004) Why are African honey bees and not European bees invasive? Pollen diet diversity in community experiments. *Apidologie* **35**: 481-491
- Visscher, P.K. and Seeley, T.D. (1982) Foraging strategy of honeybee colonies in a temperate deciduous forest. *Ecology* **63**: 1790-1801
- Walker, B. (2005) Field guide – The birds of Machu Picchu and the Cusco region. *Nuevas Imágenes*, Lima, Perú
- Warren, S.D., Harper, K.T. and Booth, G.M. (1988) Elevational distribution of insect pollinators. *American Midland Naturalist* **120**: 325-330
- Waser, N.M. and Price, M.V. (1990) Pollination efficiency and effectiveness of bumble bees and hummingbirds visiting *Delphinium nelsonii*. *Coll Bota (Barcelona)* **19**: 9-20
- Waser, N.M., Chittka, L., Price, M.V., Williams, N.M., Ollerton, J. (1996) Generalization in pollination systems, and why it matters. *Ecology* **77**: 1043-1060
- Waser, N.M. and Ollerton, J. (2006) Community and biogeographic perspectives. *Plant-Pollinator Interactions: from Specialization to Generalization* (eds N.M. Waser and J. Ollerton), pp. 167-172. The University of Chicago Press, Chicago
- Waser, N.M. (2006) Specialization and generalization in plant-pollinator interactions: a historical perspective. *Plant-Pollinator Interactions: from Specialization to Generalization* (eds N.M. Waser and J. Ollerton), pp. 3-17. The University of Chicago Press, Chicago
- Wege, D.C. and Long, A.J. (1995) Key area of threatened birds in the neotropics. Birdlife International, series 5. Cambridge UK
- Westphal, C., Steffan-Dewenter, I. and Tschardtke, T. (2003) Mass flowering crops enhance pollinator densities at a landscape level. *Ecology Letters* **6**: 961-965
- Whittaker, R. H. (1956) Vegetation of the Great Smoky Mountains. *Ecological Monographs* **26**: 1-80
- Wilson, P. and Thomson, J.D. (1991) Heterogeneity among floral visitors leads to discordance between removal and deposition of pollen. *Ecology* **72**: 1503-1510
- Wolda, H. and Roubik, D.W. (1986) Nocturnal bee abundance and seasonal bee activity in a Panamanian forest. *Ecology* **67**: 426-433
- Wolf, L.L., Stiles, G. and F. R Hainsworth (1976) Ecological organization of a tropical, highland hummingbird community. *The Journal of Animal Ecology*. **45**: 349-379
- World Conservation Monitoring Centre (1983) Cambridge
<http://www.unepwcmc.org/sites/wh/macchu.html>.
- Wright, D.H. and Reeves, J.J (1992) On the meaning and measurement of nestedness of species assemblages. *Oecologia* **92**: 416-428

Yensen, E. and Tarifa, T. (2002) Mammals of Bolivian *Polylepis* woodlands: guild structure and diversity patterns in the world's highest woodlands. *Ecotropica* **8**: 145-162

Young, H.J., Stanton, M.L. (1990) Influences of floral variation on pollen removal and seed production in wild radish. *Ecology* **7**: 536-547

Young, K.R. (1992) Biogeography of the montane forest zone of the Eastern slopes of Perú
In: Biogeografía, Ecología y Conservación del Bosque Montano en el Perú. Memorias de Museo Historia Natural **21**: 119-140 (Young K.R. and Valencia, R., eds). Universidad Nacional Mayor de San Marcos, Lima

