

Movement in the Matrix: pollination and dispersal processes in a tropical coffee and forest landscape mosaic

by

Shalene Jha

**A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Ecology and Evolutionary Biology)
in The University of Michigan
2009**

Doctoral Committee:

**Professor John H. Vandermeer, Co-Chair
Assistant Professor Christopher W. Dick, Co-Chair
Professor Ivette Perfecto
Associate Professor Beverly J. Rathcke**



© Shalene Jha
2009

I would like to dedicate this thesis to my mother and father who, from my earliest days, have suffered through innumerable bedroom bug-collections, terrariums, and windowsill gardens. With their limitless encouragement and patience, they single-handedly fueled my love for science. They have been my greatest supporters, through all of my ecological-endeavors, even when it meant months abroad and a long hike to the nearest phone. I can never thank them enough for their unconditional love and support.

Table of Contents

Dedication	ii
Acknowledgements	iii
List of Tables	vi
List of Figures	vii
Abstract	ix
Chapter	
I. Introduction	1
II. Foraging patterns of Africanized honeybees and native bees and wasps in a tropical agroforestry landscape	9
III. Contrasting bee foraging on coffee in response to resource scale and local habitat management	31
IV. Local agroforestry landscapes mediate bee community composition	52
V. Isolation and characterization of nine microsatellite loci for the tropical understory tree <i>Miconia affinis</i> Wurdack (Melastomataceae)	87
VI. Shade coffee farms promote the genetic diversity of native trees	93
VII. Extensive plant gene flow mediated by native bees across a shade coffee landscape mosaic	101
VIII. Conclusion	112

List of Tables

Tables

2.1	We utilized an Analysis of Covariance to measure the influence of all covariates and factors on the number of visiting insects for each foraging group.....	22
3.1	Pearson correlation coefficient matrix of floral resources at bush, 5 m, 10 m, and 100 m scales.....	42
3.2	Results of repeated-measures ANCOVA, testing the effects of floral resource levels (at 10m scale), cloud cover, temperature, distance to the forest, vegetation management (high or low-shade coffee), and bee group (honeybees, native social bees, native solitary bees) on the number of bee visits per observation period.....	43
3.3	Species present in high-shade coffee (HSC) and low-shade coffee (LSC) agroforestry habitats and their functional group (FG), classified as an Africanized honeybee (AHB), native solitary bee (NSOL), or native social bee (NSOC).	44
4.1	Local seasonal agroforestry characteristics at each study site.	68
4.2	Species list of overstory trees (OT), overstory trees in flower (OTF) and understory plants in flower (UPF) recorded over the 12 week sampling period.	69
4.3	Species list of bees captured over the 12 week sampling period. Individuals listed as a ‘msp. MX#’ did not match any species within reference collections while ‘msp. # (T.G.)’ matched individuals within reference collections. Nesting (Nest) and Sociality (Soc) type are indicated as follows: ground (g), cavity (c), wood (w), social (x) and solitary (o).....	70
5.1	Forward (F) and reverse (R) sequences with fluorescent dye label (used for genotyping), repeat motifs, annealing temperatures in °C (T_a), number of alleles (N_a), allelic size range in bp (Size Range), observed heterozygosity (H_O), expected heterozygosity (H_E), and GenBank Accession number (Ac. No.) for nine microsatellite loci for <i>Miconia affinis</i>	91
6.1	Number of <i>M. affinis</i> individuals (N), allelic richness (A), expected heterozygosity (H_E), and observed heterozygosity (H_O).....	96

List of Figures

Figures

2.1	Map of the study region with concentric circles indicating feeder locations at 400 m, 500 m, 600 m, and 800 m from the apiary, upon which nectar feeders were randomly placed in each habitat.	23
2.2	Species richness of plants flowering and light levels in each habitat and across the experimental season.	24
2.3	Mean number of visits in the two coffee habitats on day one and day two for Africanized honeybees (a-b) native social bees (c-d) native solitary vespids (e-f) and native solitary bees (g-h).....	25
2.4	Visitation over the 8-wk experimental period for all visitor groups (a-d).	26
3.1	Species richness of bee visitors in high-shade coffee (HSC) and low-shade coffee (LSC) habitats.	45
3.2	Visitation was significantly higher in high-shade coffee habitats (HSC) than low-shade habitats (LSC) for (A) native solitary bees, (B) native social bees, and (C) Africanized honeybees.	46
3.3	Contrasting responses of native and exotic bees under differing habitat management styles.	47
4.1	Study sites in Nueva Alemania, a region in the southern portion of the state of Chiapas, outlined in black within the inserted map of Mexico.....	72
4.2	Photo of one study site, Finca Irlanda, where flowering coffee bushes line the understory, and shade trees fill the canopy.	73
4.3	Observed species accumulation curve reaching 46 species.	74
4.4	Bees are more abundant and their communities are more species rich in habitats with a greater number of tree species (linear regression for illustration).	75
4.5	Bee community composition across a gradient of (A) tree species and (B) local forest cover.....	76
4.6	Optimal regression trees for (A) solitary bees and (B) social bees.....	77
4.7	Optimal regression trees for each bee tribe, (A) Halictini, (B) Augochlorini, and (C) Ceratinini.	78
4.8	Optimal regression trees for each nesting guild, (A) cavity-nesting bees, (B) wood-nesting bees, and (C) ground-nesting bees... ..	79
6.1	(A , B) Spatial autocorrelation diagrams showing kinship coefficient F_{ij} (solid lines) averaged across all pair wise comparisons within distance categories.	97
7.1	Study region in Chiapas, Mexico, where <i>M. affinis</i> individuals in forest and coffee habitats are labeled as green and brown squares, respectively.	105
7.2	(A) Bee visitation to <i>M. affinis</i> in forest (green) and coffee (brown) habitats and photos (top to bottom) of the native solitary bee <i>Xylocopa tabaniformis</i> ,	

<i>tabaniformis</i> , native social bee <i>Trigona fulviventris</i> , and exotic social bee <i>Apis mellifera scutellata</i>	106
7.3 Pollen dispersal patterns for <i>M. affinis</i> mothers in (A) forest and (B) coffee habitats (photo of <i>M. affinis</i> mother in each habitat) in 100m binned distance categories.	107

ABSTRACT

Movement in the Matrix: pollination and dispersal processes in a tropical coffee and forest landscape mosaic

by

Shalene Jha

Co-Chairs: John H. Vandermeer and Christopher W. Dick

Despite efforts at conservation, tropical forests continue to face destruction, threatening many tropical tree species, especially those that depend on a narrow guild of pollen and seed dispersers. My dissertation investigates essential pollen and seed dispersal processes for *Miconia affinis* (Melastomataceae), a native bee-pollinated tree that primarily inhabits tropical forests, but can be found as a colonist in shade coffee systems. I quantified bee diversity, foraging behavior, and pollination success of *M. affinis* in different coffee plantations and determined that honeybee visitation was significantly higher and ambient fruit set was significantly lower for *M. affinis* in coffee habitats compared to forest habitats. I found that native bees and exotic bees exhibited contrasting foraging patterns in response to coffee management style and foraging scale, and that native bees were more abundant and diverse in coffee farms with greater overstory tree diversity. Using eight microsatellite loci, I conducted molecular analyses that revealed recent colonization of coffee fields by *M. affinis* from four sources. Levels of allelic richness and heterozygosity in forest and coffee habitats were high. Forest populations showed strong spatial genetic structure at the 100 and 200m distance class, while no spatial genetic structure was detected in the coffee habitats. Molecular-based pollen dispersal analyses revealed long-distance pollen movement across the landscape (up to 1800 m) with extensive dispersal between coffee and forest habitats. Overall, these results provide strong evidence that shade coffee farms can serve as permeable habitat matrices for critical native plant pollinators and seed dispersers.

Chapter I: Introduction

The world's tropical forests continue to face habitat destruction and fragmentation, largely due to human-driven land use change (Laurance 1998, Geist and Lambin 2002). The result is a loss in both floral and faunal biodiversity, which can lead to a breakdown in ecological processes within the landscape (Redford 1992, Daily 1997, Bawa et al. 2004, Wright 2005, Ricketts et al. 2008). This loss of faunal biodiversity can severely impact tropical trees, in particular, because most tropical trees require animals to disperse their pollen and seeds (reviewed in Bawa 1990, 1992, Levey et al. 2002, Dick et al. 2008). Reproductively specialized tropical trees, those which are dependent on a narrow guild of pollen and seed dispersers, may be particularly vulnerable to habitat alteration, yet little is known about their ecology (Ashworth et al. 2004, Aguilar et al. 2006, Jones and Muller-Landau 2008). My dissertation examines how forest fragmentation and agricultural land use affect pollinators, seed dispersers, and pollen and seed dispersal processes in a widespread but understudied tropical habitat: the coffee and forest landscape mosaic. Specifically, my research investigates the pollination ecology, population genetics, and gene flow processes for the buzz-pollinated neotropical tree, *Miconia affinis* (Melastomataceae).

My dissertation is divided into six studies (chapters), all of which examine ecological processes within shade coffee farms and forest fragments in Chiapas, Mexico. The second and third chapters explore the foraging patterns of native and exotic bees within coffee agroforestry systems. The fourth chapter examines the impact of local and regional agroforestry management on native bee communities. The fifth chapter briefly describes the development of microsatellite primers for *M. affinis*. The sixth and seventh chapters explore pollen and seed mediated gene flow (respectively) as revealed by microsatellite based molecular analyses. The last chapter is a synthesis of my thesis research and discusses the broader impacts of the work.

Chapter II: Foraging patterns of Africanized honeybees and native bees and wasps in a tropical agroforestry landscape

Recent studies examining the influence of Africanized honeybees on native bee foraging patterns have presented conflicting findings (Robertson et al 1989, Butz-Huryn 1999, Paini 2004). While some studies suggest that Africanized honeybees physically attack native bees, exhibiting ‘interference competition’, others posit that Africanized honeybees only exhibit ‘resource competition’, by foraging on the most productive patches in the community, thus eliminating these high resource sites for use by native bees (Schaffer et al 1979, 1983, Ginsberg 1983).

Though both ecological and temporal factors can influence bee foraging patterns (e.g., Kunin 1997, Steffan-Dewenter et al. 2002, Veddeler et al. 2006), no studies have yet examined whether competitive foraging interactions between native and invasive bees vary with habitat composition. In this study we examined how environmental and temporal factors affect the foraging patterns of Africanized honeybees, native bees, and native wasps. We measured the visitation of native and exotic bees and wasps at nectar feeders within coffee systems, in order to determine the role of vegetation management, light and floral resource levels, and recruiting ability in predicting the abundance of each foraging group.

Chapter III: Contrasting bee foraging on coffee in response to resource scale and local habitat management

Pollination is a potentially vulnerable ecological interaction that merits close examination in coffee agroforestry systems, where pollination can significantly improve coffee bush yield (Klein et al. 2003b, Ricketts et al. 2004). Furthermore, the foraging patterns of native and exotic bees can critically influence coffee pollination success, since exotic Africanized honeybees may dominate floral resources and may not provide pollinator services comparable to native bees (Aizen and Feinsinger 1994, reviewed in Butz-Huryn 1997). It is hypothesized that two main factors drive the foraging patterns of native and exotic bees: food resource availability (e.g., Totland and Matthews 1998, Westphal et al.

2003, Veddeler et al. 2006) and habitat composition (e.g., Steffan-Dewenter et al. 2002, Klein et al. 2003c, Ricketts et al. 2008).

While coffee flower resource availability and agroforestry management can both potentially mediate native and exotic bee foraging patterns, the impacts of these factors have never been simultaneously analyzed. In this study, we measured the amount of flowering coffee available at multiple spatial scales within two distinct agroforestry habitat types (high-shade and low-shade coffee) and recorded visits to coffee flowers, documenting bee species, visit duration and visit frequency. This study is the first to examine whether foraging patterns of native bees and exotic honeybees differ in response to floral resource level and scale, and in response to local agroforestry management.

Chapter IV: Local and regional agroforestry landscapes mediate bee community composition

Given our dependence on animal-pollinated crops, it is increasingly urgent to understand how to best support diverse bee communities within agricultural systems. Approximately 35% of the global food supply relies on pollinator-dependent plants (Klein et al. 2007, Ricketts et al. 2008). Despite this dependence, major pollinator populations continue to decline, mostly as a result of human-mediated habitat destruction (reviewed in Kremen et al. 2002b, Steffan-Dewenter et al. 2005, Ricketts et al. 2008). The conservation of native pollinators requires local and regional land management that conserves nesting and foraging resources for native bees (e.g., Potts et al. 2003, Potts et al. 2005). This research investigates the influence of landscape management on native bee communities across a large coffee growing region in Chiapas, Mexico. We conducted a regional bee survey and utilized GIS imaging and machine-learning regression tree modeling to determine which local and regional habitat factors were most important for predicting native bee abundance and diversity.

Chapter V: Isolation and characterization of nine microsatellite loci for the tropical understory tree *Miconia affinis* Wurdack (Melastomataceae)

The tree *M. affinis* is found in both primary and secondary forests, ranging from southern Mexico to southern Venezuela. Understanding the population genetics of this species is

of great interest, given it is a buzz-pollinated understory tree, and thus represents both an understudied pollination syndrome and understudied plant habit. Additionally, *M. affinis* inhabits much of the world's coffee growing regions, and is often found as a colonist within coffee farms. Therefore, by examining *M. affinis* population genetics we can evaluate the effect of the coffee habitat on seed-mediated and pollinator-mediated gene flow. We isolated nine microsatellite loci from *M. affinis* and optimized them for further research on gene flow and genetic structure.

Chapter VI: Shade coffee farms promote the genetic diversity of native trees

Seed dispersal is a critical process for the regeneration of fragmented landscapes (reviewed in Levey et al. 2002, and Jones and Muller-Landau 2008) and may be limited in altered habitats if important seed dispersers are lost (Asquith et al. 1999, Wang et al. 2007, reviewed in Jones and Muller-Landau 2008). Recent molecular studies examining seed dispersal for tropical trees have revealed that seed dispersal into the pasture matrix can be limited, leading to low levels of offspring allelic diversity, and highly clumped relatedness within colonized landscapes (Aldrich and Hamrick 1998, Aldrich et al. 1998, Sezen et al. 2005, 2007). However, these studies examine a highly simplified pastoral landscape and a relatively narrow sample of tropical tree diversity. Gene flow patterns documented in pastoral landscapes are not likely representative of more complex agricultural systems, like cacao and coffee, which dominate global biodiversity hotspots (Myers et al. 2000). In this study, we used genetic markers to analyze the seed dispersal history and spatial genetic structure of *M. affinis* across coffee and forest habitats.

Chapter VII: Extensive plant gene flow mediated by native bees across a shade coffee landscape mosaic

Habitat alteration can have negative effects on plant reproduction, especially for tropical woody plants, since most tropical tree species are self-incompatible, animal pollinated, and exhibit varying degrees of floral reproductive specialization (reviewed in Bawa 1990, Bawa 1992, Ashworth et al. 2004, Aguilar et al. 2006). An estimated 8% of plant species exhibit 'buzz-pollination', a reproductively specialized syndrome where flowers have poricidal tubular anthers that limit pollen extraction to a select group of native bees

capable of vibrating the anthers in order to release the pollen (Buchmann 1983). Because Africanized and European honeybees are incapable of buzz-pollinating, plant species requiring this service may exhibit decreased reproductive success and limited pollen dispersal when visited by honeybees.

Though molecular studies have found that pollen dispersal between forest trees and isolated pasture trees may be extensive (Chase et al. 1996, Dick 2001, reviewed in Lowe et al. 2005, Dick et al. 2008), these previous studies have focused only on reproductively generalized trees, where exotic honeybees may compensate for reduced reproduction and limited pollen flow otherwise experienced in disturbed habitats (e.g., Dick 2001, Dick et al. 2003). Reproductively specialized trees, on the other hand, may experience limited reproduction and pollen-mediated gene flow if native pollinators are absent in the agricultural matrix. We examined the pollination ecology and pollen dispersal patterns of *M. affinis* by conducting a detailed breeding system study. We also examined pollinator visitation and ambient fruit set in both coffee and forest habitats. Finally, we used microsatellite markers and maternal seedling arrays to examine *M. affinis* pollen dispersal patterns across coffee and forest habitats.

Chapter I References

- Aguilar, R., L. Ashworth, L. Galetto, and M. A. Aizen. 2006. Plant reproductive susceptibility to habitat fragmentation: review and synthesis through a meta-analysis. *Ecology Letters* **9**:968-980.
- Aizen, M. A. and P. Feinsinger. 1994. Habitat fragmentation, native insect pollinators, and feral honeybees in Argentine chaco serrano. *Ecological Applications* **4**:378-392.
- Aldrich, P. R. and J. L. Hamrick. 1998. Reproductive dominance of pasture trees in a fragmented tropical forest mosaic. *Science* **281**:103-105.
- Aldrich, P. R., J. L. Hamrick, P. Chavarriga, and G. Kochert. 1998. Microsatellite analysis of demographic genetic structure in fragmented populations of the tropical tree *Symphonia globulifera*. *Molecular Ecology* **7**:933-944.
- Ashworth, L., R. Aguilar, L. Galetto, and M. A. Aizen. 2004. Why do pollination generalist and specialist plant species show similar reproductive susceptibility to habitat fragmentation? *Journal of Ecology* **92**:717-719.
- Asquith, N. M., J. Terborgh, A. E. Arnold, and C. M. Riveros. 1999. The fruits the agouti ate: *Hymenaea courbaril* seed fate when its disperser is absent. *Journal of Tropical Ecology* **15**:229-235.
- Bawa, K. S. 1990. *Reproductive Ecology of Tropical Forest Plants*. Unesco and Parthenon Press, Paris and USA.
- Bawa, K. S. 1992. Mating Systems, Genetic Differentiation and Speciation in Tropical Rain-Forest Plants. *Biotropica* **24**:250-255.
- Bawa, K. S., W. J. Kress, N. M. Nadkarni, S. Lele, P. H. Raven, D. H. Janzen, A. E. Lugo, P. S. Ashton, and T. E. Lovejoy. 2004. Tropical ecosystems into the 21st century. *Science* **306**:227-228.
- Butz-Huryn, V. 1997. Ecological impacts of introduced honey bees. *Quarterly Review of Biology* **72**:275-297
- Chase, M. R., C. Moller, R. Kesseli, and K. S. Bawa. 1996. Distant gene flow in tropical trees. *Nature* **383**:398-399.
- Daily, G. C. 1997. *Nature's services: Societal dependence on natural ecosystems*. Island Press, Washington, D.C., USA.
- Dick, C. W. 2001. Genetic rescue of remnant tropical trees by an alien pollinator. *Proceedings of the Royal Society of London Series B-Biological Sciences* **268**:2391-2396.
- Dick, C. W., G. Etchelecu, and F. Austerlitz. 2003. Pollen dispersal of tropical trees (*Dinizia excelsa*: Fabaceae) by native insects and African honeybees in pristine and fragmented Amazonian rainforest. *Molecular Ecology* **12**:753-764.
- Dick, C. W., F. A. Jones, O. J. Hardy, and R. J. Petit. 2008. Spatial scales of seed and pollen-mediated gene flow in tropical forest trees. *Tropical Plant Biology* **1**:20-33.
- Donald, P. F. 2004. Biodiversity impacts of some agricultural commodity production systems. *Conservation Biology* **18**:17-37.
- Geist, H. J. and E. F. Lambin. 2002. Proximate causes and underlying driving forces of tropical deforestation. *Bioscience* **52**:143-150.

- Greenberg, R., P. Bichier, and J. Sterling. 1997. Bird populations in rustic and planted shade coffee plantations of eastern Chiapas, Mexico. *Biotropica* **29**:501-514.
- Hanski, I. 1999. *Metapopulation ecology*. Oxford University Press, New York, NY.
- Jones, F. A. and H. C. Muller-Landau. 2008. Measuring long-distance seed dispersal in complex natural environments: an evaluation and integration of classical and genetic methods. *Journal of Ecology* **96**:642-652.
- Klein, A. M., I. Steffan-Dewenter, and T. Tschardt. 2003a. Fruit set of highland coffee increases with the diversity of pollinating bees. *Proceedings of the Royal Society of London Series B-Biological Sciences* **270**:955-961.
- Klein, A. M., I. Steffan-Dewenter, and T. Tschardt. 2003b. Pollination of *Coffea canephora* in relation to local and regional agroforestry management. *Journal of Applied Ecology* **40**:837-845.
- Klein, A. M., B. E. Vaissiere, J. H. Cane, I. Steffan-Dewenter, S. A. Cunningham, C. Kremen, and T. Tschardt. 2007. Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B-Biological Sciences* **274**:303-313.
- Kremen, C., N. M. Williams, and R. W. Thorp. 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.
- Kunin, W. E. 1997. Population size and density effects in pollination: Pollinator foraging and plant reproductive success in experimental arrays of *Brassica kaber*. *Journal of Ecology* **85**:225-234.
- Laurance, W. F. 1998. A crisis in the making: responses of Amazonian forests to land use and climate change. *Trends in Ecology & Evolution* **13**:411-415.
- Levey, D. J., W. R. Silva, and M. Galetti. 2002. *Seed Dispersal and Frugivory: Ecology, Evolution and Conservation* CAB International, Wallingford, UK.
- Lowe, A. J., D. Boshier, M. Ward, C. F. E. Bacles, and C. Navarro. 2005. Genetic resource impacts of habitat loss and degradation; reconciling empirical evidence and predicted theory for neotropical trees. *Heredity* **95**:255-273.
- Myers, N., R. A. Mittermeier, C. G. Mittermeier, G. A. B. da Fonseca, and J. Kent. 2000. Biodiversity hotspots for conservation priorities. *Nature* **403**:853-858.
- Nee, S. and R. M. May. 1992. Dynamics of metapopulations- habitat destruction and competitive coexistence. *Journal of Animal Ecology* **61**:37-40.
- Perfecto, I., R. A. Rice, R. Greenberg, and M. E. VanderVoort. 1996. Shade coffee: A disappearing refuge for biodiversity. *Bioscience* **46**:598-608.
- Perfecto, I. and J. Vandermeer. 2008. Biodiversity conservation in tropical agroecosystems - A new conservation paradigm. Pages 173-200 *Year in Ecology and Conservation Biology 2008*. Blackwell Publishing, Oxford.
- Potts, S. G., B. Vulliamy, A. Dafni, G. Ne'eman, and P. Willmer. 2003. Linking bees and flowers: How do floral communities structure pollinator communities? *Ecology* **84**:2628-2642.
- Potts, S. G., B. Vulliamy, S. Roberts, C. O'Toole, A. Dafni, G. Ne'Eman, and P. Willmer. 2005. Role of nesting resources in organising diverse bee communities in a Mediterranean landscape. *Ecological Entomology* **30**:78-85.
- Redford, K. H. 1992. *The Empty Forest*. *Bioscience* **42**:412-422.

- Ricketts, T. H., G. C. Daily, P. R. Ehrlich, and C. D. Michener. 2004. Economic value of tropical forest to coffee production. *Proceedings of the National Academy of Sciences of the United States of America* **101**:12579-12582.
- Ricketts, T. H., J. Regetz, I. Steffan-Dewenter, S. A. Cunningham, C. Kremen, A. Bogdanski, B. Gemmill-Herren, S. S. Greenleaf, A. M. Klein, M. M. Mayfield, L. A. Morandin, A. Ochieng, and B. F. Viana. 2008. Landscape effects on crop pollination services: are there general patterns? *Ecology Letters* **11**:499-515.
- Sezen, U. U., R. L. Chazdon, and K. E. Holsinger. 2005. Genetic consequences of tropical second-growth forest regeneration. *Science* **307**:891-891.
- Sezen, U. U., R. L. Chazdon, and K. E. Holsinger. 2007. Multigenerational genetic analysis of tropical secondary regeneration in a canopy palm. *Ecology* **88**:3065-3075.
- Steffan-Dewenter, I., U. Munzenberg, C. Burger, C. Thies, and T. Tscharntke. 2002. Scale-dependent effects of landscape context on three pollinator guilds. *Ecology* **83**:1421-1432.
- Steffan-Dewenter, I., S. G. Potts, and L. Packer. 2005. Pollinator diversity and crop pollination services are at risk. *Trends in Ecology & Evolution* **20**:651-652.
- Totland, O. and I. Matthews. 1998. Determinants of pollinator activity and flower preference in the early spring blooming *Crocus vernus*. *Acta Oecologica-International Journal of Ecology* **19**:155-165.
- Veddeler, D., A. M. Klein, and T. Tscharntke. 2006. Contrasting responses of bee communities to coffee flowering at different spatial scales. *Oikos* **112**:594-601.
- Wang, B. C., V. L. Sork, M. T. Leong, and T. B. Smith. 2007. Hunting of mammals reduces seed removal and dispersal of the afrotropical tree *Antrocaryon klaineanum* (Anacardiaceae). *Biotropica* **39**:340-347.
- Westphal, C., I. Steffan-Dewenter, and T. Tscharntke. 2003. Mass flowering crops enhance pollinator densities at a landscape scale. *Ecology Letters* **6**:961-965.
- Wright, S. J. 2005. Tropical forests in a changing environment. *Trends in Ecology & Evolution* **20**:553-560.

Chapter II: Foraging patterns of Africanized honeybees and native bees and wasps in a tropical agroforestry landscape

ABSTRACT

Ecological and temporal factors can influence animal foraging patterns and may obscure our understanding of how native and exotic species interact. To understand how such factors affect foraging, the visitation of native and exotic bees and wasps was observed at nectar feeders within Mexican agroforestry systems, while documenting (1) vegetation management (low-shade vs. high-shade coffee), (2) light and floral resource levels and (3) recruiting ability, as measured by the change in visitation between two consecutive experimental days. On day one, Africanized honeybee visitation was significantly greater in low-shade habitats, and native solitary bee abundance was significantly greater in high-shade habitats, while native social bee and solitary wasp visitation were not significantly different between habitat types. After 24 h, Africanized honeybee visitation increased significantly in both habitat types, while native social bee visitation increased significantly only in high-shade coffee. In contrast, native solitary bee and native solitary wasp visitation decreased in both habitat types. Overall, this study reveals that Africanized honeybees exhibit only initial foraging preference for low-shade habitats, while native bees exhibit both initial and delayed recruitment-based foraging preferences for high-shade habitats.

INTRODUCTION

A large number of studies have revealed that exotic species can negatively impact native species via interference or resource-driven competitive interactions (reviewed in Vitousek et al. 1996, Wilcove et al. 1998, Stein et al. 2000, Clavero and Garcia-Berthou 2005). However, if the native species are inherently sensitive to environmental factors, such as food resource levels, temperature and seasonality, as seen in many bees (Ackerman 1983, Wolda and Roubik 1986, Stone 1994, Hingston 1998), the impact of an introduced species upon native species may be difficult to characterize (Tepedino and Stanton 1981). One exotic species widely debated as having a negative impact on native bees and wasps is the Africanized honeybee, a hybrid between the African honeybee, *Apis mellifera scutellata* (Lepeletier), and the European honeybee *Apis mellifera mellifera*. The success of the Africanized honeybee is largely attributed to its large colony size, long-range foraging and aggressive behaviour (Kerr 1967, Roubik 1978, 1980, Kerr et al. 1982, Schneider et al. 2004). While most studies agree that aggressive physical interactions (i.e. interference competition) do not frequently take place between Africanized honeybees and native insects during foraging (Schaffer et al. 1983, Thorp 1987, Butz-Huryn 1997), much debate remains about whether Africanized honeybees negatively affect native insects via resource-driven competitive interactions (i.e. exploitation competition)(Schaffer et al. 1983, Thorp 1987, reviewed in Butz-Huryn 1997, Roubik and Wolda 2001, reviewed in Goulson 2003, Thomson 2004, 2006).

Ultimately, understanding interactions between Africanized honeybees, native bees and wasps also requires an examination of the ecological factors which mediate their foraging. Bees vary widely in their ecology (Michener 1979, 2000) and nest-site preferences (Potts *et al.* 2005), as do wasps (Evans and West-Eberhard 1970, Ross and Matthews 1991, Richter 2000); thus their foraging patterns are dependent on important ecological and behavioural characteristics, including colony size and recruitment strategy. Africanized honeybees are extremely effective at recruiting colony mates to forage at a high-quality resource sites (Schaffer et al. 1979, Roubik 1991). Though social bees use recruitment strategies, they may avoid foraging at sites that are occupied by another species (i.e. meliponine bees, Johnson and Hubbell 1974, Nagamitsu and Inoue

1997), as may wasp species (Richter 1990, Richter and Tisch 1999). Native bees and wasps that cannot tolerate intense recruitment by other species, like Africanized honeybees, may not be able to forage extensively at sites of high quality resources.