APPENDICES

Appendix I

List of visited plant species in the Sacred Valley

Family	Scientific name
Amaryllidaceae	<i>Furcraea andina</i> Trelease
Apiaceae	Apiaceae sp.
Asclepiadaceae	Asclepiadaceae sp. 1
Asclepiadaceae	Asclepiadaceae sp. 2
Asteraceae	<i>Jungia rugosa</i> Lessing
Asteraceae	<i>Bidens</i> sp. 1
Asteraceae	<i>Baccharis odorata</i> H.B.K.
Asteraceae	<i>Baccharis buxifolia</i> (Lamarck) Persoon
Asteraceae	<i>Bidens triplinervia</i> H.B.K.
Asteraceae	<i>Senecio panticallensis</i> Cabrera
Asteraceae	<i>Eupatorium</i> sp. 1
Asteraceae	<i>Ageratina</i> sp. 1
Asteraceae	<i>Aristeguetia anisodonoton</i> (D. C.) King H. Robinson
Asteraceae	<i>Bidens andicola</i> H.B.K.
Asteraceae	<i>Bidens pilosa</i> L.
Asteraceae	<i>Baccharis boliviensis</i> (Weddell) Cabrera
Asteraceae	<i>Baccharis salicifolia</i> (R. & P.) Persoon
Asteraceae	<i>Cronquistianthus</i> cf. <i>urubambensis</i> (B. Robinson) King H. Robinson
Asteraceae	<i>Aristeguetia discolor</i> (D. C.) King H. Robinson
Asteraceae	<i>Berbesina</i> sp.
Asteraceae	<i>Barnadesia horrida</i> Muschler
Asteraceae	<i>Cronquistianthus</i> sp.
Asteraceae	<i>Baccharis</i> sp. 6
Asteraceae	<i>Ageratina stenbergiana</i> (D. C.) King & H. Robinson
Asteraceae	Asteraceae sp. 2
Asteraceae	Asteraceae sp. 4
Asteraceae	Asteraceae sp. 5
Asteraceae	Asteraceae sp. 6
Asteraceae	Asteraceae sp. 7
Asteraceae	Asteraceae sp. 8
Asteraceae	Asteraceae sp. 14
Asteraceae	Asteraceae sp. 15
Asteraceae	Asteraceae sp. 16
Asteraceae	Asteraceae sp. 17
Asteraceae	Asteraceae sp. 37

Asteraceae	Asteraceae sp. 45
Asteraceae	Asteraceae sp. 46
Asteraceae	Asteraceae sp. 52
Asteraceae	<i>Taraxacum</i> sp.
Asteraceae	<i>Gynoxys longiflora</i>
Berberidaceae	<i>Berberis humbertiana</i> J. F. Macbride
Berberidaceae	<i>Berberis carinata</i> Lechler
Bignoniaceae	<i>Tecoma sambucifolia</i> H.B.K.
Brassicaceae	Brassicaceae sp.
Bromeliaceae	<i>Puya ferruginea</i> (R. & P.) L. D. Smith
Campanulaceae	<i>Syphocampilus</i> sp.
Campanulaceae	<i>Syphocampilus actinothrix</i>
Campanulaceae	<i>Lobelia tenera</i> H. B. K.
Caryophyllaceae	<i>Drimaria</i> sp.
Caryophyllaceae	<i>Arenaria lanuginosa</i> (Michaux) Rohrbach
Caryophyllaceae	<i>Stellaria media</i> (L.) Vill.
Cunoniaceae	<i>Weinmannia pentaphyla</i> R.& P.
Cuscutaceae	<i>Cuscuta grandiflora</i> H.B.K.
Fabaceae	<i>Desmodium rotundus</i>
Fabaceae	<i>Lupinus</i> sp. 1
Fabaceae	<i>Lupinus</i> sp. 2
Fabaceae	<i>Trifolium amabile</i> var. <i>pentlandianum</i> Ball
Fabaceae	<i>Melilotus alba</i> Medikus
Fabaceae	Fabaceae sp.
Fabaceae	<i>Lupinus mutabilis</i> (Sweet)
Fabaceae	<i>Lupinus</i> sp. 3
Fabaceae	<i>Platymiscium</i> sp.
Gentianaceae	<i>Gentianella</i> sp.1
Gentianaceae	<i>Gentianella</i> sp. 2
Gentianaceae	Gentianaceae sp. 1
Geraniaceae	<i>Geranium</i> sp.
Grossulariaceae	<i>Escallonia resinosa</i> (Ruiz & Pav.)
Lamiaceae	<i>Salvia oppositiflora</i> R. & P.
Lamiaceae	<i>Lamium amplexicaule</i> L.
Lamiaceae	<i>Minthostachys spicata</i> (Bentham) Epling
Leguminosae	<i>Senna birostris</i> (Vogel) H. S. Irwin & Barneby var. <i>hookeriana</i>
Loasaceae	<i>Mentzelia fendleriana</i> Urbant & Gilg
Loranthaceae	<i>Gaiadendrum</i> cf. <i>punctatum</i> (R. & P.) G. Don
Melastomataceae	<i>Brachyotum nutans</i> Gleason
Melastomataceae	Melastomataceae sp.
Myrtaceae	<i>Myrcianthes orephila</i> (Diels) McVaugh
Onagraceae	<i>Oenothera rosea</i> Aiton
Onagraceae	<i>Fuchsia apetala</i> R.& P.
Onagraceae	<i>Oenothera versicolor</i> Lehman
Onagraceae	<i>Fuchsia boliviana</i> Carriere
Oxalidaceae	<i>Oxalis urubambensis</i>
Oxalidaceae	<i>Oxalis lotoides</i> (Knuth)
Passifloraceae	<i>Passiflora tripartita</i> (A. L. Jussieu) Poiret