Local land management directly affects floral and nest-site availability, and a number of studies have documented decreases in native bee abundance and increases in Africanized honeybees abundance in less-forested habitats (Aizen and Feinsinger 1994, Cairns et al. 2005, Brosi et al. 2007). However, few studies have attempted to quantify how local habitat composition influences bee foraging patterns. Shade coffee agroforestry systems provide an ideal system in which to examine the impact of local habitat on native and exotic bee foraging, since coffee flowers are predominantly pollinated by both native and exotic bees (Roubik 2002, Klein et al. 2003a, Ricketts 2004, 2008). Additionally, shade coffee agroforestry systems are widespread in the tropics, vary widely in their vegetation management styles, and have gained recent attention for their potential role as biodiversity refuges (Perfecto et al. 1996, Moguel and Toledo 1999, Donald 2004). In southern Mexico, a number of coffee agroforestry regions are uniquely suited for the study of Africanized honeybee and native insect foraging. In these regions, farmers trap and manage feral Africanized honeybees and therefore maintain a controlled number of Africanized honeybee colonies within a region. In this study, we investigate the influence of a number of environmental factors on the foraging patterns of Africanized honeybees and native bees and wasps. Specifically, we examine the hypothesis that differences in vegetation management, floral resource levels and species recruiting ability, will contribute to contrasting habitat preferences and foraging patterns for native bees and wasps and exotic honeybees.

METHODS

Study site

The study was conducted between 4 May and 22 June 2006, in the highlands of the Soconusco, in the state of Chiapas, Mexico (92°18'55'' - 92°20'24''W, 15°09'38'' - 15°10'48''N) at an altitude ranging between 1125 and 1300m. The study site is

composed of a low-shade coffee region, which has an average of 16.1 trees ha⁻¹, resulting in 20%-30% canopy cover, and a high-shade coffee region, which has an average of 42.3 trees ha⁻¹, resulting in 55%-65% canopy cover, and a small uncut forest reserve, *La Montañita*, located between the two regions (Figure 2.1). The two coffee regions investigated are extremely large; each is greater than 300 ha, and each equal in size to a dozen or more coffee farms typical of the Soconusco area. Also located between the two coffee regions is an Africanized honeybee apiary, which was constructed in November 2004. The apiary contains an average of 70-100 colonies and had 83 colonies at the time of the study. All nests in the apiary were obtained by trapping for feral Africanized honeybees within the two neighboring coffee regions consistently for 3 y before this study. Throughout the 2006 experimental season, nests found within the two coffee regions were continuously trapped and added to the apiary. Few feral nests (2) were found in the coffee fields after the intensive collection between 2002 and 2005.

Bee visitation at nectar feeders

Bee foraging rates were measured for two consecutive days at high-quality-resource 'nectar feeders'. Each nectar feeder was composed of a small 12-cm-diameter dish with a 6-cm-diameter foam landing platform and an inexhaustible supply of a 1:1 honey:water solution, a standard bee attractant and feeder (Johnson and Hubbell 1974, Roubik 1980, Slaa 2003). Nectar feeders were placed in groups of three, representing a nectar-feeder site. The three feeders within a site were hung 1m off the ground in the outer branches of coffee bushes, forming a line of three dishes separated from one another by 1 m. Nectar feeder sites were selected by randomly choosing a location along a series of concentric circles located around the managed Africanized honeybee apiary at 400 m, 500 m, 600 m and 800 m away from the apiary, in both the low-shade and high-shade coffee habitats (Figure 2.1). Because many of the insects studied are social and employ recruitment strategies which can only be observed after an extended time period, nectar feeders were monitored for two consecutive days during peak bee and wasp foraging hours, between 08h00 and 10h00. These observations were repeated fortnightly for a total of 8 wk (four replicates per habitat). Because individual bees and wasps did not forage at the bait for more than 10-15 s (initial trials involved documentation of visit duration), the number of

bees and wasps visiting each of the three nectar feeders was recorded every minute for a 10-min period. The visitation at the three nectar feeders was then averaged and summed across the 10 min. Field identifications of bees were made with reference to voucher specimens, which were identified and stored at El Colegio de la Frontera Sur, Tapachula, Mexico and the USDA bee laboratory in Logan, Utah, USA. To minimize field identification errors, morphologically similar species were grouped into single morphospecies in the field (e.g. *Ceratina* sp. refers to *C. trimaculata*, *C. ignara* and *C. sp*)

Coffee plants were not blooming during the study season, and very few understory herbs were in bloom over the 8-wk period. The species richness of the understory plants located within a 10-m radius were recorded for each nectar-feeder site, and light readings were taken at the height of each feeder across the sampling dates using a digital light meter to measure incident light at 633 nm (Pocket Light Meter 840010, Technika Inc) and to assess potential effects of local vegetation and light on visitation levels. In order to measure the abundance of plants flowering for each coffee region at the beginning of the experimental season, we randomly placed six 1 x 50-m transects in each habitat, low-shade and high-shade coffee, and recorded the proportion of each transect which contained at least five flowering inflorescences per 10-m² section (proportion with ≥ 0.5 flowering plants m⁻²).

Statistical analyses

To meet conditions of normality, all count data were square root-transformed and all proportions were arcsine-square root-transformed. All statistical analyses were conducted with the software R (R Development Core Team, URL <http://www.r-project.org>, Vienna, Austria). Visiting insects were grouped into ‘foraging group’ based on foraging strategies and origin; these foraging groups include Africanized honeybees, native social bees, native solitary bees and native solitary wasps.

To test the effect of the wet season’s progression on light and flowering levels, we used a standard Analysis of Variance, ANOVA. To examine the influence of all factors on the number of visiting insects for each foraging group, we utilized an Analysis of Covariance, ANCOVA, using a non-sequential test for examining individual coefficients

(also known as a Type III sum of squares test). The covariates include (1) distance from Africanized honeybee apiary, (2) local light conditions, (3) species richness of local plants in flower and (4) the week of the experiment. The fixed factors include (5) habitat (low-shade vs. high-shade coffee), (6) foraging group and (7) day of visitation (day one or day two). In this case, the ANCOVA method is the most suitable because it provides a descriptive comparison between the visitation levels in different habitats, on the different days and between foraging groups, so it can test hypotheses concerning the significant differences between these categorical variables. Also, the ANCOVA, unlike the Analysis of Variance (ANOVA), disentangles differences in the effects of the fixed factors from the covariates which change in value and unlike the analyses of deviance, the ANCOVA is based on exact F and t distributions for testing the significance of factors and covariates, also yielding measures of R^2 that can be interpreted as the percentage of variance explained (Faraway 2005). The model error was independent and normally distributed.

RESULTS

Flowering plants and light levels

During the study, less than 2% of the understorey plant species were in flower, therefore few flowering species were recorded and most species counts represent single plants with few flowering heads. In both systems we found, *Borreria laevis* Lamk. (Rubiaceae), *Desmodium* sp. (Fabaceae), *Cyperus ferax* Rich. (Cyperaceae), *Impatiens walleriana* Hook (Balsaminaceae), *Solanum nigrum* L. (Solanaceae) and *Lantana camara* L. (Verbenaceae) in very low abundances. The flowering plant *Ipomoea* sp. (Convolvulaceae) was only found in the high-shade coffee system, while *Commelina diffusa* Burm. (Commelinaceae) was only found in the low-shade coffee system, though both were rare. The proportion of transects with flowering plants was not significantly different between low-shade and high-shade coffee habitats (paired t-test, $t_{10} = 10.8$, $P = 0.581$).

Across the eight experimental weeks, there was no significant difference between the average number of plant species flowering in the low-shade coffee habitats (1.5 species per site) and the high-shade coffee habitats (1.9 species per site) (t-test, $t_{30} = 0.53$, $P = 0.087$). However, the average number of flowering plant species remained relatively constant over the eight experimental weeks in the low-shade coffee habitats (ANOVA, $F_{1,30} = 0.076$, $P = 0.78$), while the number increased between week 1 and week 3 and then remained very consistent in shade coffee habitats (ANOVA, $F_{1,30} = 14.2$, $P = 0.002$, Figure 2.2a). Light levels were low in both habitats because readings were taken in the morning at the time of visitation and week 5 observations were lower than expected due to unusual weather conditions; however, across the eight experimental weeks, light levels were significantly lower in the high-shade coffee habitats, with an average of 24.6 W m^{-2} , than in the low-shade coffee habitats, which had an average 46.6 W m^{-2} (t-test, $t_{30} = 1.67$, $P < 0.0001$). Increases or decreases in light level across the experimental season were not significant for either the high-shade coffee habitats (ANOVA, $F_{1,30} = 1.76$, $P = 0.187$) or the low-shade coffee habitats (ANOVA, $F_{1,30} = 2.36$, $P = 0.127$, Figure 2.2b) and probably reflected changes in light associated with the onset of the rainy season.

Environmental and temporal effects on visitation

Africanized honeybees, native social and solitary bees and native solitary wasps were observed visiting the nectar feeders. No aggressive physical interactions were observed between individuals at the nectar feeders. Native solitary bees mostly comprised bees within the tribe Ceratinini (Xylocopinae), predominantly *Ceratina trimaculata* Friese and *Ceratina ignara* Cresson. Social bees comprised of bees with the subfamily Meliponinae (Apidae), predominantly *Trigona fulviventris* Guerin, *Trigona schulthessi* Friese and *Plebeia frontalis* Friese. Native solitary wasps visiting the nectar feeders were within the family Vespidae.

Africanized honeybees

On the first day of the experiment, across all sites, Africanized honeybee visitation was significantly higher in the low-shade coffee habitats than in the high-shade coffee habitats (Figure 2.3a). From the first day to the second day of the experiment, Africanized

honeybee visitation increased significantly in both coffee habitats (Figure 2.4a); however, on the second day, no significant difference in visitation was evident between coffee habitats, indicating that greater second day recruitment took place in the high-shade coffee system (Figure 2.3b).

Native social bees

On the first day of the experiment across all sites, native social bee visitation was not significantly different between high-shade and low-shade coffee habitats (Figure 2.3c). From the first day to the second day of the experiment, native social bee visitation increased significantly only in the high-shade coffee habitats (Figure 2.4b) and on the second day, visitation was also significantly higher in the high-shade coffee habitats than in the low-shade coffee habitats (Figure 2.3d).

Native solitary bees

On the first experimental day across all sites, native solitary bee visitation was significantly higher in the high-shade coffee habitats than the low-shade coffee habitats (Figure 2.3e); however, on the second day of the experiment, in the high-shade coffee habitats, native solitary bee visitation was significantly lower than the previous day (Figure 2.4c). On the second day of the experiment, there was also no significant difference in native solitary bee visitation between coffee habitats (Figure 2.3f).

Native solitary wasps

On the first day of the experiment, native solitary wasp visitation was not significantly different between the low-shade and high-shade coffee habitats across all sites (Figure 2.3g). Visitation did not change significantly from the first day to the second day in either coffee habitats (Figure 2.4d) and on the second day, native solitary wasp visitation was not significantly different between coffee habitats (Figure 2.3h).

An ANCOVA revealed that foraging group type, distance from the apiary, the day of the experiment and the week of the experiment were significant variables, as were the interaction between these variables, and the adjusted $R^2 = 0.799$ (Table 2.1a). According

to the ANCOVA, distance from the apiary significantly influenced visitation rates but the coefficient for this variable was very small (<-0.005), rendering it biologically insignificant. Backward elimination of factors within the model confirmed that light levels, flowering plants and distance from the apiary were not important variables for predicting visitation rates. The adjusted $R^2 = 0.796$, indicating that the simplified model captures most of the variance and the most important predictor variables for visitation are habitat type, week of experiment, day of experiment and the foraging group type (Table 2.1b). The final model is described below:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + \delta_k(\text{week}) + \alpha_i \times \beta_j + \alpha_i \times \gamma_k + \beta_j \times \gamma_k + \alpha_i \times \beta_j \times \gamma_k + E_{ijkl}$$

where α is habitat ($i = 1, 2$ for low-shade/ high-shade coffee habitats), γ is visitor group ($k = 1, 2, 3, 4$ for Africanized honeybees, native social bees, native solitary bees and native solitary wasps, respectively), β is the day of visitation ($j = 1, 2$ for day one or two, respectively) and δ_k is the species group specific coefficient for the effect of sampling at different times. In the model, ‘week’ represents the order of the experimental week, so that the value in the model for experimental week number is 1, 3, 5 and 7. For all groups of visitors, an increase in visitation at the feeders was observed across the experimental season (Table 2.1, Figure 2.4). Native social bees exhibited the greatest increase across the experimental season, followed by Africanized honeybees, native solitary wasps and native solitary bees (Figure 2.4).

DISCUSSION

The tropics are full of naturally occurring resource bursts, such as the sporadic mass flowering of tropical trees and the ephemeral coffee bloom. These large floral resource bursts can have major implications on the foraging behaviour of social bees, which may have evolved recruiting strategies specifically for the exploitation of mass-flowering patches (Dornhaus and Chittka 1999, 2004, Raine et al. 2006). In this study, we used nectar feeders to examine foraging levels at a simulated resource burst, and we found

marked differences in foraging levels for visiting species, across time and between habitats types.

Visitation: day 1

There was significantly higher Africanized honeybee visitation in low-shade coffee habitats than high-shade coffee habitats on the first day of the experiment. This finding is in accordance with previous studies which have documented greater abundances of foraging Africanized honeybees in less-forested landscapes (Aizen and Feinsinger 1994, Rincon et al. 1999, Steffan-Dewenter et al. 2002, Cairns et al. 2005, Brosi et al. 2007). Unlike Africanized honeybees, both native social and solitary bee visitation was high in high-shade coffee habitats on the first day of the experiment. In our study, most of the native solitary bees observed were in the Ceratinini, a wood-nesting tribe, thus solitary bee visitation to feeders is likely linked with wood-based nest-site availability, which is more abundant in the high-shade coffee system. Likewise, visitation of native social bees in high-shade coffee habitats is most likely due to greater nest-site availability, as many of tropical social bees (especially within the Meliponinae) nest in old trees (Michener 2000, Slaa 2003). Similar patterns of increased visitation with increased proximity to forested and semi-natural habitats have been documented in coffee agroforestry systems for native solitary and social bees in Costa Rica (Ricketts 2004), for native social bees in Indonesia (Klein et al. 2003c, 2006). This relationship also exists for social bees in less-forested agricultural ecosystems in Germany (Steffan-Dewenter *et al.* 2002) and the western United States (Kremen et al. 2002b, 2004, Kim et al. 2006, reviewed in Klein et al. 2007, 2007).

Native solitary vespid wasp visitation was not significantly influenced by habitat type. Previous studies within coffee regions have found that solitary vespid wasps in the family Eumenidae are in fact more often found nesting in the less-forested coffee plantations (Klein *et al.* 2004, 2006). These studies have suggested that wasp prey (i.e. caterpillars) is more abundant in less-forested coffee, and wasp nesting density responds strongly to prey availability. In Mexican coffee agroforestry systems, it is possible that wasp prey choice is unaffected by differing coffee management styles, but further study is necessary to examine this hypothesis.

Visitation: day 2

After 24 h had passed, Africanized honeybees and native social bees exhibited significantly higher visitation levels at the nectar feeders than the previous day. This is a reflection of the highly developed recruitment strategies utilized by honeybees (Vonfrisch et al. 1967, Roubik 1980) and native social meliponine bees (Lindauer 1971, Hubbell and Johnson 1978, Slaa 2003). Also, Africanized honeybees and meliponine bees both often support large brood sizes (Spivak et al. 1991, Michener 2000) and thus have greater colony-wide nectar demands. On the second day, Africanized honeybee visitation was equally high in both habitats, indicating that greater second day recruitment took place in the high shade coffee system and that foragers had likely saturated all available sites. In contrast, for native social bees, increased visitation in later foraging bouts occurred only in high-shade habitats. Apparent habitat-mediated recruitment for native social bees may be due to greater ease in recruitment and foraging in high-shade habitats, or to greater nest site proximity. Many social bees, including Africanized honeybees and meliponines, also use scents to mark forage sites (Free and Williams 1983, Nieh 2004), and more densely vegetated coffee habitats may allow for greater scent permanence.

Unlike social bees, visitation by solitary bees and wasps decreased on the second day of the experiment. Decreases in the number of foraging solitary bees and wasps are likely the result of resource competition, due to large numbers of foraging social bees. Solitary bees and wasps forage for more diverse resources (food and nest resources) and have smaller brood sizes (Richter and Tisch 1999, Michener 2000), but there their nectar demands cannot be satiated by a single day of foraging. Solitary bee and wasp species have high energetic needs, especially during warm wet-season months, and thus could be benefited by maintained foraging levels (Stone 1994, Willmer and Stone 2004). Intense foraging of Africanized honeybees and native social bees may prevent solitary bees from revisiting high-resource sites, leading to the significant declines in solitary bee visitation after 24 h of resource exposure.

Species richness and density of understorey plants flowering were not significantly different between habitat types and did not influence bee or wasp foraging at

nectar feeders. However, we detected visitation increases for all foraging groups across the experimental period. The consistent increase in bee and wasp visitation is best explained by decreasing regional ambient floral resource availability during the late wet-season months. In the highlands of Mexico, there is a peak in native tree flowering from mid-April to early June, followed by a decline in floral resource availability (Croat 1978, Foster 1982, Bullock and Solis-Magallanes 1990), which leads bees and wasps to forage more extensively at feeders during this time period.

Conclusions

Though we did not observe any physical competitive interactions between Africanized honeybees and native bees and wasps, our results suggest that marked decreases in native solitary bee visitation may be due to high numbers of Africanized honeybees foraging at resource sites. Previous studies have suggested that competitive interactions between bee foraging groups would be more common for similarly recruiting social bees (Schaffer *et al.* 1979), and only social bees have been documented to exhibit foraging shifts in order to avoid competition with the Africanized honeybee (Roubik 1980, 1986). Alternatively, in our study, native social bees exhibited extensive recruitment-based foraging in high-shade habitats, undeterred by the presence of Africanized honeybees. Findings from this study reveal that visitation levels for bees and wasps depend both on habitat composition and the duration of resource availability, and both factors must be considered in the interpretation of native and exotic species foraging.

ACKNOWLEDGEMENTS

This chapter was coauthored with John Vandermeer and published in the *Journal of Tropical Ecology* in 2009. We would like to thank Luis Fernando Chavez, Beverly Rathcke, Ivette Perfecto, Mike Reiskind, Exzequel Velasquez, the Vandermeer lab group, the Rathcke lab group and the Perfecto lab group at the University of Michigan. We would also like to thank Terry Griswold and Olivia Messenger at the USDA Bee Lab in Logan, Utah, for help with bee identifications. Finally, we would like to express our

gratitude to the people of Finca Irlanda and Finca Hamburgo for their permission and assistance in conducting this study. All experiments conducted were in compliance with current laws governing biodiversity protection in Mexico.

TABLES, FIGURES AND LEGENDS

Table 2.1 We utilized an Analysis of Covariance to measure the influence of all covariates and factors on the number of visiting insects for each foraging group. First listed are the model results for visitation rates using an ANCOVA where all relevant covariates have been included. Second listed are the model results where only significant covariates have been included. Covariates include (distance= distance to Africanized bee apiary; Flowers= species richness of blooming understorey plants; Light= light ($W m^{-2}$), Week= sampling week) and all factors (Habitat= high-shade or low-shade coffee, Group = foraging group, Day=1st or 2nd day) and relevant interaction terms.

Source	df	F	P
All covariates			
Distance	1	5.71	0.078
Flowers	1	1.38	0.241
Light	1	0.02	0.886
Week	1	38.9	< 0.001 ***
Habitat	1	6.34	0.012 *
Group	3	220	< 0.001 ***
Day	1	83.3	< 0.001 ***
Habitat × Group	3	17.8	< 0.001 ***
Week × Group	3	14.4	< 0.001 ***
Day × Group	3	51.8	< 0.001 ***
Week × Distance	1	2.71	0.101
Group × Distance	3	4.27	0.005 **
Habitat × Day × Group	3	7.81	< 0.001 ***
Residuals	229		
Significant covariates			
Week	1	36.5	< 0.001 ***
Habitat	1	5.95	0.015 *
Group	3	206	< 0.001 ***
Day	1	78.1	< 0.001 ***
Habitat × Day	1	15.4	< 0.001 ***
Habitat × Group	3	16.6	< 0.001 ***
Week × Group	3	13.5	< 0.001 ***
Day × Group	3	48.5	< 0.001 ***
Habitat × Day × Group	3	7.32	< 0.001 ***
Group			
Residuals	236		

Figure 2.1 Map of the study region with concentric circles indicating feeder locations at 400 m, 500 m, 600 m, and 800 m from the apiary, upon which nectar feeders were randomly placed in each habitat.

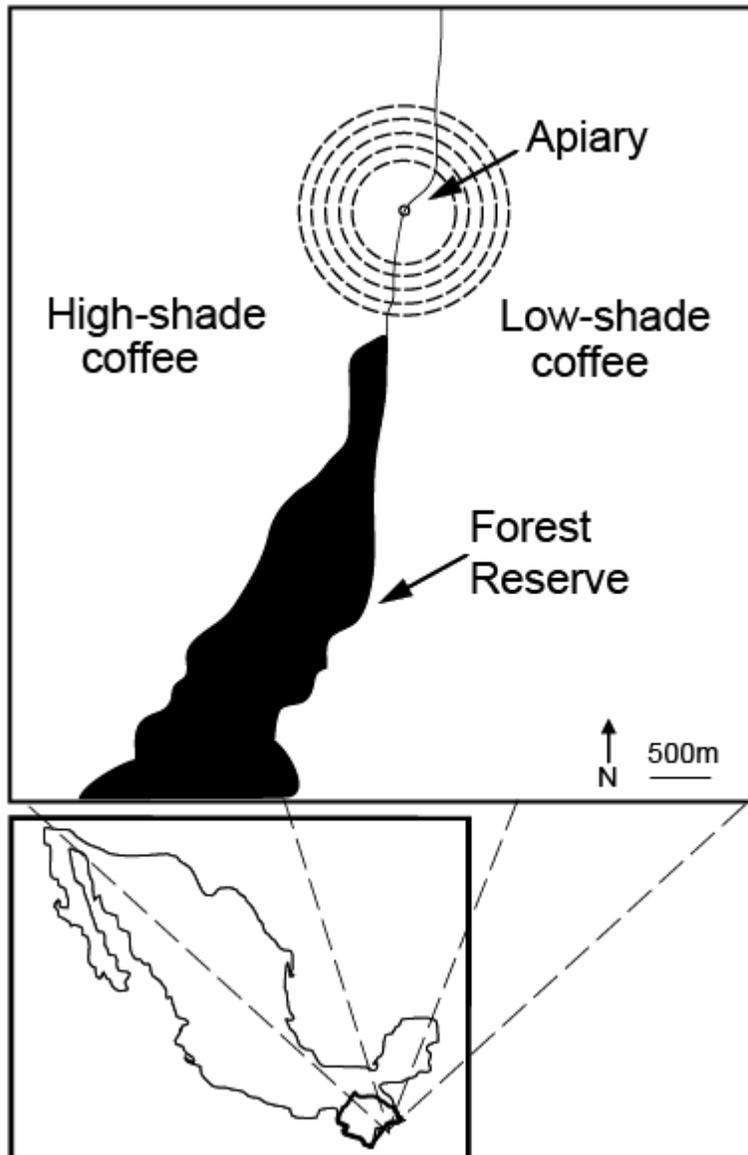


Figure 2.2 Species richness of plants flowering and light levels in each habitat and across the experimental season. Mean numbers of flowering plant species across the experimental season in the low-shade and high-shade coffee habitats (a). Mean light levels ($W m^{-2}$) across the experimental season in the low-shade and the high-shade coffee habitats (b). Error bars represent SE. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

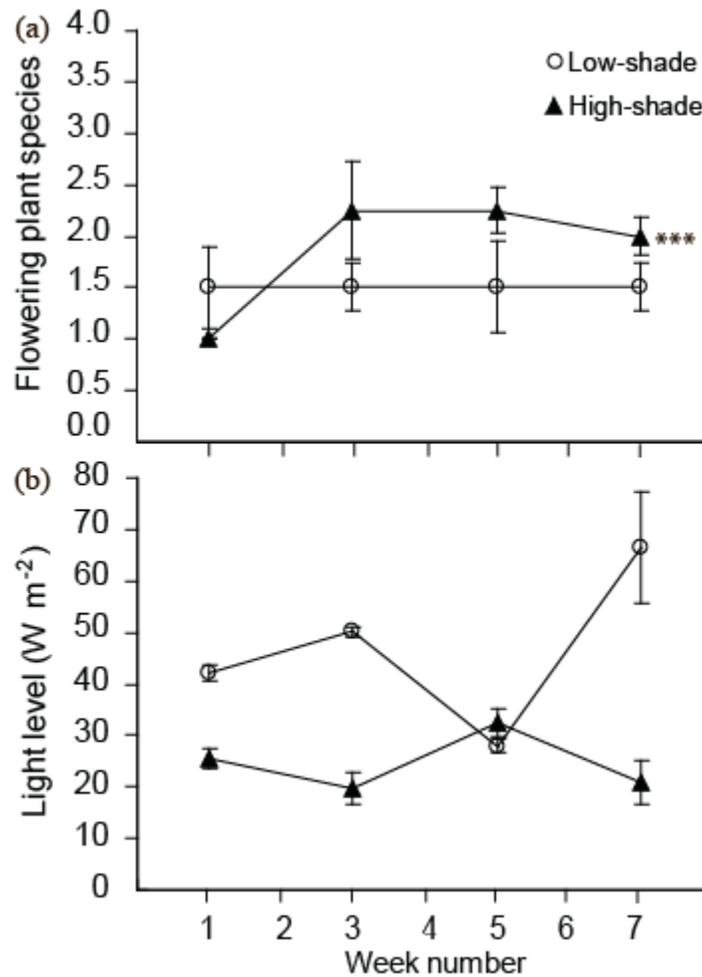


Figure 2.3 Mean number of visits in the two coffee habitats on day one and day two for Africanized honeybees (a-b) native social bees (c-d) native solitary vespid wasps (e-f) and native solitary bees (g-h). Note the scale. Error bars represent SE. Codes for significant differences in between habitats: *P < 0.05, **P < 0.01, ***P < 0.001

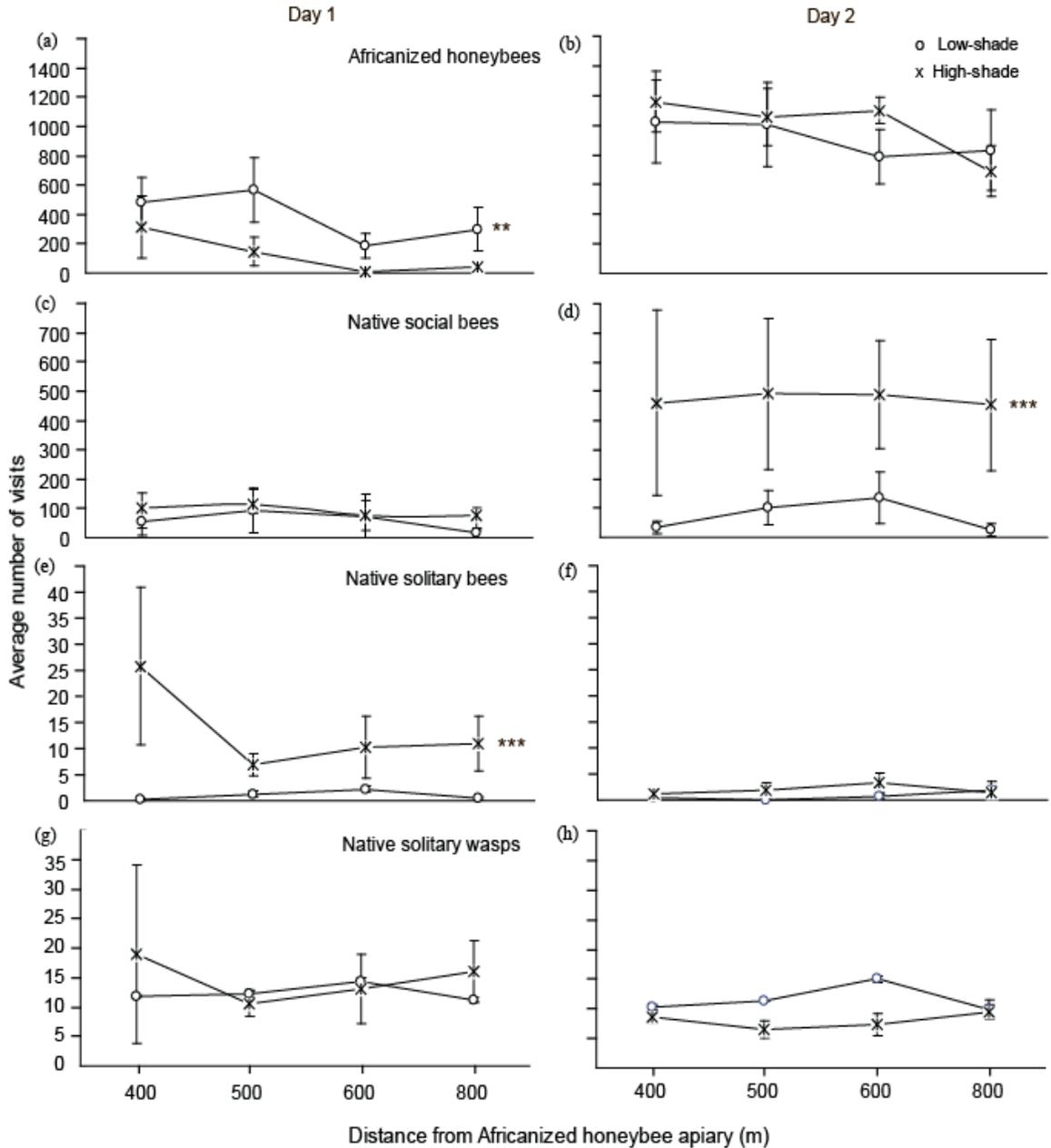
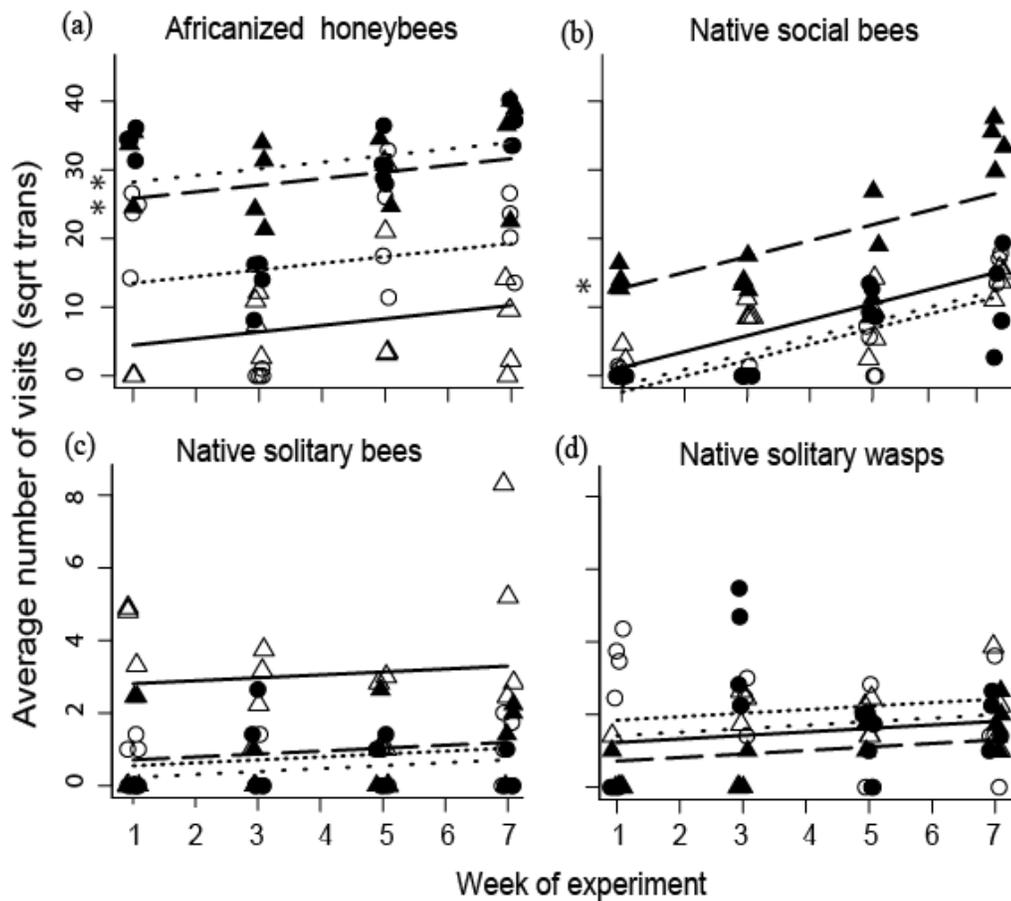


Figure 2.4 Visitation over the 8-wk experimental period for all visitor groups (a-d). Circle symbols are actual values for visitation on day one while triangle symbols are actual values for visitation on day two; unfilled symbols represent visitation in low-shade coffee, while filled symbols represent visitation in high-shade coffee. Lines are model-based regressions of visitation: solid lines represent visitation on the first day in high-shade coffee, widely dashed lines represent visitation on the second day in high-shade coffee, closely dotted lines represent visitation on the first day in low-shade coffee, and widely dotted lines represent visitation on the second day in low-shade coffee. Codes for significant differences from day one to day two: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$



Chapter II 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 P. Feinsinger. 1994. Habitat fragmentation, native insect pollinators, and feral honeybees in Argentine chaco serrano. *Ecological Applications* **4**:378-392.
- Brosi, B. J., G. C. Daily, and P. R. Ehrlich. 2007. Bee community shifts with landscape context in a tropical countryside. *Ecological Applications* **17**:418-430.
- Bullock, S. H. and J. A. Solis-Magallanes. 1990. Phenology of canopy trees of a tropical deciduous forest in Mexico. *Biotropica* **22**:22-35.
- Butz-Huryn, V. 1997. Ecological impacts of introduced honey bees. *Quarterly Review of Biology* **72**:275-297
- Cairns, C. E., R. Villanueva-Gutierrez, S. Koptur, and D. B. Bray. 2005. Bee populations, forest disturbance, and africanization in Mexico. *Biotropica* **37**:686-692.
- Clavero, M. and E. Garcia-Berthou. 2005. Invasive species are a leading cause of animal extinctions. *Trends in Ecology & Evolution* **20**:110.
- Croat, T. C. 1978. The flora of Barro Colorado Island. Stanford University Press, Palo Alto, CA, USA.
- Donald, P. F. 2004. Biodiversity impacts of some agricultural commodity production systems. *Conservation Biology* **18**:17-37.
- Dornhaus, A. and L. Chittka. 1999. Insect behaviour - Evolutionary origins of bee dances. *Nature* **401**:38-38.
- Dornhaus, A. and L. Chittka. 2004. Why do honey bees dance? *Behavioral Ecology and Sociobiology* **55**:395-401.
- Evans, H. E. and M. J. West-Eberhard. 1970. The Wasps. University of Michigan Press, Ann Arbor.
- Faraway, J. 2005. Extending the linear model with R: Generalized linear, mixed effects and nonparametric regression models. Chapman and Hall/CRC, Boca Raton.
- Foster, R. B. 1982. The seasonal rhythm of fruitfall on Barro Colorado Island. Pages 151-172 in E. G. J. Leigh, A. S. Rand, and D. M. Windsor, editors. *Ecology of a tropical forest: seasonal rhythms and long-term changes*. Smithsonian Institution Press, Washington, D.C., USA.
- Free, J. B. and I. H. Williams. 1983. Scent-marking of flowers by honeybees. *Journal of Apicultural Research* **22**:86-90.
- Goulson, D. 2003. Effects of introduced bees on native ecosystems. *Annual Review of Ecology Evolution and Systematics* **34**:1-26.
- 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.
- Hubbell, S. P. and L. K. Johnson. 1978. Comparative Foraging Behavior of 6 Stingless Bee Species Exploiting a Standardized Resource. *Ecology* **59**:1123-1136.
- Johnson, L. K. and S. P. Hubbell. 1974. Aggression and Competition among Stingless Bees - Field Studies. *Ecology* **55**:120-127.

- Kerr, W. E. 1967. The history of the introduction of African bees to Brazil. . South African Bee Journal **39**:3-5.
- Kerr, W. E., S. D. Delrio, and M. D. Barrionuevo. 1982. The Southern Limits of the Distribution of the Africanized Honey Bee in South-America. American Bee Journal **122**:196-198.
- Kim, J., N. Williams, and C. Kremen. 2006. Effects of cultivation and proximity to natural habitat on ground-nesting native bees in California sunflower fields. Journal of the Kansas Entomological Society **79**:309-320.
- Klein, A. M., S. A. Cunningham, M. Bos, and I. Steffan-Dewenter. 2008. Advances in pollination ecology from tropical plantation crops. Ecology **89**:935-943.
- Klein, A. M., I. Steffan-Dewenter, and T. Tscharrntke. 2003a. Bee pollination and fruit set of *Coffea arabica* and *C-canephora* (Rubiaceae). American Journal of Botany **90**:153-157.
- Klein, A. M., I. Steffan-Dewenter, and T. Tscharrntke. 2003b. Pollination of *Coffea canephora* in relation to local and regional agroforestry management. Journal of Applied Ecology **40**:837-845.
- Klein, A. M., I. Steffan-Dewenter, and T. Tscharrntke. 2004. Foraging trip duration and density of megachilid bees, eumenid wasps and pompilid wasps in tropical agroforestry systems. Journal of Animal Ecology **73**:517-525.
- Klein, A. M., I. Steffan-Dewenter, and T. Tscharrntke. 2006. Rain forest promotes trophic interactions and diversity of trap-nesting hymenoptera in adjacent agroforestry. Journal of Animal Ecology **75**:315-323.
- Klein, A. M., B. E. Vaissiere, J. H. Cane, I. Steffan-Dewenter, S. A. Cunningham, C. Kremen, and T. Tscharrntke. 2007. Importance of pollinators in changing landscapes for world crops. Proceedings of the Royal Society B-Biological Sciences **274**:303-313.
- Kremen, C., N. M. Williams, M. A. Aizen, B. Gemmill-Herren, G. LeBuhn, R. Minckley, L. Packer, S. G. Potts, T. Roulston, I. Steffan-Dewenter, D. P. Vazquez, R. Winfree, L. Adams, E. E. Crone, S. S. Greenleaf, T. H. Keitt, A. M. Klein, J. Regetz, and T. H. Ricketts. 2007. Pollination and other ecosystem services produced by mobile organisms: a conceptual framework for the effects of land-use change. Ecology Letters **10**:299-314.
- Kremen, C., N. M. Williams, R. L. Bugg, J. P. Fay, and R. W. Thorp. 2004. The area requirements of an ecosystem service: crop pollination by native bee communities in California. Ecology Letters **7**:1109-1119.
- Kremen, C., N. M. Williams, and R. W. Thorp. 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.
- Lindauer, M. 1971. Communication among social bees. Harvard Univ. Press, Cambridge, MA.
- Michener, C. D. 1979. Biogeography of the Bees. Annals of the Missouri Botanical Garden **66**:277-347.
- Michener, C. D. 2000. Bees of the World. Johns Hopkins University Press, Baltimore, MD.
- Moguel, P. and V. M. Toledo. 1999. Biodiversity conservation in traditional coffee systems of Mexico. Conservation Biology **13**:11-21.

- Nagamitsu, T. and T. Inoue. 1997. Aggressive foraging of social bees as a mechanism of floral resource partitioning in an Asian tropical rainforest. *Oecologia* **110**:432-439.
- Nieh, J. C. 2004. Recruitment communication in stingless bees (Hymenoptera, Apidae, Meliponini). *Apidologie* **35**:159-182.
- Perfecto, I., R. A. Rice, R. Greenberg, and M. E. VanderVoort. 1996. Shade coffee: A disappearing refuge for biodiversity. *Bioscience* **46**:598-608.
- Potts, S. G., B. Vulliamy, S. Roberts, C. O'Toole, A. Dafni, G. Ne'Eman, and P. Willmer. 2005. Role of nesting resources in organising diverse bee communities in a Mediterranean landscape. *Ecological Entomology* **30**:78-85.
- Raine, N. E., T. C. Ings, A. Dornhaus, N. Salefl, and L. Chittka. 2006. Adaptation, genetic drift, pleiotropy, and history in the evolution of bee foraging behavior. Pages 305-354 *Advances in the Study of Behavior*, Vol 36. Elsevier Academic Press Inc, San Diego.
- Richter, M. R. 1990. Hunting Social Wasp Interactions - Influence of Prey Size, Arrival Order, and Wasp Species. *Ecology* **71**:1018-1030.
- Richter, M. R. 2000. Social wasp (Hymenoptera : Vespidae) foraging behavior. *Annual Review of Entomology* **45**:121-150.
- Richter, M. R. and V. L. Tisch. 1999. Resource choice of social wasps: influence of presence, size and species of resident wasps. *Insectes Sociaux* **46**:131-136.
- Ricketts, T. H. 2004. Tropical forest fragments enhance pollinator activity in nearby coffee crops. *Conservation Biology* **18**:1262-1271.
- Rincon, M., D. W. Roubik, B. Finegan, D. Delgado, and N. Zamora. 1999. Understory bees and floral resources in logged and silviculturally treated Costa Rican rainforest plots. *Journal of the Kansas Entomological Society* **72**:379-393.
- Ross, K. and R. Matthews. 1991. *The Social Biology of Wasps*. Cornell University Press, Ithaca.
- Roubik, D. 1986. Sporadic food competition with the African honey bee: projected impact on neotropical social bees. *Journal of Tropical Ecology* **2**:97-111.
- Roubik, D. 1991. Aspects of Africanized honey bee ecology in tropical America. . Page 435 *in* F. D. Spivak M, Breed MD editor. *The "African" Honey Bee*. Westview, Boulder, CO
- Roubik, D. W. 1978. Competitive Interactions between Neotropical Pollinators and Africanized Honey Bees. *Science* **201**:1030-1032.
- Roubik, D. W. 1980. Foraging Behavior of Competing Africanized Honeybees and Stingless Bees. *Ecology* **61**:836-845.
- Roubik, D. W. 2002. Tropical agriculture - The value of bees to the coffee harvest. *Nature* **417**:708-708.
- Roubik, D. W. and H. Wolda. 2001. Do competing honey bees matter? Dynamics and abundance of native bees before and after honey bee invasion. *Population Ecology* **43**:53-62.
- Schaffer, W. M., D. B. Jensen, D. E. Hobbs, J. Gurevitch, J. R. Todd, and M. V. Schaffer. 1979. Competition, Foraging Energetics, and the Cost of Sociality in 3 Species of Bees. *Ecology* **60**:976-987.

- Schaffer, W. M., D. W. Zeh, S. L. Buchmann, S. Kleinhaus, M. V. Schaffer, and J. Antrim. 1983. Competition for Nectar between Introduced Honey Bees and Native North-American Bees and Ants. *Ecology* **64**:564-577.
- Schneider, S. S., G. D. Hoffman, and D. R. Smith. 2004. The African honey bee: Factors contributing to a successful biological invasion. *Annual Review of Entomology* **49**:351-376.
- Slaa, E. J. 2003. Foraging ecology of stingless bees: from individual behaviour to community ecology. Utrecht University, Department of Behavioural Biology., Utrecht, Germany.
- Spivak, M., D. J. C. Fletcher, and M. C. Breed. 1991. The "African" Honey Bee. Westview, Boulder, CO.
- Steffan-Dewenter, I., U. Munzenberg, C. Burger, C. Thies, and T. Tschardtke. 2002. Scale-dependent effects of landscape context on three pollinator guilds. *Ecology* **83**:1421-1432.
- Stein, B., L. Kutner, and J. Adams. 2000. Precious heritage: The status of biodiversity in the United States. Oxford University Press, New York.
- Stone, G. N. 1994. Activity Patterns of Females of the Solitary Bee *Anthophora plumipes* in Relation to Temperature, Nectar Supplies and Body-Size. *Ecological Entomology* **19**:177-189.
- Tepedino, V. J. and N. L. Stanton. 1981. Diversity and Competition in Bee-Plant Communities on Short-Grass Prairie. *Oikos* **36**:35-44.
- Thomson, D. 2004. Competitive interactions between the invasive European honey bee and native bumble bees. *Ecology* **85**:458-470.
- Thomson, D. M. 2006. Detecting the effects of introduced species: a case study of competition between *Apis* and *Bombus*. *Oikos* **114**:407-418.
- Thorp, R. 1987. World overview of the interactions between honeybees and other flora and fauna. Pages 40–47 in J. Blyth, editor. *Beekeeping and Land Management* Department of Conservation and Land Management, Perth.
- Vitousek, P. M., C. M. Dantonio, L. L. Loope, and R. Westbrooks. 1996. Biological invasions as global environmental change. *American Scientist* **84**:468-478.
- Vonfrisch, K., A. M. Wenner, and D. L. Johnson. 1967. Honeybees - Do They Use Direction and Distance Information Provided by Their Dancers. *Science* **158**:1072-&.
- Wilcove, D. S., D. Rothstein, J. Dubow, A. Phillips, and E. Losos. 1998. Quantifying threats to imperiled species in the United States. *Bioscience* **48**:607-615.
- Willmer, P. G. and G. N. Stone. 2004. Behavioral, ecological, and physiological determinants of the activity patterns of bees. *Advances in the Study of Behavior*:347-466.
- Wolda, H. and D. W. Roubik. 1986. Nocturnal Bee Abundance and Seasonal Bee Activity in a Panamanian Forest. *Ecology* **67**:426-433.

Chapter III: Contrasting bee foraging on coffee in response to resource scale and local habitat management

ABSTRACT

It is hypothesized that two main factors drive the foraging patterns of native and exotic species: food resource availability and habitat composition. These factors are particularly relevant for native bees and exotic honeybees, essential crop pollinators that are sensitive to floral resources and habitat management, and that have recently exhibited alarming population declines. Mechanisms driving native and exotic bee foraging patterns may critically depend on floral resource availability and habitat composition, yet the impacts of these factors on bee foraging have never been simultaneously analyzed. In a coffee producing region in southern Mexico, we investigated the influence of coffee floral resource levels and habitat management on native and exotic bee foraging. We measured the amount of flowering coffee available at multiple spatial scales within two distinct agroforestry habitat types (high-shade and low-shade coffee) and recorded visits to coffee flowers, documenting bee species, visit duration and visit frequency. We observed a significantly greater number of visits in high-shade coffee habitats than in low-shade coffee habitats for both native and exotic bees. In high-shade coffee habitats, native solitary bee and native social bee visitation decreased significantly in response to increasing floral resource availability, exhibiting a ‘dilution effect’ at the smallest spatial scale. In contrast, in low-shade coffee habitats, Africanized honeybees exhibited a ‘concentration effect’, increasing visitation significantly in response to increasing floral resource availability at the largest spatial scale. This study is the first to show that foraging patterns of native bees and exotic honeybees contrast in response to floral

resource level and scale and that this response is mediated by the vegetation management of the local habitat.

INTRODUCTION

Foraging animals use a number of environmental cues in order to conserve energy and maximize resource acquisition. Because most habitats have resources that are distributed in a patchy manner, depending on scale, the ability to discern between low and high quality resource sites is an essential skill for many foraging communities (e.g. Schoener 1971, Abrams 1982, Mangel and Clark 1986, Abrams 1991, Fauchald 1999, Fauchald and Tveraa 2006). Communities that are dependent on ephemeral resources may be particularly reliant on their foraging abilities, especially if there is an abundance of foragers and a limited number of resources. One such example is the native and exotic bee community, which is uniquely sensitive to food resource availability due to its dependence on pollen and nectar, the former of which is provisioned to offspring and the latter of which is mostly consumed by bees in order to sustain their high metabolic demands (i.e., Price 1984, Kunin 1993, Michener 2000, Potts et al. 2003).

Bee foraging is of great ecological and economic importance since the subsequent act of pollination provides an essential reproductive service, benefiting cultivated plants and their consumers (Buchman 1996, Daily 1997, Kremen et al. 2007, Klein et al. 2008). Approximately 35% of the global food supply relies on at least partially pollinator-dependent plants (Klein et al. 2007, Ricketts et al. 2008). Most agricultural systems are frequented and serviced by native bees and exotic honeybees; however, recent worldwide declines in honeybee populations have drawn greater attention to the study of wild native bees as crop pollinators (Kremen et al. 2002b, Steffan-Dewenter et al. 2005, Winfree et al. 2007). For many crops, native bees alone contribute substantially to increased fruit yields, highlighting the ecosystem service provided by native bees (Kremen et al. 2004, Klein et al. 2007, Kremen et al. 2007, Winfree et al. 2007).

Bee foraging is especially critical to coffee agroforestry systems. Coffee agroforestry systems are ubiquitous across the tropics (Perfecto et al. 1996, Myers et al.

2000, Donald 2004) and many varieties of coffee are partially self-incompatible and exhibit greater fruit set when visited by diverse native bee communities (Klein et al. 2003b, Ricketts et al. 2004). Foraging bee diversity and coffee pollination are heavily influenced by the local agroforestry landscape (Klein et al. 2003c, Ricketts et al. 2004, Klein et al. 2007, Ricketts et al. 2008), as seen in many other agricultural systems (Kremen et al. 2002b, Kremen et al. 2004, Kremen et al. 2007, Steffan-Dewenter and Westphal 2008), yet it is unknown how local agricultural management influences bee foraging responses. One previous study has revealed that the bee community exhibits complex and contrasting foraging responses to coffee flowering, depending on the spatial scale examined (Veddeler et al. 2006). At the largest spatial scale (field), bee foragers exhibit a ‘dilution effect’, decreasing visitation with increasing floral resource availability; while at the smallest scale (bush), bee foragers exhibit a ‘concentration effect’, increasing visitation with increasing floral resource availability (Veddeler et al. 2006). These results were among the first to provide evidence that bee foraging communities could exhibit contrasting foraging responses depending on spatial scale. However, foraging responses may differ fundamentally between native and exotic species in a community and between habitat types, and understanding these differences may yield insight into the mechanisms driving contrasting foraging patterns.

Since the 1970’s, fragmented neotropical landscapes have been dominated by exotic Africanized honeybees, and many studies have suggested that the success of these exotic bees lies in their recruitment-based foraging strategy and versatile nesting preferences (e.g., Roubik 1980, Spivak et al. 1991, Aizen and Feinsinger 1994, Cairns et al. 2005, Brosi et al. 2008). Native social bees also exhibit advanced foraging strategies which may have evolved specifically for the exploitation of mass-flowering patches (Dornhaus and Chittka 1999, 2004, Raine et al. 2006). However native social bees, native solitary bees and honeybees interpret the landscape at different scales (Steffan-Dewenter et al. 2002) and respond differently to local habitat conditions, such as light cover, floral density and distance to the nearest forest fragment (Klein et al. 2002, Klein et al. 2003a, Potts et al. 2003, Ricketts 2004). Thus, the foraging patterns exhibited by the bee community may depend critically on bee community composition and local habitat composition. In this study, we investigate the foraging responses of native bees

and exotic Africanized honeybees within Mexican coffee agroforestry systems in order to assess the influence of resource availability, resource scale and local habitat composition on bee foraging patterns.

METHODS

Study area

The study was conducted in *Nueva Alemania*, a coffee-growing region located in the southern highlands of Chiapas, Mexico (92°18'55'' to 92°20'24''W, 15°09'38'' to 15°10'48''N) at an altitude ranging between 1125 and 1300m. In the study region, *Coffea Arabica* and *Coffea robusta*, are planted in the understory (approximately 4,000 coffee bushes per hectare) under a canopy of overstorey trees that vary in density and diversity, based on the vegetation management style (Perfecto et al. 1996, Moguel and Toledo 1999, Soto-Pinto et al. 2001). The study site is composed of a low-shade coffee region, with a mean of 16.1 trees ha⁻¹ (12.1 species ha⁻¹), resulting in 20%-30% canopy cover, and a high-shade coffee region, with a mean of 42.3 trees ha⁻¹ (18.4 species ha⁻¹), resulting in 55%-65% canopy cover. Located between the two regions is a small (approximately 20 ha) uncut forest reserve, *La Montañita*. The two coffee regions investigated are extremely large; each is greater than 300 ha, and each equal in size to a dozen or more coffee farms typical of the Soconusco area. Also located between the two coffee regions is a managed Africanized honeybee apiary, which was constructed in November of 2004. The apiary contains an average of 70-100 colonies and had 83 domesticated colonies at the time of the study. All nests in the apiary were obtained by trapping for feral Africanized honeybees across the study region for 3 years before the study. Throughout the 2006 experimental season, nests found within the study site were continuously trapped and added to the apiary. Few feral nests (2) were found in the coffee fields after the intensive collection between 2002 and 2005.

Coffee plants were observed across coffee agroforestry habitat types between April 20th and April 23rd of 2006, during the peak coffee bloom, which takes place shortly after the first major winter rainfall. Coffee bushes appear to flower almost

uniformly at 2-3 km scales, but at smaller scales (bush, 5 m, 10 m, and 100 m) flowering occurs in a patchy manner over the three day flowering period. This is likely due to microsite variations in soil moisture levels. Though coffee bushes were in full flower during the study season, the flowering of understory herbs in both habitats was extremely low (less than 0.01% of the ground covering plants in flower) and was therefore not included in the study.

Bee observations

Within each coffee system, observation sessions were made between the 8:00 and 14:00, when bee foraging activity was highest. The site of each observation session was randomly selected (>300m away from any previously monitored site), and temperature, distance from the forest, and distance from the apiary were recorded at each site (distances ranged between 100 m - 2000 m from both the forest and apiary). The closest bush to the randomly selected point was chosen for bee observations, and 4 fully flowering branches (minimum of 20 blossoms) were randomly chosen on the shrub. At each site, the 4 branches were observed for 15 minutes at a time, and observations of visiting bee species, duration of visit, and number of flowers visited were noted. In addition, resource levels for each observation site were measured at four scales; shrub, 5 m, 10 m, and 100 m scales. Given the large variation in branch number per bush, we measured the proportion of fully flowering branches per bush, while for the 5 m, 10 m, and 100 m scale, the proportion of coffee bushes with greater than $\frac{1}{4}$ of their branches fully flowering was measured within a 5 m, 10 m and 100 m radius from the observation point, respectively.

Within the two agroforestry habitat types, 124 observation sessions were conducted, and 499 bee visits were recorded. When possible, bees were captured after the observation period for identification (96 specimens), and we referred to collections at El Colegio de la Frontera Sur in Tapachula, Mexico and T. Griswold, at the USDA Bee Lab in Logan, Utah, for guidance with identifications.

Statistical Analyses

To meet conditions of normality, visit number and species number per observation period were square-root transformed and proportions of floral resource at all scales were arcsin-square-root-transformed for all regression analyses (Sokal 1995). Regression analyses using absolute floral resource values (not proportions) yielded the same results. All statistical analyses were conducted with the software R (R Development Core Team, URL <http://www.r-project.org>). Species accumulation and estimation curves were generated using the Chao1 estimator (Chao 1987), utilizing the software, EstimateS (Colwell and Coddington 1994). Variation in resources levels are correlated across a range of spatial scales (Table 3.1). Therefore, linear regressions for bee visitation and bee species richness were independently examined for each resource scale. For further analysis, all bee visits were classified into bee groups based on foraging strategy (social vs. solitary) and origin (native vs. exotic Africanized honeybee), so that three bee functional groups remained: 1) native solitary bees, 2) native social bees, and 3) exotic Africanized honeybees. To examine the influence of all factors on the number of visiting bees within each functional group, we utilized an Analysis of Covariance, ANCOVA, using a non-sequential test for examining individual coefficients (also known as a Type III sum of squares test). The covariates include (1) floral resource levels, (2) cloud cover, (3) temperature, (4) distance from the forest, and (5) distance from the apiary. The fixed factors include (1) vegetation management, and (2) bee functional group. We utilized an ANCOVA because it provides a descriptive comparison between the visitation levels across different habitats and between functional groups, and can thus test hypotheses concerning the significant differences between these two categorical variables. Also, the ANCOVA, unlike the Analysis of Variance (ANOVA), disentangles differences in the effects of the covariates, which range in value, from the fixed factors. The ANCOVA is based on exact F and t distributions for testing the significance of factors and covariates, yielding measures of R^2 that can be interpreted as the percentage of variance explained (Faraway 2005). The model error was independent and normally distributed.