Passifloraceae	<i>Passiflora trifoliata</i>
Passifloraceae	<i>Passiflora</i> sp.
Polygalaceae	<i>Monnina salicifolia</i> R. & P.
Proteaceae	<i>Oreocallis grandiflora</i> (Lamarck) R. Brawn
Ranunculaceae	<i>Ranunculus praemorsus</i> H.B.K.
Rhamnaceae	<i>Colletia spinosissima</i> J. Gmelin
Rosaceae	<i>Prunus serotina</i> subsp. <i>serotina</i>
Rubiaceae	<i>Fragaria</i> sp.
Scrophulariaceae	<i>Agallinis</i> sp.
Scrophulariaceae	Scrophulariaceae sp. 1
Solanaceae	<i>Solanum</i> sp.
Solanaceae	<i>Saracha</i> sp.
Solanaceae	<i>Solanum orchrophyllum</i>
Solanaceae	Solanaceae sp. 1
Unidentified	Species 24
Unidentified	Species 55
Unidentified	Species 22
Unidentified	Species 63
Unidentified	Species 57
Unidentified	Species 62
Unidentified	Species 54
Unidentified	Species 35
Unidentified	Species 30
Unidentified	Species 31
Unidentified	Species 29
Unidentified	Species 48
Unidentified	Species 61
Verbenaceae	<i>Aegiphyla mertonii</i> Moldenke
Verbenaceae	<i>Duranta armata</i> Moldenke
Verbenaceae	<i>Duranta mandonii</i> Moldenke

Appendix II

List of species of insects in the Sacred Valley

Family	Scientific name
Diptera	
Anthomyiidae	Anthomyiidae sp. 1
	Anthomyiidae sp. 2
Bibionidae	Bibionidae sp.
Chironomidae	Chironomidae sp.
	Diptera sp. 1
	Diptera sp. 2
	Diptera sp. 3
	Diptera sp. 4
	Diptera sp. 5
	Diptera sp. 6
	Diptera sp. 7
	Diptera sp. 8
	Diptera sp. 9
	Diptera sp. 10
	Diptera sp. 11
	Diptera sp. 12
	Diptera sp. 13
	Diptera sp. 14
	Diptera sp. 15
	Diptera sp. 16
	Diptera sp. 17
Muscidae	<i>Muscina</i> sp. 1
	Muscidae sp. 1
	Muscidae sp. 2
	Muscidae sp. 3
	Muscidae sp. 4
	Muscidae sp. 5
	Muscidae sp. 6
	Muscidae sp. 7
Sciaridae	<i>Sciara</i> sp. 1
	<i>Sciara</i> sp. 2
	<i>Sciara</i> sp. 3
	<i>Sciara</i> sp. 4
	<i>Sciara</i> sp. 5
Sphaeroceridae	Sphaeroceridae sp. 1
	Sphaeroceridae sp. 2
Sarcophagidae	<i>Helicobia</i> sp. 1
Syrphidae	<i>Copestylum</i> sp. 1
	<i>Copestylum</i> sp. 2
	<i>Eristalis</i> sp. 1
	<i>Eristalis</i> sp. 2
	<i>Platycheirus</i> sp.1
	<i>Platycheirus</i> sp.2
	<i>Platycheirus</i> sp.3
	<i>Toxomerus</i> sp. 1
	<i>Toxomerus</i> sp. 2