RESULTS

Visitation and Species Richness

Across both habitat types, bee visitation per observation period did not correlate with any measures of temperature (mean =29.1°, SE=0.33), distance from the forest (mean =497 m, SE =20.2), or distance from the apiary (mean =1067 m, SE =24.9 m). Visitation was significantly influenced by bee foraging group, habitat quality, and resource levels at all scales, and the model of bee visitation in both habitat types was best fit for the 10 m resource scale (ANCOVA, Table 3.2).

On average there were 22.3 (SE =13.8) branches in flower per bush across both habitats, and the average number of branches in flower per bush was not significantly different between habitat types (t-test, $p=0.23$, $n=67$ & 57). We did not observe any aggressive physical interactions between bee visitors. Bee visitation at coffee bushes was significantly higher in high-shade coffee habitats, with a mean of 4.61 visits per observation period (SE =0.46), compared to a mean of 1.56 visits per observation period (SE =0.27) in low-shade coffee habitats (t-test, $p<0.0001$, $n=67$ & 57). The overall species richness of bee visitors was also greater in high-shade habitats (Table 3.3, Figure 3.1). Estimated species richness converged on observed species richness curves, with an estimated number of 17.5 species in high-shade habitats and an estimated number of 8.17 species in low-shade coffee habitats. Shannon Wiener diversity indices reflected the same patterns, with a value of 1.54 in high-shade habitats and 0.62 in low-shade habitats.

Visit duration was not significantly different between high-shade and low-shade habitats for native solitary bees (t-test, $p=0.61$, $n=12$ & 3), native social bees (t-test, $p=0.49$, $n=75$ & 12), or Africanized honeybees, t-test, ($p=0.70$, $n=270$ & 119). However, the number of visits was significantly greater in high-shade habitats than low-shade habitats, for native solitary bees (t-test, $p=0.048$, $n=67$ & 57), native social bees (t-test, $p<0.0001$, $n=67$ & 57), and Africanized honeybees (t-test, $p=0.002$, $n=67$ & 57) (Figure 3.2A-C). The proportion of visits conducted by solitary bees was not significantly different between the two habitat types (t-test, $p=0.911$, $n=67$ & 57 , Figure 3.2D). The proportion of visits conducted by social native bees was significantly higher in high-shade habitats (t-test, $p=0.048$, $n=67$ & 57 , Figure 3.2E), while the proportion of Africanized honeybee visits was slightly higher in low-shade habitats (t-test, $p=0.204$, $n=67$ & 57 , Figure 3.2F).

Foraging in Response to Resource Scale

Bee functional groups displayed marked differences in visitation based on resource levels at multiple scales. In addition, bee functional groups exhibited contrasting foraging responses between the two habitat types.

Native solitary bees

In low-shade coffee habitats, native solitary bees did not increase or decrease visitation levels in response to floral resources at any scale. In contrast, within high-shade habitats native solitary bees showed a significant decrease in visitation with increasing floral resources at the 5 m and 10 m resource scale, with strongest effects at the 10 m scale ($F_{1,65} = 8.454$, $r^2 = 0.112$, $p = 0.005$, $y = -0.601x + 0.539$, Figure 3.3A).

Native social bees

In low-shade coffee habitats, native social bees did not increase or decrease visitation levels in response to floral resources at any scale. In contrast, within the high-shade habitats, native social bees showed a significant decrease in visitation with increasing floral resources only at the 5 m and 10 m resource scale, with strongest effects at the 10 m scale ($F_{1,65} = 6.756$, $r^2 = 0.093$, $p = 0.012$, $y = -1.476x + 1.766$, Figure 3.3A).

Africanized honeybees

Unlike the native bees, in the low-shade habitats exotic Africanized honeybees exhibited a strong significant increase in visitation only at the 100 m scale ($F_{1,55} = 8.792$, $r^2 = 0.122$, $p = 0.004$, $y = 1.586x + 0.200$, Figure 3.3B). In contrast, within the high-shade habitats Africanized honeybees did not increase or decrease visitation levels in response to floral resources at any scale.

DISCUSSION

Forager visitation

Large floral resource bursts, such as the mass-flowering of tropical trees or the annual coffee bloom, are common in the tropics and can have major implications on the foraging behaviour of bees. As seen in previous studies, our results indicate that habitat composition and coffee floral resource availability have stronger effects on visitation levels than distance to the nearest forest fragment (Klein et al. 2003c, 2006). Past studies document that social bees often increase in density and diversity with increasing proximity to tropical forest, suggesting that bees prefer to forage near their nesting sites (Klein et al. 2003c, 2004, Ricketts 2004). In this study, greater native bee visitation in high-shade agroforestry habitats is also likely driven by nest site preferences and availability, since most native social bees in the study (e.g. *Melipona beecheii*, *Nanotrigona testaceicornis*, *Plebia* sps., *Scaptotrigona mexicana*) prefer to nest in old trees or rotting wood, most solitary bees in the study (e.g., *Ceratina eximia*, *Ceratina ignara*, *Ceratina* sp. 1) are small-bodied and nest in hollow woody stems (Michener 2000), and both substrates are more abundant in high-shade coffee habitats. The fact that local forest patches are small, and that overstorey trees managed in high-shade coffee habitats are especially diverse and dense (Moguel and Toledo 1999) could explain why regional preferences for forest proximity are particularly weak, and why local preferences for high-shade agroforestry habitats are stronger.

Though we found higher numbers of Africanized honeybee visitors in high-shade coffee habitats, we documented a greater proportion of visits conducted by Africanized honeybees in low-shade coffee habitats. This may be due to the honeybee's ability to navigate in open habitats (Barron et al. 2005), to fly greater distances from their nesting sites (Schneider and Hall 1997), or to tolerate higher temperatures (Heinrich 1979a, 1996) than native solitary and social bees. Previous studies have documented greater abundances of Africanized honeybees in more disturbed, less forested landscapes (Aizen and Feinsinger 1994, Cairns et al. 2005, Brosi et al. 2008). Our findings suggest that Africanized honeybees forage well in forested habitats, but are capable of exploiting high resources more easily than native bees in less forested habitats, likely due to their advanced foraging capabilities and high colony needs.

Foraging response to resource scale

Most importantly, this study reveals that bee functional groups respond to resources in significantly different ways across different spatial scales and habitats. While native solitary and social bees showed no foraging response to floral resources within low-shade coffee habitats, they exhibited a significant decrease in visitation with increasing floral resources at the 10 m resource scale in high-shade coffee habitats. These results suggest that native social and solitary bees perceive and respond to resources at small spatial scales, a pattern that has been documented for a number of small-bodied bee species (reviewed in Greenleaf et al. 2007). The significant negative foraging response exhibited by native solitary and social bees in our study suggests that they respond to floral resource levels by spreading out between high resource patches, a pattern known as the ‘dilution effect’ (Root and Kareiva 1984, Yamamura 1999, 2002, Veddeler et al. 2006). This pattern has been observed a number of times for bees, especially in landscapes where their numbers are assumed to be limited in relation to floral resource availability (Totland and Matthews 1998, Sargent 2003, Veddeler et al. 2006).

In contrast, in our study, only Africanized honeybees exhibited a positive foraging response to increasing coffee resource availability, and only within low-shade coffee habitats. This foraging pattern, known as the ‘concentration effect’, is usually the result of an abundance of foragers that exhibit strong preferences for high-resource sites, leading to massive recruitment (Heinrich 1979b, Rathcke 1983, Sih and Baltus 1987, Kunin 1993, Totland and Matthews 1998). While native solitary and social bees exhibit small foraging scales, honeybees exhibit relatively large foraging scales, as seen in previous studies (Steffan-Dewenter et al. 2002). Honeybees are capable of foraging outside of their usual foraging ranges in search of high-resource sites (Schneider and Hall 1997), making themselves available foragers at large spatial scales, especially when foraging for high-productive, mass-flowering sites (Schaffer et al. 1979, Schaffer et al. 1983). Because honeybees are also known to fly through more open habitats with greater speed and directness (Srinivasan et al. 1996, Tautz et al. 2004, Barron et al. 2005) the forager concentration effect best describes the foraging response for honeybees within low-shade agroforestry habitats. While both the dilution and concentration effect can be observed for foraging bee communities depending on the spatial scale examined

(Veddeler et al. 2006), our study indicates that bee functional group can explain contrasting foraging responses for bee communities across spatial scales.

We conclude that that coffee vegetation management distinctly impacts bee community composition and foraging response. Our study reveals that both vegetation management and bee functional group are critical factors driving contrasting bee foraging patterns. From a conservation perspective, we demonstrate that land management not only impacts bee abundance, but also impacts bee foraging patterns on ephemeral mass-flowering crops.

ACKNOWLEDGEMENTS

This chapter was coauthored with John Vandermeer and published in OIKOS in 2009. We would like to thank Luis Fernando Chavez, Beverly Rathcke, Ivette Perfecto, Mike Reiskind, Exzequel Velasquez, Gabriel Dominguez Martinez, the Vandermeer lab group, the Rathcke lab group, and the Perfecto lab group at the University of Michigan. We would also like to thank Terry Griswold and Olivia Messenger at the USDA Bee Lab in Logan, Utah, for help with bee identifications. Finally, we would like to express our gratitude to the people of Nueva Alemania for their permission and assistance in conducting this study. All experiments conducted were in compliance with current laws governing biodiversity protection in Mexico.

TABLES, FIGURES AND LEGENDS

Table 3.1 Pearson correlation coefficient matrix of floral resources at bush, 5 m, 10 m, and 100 m scales. ****P < 0.0001

	5 m scale	10 m scale	100 m scale
Bush scale	0.2874****	0.4711****	0.0160
5 m scale		0.7104****	0.3831****
10 m scale			0.3615****

Table 3.2 Results of repeated-measures ANCOVA, testing the effects of floral resource levels (at 10m scale), cloud cover, temperature, distance to the forest, vegetation management (high or low-shade coffee), and bee group (honeybees, native social bees, native solitary bees) on the number of bee visits per observation period. The variables floral resource levels, cloud cover, temperature, distance to forest, and distance to apiary were treated as covariates, while vegetation management and bee group were treated as main effects. Though all interaction terms were originally tested, for simplicity, only the significant interaction term is included in this model. All data were arcsin(sqrt(x)) transformed prior to analysis. N=375 observation periods, $R^2 = 0.5753$.

Source	df	F	p
Covariates			
Floral resource levels	1	7.39	0.006
Cloud cover	1	0.37	0.541
Temperature	1	2.14	0.144
Forest distance	1	3.12	0.088
Apiary distance	1	5.09	0.558
Main effects			
Vegetation management	1	147.66	<0.0001
Bee foraging group	2	46.42	<0.0001
Significant interaction terms			
Floral resource levels x Bee foraging group	2	73.67	<0.0001
Error	364	--	--

Table 3.3 Species present in high-shade coffee (HSC) and low-shade coffee (LSC) agroforestry habitats and their functional group (FG), classified as an Africanized honeybee (AHB), native solitary bee (NSOL), or native social bee (NSOC).

Species	Family	LSC	HSC	FG
<i>Apis mellifera, scutellata</i> (Lepeletier)	Apidae	x	x	AHB
<i>Ceratina eximia</i> (Smith)	Apidae		x	NSOL
<i>Ceratina ignara</i> (Cresson)	Apidae		x	NSOL
<i>Ceratina</i> sp. 1	Apidae		x	NSOL
<i>Eulaema cingulata</i> (Fabricius)	Apidae	x	x	NSOC
<i>Melipona beecheii</i> (Bennett)	Apidae		x	NSOC
<i>Nanotrigona testaceicornis</i> (Lepeletier)	Apidae	x	x	NSOC
<i>Plebia</i> sp. 1	Apidae	x	x	NSOC
<i>Scaptotrigona mexicana</i> (Guérin-Méneville)	Apidae		x	NSOC
<i>Trigona fulviventris</i> (Guérin-Méneville)	Apidae	x	x	NSOC
<i>Xylocopa tabaniformis, tabaniformis</i> (Smith)	Apidae	x	x	NSOL
<i>Augochlora aurifera</i> (Cockerell)	Halictidae		x	NSOC
<i>Augochlora nigrocyanea</i> (Cockerell)	Halictidae		x	NSOC
<i>Dialictus</i> sp. 1	Halictidae	x		NSOC
<i>Dialictus</i> sp. 2	Halictidae		x	NSOC
<i>Halictus hesperus</i> (Smith)	Halictidae		x	NSOC
<i>Halictus</i> sp. 1	Halictidae	x		NSOC

Figure 3.1 Species richness of bee visitors in high-shade coffee (HSC) and low-shade coffee (LSC) habitats. Error bars represent observed SD (Colwell and Coddington 1994).

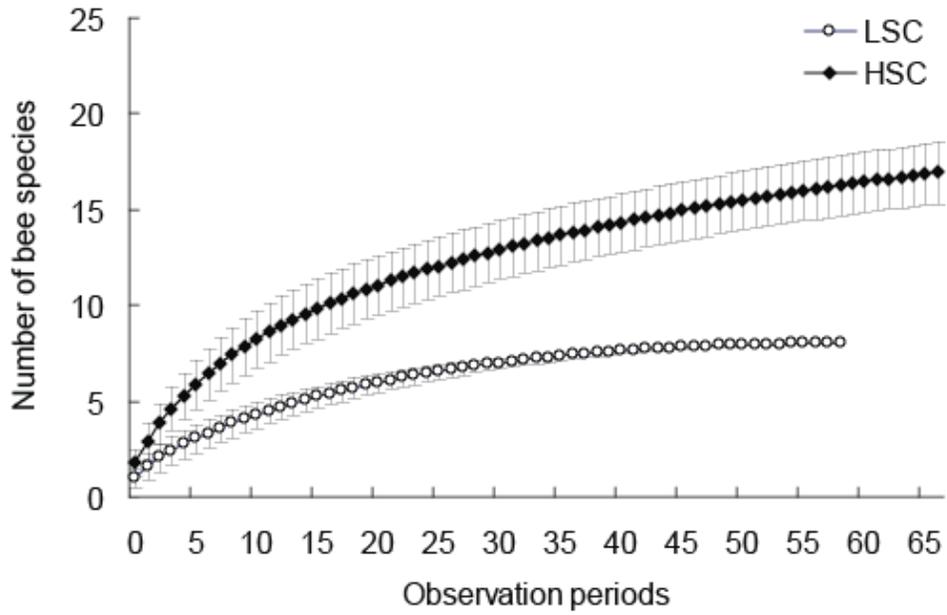


Figure 3.2 Visitation was significantly higher in high-shade coffee habitats (HSC) than low-shade habitats (LSC) for (A) native solitary bees, (B) native social bees, and (C) Africanized honeybees. The proportion of visits conducted by (D) native solitary bees was not significantly different between habitat types, (E) social native bees was significantly higher in high-shade coffee habitats, and (F) Africanized honeybees was slightly higher in low-shade coffee habitats. Error bars represent SE. Codes for significant differences in between habitats: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

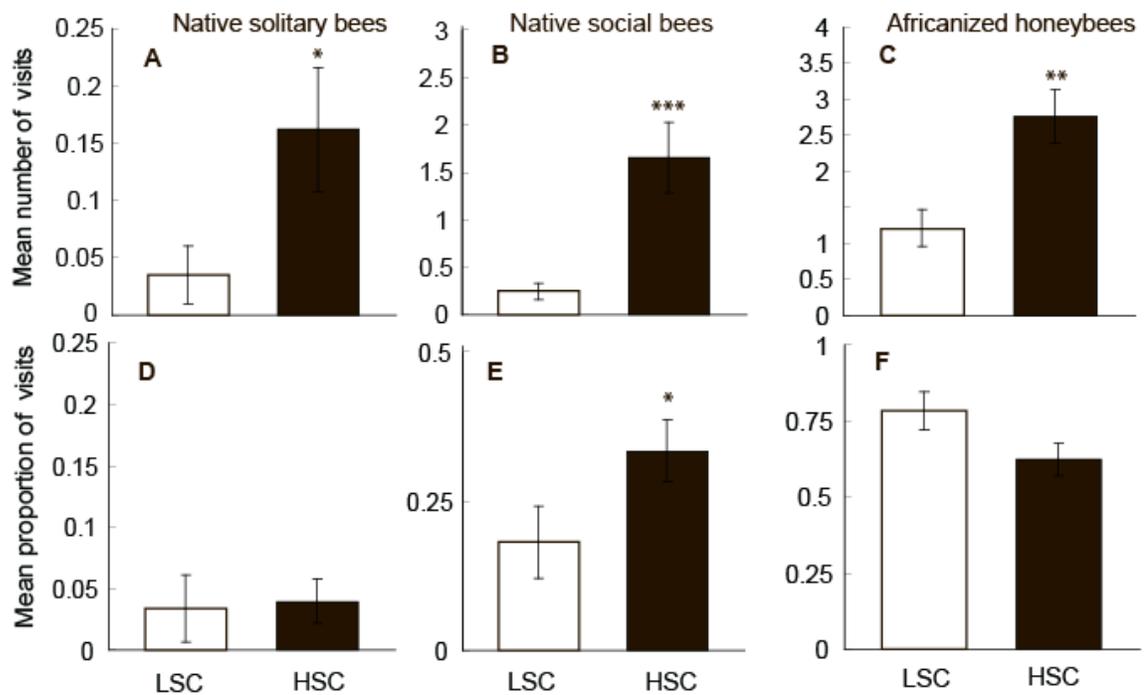
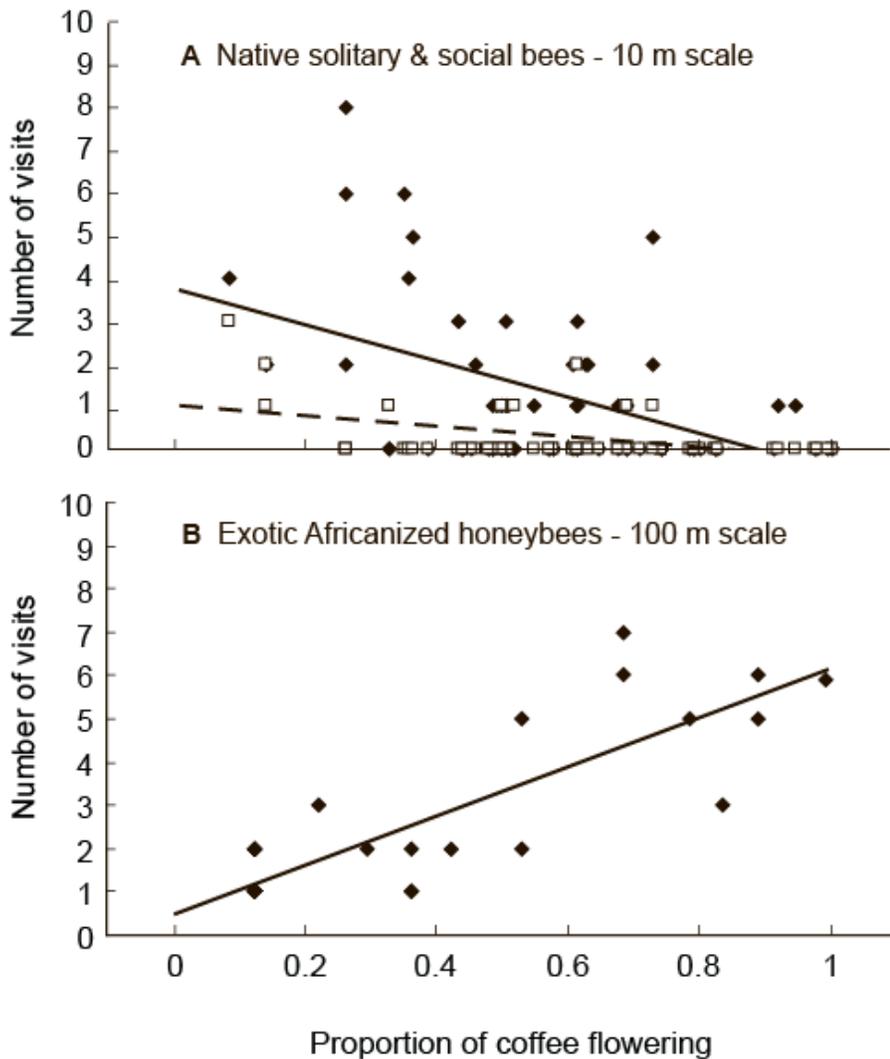


Figure 3.3 Contrasting responses of native and exotic bees under differing habitat management styles. (A) In high-shade coffee habitats, native solitary bee visitation (open squares, dashed line) and native social bee visitation (closed diamonds, solid line) decreased significantly as the floral resources decreased at the 10m resource scale. (B) In low-shade coffee habitats, Africanized honeybee visitation (closed diamonds, solid line) increased significantly as floral resources increased at the 100m resource scale.



Chapter III References

- Abrams, P. A. 1982. Functional-Responses of Optimal Foragers. *American Naturalist* **120**:382-390.
- Abrams, P. A. 1991. Life-History and the Relationship between Food Availability and Foraging Effort. *Ecology* **72**:1242-1252.
- Aizen, M. A. and P. Feinsinger. 1994. Habitat fragmentation, native insect pollinators, and feral honeybees in Argentine chaco serrano. *Ecological Applications* **4**:378-392.
- Barron, A. B., H. Zhu, G. E. Robinson, and M. V. Srinivasan. 2005. Influence of flight time and flight environment on distance communication by dancing honey bees. *Insectes Sociaux* **52**:402-407.
- Brosi, B. J., G. C. Daily, T. M. Shih, F. Oviedo, and G. Duran. 2008. The effects of forest fragmentation on bee communities in tropical countryside. *Journal of Applied Ecology* **45**:773-783.
- Buchman, G. P., and Nabhan, S.L. 1996. *The Forgotten Pollinators*. Island Press/Shearwater Books.
- Cairns, C. E., R. Villanueva-Gutierrez, S. Koptur, and D. B. Bray. 2005. Bee populations, forest disturbance, and africanization in Mexico. *Biotropica* **37**:686-692.
- Chao, A. 1987. Estimating the Population-Size for Capture Recapture Data with Unequal Catchability. *Biometrics* **43**:783-791.
- Colwell, R. K. and J. A. Coddington. 1994. Estimating Terrestrial Biodiversity through Extrapolation. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **345**:101-118.
- Daily, G. C. 1997. *Nature's services: Societal dependence on natural ecosystems*. Island Press, Washington, D.C., USA.
- Donald, P. F. 2004. Biodiversity impacts of some agricultural commodity production systems. *Conservation Biology* **18**:17-37.
- Dornhaus, A. and L. Chittka. 1999. Insect behaviour - Evolutionary origins of bee dances. *Nature* **401**:38-38.
- Dornhaus, A. and L. Chittka. 2004. Why do honey bees dance? *Behavioral Ecology and Sociobiology* **55**:395-401.
- Faraway, J. 2005. *Extending the linear model with R: Generalized linear, mixed effects and nonparametric regression models*. Chapman and Hall/CRC, Boca Raton.
- Fauchald, P. 1999. Foraging in a hierarchical patch system. *American Naturalist* **153**:603-613.
- Fauchald, P. and T. Tveraa. 2006. Hierarchical patch dynamics and animal movement pattern. *Oecologia* **149**:383-395.
- Greenleaf, S. S., N. M. Williams, R. Winfree, and C. Kremen. 2007. Bee foraging ranges and their relationship to body size. *Oecologia* **153**:589-596.
- Heinrich, B. 1979a. Keeping a Cool Head - Honeybee Thermoregulation. *Science* **205**:1269-1271.
- Heinrich, B. 1979b. Resource Heterogeneity and Patterns of Movement in Foraging Bumblebees. *Oecologia* **40**:235-245.

- Heinrich, B. 1996. How the honey bee regulates its body temperature. *Bee World* **77**:130-137.
- Klein, A. M., S. A. Cunningham, M. Bos, and I. Steffan-Dewenter. 2008. Advances in pollination ecology from tropical plantation crops. *Ecology* **89**:935-943.
- Klein, A. M., I. Steffan-Dewenter, D. Buchori, and T. Tschardtke. 2002. Effects of land-use intensity in tropical agroforestry systems on coffee flower-visiting and trap-nesting bees and wasps. *Conservation Biology* **16**:1003-1014.
- Klein, A. M., I. Steffan-Dewenter, and T. Tschardtke. 2003a. Bee pollination and fruit set of *Coffea arabica* and *C. canephora* (Rubiaceae). *American Journal of Botany* **90**:153-157.
- Klein, A. M., I. Steffan-Dewenter, and T. Tschardtke. 2003b. Fruit set of highland coffee increases with the diversity of pollinating bees. *Proceedings of the Royal Society of London Series B-Biological Sciences* **270**:955-961.
- Klein, A. M., I. Steffan-Dewenter, and T. Tschardtke. 2003c. Pollination of *Coffea canephora* in relation to local and regional agroforestry management. *Journal of Applied Ecology* **40**:837-845.
- Klein, A. M., I. Steffan-Dewenter, and T. Tschardtke. 2004. Foraging trip duration and density of megachilid bees, eumenid wasps and pompilid wasps in tropical agroforestry systems. *Journal of Animal Ecology* **73**:517-525.
- Klein, A. M., I. Steffan-Dewenter, and T. Tschardtke. 2006. Rain forest promotes trophic interactions and diversity of trap-nesting hymenoptera in adjacent agroforestry. *Journal of Animal Ecology* **75**:315-323.
- Klein, A. M., B. E. Vaissiere, J. H. Cane, I. Steffan-Dewenter, S. A. Cunningham, C. Kremen, and T. Tschardtke. 2007. Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B-Biological Sciences* **274**:303-313.
- Kremen, C., N. M. Williams, M. A. Aizen, B. Gemmill-Herren, G. LeBuhn, R. Minckley, L. Packer, S. G. Potts, T. Roulston, I. Steffan-Dewenter, D. P. Vazquez, R. Winfree, L. Adams, E. E. Crone, S. S. Greenleaf, T. H. Keitt, A. M. Klein, J. Regetz, and T. H. Ricketts. 2007. Pollination and other ecosystem services produced by mobile organisms: a conceptual framework for the effects of land-use change. *Ecology Letters* **10**:299-314.
- Kremen, C., N. M. Williams, R. L. Bugg, J. P. Fay, and R. W. Thorp. 2004. The area requirements of an ecosystem service: crop pollination by native bee communities in California. *Ecology Letters* **7**:1109-1119.
- Kremen, C., N. M. Williams, and R. W. Thorp. 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.
- Kunin, W. E. 1993. Sex and the Single Mustard - Population-Density and Pollinator Behavior Effects on Seed-Set. *Ecology* **74**:2145-2160.
- Mangel, M. and C. W. Clark. 1986. Towards a Unified Foraging Theory. *Ecology* **67**:1127-1138.
- Michener, C. D. 2000. *Bees of the World*. Johns Hopkins University Press, Baltimore, MD.
- Moguel, P. and V. M. Toledo. 1999. Biodiversity conservation in traditional coffee systems of Mexico. *Conservation Biology* **13**:11-21.

- Myers, N., R. A. Mittermeier, C. G. Mittermeier, G. A. B. da Fonseca, and J. Kent. 2000. Biodiversity hotspots for conservation priorities. *Nature* **403**:853-858.
- Perfecto, I., R. A. Rice, R. Greenberg, and M. E. VanderVoort. 1996. Shade coffee: A disappearing refuge for biodiversity. *Bioscience* **46**:598-608.
- Potts, S. G., B. Vulliamy, A. Dafni, G. Ne'eman, and P. Willmer. 2003. Linking bees and flowers: How do floral communities structure pollinator communities? *Ecology* **84**:2628-2642.
- Price, M. V. 1984. *Insect ecology*. 2nd edition. John Wiley and Sons, New York, New York, USA.
- Raine, N. E., T. C. Ings, A. Dornhaus, N. Salefl, and L. Chittka. 2006. Adaptation, genetic drift, pleiotropy, and history in the evolution of bee foraging behavior. Pages 305-354 *Advances in the Study of Behavior*, Vol 36. Elsevier Academic Press Inc, San Diego.
- Rathcke, B. 1983. Competition and facilitation among plants for pollination. Pages 305–327 in L. Real, editor. *Pollination biology*. Academic Press, New York, New York, USA. .
- Ricketts, T. H. 2004. Tropical forest fragments enhance pollinator activity in nearby coffee crops. *Conservation Biology* **18**:1262-1271.
- Ricketts, T. H., G. C. Daily, P. R. Ehrlich, and C. D. Michener. 2004. Economic value of tropical forest to coffee production. *Proceedings of the National Academy of Sciences of the United States of America* **101**:12579-12582.
- Ricketts, T. H., J. Regetz, I. Steffan-Dewenter, S. A. Cunningham, C. Kremen, A. Bogdanski, B. Gemmill-Herren, S. S. Greenleaf, A. M. Klein, M. M. Mayfield, L. A. Morandin, A. Ochieng, and B. F. Viana. 2008. Landscape effects on crop pollination services: are there general patterns? *Ecology Letters* **11**:499-515.
- Root, R. B. and P. M. Kareiva. 1984. The Search for Resources by Cabbage Butterflies (*Pieris-Rapae*) - Ecological Consequences and Adaptive Significance of Markovian Movements in a Patchy Environment. *Ecology* **65**:147-165.
- Roubik, D. W. 1980. Foraging Behavior of Competing Africanized Honeybees and Stingless Bees. *Ecology* **61**:836-845.
- Sargent, R. D. 2003. Seasonal changes in pollen-packaging schedules in the protandrous plant *Chamerion angustifolium*. *Oecologia* **135**:221-226.
- Schaffer, W. M., D. B. Jensen, D. E. Hobbs, J. Gurevitch, J. R. Todd, and M. V. Schaffer. 1979. Competition, Foraging Energetics, and the Cost of Sociality in 3 Species of Bees. *Ecology* **60**:976-987.
- Schaffer, W. M., D. W. Zeh, S. L. Buchmann, S. Kleinhans, M. V. Schaffer, and J. Antrim. 1983. Competition for Nectar between Introduced Honey Bees and Native North-American Bees and Ants. *Ecology* **64**:564-577.
- Schneider, S. S. and H. G. Hall. 1997. Diet selection and foraging distances of African and European-African hybrid honey bee colonies in Costa Rica. *Insectes Sociaux* **44**:171-187.
- Schoener, T. W. 1971. Theory of Feeding Strategies. *Annual Review of Ecology and Systematics* **2**:369-404.
- Sih, A. and M. S. Baltus. 1987. Patch Size, Pollinator Behavior, and Pollinator Limitation in Catnip. *Ecology* **68**:1679-1690.

- Sokal, R. R., Rohlf, FJ 1995. *Biometry: the principles and practice of statistics in biological research*. 3rd edition. WH Freeman, New York, N.Y.
- Soto-Pinto, L., Y. Romero-Alvarado, J. Caballero-Nieto, and G. S. Warnholtz. 2001. Woody plant diversity and structure of shade-grown-coffee plantations in Northern Chiapas, Mexico. *Revista de Biología Tropical* **49**:977-987.
- Spivak, M., D. J. C. Fletcher, and M. C. Breed. 1991. *The "African" Honey Bee*. Westview, Boulder, CO.
- Srinivasan, M. V., S. W. Zhang, M. Lehrer, and T. S. Collett. 1996. Honeybee navigation en route to the goal: Visual flight control and odometry. *Journal of Experimental Biology* **199**:237-244.
- Steffan-Dewenter, I., U. Munzenberg, C. Burger, C. Thies, and T. Tschardt. 2002. Scale-dependent effects of landscape context on three pollinator guilds. *Ecology* **83**:1421-1432.
- Steffan-Dewenter, I., S. G. Potts, and L. Packer. 2005. Pollinator diversity and crop pollination services are at risk. *Trends in Ecology & Evolution* **20**:651-652.
- Steffan-Dewenter, I. and C. Westphal. 2008. The interplay of pollinator diversity, pollination services and landscape change. *Journal of Applied Ecology* **45**:737-741.
- Tautz, J., S. W. Zhang, J. Spaethe, A. Brockmann, A. Si, and M. Srinivasan. 2004. Honeybee odometry: Performance in varying natural terrain. *PLoS Biology* **2**:915-923.
- Totland, O. and I. Matthews. 1998. Determinants of pollinator activity and flower preference in the early spring blooming *Crocus vernus*. *Acta Oecologica-International Journal of Ecology* **19**:155-165.
- Veddeler, D., A. M. Klein, and T. Tschardt. 2006. Contrasting responses of bee communities to coffee flowering at different spatial scales. *Oikos* **112**:594-601.
- Winfree, R., N. M. Williams, J. Dushoff, and C. Kremen. 2007. Native bees provide insurance against ongoing honey bee losses. *Ecology Letters* **10**:1105-1113.
- Yamamura, K. 1999. Relation between plant density and arthropod density in cabbage. *Researches on Population Ecology* **41**:177-182.
- Yamamura, K. 2002. Biodiversity and stability of herbivore populations: influences of the spatial sparseness of food plants. *Population Ecology* **44**:33-40.

Chapter IV: Local agroforestry landscapes mediate bee community composition

ABSTRACT

Though many studies have indicated that tropical bee communities are influenced by landscape composition, few have investigated the relative importance of both local and landscape-level habitat parameters in supporting diverse bee communities, especially in complex human-altered landscapes. Coffee agroforestry systems, which exhibit wide-ranging habitat management styles, are dependent on robust pollinator communities for improved coffee yields. In this study, we used pan-traps to examine tropical bee communities across seven different coffee agroforestry systems in Chiapas, Mexico. Results indicate that the most predictive factors for bee abundance and diversity are the number of tree species and the number of flowering tree species within the local agroforestry landscape. In contrast, community composition did not change substantially across local and landscape-level habitat gradients. Solitary bees were more abundant in habitats with less canopy cover and greater understory plant species richness, while social bees were more abundant in habitats with greater tree species richness and lower coffee density. For cavity-nesting and ground-nesting bees, abundance was positively affected by the number of tree species in the farm. Wood-nesting bees were most abundant in habitats with greater canopy cover and landscape-level forest cover. Overall, across sociality groups, nesting guilds, and tribes, we found that within-farm local habitat management was more predictive of bee abundance than landscape-level forest distributions. Specifically, our results highlight the importance of overstory tree management in supporting native bee abundance and species richness within shade coffee agroecosystems.

INTRODUCTION

Bee communities are critically important for ecosystem function and the maintenance of the human agricultural enterprise through their pollinating activities. For example an estimated 70% of world crops experience increased size, quality, or stability because of pollinator services (reviewed in Ricketts et al. 2008), benefitting 35% of the global food supply (reviewed in Klein et al. 2007). Animal pollination also contributes to the stability of food prices, food security, food diversity, and human nutrition (Steffan-Dewenter et al. 2005) and is estimated to be worth \$200 billion worldwide (Kearns et al. 1998). Unfortunately, native pollinator populations face many threats, and evidence of a global pollination crisis is steadily growing (Buchman 1996, Allen-Wardell et al. 1998, Kearns et al. 1998, Larsen et al. 2005, Steffan-Dewenter et al. 2005, Biesmeijer et al. 2006, Winfree 2008). Acute declines in managed honeybee populations are cause for further concern (Johnson 2007), with recent studies indicating that a reliance on managed honeybees for crop pollination is risky at best (Winfree et al. 2007, Winfree et al. 2008).

Despite fears of pollinator shortages and strong ecological and agricultural dependencies on pollination services, we are just beginning to understand how anthropogenic land use impacts wild and managed pollinators. Though crop pollinators include a wide array of insects (e.g., beetles, butterflies, flies), bees are the most important and effective of these pollinators (Roubik 1995, Klein et al. 2007). The bees (Hymenoptera: Apoidea) comprise of an extremely species-rich fauna, with an estimated 20,000 – 30,000 species worldwide (Michener 2000). Furthermore, bees are ecologically and functionally diverse, foraging on a broad array of floral forms and nesting in a wide variety of substrates (Michener 2000). Many of the world's crops are primarily and effectively pollinated by wild native bees, rather than managed honeybees (Kremen et al. 2002b, Kremen et al. 2004, Steffan-Dewenter et al. 2005, Klein et al. 2007, Kremen et al. 2007, Ricketts et al. 2008, Steffan-Dewenter and Westphal 2008). Moreover, recent studies have revealed that crops experience higher or more stabilized fruit set in habitats with greater native bee diversity (Kremen et al. 2002b, Klein et al. 2003b, reviewed in Klein et al. 2007, Hoehn et al. 2008, Vergara and Badano 2009, Winfree and Kremen

2009), a pattern explained by the greater niche complementarity existing between functional groups within diverse communities (reviewed in Fenster et al. 2004, Hooper et al. 2005, Cardinale et al. 2006, Hoehn et al. 2008).

Diverse pollinator communities are especially essential in the tropics, where the vast majority of plant species are animal pollinated (Bawa et al. 1985, Bawa 1990). Tropical regions are home to immense faunal and floral diversity, and encompass many of the world's biodiversity hotspots (Myers et al. 2000). Tropical regions also contribute substantially to global agriculture and are predominantly comprised of a mosaic of forest patches and agricultural lands (Lambin et al. 2003, Donald 2004, Perfecto and Vandermeer 2008). In particular, coffee (*Coffea arabica* and *Coffea robusta*) are a cornerstone of tropical export agriculture and are found almost exclusively in tropical regions (Perfecto et al. 1996, Donald 2004). Coffee covers over 11 million ha worldwide and provides income for 25 million people, most of whom are small landholders and laborers (Donald 2004).

Traditionally, coffee is grown under a canopy of shade trees, which provide food resources, nitrogen fixation (Perfecto et al. 1996, Moguel and Toledo 1999), soil erosion prevention (Lin 2007, Lin and Richards 2007, Philpott et al. 2008b) and habitat for birds and bats that serve as coffee pest predators (Greenberg et al. 2000, Perfecto et al. 2004, Van Bael et al. 2008, Williams-Guillen et al. 2008). However, in recent years, with the increased availability of fertilizers, pesticides, and machines, many coffee farms have cut their shade trees and intensified their farming practices, creating homogenous fields of coffee that lack vegetation structure or diversity (Perfecto et al. 1996, Donald 2004). Tropical forest destruction threatens forest-dwelling organisms (Challenger 1998, Geist and Lambin 2002, Lambin et al. 2003) and can also disturb bee communities within adjacent agricultural systems, if the communities are dependent on local forest patches or natural areas (e.g., Steffan-Dewenter et al. 2002, Kremen et al. 2004, Greenleaf and Kremen 2006, Klein et al. 2006, Klein et al. 2008).

In addition to regional forest distributions, local agroforestry habitat can be critical in organizing native bee communities within coffee regions (reviewed in Klein et al. 2008). Studies have found that understory flowering, including coffee and herbaceous plants, is one of the most predictive local factors of bee abundance and diversity (Klein et

al. 2003c, De Marco and Coelho 2004, Veddeler et al. 2006). However, because of a research emphasis on coffee pollination services, many bee surveys have documented habitat factors primarily within the understory, and have examined bee communities only during the coffee blooming period. Additionally, while it is established that bee communities respond to habitat factors at multiple scales (e.g., Steffan-Dewenter et al. 2002, Westphal et al. 2003, Tylianakis et al. 2006, Veddeler et al. 2006), little is known about the relative importance of local vs. landscape-level agroforestry factors in predicting native bee diversity and abundance.

In this study, we investigate the effects of local and landscape-level habitat on bee abundance and diversity across a coffee growing region in Chiapas, Mexico, using regression trees (Morgan and Sonquist 1963, Breiman et al. 1984) to examine the most critical habitat variables driving bee abundance and diversity. We also separately examine the responses of multiple functional groups within the bee community (based on sociality, evolutionary history, and nesting) in order to reveal group-specific habitat requirements. We hypothesize that overall bee diversity and abundance will respond primarily to forest cover at both local and landscape-level scales. Based on previous studies, we conjecture that bee functional groups will differ in their responses to local and landscape habitat factors, but will share strong responses to coffee flowering and forest cover.

METHODS

Study sites

The study was conducted in the *Soconusco*, a coffee-growing region located in the southern foothills of Chiapas, Mexico (15°10'59''N, 92°20'44''W (NW corner) to 15°08'52''N, 92°18'33''W (SE corner)) at an altitude ranging between 825 and 1300m (Figure 4.1, Table 4.1). The study area covers approximately 51.8 km² and is comprised of a mosaic of coffee farms and forest fragments. In the study region, coffee is predominately cultivated in the semi-traditional style, under a canopy of overstory trees that varies in density and diversity depending on the vegetation management style

employed in each agroforestry system (Perfecto et al. 1996, Moguel and Toledo 1999, Soto-Pinto et al. 2001). We examined seven agroforestry systems which vary substantially in their overstory and understory vegetation management styles. The sites range from traditional/commercial polycultures to shade monocultures (Moguel and Toledo 1999, Philpott et al. 2008a)(Figure 4.2).

In each site, we established a 100m x 100m (1 ha) plot, and measured the following seasonal agroforestry habitat variables: 1) the density and diversity of overstory trees (trees), 2) the DBH of shade trees (for basal area), 3) the density of understory coffee bushes, and 4) the plot elevation. We counted all coffee plant individuals in the 1-ha plot, as well as all trees, which were identified to species and measured for diameter at breast height. Elevation was measured with a GPS unit (Trimble GeoExplorer 3) at the SE corner of each plot.

Bee sampling

Within each plot, bees were captured using a pan trap methodology, according to the Bee Inventory Plot guidelines (LeBuhn et al. 2005). Pan traps are easy to standardize across sites, decrease the risk of collector's bias, and are extremely effective at trapping, thus they are considered the most practical and informative sampling methodology for bees (Westphal et al. 2008). Using the Bee Inventory Plot pan trap methodology, we laid 30 pan traps made from 6 oz plastic bowls (SOLO model number: PB6-0099) across two intersecting 50m transects located in the center of each plot. Before the experiment, 1/3 of the bowls were painted fluorescent blue, 1/3 painted fluorescent yellow and 1/3 left white, and the colors were alternated along the two transects. Bowls were then filled with a soapy water solution (1 tsp Dawn® blue soap per gallon water). The fluorescent colors attract bees, and the soapy water traps them in the pan (e.g., Kearns and Inouye 1993, LeBuhn et al. 2005). To ensure pan trap visibility, coffee branches hanging above the pan traps were trimmed.

Pan traps were set out at all plots at 9am and collected at 5pm once every two weeks from Feb 2nd - April 13th 2006. This period extends from the middle of the dry season to the beginning of the wet season in southern Mexico. We processed the specimens on each sampling day by pinning and identifying them to genus or subgenus

level. For species-level identifications, we referred to local collections at *El Colegio de la Frontera Sur* in Tapachula, Mexico and the expertise of T. Griswold, at the USDA Bee Lab in Logan, Utah. For some individuals, we could not arrive at species-level classifications, thus we left their classification at the genus level (with a morphospecies label). Because many bee groups do not have adequate taxonomic treatments (Michener 2000), especially in the tropics, identification to the morphospecies level was necessary, and previous work shows that morphospecies-level identifications can serve as a good proxy in the estimation of species richness (Oliver and Beattie 1996).

Local agroforestry management

Plots were also sampled for daily agroforestry habitat variables once every two weeks from Feb 2nd- April 13th 2006, one day after bee sampling days. On each sampling day, we measured: 5) the density and richness of understory plants in flower, 6) the percent of coffee bushes in flower, 7) the percent of canopy cover, 8) the percent ground cover, and 9) the density and richness of angiosperm trees in flower (hereafter flowering trees). To monitor the density and richness of understory plants in flower and the percent of coffee bushes in flower per plot, we established four 10 x 4m transects, starting with a random starting point, continuing 10m in the four cardinal directions for each of the four plots. Transects did not overlap or extend outside of the plot. On each sampling day, we counted and identified to species all individual understory plants in flower within each transect. For each transect, we calculated the percent of coffee bushes in flower by dividing the number of branches in flower by the total number of branches for each bush, and then averaging across all bushes in each transect. Canopy cover and ground cover were recorded along the diagonals of each 1 ha plot using a gridded densiometer, recording the vegetation cover overhead (canopy cover) and the vegetation cover on the ground (ground cover) every three meters for a total of 46 sampling points for each index. This was then averaged per plot. We measured the density and richness of trees in flower throughout the entire plot based on counts taken using binoculars.

Forest cover

For each plot, we also measured the percent of primary forest cover within 100m, 500m, and 1km radii, based on a panchromatic IKONOS® image with 1m spatial resolution (Land Info Worldwide Mapping, 2007). The image was taken on 10 December 2005 and had negligible cloud cover. The image was orthorectified and ground-truthed using a 20-30 points taken at all sampling sites within the region. Boundaries between coffee farms and forest fragments were measured using the visual aid of the panchromatic image and with the textural and reflectance patterns of a chlorophyll composite provided by the multispectral image (spectral bands 4-3-1, 4m spatial resolution). Area of forest within each radius was calculated using the software ArcMap9.2 (ESRI, 1999-2006).

Statistical Analyses

To meet conditions of normality, all counts and proportions were $\log(x+1)$ transformed, and all variables were first tested for colinearity (Sokal 1995). Density of trees and basal area were colinear, so they were not tested simultaneously in the analyses. Species accumulation and estimation curves were generated using the Chao1 estimator (Chao 1987), utilizing the EstimateS software (Colwell and Coddington 1994). To determine the most important factors predictive of bee abundance at the sociality, nesting guild and tribe level, we used regression trees. We built regression trees utilizing the rpart package in the programming language R (R Development Core Team, 2005).

The regression tree is a non-parametric modeling approach that utilizes a recursive data-partitioning algorithm that chooses the single best predictor variable, and splits the data into two sections (nodes) based on that variable. Regression trees are commonly used to examine patterns in ecological data (Recknagel 2001), and are especially useful for developing habitat models where factors may interact in a hierarchical fashion (reviewed in De'ath and Fabricius 2000, Olden et al. 2008). Regression trees are not violated by repeated measures and do not assume linearities in response variables. Each regression tree model began by including all seasonal and daily agroforestry habitat variables and forest cover at each of the three radii. After the first partition, each node is then further split based on the next best predictor variable (Breiman et al. 1984). To prevent over-fitting, the rpart subroutine splits branches based

on a cost complexity value which balances model complexity with model fit. In the graphical depictions of the regression tree analyses (Figs. 4.6-4.8) the importance of each predictor variable corresponds to the length of the vertical line (branch) below each split.

RESULTS

Local and landscape-level agroforestry management

Sites exhibited a wide range of seasonal agroforestry management levels, varying between 13.2% and 69.4% canopy cover and 162 to 258 trees per ha (Table 4.1). Across all sites and sampling periods, we documented 25 tree species (6 flowered during the season) and 16 understory flowering plant species (Table 4.2). Coffee flowering began in the 6th week of the survey and lasted 9 days across the entire region. Coffee cultivation accounted for approximately 93.7% of the entire study area and forest for the remaining 6.3%. For the each plot, local forest cover within a 100m to 1km radius ranged between 0.0% and 14.2%.

Bee communities

We trapped a total of 648 bees, including 46 different species representing three families: Halictidae (72.2%), Apidae (27.5%), and Anthophoridae (0.3%)(Table 4.3). Observed species accumulation curves (Figure 4.3) approach but do not reach a plateau, and the estimated species richness in the region is 59 species. For the entire bee community, the most critical variable for predicting both bee abundance and bee species richness is the total number of tree species (Figure 4.4). Based on regression tree analyses, tree diversity explained 46.98% of the variation for bee abundance, and explained 33.02% of the variation for species richness (regression trees not shown). Overall bee community composition (tribe level) did not vary substantially based on a gradient of forest cover or tree diversity, but did show a weak trend of increasing tribe diversity with increasing tree diversity (Figure 4.5).

Given the diverse composition of the sampled bee community, and the likely divergence in responses to agroforestry management depending on 1) sociality, 2) evolutionary history (tribe), and 3) nesting guild, we separately examined bee abundance in response to habitat factors within these three groups. All optimal trees at the sociality, tribe, and nesting guild levels were 3-branched. Of the nine predictor variables measured, six variables were selected in the construction of all six regression trees. The variation explained by each model was relatively high for bee groups, ranging between 20.0% and 56.9%.

Sociality, Tribes and Nesting Guilds

The most predictive factors for solitary bee abundance were percent canopy cover and the number of herb species flowering, while the most predictive factors for social bee abundance were the number of tree species, followed by the coffee bush density (Figure 4.6). For the tribe Halictini, the factors with the greatest effect on bee abundance were the number of tree species and the number of flowering tree species. For the tribe Augochlorini, the factors showing the greatest effect on bee abundance were the density of trees, followed by the density of coffee bushes. For the tribe Ceratinini, the traits that showed the greatest effect on bee abundance were percent canopy cover and the number of flowering tree species (Figure 4.7).

The most predictive factors for cavity-nesting bee abundance were the number of tree species and the percent canopy cover within a plot. The factors that showed the greatest effect on wood-nesting bee abundance were the percent canopy cover, followed by the percent of forest cover within a 500m radius. The most predictive factors for ground-nesting bee abundance were the number of tree species, followed by the number of flowering tree species (Figure 4.8).

DISCUSSION

Bee communities

We found that within-farm local habitat management was more predictive of bee abundance than native forest cover at all scales. A number of other studies have also found that bee diversity levels may not respond to native habitat fragment size or availability (Donaldson et al. 2002, Cane et al. 2006, Brosi et al. 2008), a pattern potentially explained by enhanced nesting opportunities in the human-managed matrix (Cane et al. 2006). These findings and ours contrast many previous studies which emphasize the importance of forest proximity and size in providing resources for bees within agroforestry systems (Aizen and Feinsinger 1994, Klein et al. 2003c, b, De Marco and Coelho 2004, Ricketts 2004, Ricketts et al. 2004, reviewed in, Klein et al. 2008, Ricketts et al. 2008, Vergara and Badano 2009). This distinction in response is likely due to differences in bee community composition and analytical approach (Cane 2001, Brosi et al. 2008), as well as differences in sampling duration and floral resource levels between these studies.

To begin, the bee community in this study is dominated by small-bodied bees within the Halictini, Augochlorini, and Ceratinini, which likely respond differently to habitat than larger bodied, more forest dependent tribes like the Apini or Meliponini. Many previous studies documenting changes in bee diversity or community composition in response to local forest distributions have examined bee communities comprised largely of bees within the Apini and Meliponini tribes (Brown and Albrecht 2001, Klein et al. 2003c, Ricketts 2004, Brosi et al. 2007, Brosi et al. 2008). Second, the strong dependencies on forest proximity seen in studies conducted within coffee systems may exist only during the ephemeral coffee bloom, but may not be as critical for bee communities across a longer sampling season. Non-linear dependencies on alternative floral and nesting resources across the season may also be more easily revealed by regression tree analyses than by linear regression-based statistical analyses.

Additionally, it is possible that traditional Mexican shade coffee farms exhibit higher levels of overstory and understory plant richness than other agroforestry systems (Moguel and Toledo 1999), potentially making local nesting and floral resource levels more abundant in this study system. Due to a complex history of agriculture-related deforestation in Chiapas (reviewed in Ramirez-Marcial et al. 2001) forest cover is low

and therefore may not play a critical role in providing bee habitat within this region. Many coffee regions across the tropics are similarly deforested (Perfecto et al. 1996, Donald 2004), and while native forest preservation is paramount to biodiversity conservation, most coffee cultivators can only implement land-use change within their own farms (Philpott et al. 2008b). Our study indicates that local habitat factors, managed within agroforestry systems, can have strong impacts on local bee abundance and diversity, potentially improving coffee yields.

Sociality groups

Overall, solitary bees showed the greatest response to low levels of canopy cover and high understory flowering plant richness. These results echo the findings of many studies conducted within tropical agricultural systems, all of which document increasing solitary bee abundance with increasing light intensity and flowering herb cover (Klein et al. 2002, Klein et al. 2003c, Tylianakis et al. 2006). Reduced canopy cover often leads to increased understory plant growth and bloom, especially in farms that do not frequently cut back herbaceous growth. In contrast, for social bees, we documented strong preferences for high tree species richness and low coffee bush density. Higher tree species richness and sparser coffee cover may provide social bees with more suitable nesting options, given that many social bees are wood or ground-nesters (Michener 2000). We did not find that forest cover influenced social bee abundance, as noted in some studies (Klein et al. 2003c, Ricketts 2004, Brosi in press). Similar to the patterns documented by Klein and colleagues (2002), our study suggests that social bee abundance increases with increasing vegetation diversity and density. Additionally, our results demonstrate that social bee abundance is specifically related to the species richness of shade trees within an agroecosystem, a novel result to our knowledge.

Tribes

Across all three tribes, nest and forage resource availability were both key factors predictive of bee abundance. The tribe Halictini includes both solitary and social species that usually nest within the ground in simple burrows. They are polylectic and provision their offspring with a large quantity of food resources at the time of oviposition (reviewed

in Sakagami and Michener 1962, Michener 2000, Plateaux-Quenu 2008). We found that the key variables influencing halictine bee abundance were high tree species richness and high species richness of trees in flower. Given their ground-nesting behavior, these results indicate that floral resources are the primary drivers of halictine bee abundance within coffee growing regions. Like the Halictini, the Augochlorini are a polylectic tribe (Engel 2000) including both solitary and social species. The augochlorines nest both in the ground and in dead or decaying plant matter (e.g., Eickwort and Sakagami 1979, Wcislo et al. 2003, Brosi et al. 2006). The key factors predictive of augochlorine abundance in this study were high tree density, followed by high coffee density. Both trees and coffee bushes provide potential nesting resources, thus augochlorine bee response appears to be driven primarily by local nest site availability. The Ceratinini tribe includes small bees which nest in pithy stems (Okazaki 1992, McIntosh 1996, Michener 2000). They are polylectic and could potentially provide crop pollination services (Sakagami and Maeta 1977). We found that ceratinines responded most to high canopy cover and high species richness of flowering trees. The strong response of ceratinines to increased canopy cover is expected, given that dense canopy cover provides greater access to stem nesting sites. Increased abundance in the presence of high flowering tree species richness indicates that diverse floral resources are also critical in sustaining high local densities of ceratinine bees.

Nesting guilds

Bee nesting guilds were distinct in their responses to habitat factors, but did not show a universal response to coffee flowering or forest cover, as hypothesized. Cavity-nesting bees were most abundant in habitats with high tree species richness and high canopy cover, indicating that nesting resources are the key factors influencing their local abundance. Previous studies have shown that cavity-nesting bees may be influenced by cavity size and shape (Schmidt and Thoenes 1992) and exhibit greater abundance in habitats with greater cavity availability (Potts et al. 2005). In our study, higher canopy cover and tree species richness lead to greater diversity in canopy complexity, and potentially greater cavity availability and size variation. Because dense canopy cover is a

result of little to no overstory tree pruning, our results suggest that diverse and relatively unpruned shade tree management can lead to greater cavity-nesting bee abundance.

We found that high canopy cover was the habitat factor most predictive of wood-nesting bee abundance. These results echo those of past studies, which have shown that wood-nesting bees display preferences for sites with greater woody vegetation density, such as regenerating scrub lands (Potts et al. 2005) and abandoned coffee farms (Tylianakis et al. 2005). Additionally, many of the wood-nesting species in our study are stem-nesting bees within the Ceratinini, a tribe which exhibits distinct preferences for old stems and branches that are often found in the canopy. Our analysis revealed that the second most important factor for wood-nesting bees is forest coverage within a 500m radius. Wood-nesting bees were the only guild which exhibited a response to landscape-level forest resources, likely due to the presence of large, and thus long ranging (Greenleaf et al. 2007) guild members. We observed a number of large-bodied carpenter bees (genus *Xylocopa*), a genus known for extensive foraging ranges, often averaging over 1 km (Pasquet et al. 2008). Carpenter bees also show a strong preference for nesting in decaying trees and branches (Michener 2000), a habitat more abundant in forest fragments. Similar dependences on forest proximity have been documented for other wood-nesting bees (Tylianakis et al. 2005, Klein et al. 2006, Brosi et al. 2008, Brosi in press) and for bee communities across a wide range of ecological settings (Steffan-Dewenter et al. 2001, Kremen et al. 2002b, Steffan-Dewenter et al. 2002, Klein et al. 2003b, Ricketts 2004, Chacoff and Aizen 2006, reviewed in Ricketts et al. 2008).

Ground-nesting bee abundance responded to high tree species richness and high species richness of flowering trees, but did not show any response to ground vegetation cover. Thus our results depart from previous studies, where ground-nesting bees demonstrate preferences for nest sites with minimal vegetation (Wuellner 1999) and exhibit increased numbers in habitats with greater bare ground availability (Potts et al. 2005). However, other studies conducted within agricultural systems have found that ground-nesting bee abundance is often highest near forested semi-natural habitat, due to better nest site availability and less disturbance (Kim et al. 2006). These forested areas also provide bees with floral resources that are critical when local crops are not flowering (Kremen et al. 2002a, Kim et al. 2006). In our study, response to non-crop flowering

trees suggests that diverse local floral resources are essential for robust ground-nesting bee communities.