	<i>Toxomerus</i> sp. 3
	Syrphidae sp. 1
	Syrphidae sp. 2
	Syrphidae sp. 3
	Syrphidae sp. 4
	Syrphidae sp. 5
	Syrphidae sp. 6
	Syrphidae sp. 7
Tachinidae	<i>Tuberculanostoma</i> sp. 1
	Tachinidae sp. 1
	Tachinidae sp. 2
	Tachinidae sp. 3
	Tachinidae sp. 4
	Tachinidae sp. 5
	Tachinidae sp. 6
	Tachinidae sp. 7
	Tachinidae sp. 8
	Tachinidae sp. 9
	Tachinidae sp. 10
	Tachinidae sp. 11
	Tachinidae sp. 12
	Tachinidae sp. 13
	Tachinidae sp. 14
	Tachinidae sp. 15
	Tachinidae sp. 16
	Tachinidae sp. 17
	Tachinidae sp. 18
	Tachinidae sp. 19
Hymenoptera	
Apidae	<i>Apis mellifera caucasica</i> (Gorbachev 1916)
	<i>Apis mellifera ligustica</i> (Spinola)
	<i>Apis mellifera carnica</i> (Pollman 1879)
	<i>Bombus (Funebribombus) funebris</i> Smith, 1854
	<i>Bombus (Robustobombus) melaleucus</i> Handlirsch, 1888
	<i>Bombus</i> sp.
Halictidae	<i>Lasioglossum</i> sp.
Ichneumonidae	<i>Ophion</i> sp.
	Hymenoptera sp. 1
	Hymenoptera sp. 2
	Hymenoptera sp. 3
	Hymenoptera sp. 4
	Hymenoptera sp. 5
Vespidae	Vespidae sp. 1
	Vespidae sp. 2
	Vespidae sp. 3
	Vespidae sp. 4
	Vespidae sp. 5
	Vespidae sp. 6
Coleoptera	
Melyridae	<i>Astylus</i> sp. 1
	<i>Astylus</i> sp. 2
	<i>Asylus</i> sp. 3
Bruchidae	Bruchidae sp. 1
	Bruchidae sp. 2

Chrysomelidae	Bruchidae sp. 3
	Chrysomelidae sp. 1
	Chrysomelidae sp. 2
	Coleoptera sp. 1
	Coleoptera sp. 2
	Coleoptera sp. 3
	Coleoptera sp. 4
	Coleoptera sp. 5
	Coleoptera sp. 6
	Coleoptera sp. 7
	Coleoptera sp. 8
	Coleoptera sp. 9
	Coleoptera sp. 10
	Coleoptera sp. 11
	Coleoptera sp. 12
	Coleoptera sp. 13
Curculionidae	Curculionidae sp. 1
	Curculionidae sp. 2
	Curculionidae sp. 3
	Curculionidae sp. 4
Coccinellidae	<i>Eropis</i> sp. 1
	<i>Eropis</i> sp. 2
	<i>Eropis</i> sp. 3
Hemiptera	
Lygaeidae	<i>Lygaeus albornatus</i> Blanchard
Cicadellidae	Cicadellidae sp.
Lepidoptera	Lepidoptera sp. 1
	Lepidoptera sp. 2
	Lepidoptera sp. 3
	Lepidoptera sp. 4
	Lepidoptera sp. 5
	Lepidoptera sp. 6
	Lepidoptera sp. 7
	Lepidoptera sp. 8
	Lepidoptera sp. 9
	Lepidoptera sp. 10
	Lepidoptera sp. 11
	Lepidoptera sp. 12
	Lepidoptera sp. 13
	Lepidoptera sp. 14
Hesperidae	<i>Metardaris cosinga</i> (Hewiston 1874)

Appendix III

List of hummingbird species observed in the Sacred Valley

	IUCN Status	Habitat	Elevational range	Forest position	Abundance
Sparkling Violetear*	Lc	< 4	2000-4000m	U/M/C	C
Green-and-white Hummingbird	Lc	SG/HPF	1200-2900m	U/C	R
White-bellied Hummingbird	Lc	HMS/AMS/AA	1000-2900m	U/C	U
Andean Hillstar*	Lc	PW/PG	3500-4500m	T/U	FC
Giant Hummingbird	Lc	HMS/AMS/EF	2500-4000m	U/C	C
Shining Sunbeam*	Lc	PW/HMS	2500-4200m	U/C	C
White-tufted Sunbeam*	Lc	PW/HMS	2500-4300m	U/C	U
Giant Sapphire Wing*	Lc	PW/EF/HMF	2600-3900m	U/M	U
Sword-billed Hummingbird	Lc	EF/HMS/VT	1700-3600m	C	FC
Tyrian Metaltail*	Lc	HMS/EF	2500-3600m	U/M	C
Bearded Mountaineer*	Lc	HMS/AMS/S	2500-3900m	U/M	U
Black-tailed trainbearer	Lc	AA/AMS/PW	2600-4000m	U/M	FC
Green-tailed trainbearer	Lc	AA/AMS	2000-3800m	U/C	FC
Violet-throated Starfrontlet	Lc	SG/HMF/HMS	2800-3330M	U/M	R
Sapphire-vented Puffleg	Lc	HMF	2000-3500m	U/M	R