General patterns

Overall, our results suggest that coffee flowering alone does not drive neotropical bee abundance and diversity within coffee agroforestry systems. Though coffee flowering provides a diverse spectrum of bees with abundant floral resources, these resources are only available in short bursts, often for only a few days at a time (Klein et al. 2003b, c, a, Ricketts 2004, Veddeler et al. 2006). Alternatively, non-crop vegetation, such as flowering field margins and hedge rows, have been shown to provide pollinators with floral resources during and outside of the crop flowering season (Marshall 2002, Carvell et al. 2004, Ockinger and Smith 2007, Dover and Settele 2009). Likewise, we found that shade trees were critical in supporting native bee diversity and abundance by providing alternative floral and nesting resources within agroforestry systems. While previous studies demonstrate that the flowering of understory plants (coffee and herbaceous plants) affects bee abundance and diversity within coffee farms (Klein et al. 2003c, De Marco and Coelho 2004, Veddeler et al. 2006), no studies have examined the role of shade tree management in supporting native bee communities.

Specifically, we found greater bee abundance and species richness in habitats with greater flowering tree richness. Across agricultural and non-agricultural landscapes, many studies have also shown that the diversity of flowering plant species is positively correlated with bee abundance and species richness (Banaszak 1996, Steffan-Dewenter and Tscharrntke 2001, Potts et al. 2003). In contrast, we did not find that floral density played a critical role in driving local bee abundance or diversity, as seen in a number of studies (Klein et al. 2003b, c, Hegland and Boeke 2006, Ebeling et al. 2008). Pollinators in our study may be more specialist in their floral preferences, as is common in tropical systems (Bawa et al. 1985, Bawa 1990), thus their abundance and diversity responds to increasing floral diversity more consistently than to increasing floral density. Given that increased bee diversity leads to increased fruit set for coffee (Klein et al. 2003b, Vergara and Badano 2009), our results indicate that diversifying trees within shade coffee farms may increase per bush coffee yields.

Conclusions

Positive responses to resource diversity and habitat heterogeneity abound in ecology. Across ecological systems and organisms, habitat heterogeneity is a key driver of patch colonization (Gustafson and Gardner 1996, Kindlmann and Burel 2008) and increased species richness and abundance (e.g., Macarthur and Macarthur 1961, Williams 1964, Murdoch et al. 1972, Shmida and Wilson 1985, Loyola and Martins 2008, Tylianakis et al. 2008). Bees and other animals that forage on multiple food sources across the year can use heterogeneous environments to meet seasonal requirements and maintain their foraging needs over extended periods of time (Rice et al. 1980, Karr and Freemark 1983, Griffis-Kyle and Beier 2005). For bees in particular, high habitat heterogeneity can lead to high species richness and abundance across the landscape (Steffan-Dewenter et al. 2002, Potts et al. 2003, Brosi et al. 2007, Ebeling et al. 2008, Loyola and Martins 2008, Krauss et al. 2009). Not surprisingly, across taxa, declines in habitat heterogeneity are the key drivers of biodiversity loss within agricultural landscapes (Benton et al. 2003).

For native bee communities within Mexican agroforestry systems, we found that floral and nesting resource heterogeneity was more critical than landscape-level forest cover. The most critical factors predictive of bee abundance were related to vegetation management within coffee farms, indicating that coffee farmers do not need to rely solely on landscape-level forest patches to provide pollinator resources. Instead, farmers can contribute to biodiversity conservation by creating more hospitable and resource rich agricultural matrices (i.e., Perfecto and Vandermeer 2008). Specifically, shade coffee farmers can promote bee abundance and diversity within their own farms by diversifying their shade trees, allowing these trees to age, and by creating a mosaic of light gaps and flowering herb patches that will attract foraging pollinators. Given the tremendous benefit of insect pollination to coffee yields, there is great incentive for farmers to improve agroforestry management, dually garnering ecosystem services and supporting biodiversity conservation.

ACKNOWLEDGEMENTS

We would like to thank Berry, Brosi, Luis Fernando Chavez, Beverly Rathcke, Ivette Perfecto, Mike Reiskind, Exzequel Velasquez, Gabriel Dominguez Martinez, the Vandermeer lab group, the Rathcke lab group, and the Perfecto lab group at the University of Michigan for help in the design and writing of this project. We would also like to thank Terry Griswold and Olivia Messenger at the USDA Bee Lab in Logan, Utah, for their generous assistance with bee identifications. Finally, we would like to express our gratitude to the people of *Nueva Alemania* for their permission and assistance in conducting this study. All experiments conducted were in compliance with current laws governing biodiversity protection in Mexico.

TABLES, FIGURES AND LEGENDS

Table 4.1 Local seasonal agroforestry characteristics at each study site.

Site (Map name)	Land Use Classification*	% Canopy cover	# of tree species (per m ²)	Tree density (per m ²)	Coffee density (per m ²)	Basal area overstory trees (per ha)
Irlanda (IR)	Traditional/ Commercial Polyculture	62.6	0.0018	0.0167	0.36	6.55
Rancho Allegre (RA)	Traditional/ Commercial Polyculture	59.5	0.0017	0.0216	0.32	3.44
Chiripa (CH)	Traditional/ Commercial Polyculture	28.4	0.0017	0.0162	0.43	4.44
Santa Anita (SA)	Commercial Polyculture	69.4	0.0013	0.0258	0.32	6.53
Genova (GE)	Commercial Polyculture/ Shade monoculture	13.2	0.0017	0.0177	0.26	4.03
Hamburgo North (HN)	Shade monoculture	36.0	0.0014	0.0153	0.45	3.50
Hamburgo South (HS)	Shade monoculture	29.8	0.0012	0.0151	0.46	3.96

* based on vegetation management (for additional details, see Philpott et al. 2008b)

Table 4.2 Species list of overstory trees (OT), overstory trees in flower (OTF) and understory plants in flower (UPF) recorded over the 12 week sampling period.

Family	Genus	Habit and Flowering
Agavaceae	<i>Yucca elephantipes</i>	OT
Anacardiaceae	<i>Mangifera indica</i>	OT
Apocynaceae	<i>Aspidosperma cruentum</i>	OT
Asteraceae	<i>Veronia deppeana</i>	OTF
	<i>Bidens pilosa</i>	UPF
	<i>Elephantopus mollis</i>	UPF
	<i>Galinsoga quadriradiata</i>	UPF
	<i>Impatiens walleriana</i>	UPF
	<i>Commelina diffusa</i>	UPF
	<i>Ipomoea sp.</i>	UPF
Balsaminaceae	<i>Ipomoea sp.</i>	UPF
Commelinaceae	<i>Bidens pilosa</i>	UPF
Convolvulaceae	<i>Elephantopus mollis</i>	UPF
Euphorbiaceae	<i>Alchornea latifolia</i>	OT
Fabaceae	<i>Desmodium sp.</i>	UPF
	<i>Schizolobium parahybum</i>	OT
	<i>Inga lauriana</i>	OT
	<i>Inga michelieae</i>	OTF
	<i>Inga vera</i>	OTF
	<i>Inga rodrigueziana</i>	OT
	<i>Pithecellobium sp</i>	OT
	<i>Persea americana</i>	OT
	<i>Cinnamomum sp.</i>	OT
	<i>Nectandra ambligens</i>	OT
Malvaceae	<i>Belotia mexicana</i>	OT
Melastomataceae	<i>Conostegia xalapensis</i>	OTF
	<i>Miconia argentea</i>	OTF
	<i>Melastoma sp.</i>	UPF
Meliaceae	<i>Cedrela odorata</i>	OT
	<i>Trichilia mauritiana</i>	OT
Moraceae	<i>Ficus glabrata</i>	OT
Myrtaceae	<i>Syzygium jambos</i>	OTF
Rubiaceae	<i>Borreria laevis</i>	UPF
Rutaceae	<i>Citrus sinesis</i>	OT
Solanaceae	<i>Cestrum nocturnum</i>	OTF
	<i>Browallia americana</i>	UPF
	<i>Solanum sp.</i>	UPF
Ulmaceae	<i>Trema micrantha</i>	OT
Verbenaceae	<i>Lantana camara</i>	UPF

Table 4.3 Species list of bees captured over the 12 week sampling period. Individuals listed as a ‘msp. MX#’ did not match any species within reference collections while ‘msp. # (T.G.)’ matched individuals within reference collections. Nesting (Nest) and Sociality (Soc) type are indicated as follows: ground (g), cavity (c), wood (w), social (x) and solitary (o).

Family	Tribe	Genus	Species	Nest	Soc
Anthophoridae	Eucerini	<i>Mellissodes</i>	<i>tapaneca</i>	g	o
Apidae	Apini	<i>Apis</i>	<i>mellifera, scutellata</i>	c	x
	Ceratinini	<i>Ceratina</i>	<i>eximia</i>	w	o
	Ceratinini	<i>Ceratina</i>	<i>ignara</i>	w	o
	Ceratinini	<i>Ceratina</i>	<i>msp. 4 (T.G.)</i>	w	o
	Ceratinini	<i>Ceratina</i>	<i>msp. MX1</i>	w	o
	Ceratinini	<i>Ceratina</i>	<i>trimaculata</i>	w	o
	Euglossini	<i>Euglossa</i>	<i>viridissima</i>	c	x
	Emphorini	<i>Melitoma</i>	<i>marginella</i>	g	o
	Emphorini	<i>Melitoma</i>	<i>msp. MX1</i>	g	o
	Meliponini	<i>Plebia</i>	<i>msp. 2 (T.G.)</i>	w	x
	Meliponini	<i>Trigona</i>	<i>fulviventris</i>	g	x
	Meliponini	<i>Trigonisca</i>	<i>schulthess</i>	c	x
Halictidae	Augochlorini	<i>Augochlora</i>	<i>aurifera</i>	w	x
	Augochlorini	<i>Augochlora</i>	<i>nigrocyanea</i>	w	x
	Augochlorini	<i>Augochlora</i>	<i>nominata</i>	w	x
	Augochlorini	<i>Augochlora</i>	<i>quiriguensis</i>	w	x
	Augochlorini	<i>Augochlora</i>	<i>sidaefoliae</i>	w	x
	Augochlorini	<i>Augochlora</i>	<i>msp. 1 (T.G.)</i>	w	x
	Augochlorini	<i>Augochlora</i>	<i>msp. 17 (T.G.)</i>	w	x
	Augochlorini	<i>Augochlora</i>	<i>msp. 6 (T.G.)</i>	w	x
	Augochlorini	<i>Augochlora</i>	<i>msp. 6 (T.G.)</i>	w	x
	Augochlorini	<i>Augochlorella</i>	<i>comis</i>	g	x
	Augochlorini	<i>Augochloropsis</i>	<i>msp. 10 (T.G.)</i>	g	x
	Augochlorini	<i>Augochloropsis</i>	<i>msp. 5 (T.G.)</i>	g	x
	Augochlorini	<i>Caenaugochlora</i>	<i>gemella</i>	g	x
	Halictini	<i>Dialictus</i>	<i>picadensis</i>	g	x
	Halictini	<i>Dialictus</i>	<i>msp. 12 (T.G.)</i>	g	x
	Halictini	<i>Dialictus</i>	<i>msp. 17 (T.G.)</i>	g	x
	Halictini	<i>Dialictus</i>	<i>msp. 19 (T.G.)</i>	g	x
	Halictini	<i>Dialictus</i>	<i>msp. 21 (T.G.)</i>	g	x
	Halictini	<i>Dialictus</i>	<i>msp. 37 (T.G.)</i>	g	x
	Halictini	<i>Dialictus</i>	<i>msp. 42 (T.G.)</i>	g	x
	Halictini	<i>Dialictus</i>	<i>msp. 49 (T.G.)</i>	g	x
	Halictini	<i>Dialictus</i>	<i>msp. MX1</i>	g	x
	Halictini	<i>Dialictus</i>	<i>msp. MX2</i>	g	x
	Halictini	<i>Dialictus</i>	<i>msp. MX3</i>	g	x
	Halictini	<i>Dialictus</i>	<i>msp. MX4</i>	g	x

Halictini	<i>Dialictus</i>	<i>sp. MX5</i>	gg	X
Halictini	<i>Dialictus</i>	<i>sp. MX6</i>	gg	X
Halictini	<i>Dialictus</i>	<i>sp. MX7</i>	gg	X
Halictini	<i>Dialictus</i>	<i>sp. MX8</i>	gg	X
Halictini	<i>Halictus</i>	<i>hesperus</i>	gg	X
Halictini	<i>Halictus</i>	<i>sp. MX1</i>	g	X

Figure 4.1 Study sites in *Nueva Alemania*, a region in the southern portion of the state of Chiapas, outlined in black within the inserted map of Mexico.

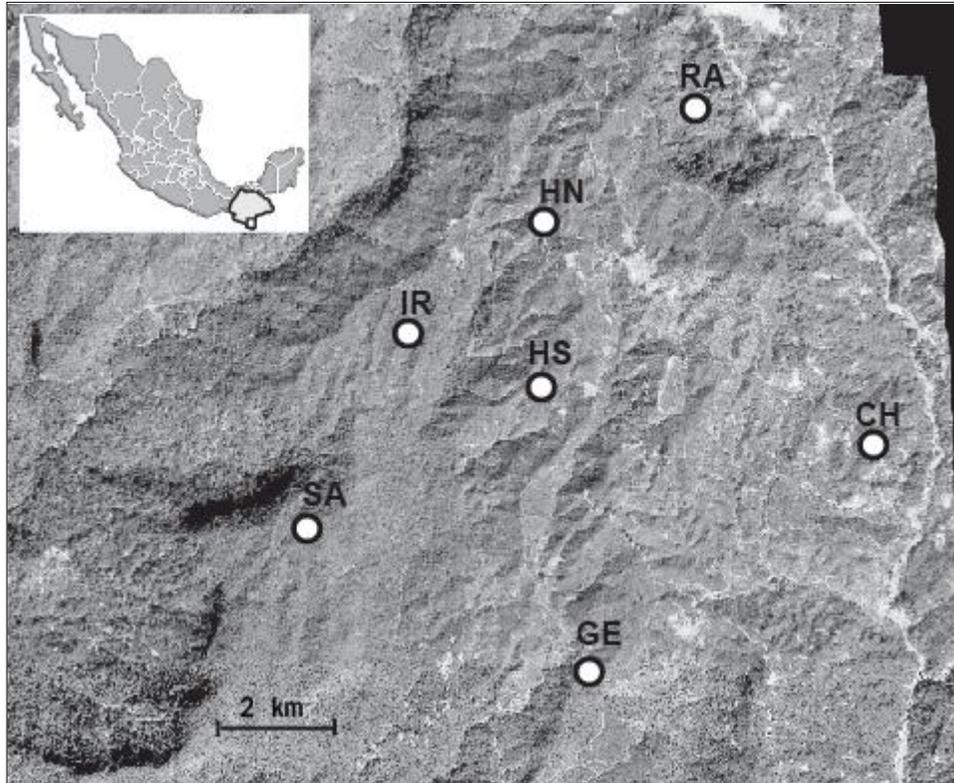


Figure 4.2 Photo of a shaded coffee agroforestry system, Finca Irlanda, where coffee bushes line the understory, and shade trees fill the canopy. For scale, the author stands on the bottom center of the photo.

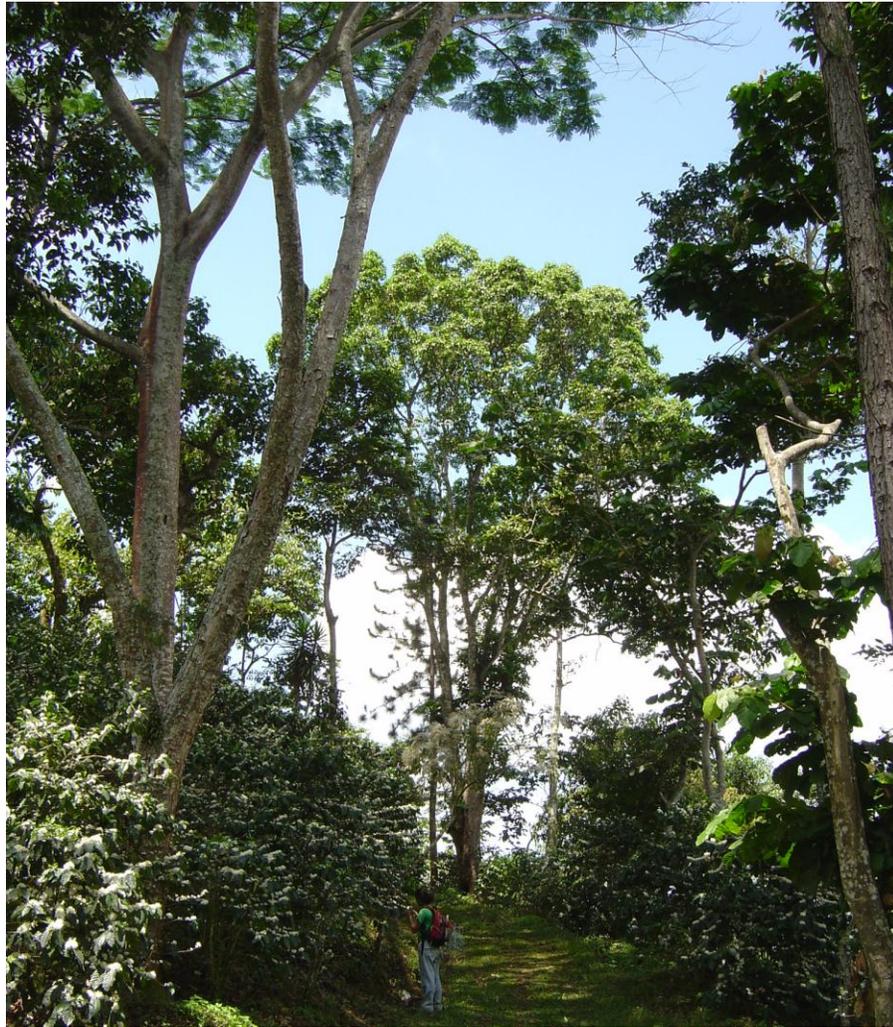


Figure 4.3 Observed species accumulation curve reaching 46 species.

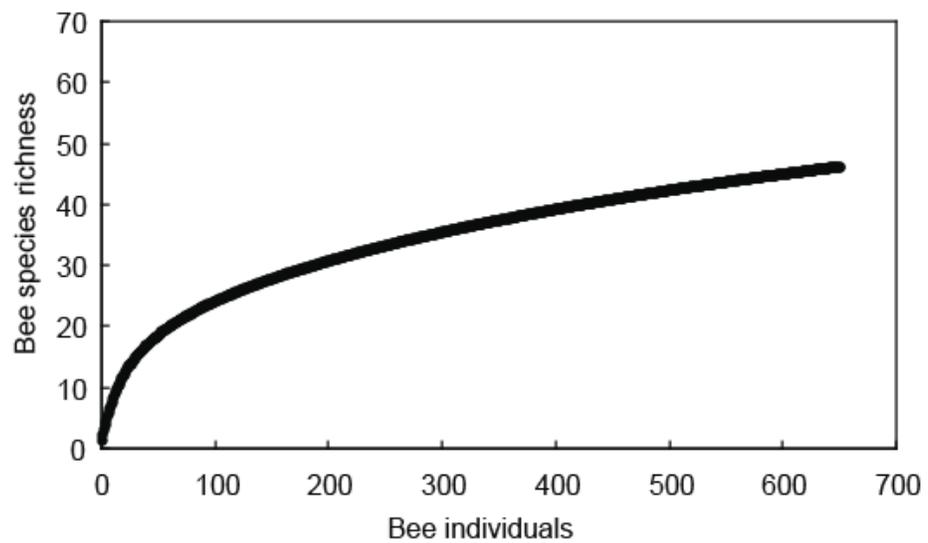


Figure 4.4 Bees are more abundant and their communities are more species rich in habitats with a greater number of tree species (linear regression for illustration)

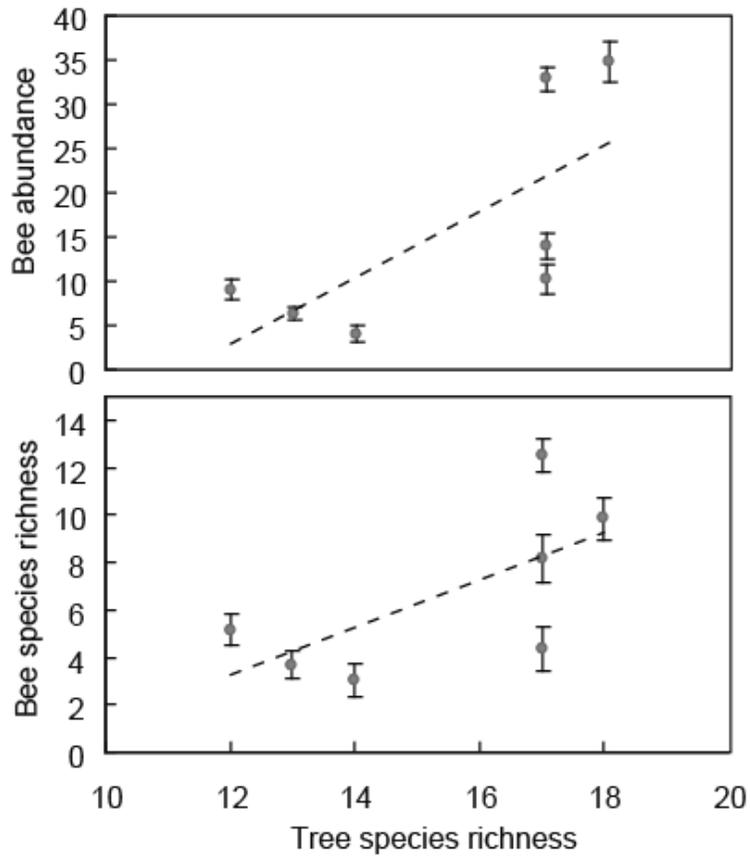


Figure 4.5 Bee community composition across a gradient of (A) tree species and (B) local forest cover. The three most dominant tribes are labeled, while the total number of tribes in each group is noted at the top right of each column.

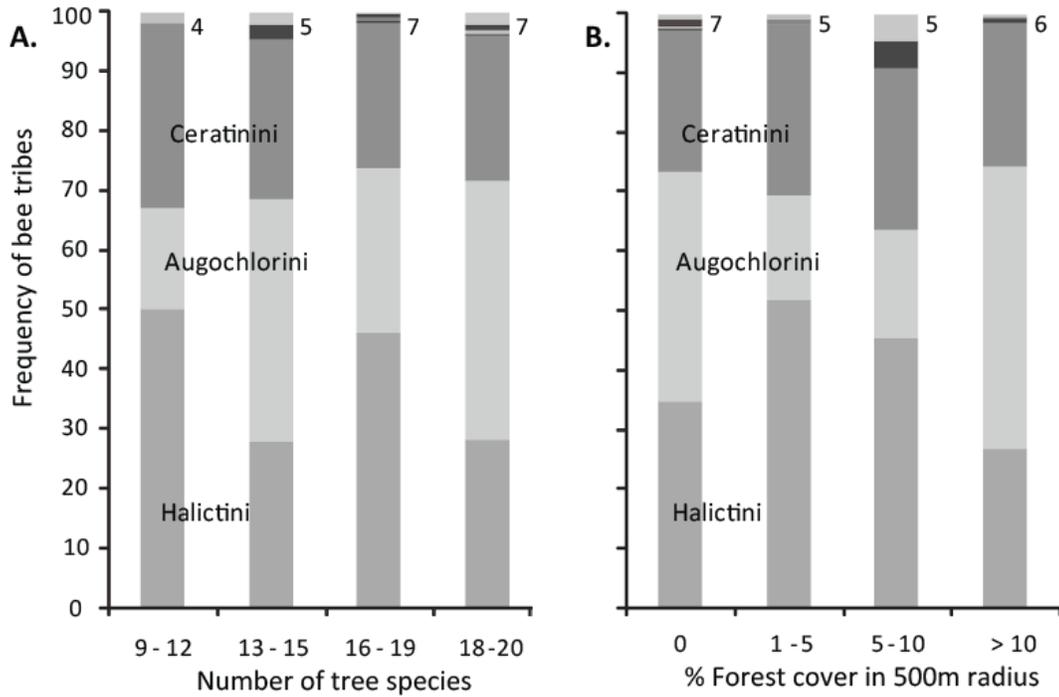


Figure 4.6 Optimal regression trees for (A) solitary bees and (B) social bees. The terminal nodes are labeled with the mean rating and the actual bee abundance (in parenthesis) while the importance of each predictor variable corresponds to the length of the vertical line below each split. The criterion listed at each split designates the left hand side of the branch, while observations falling outside of the criterion designate the right side of the branch. The total variation explained (sums of squares for each node) is included at the bottom of each tree.

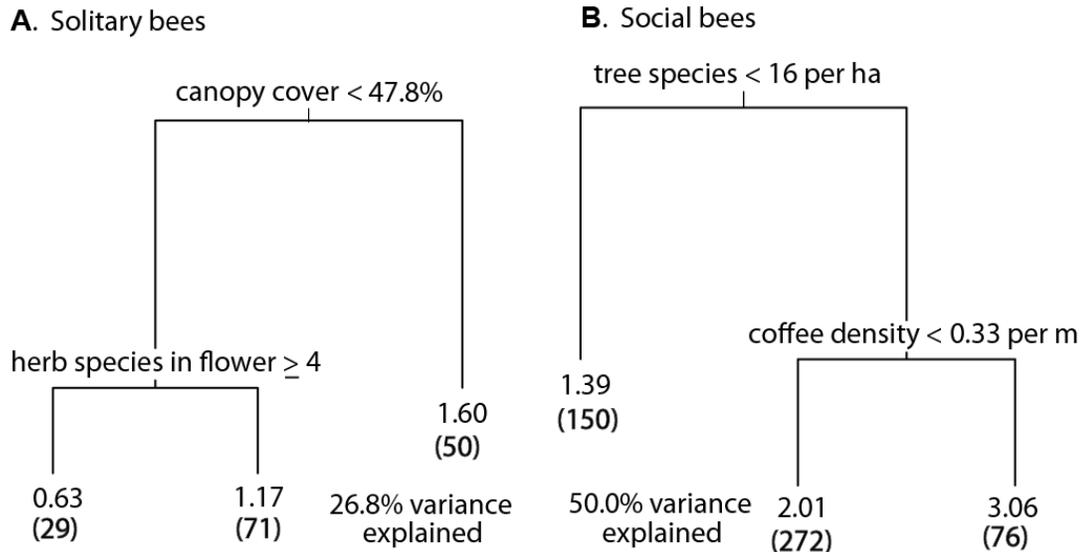


Figure 4.7 Optimal regression trees for each tribe, (A) Halictini, (B) Augochlorini, and (C) Ceratinini. The terminal nodes are labeled with the mean rating and the actual bee abundance in each group (in parenthesis) while the importance of each predictor variable corresponds to the length of the vertical line below each split. The criterion listed at each split designates the left hand side of the branch, while observations falling outside of the criterion designate the right side of the branch. The total variation explained (sums of squares for each node) is included at the bottom of each tree.

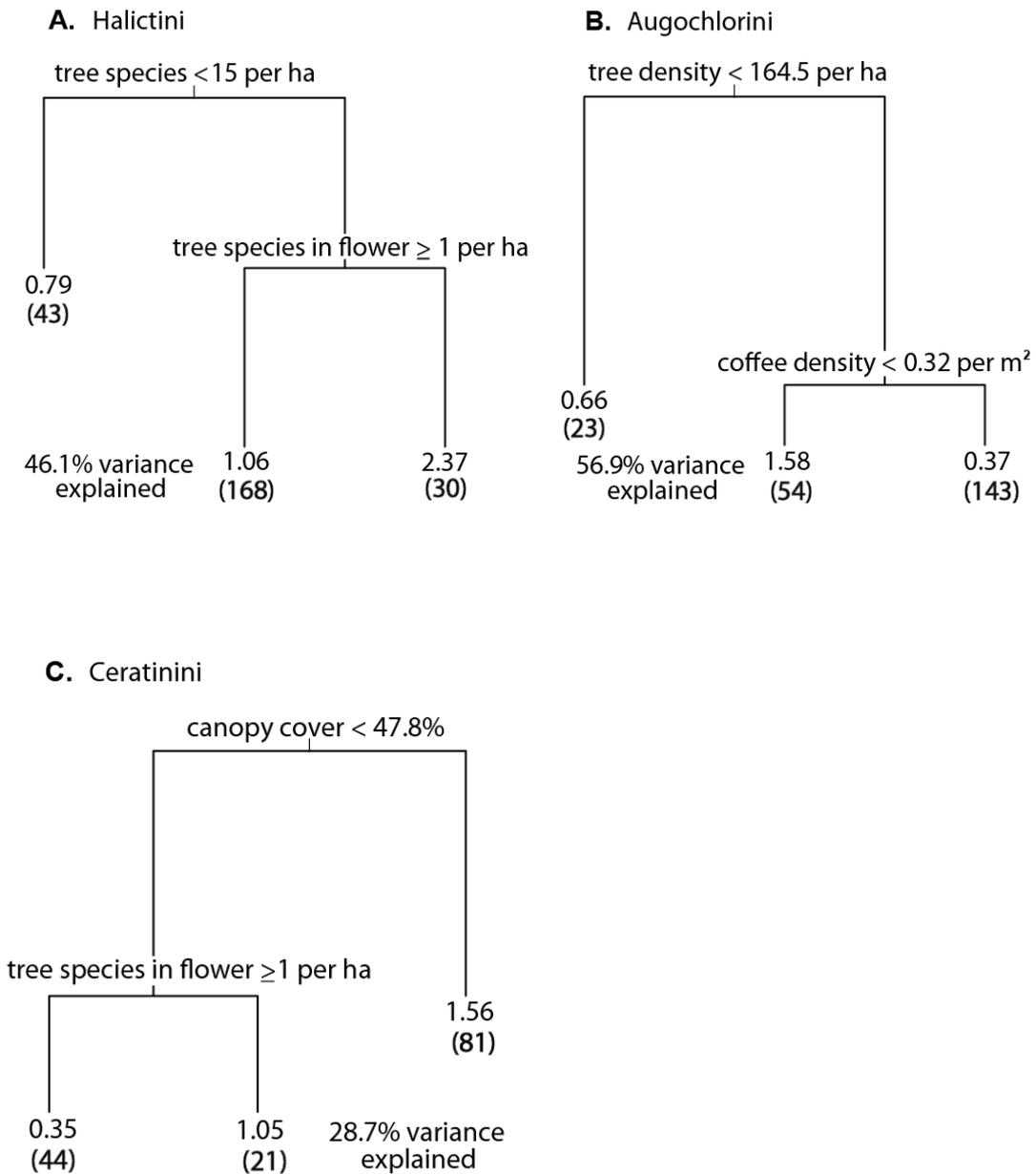
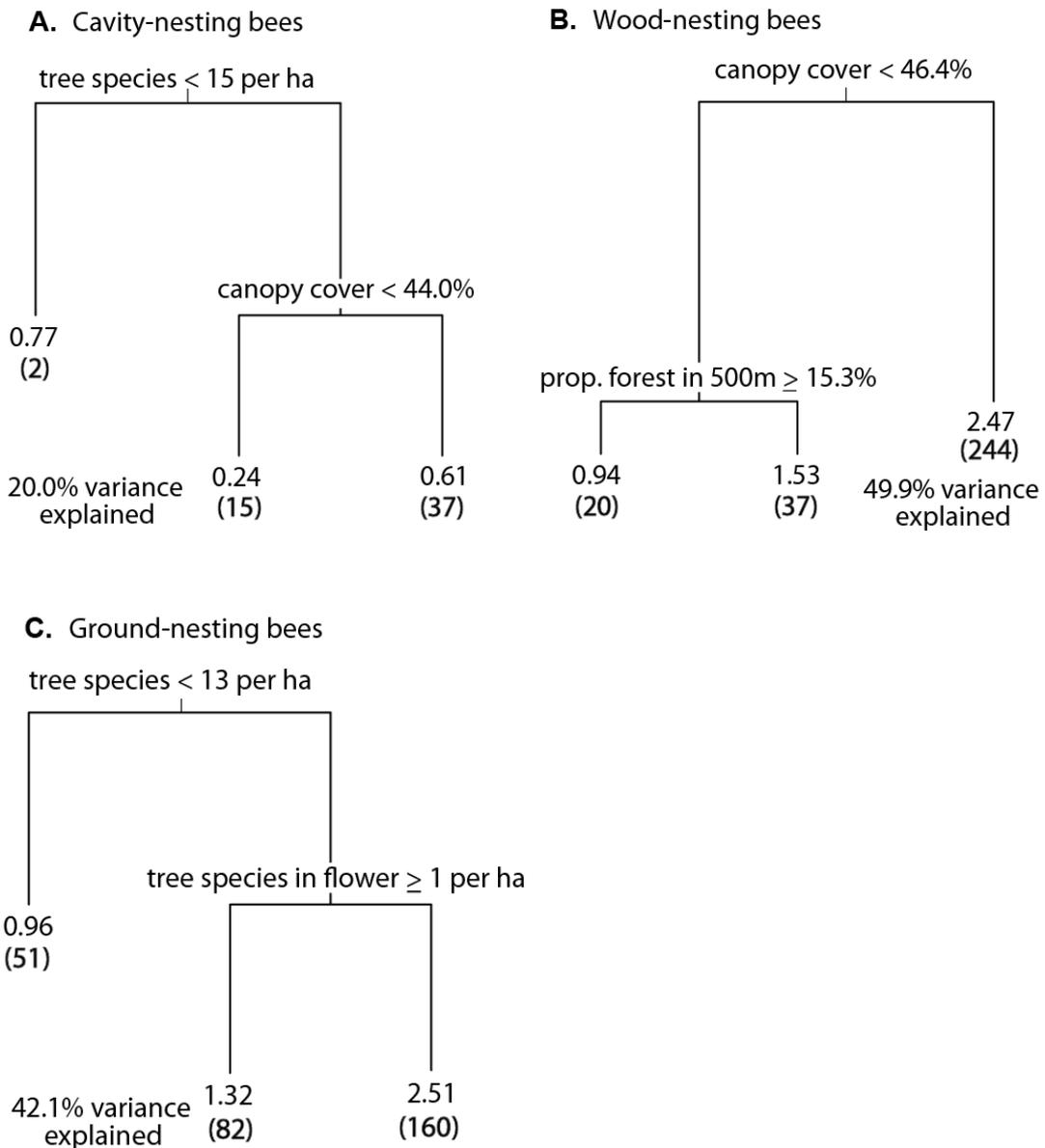


Figure 4.8 Optimal regression trees for each nesting guild, (A) cavity-nesting bees, (B) wood-nesting bees, and (C) ground-nesting bees. The terminal nodes are labeled with the mean rating and the actual bee abundance in each group (in parenthesis) while the importance of each predictor variable corresponds to the length of the vertical line below each split. The criterion listed at each split designates the left hand side of the branch, while observations falling outside of the criterion designate the right side of the branch. The total variation explained (sums of squares for each node) is included at the bottom of each tree.



Chapter IV References

2005. R: A language and environment for statistical computing. R Development Core Team. R Development Core Team, R Foundation for Statistical Computing, URL <http://www.R-project.org>, Vienna, Austria.
- Allen-Wardell, G., P. Bernhardt, R. Bitner, A. Burquez, S. Buchmann, J. Cane, P. A. Cox, V. Dalton, P. Feinsinger, M. Ingram, D. Inouye, C. E. Jones, K. Kennedy, P. Kevan, H. Koopowitz, R. Medellin, S. Medellin-Morales, G. P. Nabhan, B. Pavlik, V. Tepedino, P. Torchio, and S. Walker. 1998. The potential consequences of pollinator declines on the conservation of biodiversity and stability of food crop yields. *Conservation Biology* **12**:8-17.
- Banaszak, J. 1996. Ecological bases of conservation of wild bees. Pages 55-62 in A. Matheson, S. Buchmann, C. O'Toole, P. Westrich, and I. Williams, editors. *The Conservation of Bees*. Academic Press, London.
- Bawa, K. S. 1990. *Reproductive Ecology of Tropical Forest Plants*. Unesco and Parthenon Press, Paris and USA.
- Bawa, K. S., S. H. Bullock, D. R. Perry, R. E. Coville, and M. H. Grayum. 1985. Reproductive-Biology of Tropical Lowland Rain-Forest Trees .2. Pollination Systems. *American Journal of Botany* **72**:346-356.
- Benton, T. G., J. A. Vickery, and J. D. Wilson. 2003. Farmland biodiversity: is habitat heterogeneity the key? *Trends in Ecology & Evolution* **18**:182-188.
- Biesmeijer, J. C., S. P. M. Roberts, M. Reemer, R. Ohlemuller, M. Edwards, T. Peeters, A. P. Schaffers, S. G. Potts, R. Kleukers, C. D. Thomas, J. Settele, and W. E. Kunin. 2006. Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science* **313**:351-354.
- Breiman, L., J. Freidman, R. Olshen, and C. Stone. 1984. *Classification and Regression Trees*. Wadsworth, Belmont, CA.
- Brosi, B. J., A. Smith-Pardo, and V. H. Gonzalez. 2006. A new wood-nesting *Neocorynura* (Hymenoptera : Halictidae : Augochlorini) from Costa Rica, with notes on its biology. *Zootaxa*:55-68.
- Buchman, G. P., and Nabhan, S.L. 1996. *The Forgotten Pollinators*. Island Press/Shearwater Books.
- Cardinale, B. J., J. J. Weis, A. E. Forbes, K. J. Tilmon, and A. R. Ives. 2006. Biodiversity as both a cause and consequence of resource availability: a study of reciprocal causality in a predator-prey system. *Journal of Animal Ecology* **75**:497-505.
- Carvell, C., W. R. Meek, R. F. Pywell, and M. Nowakowski. 2004. The response of foraging bumblebees to successional change in newly created arable field margins. *Biological Conservation* **118**:327-339.
- Chacoff, N. P. and M. A. Aizen. 2006. Edge effects on flower-visiting insects in grapefruit plantations bordering premontane subtropical forest. *Journal of Applied Ecology* **43**:18-27.
- Challenger, A. 1998. *Utilización y conservación de los ecosistemas terrestres de México. Pasado, presente y futuro*. México. Conabio, IBUNAM y Agrupación Sierra Madre.
- Chao, A. 1987. Estimating the Population-Size for Capture Recapture Data with Unequal Catchability. *Biometrics* **43**:783-791.

- Colwell, R. K. and J. A. Coddington. 1994. Estimating Terrestrial Biodiversity through Extrapolation. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **345**:101-118.
- De'ath, G. and K. E. Fabricius. 2000. Classification and regression trees: A powerful yet simple technique for ecological data analysis. *Ecology* **81**:3178-3192.
- De Marco, P. and F. M. Coelho. 2004. Services performed by the ecosystem: forest remnants influence agricultural cultures' pollination and production. *Biodiversity and Conservation* **13**:1245-1255.
- Donald, P. F. 2004. Biodiversity impacts of some agricultural commodity production systems. *Conservation Biology* **18**:17-37.
- Dover, J. and J. Settele. 2009. The influences of landscape structure on butterfly distribution and movement: a review. *Journal of Insect Conservation* **13**:3-27.
- Ebeling, A., A. M. Klein, J. Schumacher, W. W. Weisser, and T. Tschardtke. 2008. How does plant richness affect pollinator richness and temporal stability of flower visits? *Oikos* **117**:1808-1815.
- Eickwort, G. C. and S. F. Sakagami. 1979. Classification of nest architecture of bees in the trip Augochlorini(Hymenoptera, Halictidae, Halictinae), with description of a Brazilian nest of *Rhinocorynura inflaticeps*. *Biotropica* **11**:28-37.
- Engel, M. S. 2000. Classification of the bee tribe Augochlorini (Hymenoptera : Halictidae). *Bulletin of the American Museum of Natural History*:4-89.
- Fenster, C. B., W. S. Armbruster, P. Wilson, M. R. Dudash, and J. D. Thomson. 2004. Pollination syndromes and floral specialization. *Annual Review of Ecology Evolution and Systematics* **35**:375-403.
- Geist, H. J. and E. F. Lambin. 2002. Proximate causes and underlying driving forces of tropical deforestation. *Bioscience* **52**:143-150.
- Ghazoul, J. 2005. Response to Steffan-Dewenter et al.: Questioning the global pollination crisis. *Trends in Ecology & Evolution* **20**:652-653.
- Greenberg, R., P. Bichier, A. C. Angon, C. MacVean, R. Perez, and E. Cano. 2000. The impact of avian insectivory on arthropods and leaf damage in some Guatemalan coffee plantations. *Ecology* **81**:1750-1755.
- Greenleaf, S. S. and C. Kremen. 2006. Wild bee species increase tomato production and respond differently to surrounding land use in Northern California. *Biological Conservation* **133**:81-87.
- Greenleaf, S. S., N. M. Williams, R. Winfree, and C. Kremen. 2007. Bee foraging ranges and their relationship to body size. *Oecologia* **153**:589-596.
- Griffis-Kyle, K. L. and P. Beier. 2005. Migratory strategy and seasonal patterns of bird diversity in relation to forest habitat. *American Midland Naturalist* **153**:436-443.
- Gustafson, E. J. and R. H. Gardner. 1996. The effect of landscape heterogeneity on the probability of patch colonization. *Ecology* **77**:94-107.
- Hegland, S. J. and L. Boeke. 2006. Relationships between the density and diversity of floral resources and flower visitor activity in a temperate grassland community. *Ecological Entomology* **31**:532-538.
- Hoehn, P., T. Tschardtke, J. M. Tylianakis, and I. Steffan-Dewenter. 2008. Functional group diversity of bee pollinators increases crop yield. *Proceedings of the Royal Society B-Biological Sciences* **275**:2283-2291.

- Hooper, D. U., F. S. Chapin, J. J. Ewel, A. Hector, P. Inchausti, S. Lavorel, J. H. Lawton, D. M. Lodge, M. Loreau, S. Naeem, B. Schmid, H. Setälä, A. J. Symstad, J. Vandermeer, and D. A. Wardle. 2005. Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. *Ecological Monographs* **75**:3-35.
- Johnson, R. 2007. CRS report for congress: recent honey bee colony declines (Government report prepared for members of committees of congress RL33938). *in* R. AiAE, Science and Industry Division, editor., Washington, DC.
- Karr, J. R. and K. E. Freemark. 1983. Habitat selection and environmental gradients - dynamics in the stable tropics. *Ecology* **64**:1481-1494.
- Kearns, C. A. and D. W. Inoué. 1993. *Techniques for Pollination Biologists*. University Press of Colorado, Niwot, Colorado, USA.
- Kearns, C. A., D. W. Inoué, and N. M. Waser. 1998. Endangered mutualisms: The conservation of plant-pollinator interactions. *Annual Review of Ecology and Systematics* **29**:83-112.
- Kim, J., N. Williams, and C. Kremen. 2006. Effects of cultivation and proximity to natural habitat on ground-nesting native bees in California sunflower fields. *Journal of the Kansas Entomological Society* **79**:309-320.
- Kindlmann, P. and F. Burel. 2008. Connectivity measures: a review. *Landscape Ecology* **23**:879-890.
- Klein, A. M., S. A. Cunningham, M. Bos, and I. Steffan-Dewenter. 2008. Advances in pollination ecology from tropical plantation crops. *Ecology* **89**:935-943.
- Klein, A. M., I. Steffan-Dewenter, D. Buchori, and T. Tschardt. 2002. Effects of land-use intensity in tropical agroforestry systems on coffee flower-visiting and trap-nesting bees and wasps. *Conservation Biology* **16**:1003-1014.
- Klein, A. M., I. Steffan-Dewenter, and T. Tschardt. 2003a. Bee pollination and fruit set of *Coffea arabica* and *C. canephora* (Rubiaceae). *American Journal of Botany* **90**:153-157.
- Klein, A. M., I. Steffan-Dewenter, and T. Tschardt. 2003b. Fruit set of highland coffee increases with the diversity of pollinating bees. *Proceedings of the Royal Society of London Series B-Biological Sciences* **270**:955-961.
- Klein, A. M., I. Steffan-Dewenter, and T. Tschardt. 2003c. Pollination of *Coffea canephora* in relation to local and regional agroforestry management. *Journal of Applied Ecology* **40**:837-845.
- Klein, A. M., I. Steffan-Dewenter, and T. Tschardt. 2006. Rain forest promotes trophic interactions and diversity of trap-nesting hymenoptera in adjacent agroforestry. *Journal of Animal Ecology* **75**:315-323.
- Klein, A. M., B. E. Vaissière, J. H. Cane, I. Steffan-Dewenter, S. A. Cunningham, C. Kremen, and T. Tschardt. 2007. Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B-Biological Sciences* **274**:303-313.
- Krauss, J., T. Alfert, and I. Steffan-Dewenter. 2009. Habitat area but not habitat age determines wild bee richness in limestone quarries. *Journal of Applied Ecology* **46**:194-202.
- Kremen, C., R. Bugg, N. Nicola, S. SA, R. Thorp, and N. Williams. 2002a. Native bees, native plants and crop pollination in California. *Fremontia* **30**:41-49.

- Kremen, C., N. M. Williams, M. A. Aizen, B. Gemmill-Herren, G. LeBuhn, R. Minckley, L. Packer, S. G. Potts, T. Roulston, I. Steffan-Dewenter, D. P. Vazquez, R. Winfree, L. Adams, E. E. Crone, S. S. Greenleaf, T. H. Keitt, A. M. Klein, J. Regetz, and T. H. Ricketts. 2007. Pollination and other ecosystem services produced by mobile organisms: a conceptual framework for the effects of land-use change. *Ecology Letters* **10**:299-314.
- Kremen, C., N. M. Williams, R. L. Bugg, J. P. Fay, and R. W. Thorp. 2004. The area requirements of an ecosystem service: crop pollination by native bee communities in California. *Ecology Letters* **7**:1109-1119.
- Kremen, C., N. M. Williams, and R. W. Thorp. 2002b. 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.
- Lambin, E. F., H. J. Geist, and E. Lepers. 2003. Dynamics of land-use and land-cover change in tropical regions. *Annual Review of Environment and Resources* **28**:205-241.
- Larsen, T. H., N. Williams, and C. Kremen. 2005. Extinction order and altered community structure rapidly disrupt ecosystem functioning. *Ecology Letters* **8**:538-547.
- LeBuhn, G., S. Droege, N. Williams, R. Minckley, T. Griswold, C. Kremen, O. Messinger, J. Cane, T. Roulston, F. Parker, V. Tepedino, and S. Buchmann. 2005. A standardized method for monitoring bee populations- The Bee Inventory Plot. available online at <http://online.sfsu.edu/~beeplot/>.
- Levin, S. A. 1998. Ecosystems and the biosphere as complex adaptive systems. *Ecosystems* **1**:431-436.
- Lin, B. B. 2007. Agroforestry management as an adaptive strategy against potential microclimate extremes in coffee agriculture. *Agricultural and Forest Meteorology* **144**:85-94.
- Lin, B. B. and P. L. Richards. 2007. Soil random roughness and depression storage on coffee farms of varying shade levels. *Agricultural Water Management* **92**:194-204.
- Loyola, R. D. and R. P. Martins. 2008. Habitat structure components are effective predictors of trap-nesting Hymenoptera diversity. *Basic and Applied Ecology* **9**:735-742.
- MacArthur, R. and J. W. MacArthur. 1961. On bird species-diversity. *Ecology* **42**:594-&.
- Marshall, E. J. P. 2002. Field margins in northern Europe: Integrating agricultural, environmental and biodiversity functions. Pages 39-67 in *Symposium on Field Boundary Habitats*. Canadian Weed Science Soc, Soc Canadienne Malherbologie, Saskatoon, CANADA.
- McIntosh, M. 1996. Nest-substrate preferences of the twig-nesters *Ceratina acantha*, *Ceratina nanula* (Apidae) and *Pemphredon lethifer* (Sphecidae). *Journal of the Kansas Entomological Society* **69**:216-231.
- Michener, C. D. 2000. *Bees of the World*. Johns Hopkins University Press, Baltimore, MD.
- Moguel, P. and V. M. Toledo. 1999. Biodiversity conservation in traditional coffee systems of Mexico. *Conservation Biology* **13**:11-21.

- Morgan, J. N. and J. A. Sonquist. 1963. Problems in analysis of survey data, and a proposal. *Journal of the American Statistical Association* **58**:415-&.
- Murdoch, W. W., C. H. Peterson, and F. C. Evans. 1972. Diversity and Pattern in Plants and Insects. *Ecology* **53**:819-&.
- Myers, N., R. A. Mittermeier, C. G. Mittermeier, G. A. B. da Fonseca, and J. Kent. 2000. Biodiversity hotspots for conservation priorities. *Nature* **403**:853-858.
- Ockinger, E. and H. G. Smith. 2007. Semi-natural grasslands as population sources for pollinating insects in agricultural landscapes. *Journal of Applied Ecology* **44**:50-59.
- Okazaki, K. 1992. Nesting habits of the small carpenter bee, *Ceratina dentipes*, in Hengchun Peninsula, Southern Taiwan. *Journal of the Kansas Entomological Society* **65**:190-195.
- Olden, J. D., J. J. Lawler, and N. L. Poff. 2008. Machine learning methods without tears: A primer for ecologists. *Quarterly Review of Biology* **83**:171-193.
- Oliver, I. and A. J. Beattie. 1996. Invertebrate morphospecies as surrogates for species: a case study. *Conservation Biology* **10**:99-109.
- Pasquet, R. S., A. Peltier, M. B. Hufford, E. Oudin, J. Saulnier, L. Paul, J. T. Knudsen, H. R. Herren, and P. Gepts. 2008. Long-distance pollen flow assessment through evaluation of pollinator foraging range suggests transgene escape distances. *Proceedings of the National Academy of Sciences of the United States of America* **105**:13456-13461.
- Perfecto, I., R. A. Rice, R. Greenberg, and M. E. VanderVoort. 1996. Shade coffee: A disappearing refuge for biodiversity. *Bioscience* **46**:598-608.
- Perfecto, I. and J. Vandermeer. 2008. Biodiversity conservation in tropical agroecosystems - A new conservation paradigm. Pages 173-200 *Year in Ecology and Conservation Biology 2008*. Blackwell Publishing, Oxford.
- Perfecto, I., J. H. Vandermeer, G. L. Bautista, G. I. Nunez, R. Greenberg, P. Bichier, and S. Langridge. 2004. Greater predation in shaded coffee farms: The role of resident neotropical birds. *Ecology* **85**:2677-2681.
- Philpott, S. M., W. J. Arendt, I. Armbrecht, P. Bichier, T. V. Diestch, C. Gordon, R. Greenberg, I. Perfecto, R. Reynoso-Santos, L. Soto-Pinto, C. Tejada-Cruz, G. Williams-Linera, J. Valenzuela, and J. M. Zolotoff. 2008a. Biodiversity Loss in Latin American Coffee Landscapes: Review of the Evidence on Ants, Birds, and Trees. *Conservation Biology* **22**:1093-1105.
- Philpott, S. M., B. Lin, S. Jha, and S. J. Brines. 2008b. A multi-scale assessment of hurricane impacts on agricultural landscapes based on landuse and topographic features. *Agriculture, Ecosystems and Environment* **128**:12-20.
- Plateaux-Quenu, C. 2008. Subsociality in halictine bees. *Insectes Sociaux* **55**:335-346.
- Potts, S. G., B. Vulliamy, A. Dafni, G. Ne'eman, and P. Willmer. 2003. Linking bees and flowers: How do floral communities structure pollinator communities? *Ecology* **84**:2628-2642.
- Potts, S. G., B. Vulliamy, S. Roberts, C. O'Toole, A. Dafni, G. Ne'Eman, and P. Willmer. 2005. Role of nesting resources in organising diverse bee communities in a Mediterranean landscape. *Ecological Entomology* **30**:78-85.

- Ramirez-Marcial, N., M. Gonzalez-Espinosa, and G. Williams-Linera. 2001. Anthropogenic disturbance and tree diversity in Montane Rain Forests in Chiapas, Mexico. *Forest Ecology and Management* **154**:311-326.
- Recknagel, F. 2001. Applications of machine learning to ecological modelling. *Ecological Modelling* **146**:303-310.
- Rice, J., B. W. Anderson, and R. D. Ohmart. 1980. Seasonal habitat selection by birds in the lower Colorado river valley. *Ecology* **61**:1402-1411.
- Ricketts, T. H. 2004. Tropical forest fragments enhance pollinator activity in nearby coffee crops. *Conservation Biology* **18**:1262-1271.
- Ricketts, T. H., G. C. Daily, P. R. Ehrlich, and C. D. Michener. 2004. Economic value of tropical forest to coffee production. *Proceedings of the National Academy of Sciences of the United States of America* **101**:12579-12582.
- Ricketts, T. H., J. Regetz, I. Steffan-Dewenter, S. A. Cunningham, C. Kremen, A. Bogdanski, B. Gemmill-Herren, S. S. Greenleaf, A. M. Klein, M. M. Mayfield, L. A. Morandin, A. Ochieng, and B. F. Viana. 2008. Landscape effects on crop pollination services: are there general patterns? *Ecology Letters* **11**:499-515.
- Roubik, D. 1995. *Pollination of cultivated plants in the tropics*. Food and Agriculture Organization of the United Nations, Rome, Italy.
- Sakagami, S. and C. Michener. 1962. *The Nest Architecture of Sweat Bees*. University of Kansas Press, Lawrence, Kansas, U.S.A.
- Sakagami, S. F. and Y. Maeta. 1977. Some presumably pre-social habits of Japanese *Ceratina* bees, with notes on various social types in Hymenoptera. *Insectes Sociaux* **24**:319-343.
- Schmidt, J. O. and S. C. Thoenes. 1992. Criteria for nest site selection in honey-bees (Hymenoptera, Apidae) - preferences between pheromone attractants and cavity shapes. *Environmental Entomology* **21**:1130-1133.
- Shmida, A. and M. V. Wilson. 1985. Biological determinants of species-diversity. *Journal of Biogeography* **12**:1-20.
- Sokal, R. R., Rohlf, FJ 1995. *Biometry: the principles and practice of statistics in biological research*. 3rd edition. WH Freeman, New York, N.Y.
- Soto-Pinto, L., Y. Romero-Alvarado, J. Caballero-Nieto, and G. S. Warnholtz. 2001. Woody plant diversity and structure of shade-grown-coffee plantations in Northern Chiapas, Mexico. *Revista de Biología Tropical* **49**:977-987.
- Steffan-Dewenter, I., U. Munzenberg, C. Burger, C. Thies, and T. Tschardtke. 2002. Scale-dependent effects of landscape context on three pollinator guilds. *Ecology* **83**:1421-1432.
- Steffan-Dewenter, I., U. Munzenberg, and T. Tschardtke. 2001. Pollination, seed set and seed predation on a landscape scale. *Proceedings of the Royal Society of London Series B-Biological Sciences* **268**:1685-1690.
- Steffan-Dewenter, I., S. G. Potts, and L. Packer. 2005. Pollinator diversity and crop pollination services are at risk. *Trends in Ecology & Evolution* **20**:651-652.
- Steffan-Dewenter, I. and T. Tschardtke. 2001. Succession of bee communities on fallows. *Ecography* **24**:83-93.
- Steffan-Dewenter, I. and C. Westphal. 2008. The interplay of pollinator diversity, pollination services and landscape change. *Journal of Applied Ecology* **45**:737-741.