BirdLife International (2006). IUCN Red List of Threatened Species. *Hummingbird species from transect records, remaining species observed from December 2001-November 2002. Habitats: SG: Secondary Growth; HPF: Humid pre-Montane Forest; HMS: Semi-humid /Humid Montane Scrub; AMS: Arid Montane Scrub; AA: Agricultural Areas; PW: *Polylepis* Woodland; PG: Puna Grasslands; EF: Elfin Forest; VT: Vine Tangles; S: Streams. Forest Position: U: Understorey; M: Midstorey; C: Canopy; T: Terrestrial. Abundance based on SW personal observations mainly during transect surveys. Adapted from Fjeldså and Krabbe (1990) and Walker (2005) and from personal observations.

Appendix IV

Comparisons of abundance of functional groups of pollinators from eight montane and alpine studies. Numbers were calculated as percentages from number of individuals, except Cordón de Cepo, where values are equivalent to percentages of number of species. For Central Chile and California values are equivalent to insect species richness according to altitude and number (in parentheses).

Authors	Location	Functional group	%
This present study	Perú Sacred Valley 2900 - 4050m	Hymenoptera	33
		Diptera	50
		Lepidoptera	4
		Coleoptera	7
		Trochilidae	6
Warren <i>et al.</i> (1988)	Utah Bald Mountain Pass 3170m	Hymenoptera	27
		Diptera	62
		Lepidoptera	5
		Coleoptera	6
Medan <i>et al.</i> (2002)	Argentina Río Blanco 1900m	Hymenoptera	34
		Diptera	46
		Lepidoptera	4
		Coleoptera	9
		Trochilidae	7
Medan <i>et al.</i> (2002)	Argentina Laguna Diamante 3330m	Hymenoptera	35
		Diptera	48
		Lepidoptera	12
		Coleoptera	5
Arroyo <i>et al.</i> (1982)	Central Chilean Andes Cordón del Cepo Sub-Andean scrub 2200-2600m	Hymenoptera	40
		Diptera	45
		Lepidoptera	11
		Coleoptera	4
Arroyo <i>et al.</i> (1982)	Central Chilean Andes Cordón del Cepo Subnivel 3200-3600m	Hymenoptera	14
		Diptera	58
		Lepidoptera	21
		Coleoptera	7
Smith (1975)	Venezuelan Andes 3500m	Hymenoptera	8
		Diptera	83
		Lepidoptera	9
Primack (1983)	New Zealand: Mount Cook	Hymenoptera	27

Alpine grassland	1100m	Diptera	65
Plant group	<i>Hebe</i>	Lepidoptera	5
		Coleoptera	3
Plant group	Asteraceae	Hymenoptera	17
		Diptera	73
		Lepidoptera	9
		Coleoptera	1
	Remaining plant species	Hymenoptera	19
		Diptera	50
		Lepidoptera	12
		Coleoptera	19
Primack (1983)	New Zealand	Hymenoptera	4
Alpine grassland/scrub	Craigieburn Mountains	Diptera	76
	900m	Lepidoptera	9
Plant group	<i>Hebe</i>	Coleoptera	11
	Asteraceae	Hymenoptera	10
		Diptera	71
		Lepidoptera	4
		Coleoptera	15
	Remaining plant species	Hymenoptera	13
		Diptera	80
		Lepidoptera	3
		Coleoptera	4
Moldenke (1975)*	California	Hymenoptera	59 (176)
	1000-1999m	Diptera	19 (56)
		Lepidoptera	7 (21)
		Coleoptera	15 (45)
Moldenke (1975)*	California	Hymenoptera	41 (67)
	>3000m	Diptera	33 (54)
		Lepidoptera	17 (28)
		Coleoptera	9 (14)
Müller (1880)* (cited in Warren <i>et al.</i> 1988)	European Alps	Hymenoptera	22 (183)
	2000-2999m	Diptera	42 (348)
		Lepidoptera	26 (220)
		Coleoptera	10 (83)
	European Alps	Hymenoptera	18 (88)
	≥ 3000m	Diptera	44 (210)
		Lepidoptera	31 (148)
		Coleoptera	7 (33)

* Species richness calculations (from Warren *et al.* 1988)

Raw data available from the author (email: hummingbird_pe@yahoo.com)