- Tylianakis, J. M., A. M. Klein, T. Lozada, and T. Tscharntke. 2006. Spatial scale of observation affects alpha, beta and gamma diversity of cavity-nesting bees and wasps across a tropical land-use gradient. *Journal of Biogeography* **33**:1295-1304.
- Tylianakis, J. M., A. M. Klein, and T. Tscharntke. 2005. Spatiotemporal variation in the diversity of hymenoptera across a tropical habitat gradient. *Ecology* **86**:3296-3302.
- Tylianakis, J. M., T. A. Rand, A. Kahmen, A. M. Klein, N. Buchmann, J. Perner, and T. Tscharntke. 2008. Resource heterogeneity moderates the biodiversity-function relationship in real world ecosystems. *PLoS Biology* **6**:947-956.
- Van Bael, S. A., S. M. Philpott, R. Greenberg, P. Bichier, N. A. Barber, K. A. Mooney, and D. S. Gruner. 2008. Birds as predators in tropical agroforestry systems. *Ecology* **89**:928-934.
- Veddeler, D., A. M. Klein, and T. Tscharntke. 2006. Contrasting responses of bee communities to coffee flowering at different spatial scales. *Oikos* **112**:594-601.
- Vergara, C. H. and E. I. Badano. 2009. Pollinator diversity increases fruit production in Mexican coffee plantations: The importance of rustic management systems. *Agriculture Ecosystems & Environment* **129**:117-123.
- Waser, N. M., L. Chittka, M. V. Price, N. M. Williams, and J. Ollerton. 1996. Generalization in pollination systems, and why it matters. *Ecology* **77**:1043-1060.
- Wcislo, W. T., V. H. Gonzalez, and M. S. Engel. 2003. Nesting and social behavior of a wood-dwelling neotropical bee, *Augochlora isthmii* (Schwarz), and notes on a new species, *A-alexanderi* Engel (Hymenoptera : Halictidae). *Journal of the Kansas Entomological Society* **76**:588-602.
- Westphal, C., R. Bommarco, G. Carre, E. Lamborn, N. Morison, T. Petanidou, S. G. Potts, S. P. M. Roberts, H. Szentgyorgyi, T. Tscheulin, B. E. Vaissiere, M. Woyciechowski, J. C. Biesmeijer, W. E. Kunin, J. Settele, and I. Steffan-Dewenter. 2008. Measuring bee diversity in different European habitats and biogeographical regions. *Ecological Monographs* **78**:653-671.
- Westphal, C., I. Steffan-Dewenter, and T. Tscharntke. 2003. Mass flowering crops enhance pollinator densities at a landscape scale. *Ecology Letters* **6**:961-965.
- Williams-Guillen, K., I. Perfecto, and J. Vandermeer. 2008. Bats limit insects in a neotropical agroforestry system. *Science* **320**:70-70.
- Williams, C. 1964. Patterns in the balance of nature and related problems in quantitative ecology. Academic Press, London.
- Winfree, R. 2008. Pollinator-Dependent Crops: An Increasingly Risky Business. *Current Biology* **18**:R968-R969.
- Winfree, R. and C. Kremen. 2009. Are ecosystem services stabilized by differences among species? A test using crop pollination. *Proceedings of the Royal Society B-Biological Sciences* **276**:229-237.
- Winfree, R., N. M. Williams, J. Dushoff, and C. Kremen. 2007. Native bees provide insurance against ongoing honey bee losses. *Ecology Letters* **10**:1105-1113.
- Winfree, R., N. M. Williams, H. Gaines, J. S. Ascher, and C. Kremen. 2008. Wild bee pollinators provide the majority of crop visitation across land-use gradients in New Jersey and Pennsylvania, USA. *Journal of Applied Ecology* **45**:793-802.
- Wuellner, C. T. 1999. Nest site preference and success in a gregarious, ground-nesting bee *Dieunomia triangulifera*. *Ecological Entomology* **24**:471-479.

Chapter V: Isolation and characterization of nine microsatellite loci for the tropical understory tree *Miconia affinis* Wurdack (Melastomataceae)

ABSTRACT

We isolated nine microsatellite loci from the Neotropical understory tree *Miconia affinis* (Melastomataceae) and optimized them for research on gene flow and genetic structure. Loci screened in 43 individuals from a 2.26 km² region were shown to be unlinked and polymorphic, with 5 to 14 alleles per locus and observed heterozygosity ranging from 0.394 to 0.810.

INTRODUCTION

The neotropical understory tree *Miconia affinis* (Melastomataceae) is broadly distributed in the Neotropics, ranging from southern Mexico to southern Venezuela. *Miconia affinis* is known in Mexico and Central America as *Cinco Negritos* (five black berries) because of its conspicuous berries, which are borne on magenta stalks during the summer rainy season. Seeds of *M. affinis* are broadly dispersed by neotropical birds and bats.

Although *M. affinis* is most often found in remnant forest, widespread seed dispersal facilitates colonization of *M. affinis* into adjacent agricultural lands. Like many other melastomes, *M. affinis* has deep poricidal anthers which must be vibrated by a pollinator in order for pollen to be released. "Buzz-pollination" is effected by a select group of bee pollinators (Buchmann 1983). Therefore, the study of *M. affinis* provides an opportunity to evaluate effects of habitat changes on gene flow and reproductive success of a reproductively specialized understory tree, as well as the pattern of seed dispersal between forest remnants and adjacent agricultural habitat. The purpose of the present research was to develop microsatellite markers to examine the breeding structure of *M. affinis* in remnant forest patches and shade coffee farms in Chiapas, Mexico.

DNA was extracted from a single plant using the DNeasy Plant kit (QIAGEN). A library of microsatellite repeats was constructed using a combination of procedures described in Weising et al. (2005) and Glen and Schable (2005). First, the genomic DNA (6µg) was digested with restriction enzyme *RsaI* and ligated with forward and reverse SuperSNX24 adaptors (SuperSNX24 forward 5'-GTTTAAGGCCTAGCTAGCAGCAGAATC and SuperSNX24 reverse 5'-GATTCTGCTAGCTAGGCCTTAAACAAA). The restriction-ligation product was purified (GeneClean kit, QBiogene), and hybridized with biotinylated di- and trinucleotide-specific oligonucleotides [oligo mix 2 = (AG)₁₂ (TG)₁₂ (AAC)₆ (AAG)₈ (AAT)₁₂ (ACT)₁₂ (ATC)₈] as described in Glenn & Schable (2005). Hybridized fragments were captured twice with streptavidin-coated magnetic beads (Dynal), filtered using a Microcon YM-30 spin filter (Millipore), and then amplified with adaptor-specific primers. The PCR products were ligated into a TOPO TA Cloning Kit (Invitrogen) and

selected colonies were amplified and sequenced (ABI Model 3730 Sequencer). Twelve of the 50 clones sequenced (24%) contained microsatellites. Primers were designed for the flanking sequences using PRIMER3 (Rozen & Skaletsky 2000).

We initially screened all 12 loci in 43 individuals sampled across a 2.26 km² shade coffee farm in Chiapas, Mexico, which has been colonized by *M. affinis* since establishment of the farm approximately 100 years ago. PCR was performed in a final volume of 20µL, containing approximately 2 ng of DNA, 2 µL of 10x PCR buffer, 1,5mM MgCl₂, 300 µM of each dNTP, 1 U of Hotstart*Taq* Polymerase (QIAGEN) and 0.25 µM of each primer. The thermal cycle began with a 15 minute denaturation step at 95 °C, and was followed by 45 cycles: 30s at 94 °C, 60s at the locus-specific annealing temperature (Table 1), and 30s at 72 °, followed by a final extension at 72 °C for 20 mins. Nine of the 12 primer pairs generated consistent and scorable amplification products of the expected size across all individuals tested. For these nine primer pairs, one primer from each primer pair was end-labelled with a fluorescent dye, either FAM, HEX or ROX, and genotyped on an ABI 3730 Sequencer. The genotypes were analyzed using the ABI Peak Scanner Software v.1.0.

Deviations from Hardy Weinberg Equilibrium (HWE) and linkage disequilibrium were tested in GENEPOP v.4.0 (Raymond and Rousset 1995) using the Markov chain method (Guo and Thompson 1992) with 1000 dememorizations, 100 batches and 1000 iterations per batch. A Bonferroni correction for multiple comparisons was applied in both cases to a significance level of $p < 0.05$. The probability of null alleles was calculated using the software MICROCHECKER (Oosterhout et al. 2004).

All of the nine loci were polymorphic (Table 5.1). Four loci exhibited significant deviations from Hardy-Weinberg equilibrium. This may be due to bi-parental inbreeding and/or null alleles, although only a single locus, Micaff-19, was indicated as having null alleles in Microchecker. No significant linkage disequilibrium was detected between any of the loci. The cumulative exclusion probability estimated from sampled adult individuals reached 0.995 for the first parent and 0.9999 for the second parent. This high level of polymorphism makes these powerful markers for direct and indirect estimates of gene flow and population structure. These are the first microsatellite DNA markers for the Melastomataceae, which contain over 3,000 tropical woody plant species.

ACKNOWLEDGEMENTS

This chapter was coauthored with Christopher Dick and published in *Molecular Ecology Resources* in 2009. This project was supported by the Helen Olson Brower Fellowship at the University of Michigan (to S. Jha) and the National Science Foundation (award DEB 043665 to C. Dick). The authors would like to thank Susana Pereira for assistance in the lab. The authors would also like to thank the farmers in Nueva Alemania, Chiapas for the permission to collect samples on their land.

TABLES, FIGURES AND LEGENDS

Table 5.1 Forward (F) and reverse (R) sequences with fluorescent dye label (used for genotyping), repeat motifs, annealing temperatures in °C (T_a), number of alleles (N_a), allelic size range in bp (Size Range), observed heterozygosity (H_o), expected heterozygosity (H_E), and GenBank Accession number (Ac. No.) for nine microsatellite loci for *Miconia affinis*.

Locus	Primer Sequence (5' to 3')	Repeat motif	T_a (°C)	N_a	Size Range	H_o	H_E	Ac. No.
Micaff-5	R: ^{ROX} GGAGGAGAACTCAACACAACCTCAA F:CCACCATGAAAAAGGAGAGACAATG	(TG) ₁₁	57.0	8	258-276	0.394 ***	0.618	EU67 5265
Micaff-7	R:GACAGTCACGGAGCAGTGGAAAT F: ^{ROX} TCGATTAATGAACITTTGAAATCAGG	(CT) ₁₁	53.3	10	248-298	0.541 ***	0.804	EU67 5266
Micaff-8	R:CGTTACAACGTGTCCTGCTTGTTG F: ^{HEX} AGGACAATGAATTAGCGTTGAACCA	(CT) ₁₂	56.9	10	278-300	0.800	0.774	EU67 5267
Micaff-9	R: ^{6-FAM} CGTTCGTCGTCTTCTTTCATCTTCT F:GGCGAGTCAATTCCTATTCAAAAA	(AG) ₁₉	59.8	7	266-290	0.810	0.783	EU67 5268
Micaff-14	R: ^{6-FAM} CCCCTTTTCTTTATCCTGTCTGT F:GAACATGTTATTGGATGGGCTACC	(TG) ₁₁	54.7	5	214-222	0.400 ***	0.677	EU67 5269
Micaff-16	R: ^{HEX} TGGGAAATGGGAAGAAACAAGTAAG F:GCAAAGGTGAGAGCTGTTGAGATTT	(AG) ₁₀ (TG) ₁₀	56.2	8	242-270	0.682	0.703	EU67 5270
Micaff-17	R:CGTCCGTCGAAGCAACCTATAAAA F: ^{6-FAM} ATCACGTCCAATTCACGTC	(CT) ₉	56.3	14	272-300	0.775	0.834	EU67 5271
Micaff-18	R: ^{6-FAM} ACCATCCGAACAACACAACACAAA F:GCGTCCGTCGAAGCAACCTATAAAA	(AG) ₁₁	57.4	9	206-226	0.464 ***	0.813	EU67 5272
Micaff-19	R: ^{HEX} CGTTCCTTGACTTTTCCCTCGACTC F:GAACGTAGGAACGGGAGTTGGAAT	(AG) ₆ G (GA) ₉	57.9	9	260-276	0.480	0.814	EU67 5273

***deviation from Hardy–Weinberg equilibrium at p=0.001

Chapter V References

- Buchmann, S. 1983. Buzz pollination in angiosperms. . Pages 73-113 *in* C. E. R. Jones, editor. Little Handbook of experimental pollination biology Van Nostrand, New York.
- Glen, T. C. and N. A. Schable. 2005. Isolating microsatellite DNA loci. *Methods in Enzymology* **395**:202-222.
- Guo, S. and E. Thompson. 1992. Performing the exact test of Hardy–Weinberg proportion from multiple alleles. *Biometrics*:361–372.
- Oosterhout, C., W. F. Hutchinson, D. P. M. Wills, and P. Shipley. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*:535–538.
- Raymond, M. and F. Rousset. 1995. GENEPOP version 1.2: a population genetics software for exact test and ecumenicism. *Journal of Heredity* **86**:248–249.
- Weising, K., H. Nybom, K. Wolff, and G. Kahl. 2005. DNA Fingerprinting in Plants. CRC Press, Boca raton.

Chapter VI: Shade coffee farms promote the genetic diversity of native trees

Coffee is cultivated across 11 million ha of land within the world's richest centers of terrestrial biodiversity (Myers et al. 2000). In tropical America, coffee is traditionally grown under a diverse canopy of overstory shade trees, which enhances the quality of the coffee farm as a conservation matrix and supports a broad spectrum of pollinators that increase fruit set per bush (Perfecto et al. 1996, Donald 2004, Klein et al. 2008). Unlike sun coffee monocultures, shade coffee also sustains a diverse array of vertebrates including bats and migratory birds, which provide farmers with many ecological services, such as insect predation (Williams-Guillen et al. 2008) and may also conserve seed dispersal processes necessary for native tree re-establishment (Sekercioglu 2006). However, little is known about the capacity of shade coffee farms to maintain gene flow and genetic diversity of remnant tree populations across this common tropical landscape. In this study, we conducted genetic analyses that reveal recent colonization and extensive gene flow of a native tree species in shade coffee farms in Chiapas, Mexico. The high genetic diversity and overlapping deme structure of the colonizing trees also show that traditional coffee farms maintain genetic connectivity with adjacent habitats and can serve as foci of forest regeneration.

Previous genetic studies of forest regeneration in degraded tropical landscapes have focused on canopy trees and have found pronounced fine scale spatial genetic structure within colonizing tree populations (e.g. Aldrich and Hamrick 1998, Sezen et al. 2005) as a result of limited seed dispersal from scattered maternal trees. Such population bottlenecks can lead to inbreeding depression and demographic declines for plants in fragmented landscapes. Alternatively, agricultural matrices that facilitate the movement

of seed and pollen dispersers among habitats can increase the breeding size and genetic diversity of native plant populations.

We used genetic markers to analyze the dispersal history and spatial genetic structure of the understory tree, *Miconia affinis* (Melastomataceae) within a network of coffee farms and forest fragments in Chiapas, Mexico. Though an integral part of tropical forests, relatively little is known about the population genetics of understory trees compared to canopy trees. Like many tropical understory trees, the seeds of the genus *Miconia* are dispersed by widespread large-bodied birds, such as the Clay-colored Thrush (*Turdus grayi*), as well as small-bodied scrub and forest specialists such as the Chestnut-sided Warbler (*Dendroica pensylvanica*) (Luck and Daily 2003); thus its colonization patterns are likely representative of other understory vertebrate-dispersed trees. *Miconia affinis* is an obligate outcrosser and requires native bees (e.g. *Trigona* and *Scaptotrigona* spp.) for cross-fertilization through buzz-pollination, of which the ubiquitous Africanized honeybees are incapable. The survey area of 1200 ha encompasses an uncut primary forest and three adjacent shade coffee farms (Figure 1). The three farms were clear-cut and burned in the late 1930's and immediately replanted with coffee bushes and canopy tree species, including nitrogen-fixing legumes and many other fruit trees (means of 157.21 ha⁻¹ overstory trees, and 14.67 ha⁻¹ tree species). Since the creation of these coffee farms, farmers have allowed native understory trees, like *M. affinis*, to colonize the coffee plots because they help to reduce soil erosion.

Strong spatial genetic structure of *M. affinis* found in the forest plots (*Sp* statistic = 0.020) is similar to levels reported in species with extreme seed dispersal limitation (Vekemans and Hardy 2004); in contrast, no spatial genetic structure was detected for any distance class within the coffee farms (*Sp* statistic = 0.006), revealing more extensive gene flow across the shade coffee matrix (Figure 1). Limited seed dispersal in the forest, relative to the coffee farm, may be due to the short foraging ranges of forest specialist birds. Using an individual-based clustering approach, *M. affinis* individuals in the landscape were assigned to four well-supported demes (96% posterior probability), which were overlapping in space and not aggregated by habitat or age class, indicating that colonization into the farms was recent and from multiple source populations (Figure 6.1). Levels of *M. affinis* genetic diversity within the coffee farms did not differ from forest

populations (Table 6.1). These results depart from previous agricultural colonization studies which report strong spatial genetic structure and reduced genetic diversity in the founding canopy tree populations, usually caused by the reproductive dominance of just a few maternal trees in the early colonizing stages (Sezen et al. 2005).

By conserving resident bird populations, our results reveal that shade coffee farms support extensive dispersal processes crucial for the connectivity of remnant forest and agricultural habitats. The colonization pattern and high genetic diversity of *M. affinis* also points to the role of shade-coffee farms as potential foci of native forest regeneration, as coffee farms typically fall out of production in less than a century. However, despite the clear ecological benefits of traditional shade coffee farms, these farms are rapidly being converted to input-intensive monocultures (Perfecto et al. 1996, Donald 2004). Thus it is imperative to highlight the ecological function of shade coffee farms, not only in providing refuge for native fauna, but also in preserving habitat connectivity and gene-flow processes essential for reforestation by native tree species.

ACKNOWLEDGEMENTS

This chapter was coauthored with Christopher Dick and published in *Current Biology* in 2008. This project was supported by the Helen Olson Brower Fellowship at the University of Michigan (to S. Jha) and the National Science Foundation (award DEB 043665 to C. Dick). The authors would like to thank John Vandermeer for help in the development and implementation of this project. The authors would also like to thank the farmers in Nueva Alemania, Chiapas for the permission to collect samples on their land.

TABLES, FIGURES AND LEGENDS

Table 6.1 Number of *M. affinis* individuals (N), allelic richness (A), expected heterozygosity (H_E), and observed heterozygosity (H_O).

	N	A (SD)		H_E (SD)		H_O (SD)	
Forest	305	12.375	1.017	0.807	0.022	0.588	0.048
Coffee	119	11.375	0.944	0.777	0.030	0.616	0.068

Figure 6.1 (A , B) Spatial autocorrelation diagrams showing kinship coefficient F_{ij} (solid lines) averaged across all pair wise comparisons within distance categories. Dashed lines show 95% confidence limits (CL) around the null expectation of no genetic structure ($F_{ij} = 0.00$). Thus values above the upper 95% CL represent significantly higher relatedness than expected at random, while values below the lower 95% CL represents significantly lower relatedness than expected for each distance class.

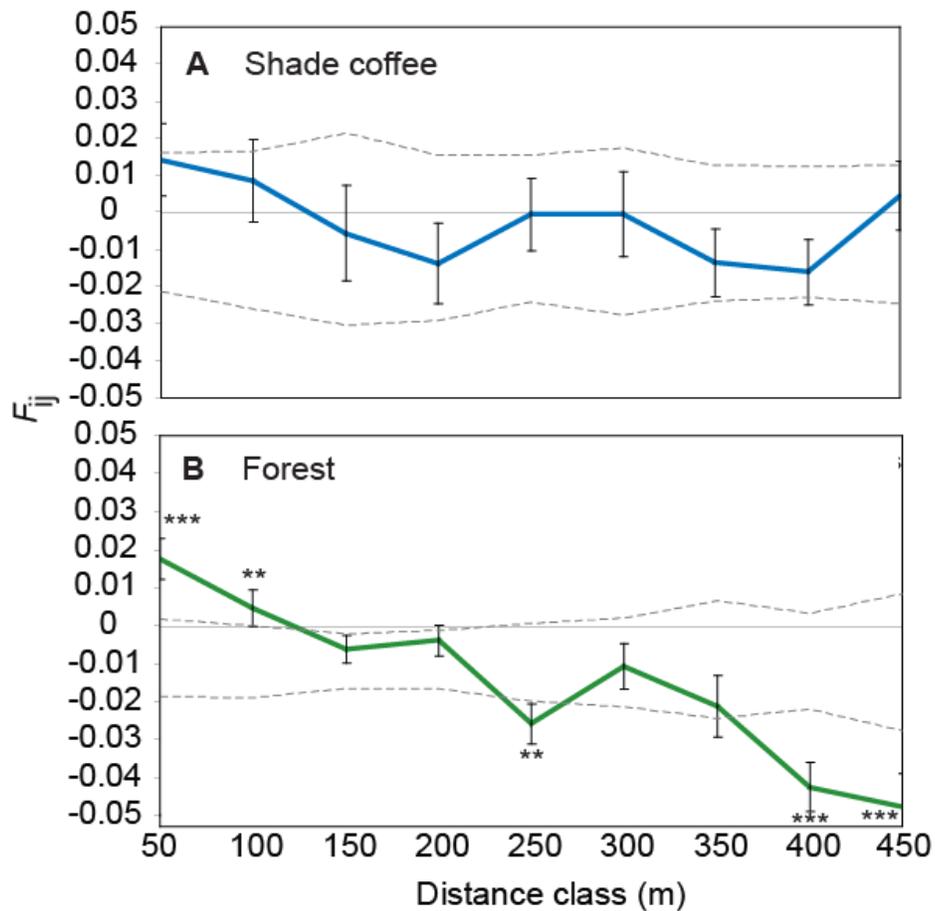
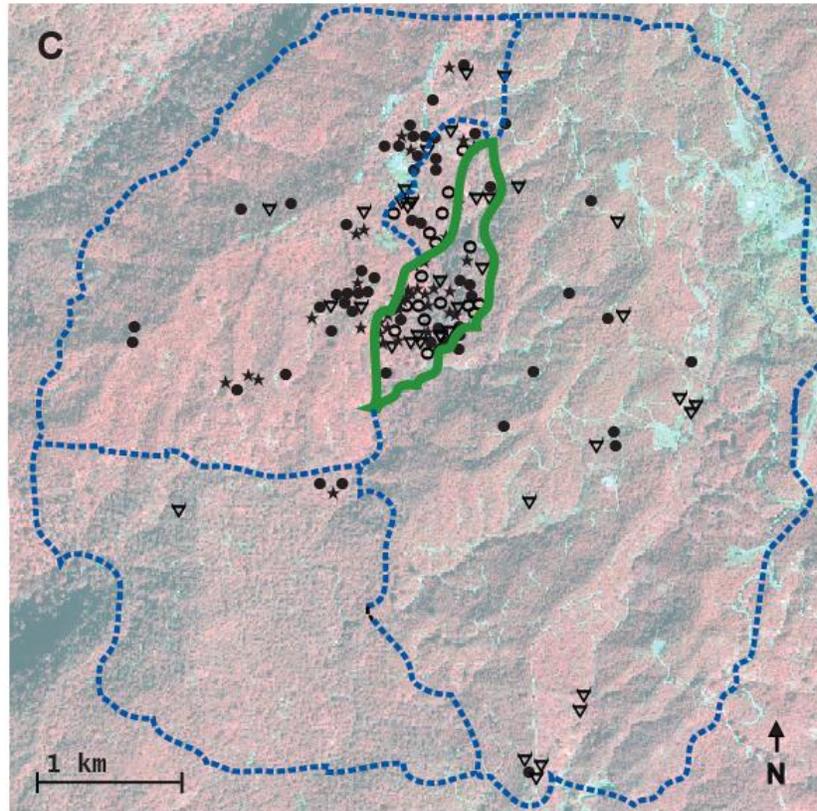


Figure 6.1 (continued) (C) Mapped trees were assigned to four demes (filled stars, filled circles, open circles, open triangles) indicating extensive, overlapping seed dispersal. Solid green lines outline the forest fragment, and dashed blue lines outline the shade coffee farms. All analyses were based on 8 highly polymorphic, unlinked microsatellite loci.



APPENDIX

Materials and Methods

Coffee farmers occasionally replant overstory leguminous trees (usually to replace dead or damaged leguminous trees), but they do not plant understory forest trees like *M. affinis* in the coffee fields. Seed dispersers of *M. affinis* were recorded in the forest and coffee using visual surveys and 24 hour mist-netting followed by fecal analyses (Williams-Guillen et al. *in prep*). GPS coordinates were measured and leaf tissue was sampled from all trees in the 1200 ha landscape from 2005-2006. DNA was extracted using the QIAGEN DNeasy kit (as described in Jha and Dick *in press*) and all individuals were genotyped at 8 unlinked microsatellite loci in order to estimate gene diversity statistics and to analyze spatial genetic structure.

We used the software SPAGedi (Hardy and Vekemans 2002) to compute the pairwise relatedness metric F_{ij} (Loiselle et al. 1995) which is based on the regression slope of relatedness b_F , and which is robust to sampling method (Vekemans and Hardy 2004). We computed relatedness at 50m distance intervals up to 450m. We obtained the 95% confidence limits (CL) around the null expectation of no genetic structure ($F_{ij} = 0.00$) by permuting multi-locus genotypes and spatial coordinates (1000 iterations)(Hardy and Vekemans 2002). We also tested for spatial genetic structure using Moran's Index and r_{ij} (Peakall and Smouse 2001), and found the same patterns with the same significance levels for the distance values analyzed.

To calculate the number of demes we used the software STRUCTURAMA (Huelsenbeck et al. *in press*), which incorporates a Bayesian clustering approach to assign individuals to K populations, and which provides posterior probabilities of observing the data under different estimates of K without prior assumptions of population structure. Individuals are assigned to populations by minimizing the squared distance among sampled partitions, where partition distance is defined as the minimum number of individuals that have to be deleted from the assignment vector in order to make the two partitions the same. In our analysis, we found the greatest support for K=4 demes, and this was additionally confirmed for each locus analyzed separately and for multiple combinations of loci using STRUCTURE (Pritchard et al. 2000).

Chapter VI References

- Aldrich, P. R. and J. L. Hamrick. 1998. Reproductive dominance of pasture trees in a fragmented tropical forest mosaic. *Science* **281**:103-105.
- Donald, P. F. 2004. Biodiversity impacts of some agricultural commodity production systems. *Conservation Biology* **18**:17-37.
- Hardy, O. J. and X. Vekemans. 2002. SPAGEDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes* **2**:618-620.
- Huelsenbeck, J. P., P. Andolfatto, and E. T. Huelsenbeck. in press. Structurama: bayesian inference of population structure. *Bioinformatics*.
- Jha, S. and C. W. Dick. *in press*. Microsatellite markers for *Miconia affinis* (Melastomataceae). *Molecular Ecology Resources*.
- Klein, A. M., S. A. Cunningham, M. Bos, and I. Steffan-Dewenter. 2008. Advances in pollination ecology from tropical plantation crops. *Ecology* **89**:935-943.
- Loiselle, B. A., V. L. Sork, J. Nason, and C. Graham. 1995. Spatial Genetic-Structure of a Tropical Understory Shrub, *Psychotria Officinalis* (Rubiaceae). *American Journal of Botany* **82**:1420-1425.
- Luck, G. W. and G. C. Daily. 2003. Tropical countryside bird assemblages: Richness, composition, and foraging differ by landscape context. *Ecological Applications* **13**:235-247.
- Myers, N., R. A. Mittermeier, C. G. Mittermeier, G. A. B. da Fonseca, and J. Kent. 2000. Biodiversity hotspots for conservation priorities. *Nature* **403**:853-858.
- Peakall, R. and P. E. Smouse. 2001. GENALEX (version 5.1): genetic analysis in Excel, Population Genetic Software for Teaching and Research, . Australian National University, Canberra, Australia.
- Perfecto, I., R. A. Rice, R. Greenberg, and M. E. VanderVoort. 1996. Shade coffee: A disappearing refuge for biodiversity. *Bioscience* **46**:598-608.
- Pritchard, J. K., M. Stephens, and P. J. Donnelly. 2000. Inference of population structure using multilocus genotype data. *Genetics* **155**:945-959.
- Sekercioglu, C. H. 2006. Ecological significance of bird populations. Pages 15-51 *Handbook of the Birds of the World*.
- Sezen, U. U., R. L. Chazdon, and K. E. Holsinger. 2005. Genetic consequences of tropical second-growth forest regeneration. *Science* **307**:891-891.
- Vekemans, X. and O. J. Hardy. 2004. New insights from fine-scale spatial genetic structure analyses in plant populations. *Molecular Ecology* **13**:921-935.
- Williams-Guillen, K., K. Goodall, and C. Taylor. *in prep*. Avian and bat seed dispersers of *Miconia affinis*.
- Williams-Guillen, K., I. Perfecto, and J. Vandermeer. 2008. Bats limit insects in a neotropical agroforestry system. *Science* **320**:70-70.

Chapter VII: Extensive plant gene flow mediated by native bees across a shade coffee landscape mosaic

ABSTRACT

Coffee farms cover much of the tropics and may critically impact native plant and animal communities. Shade coffee farms provide refuge for migratory birds and forest mammals, but their ability to sustain native plants and pollinators is not known. Our study of native tree gene flow within shade coffee farms and forest fragments revealed pollen transfer by native insects spanning more than 1800 m. Furthermore, pollen dispersal between coffee and forest habitats was extensive, constituting 43% of all pollination events. These results highlight the role that shade coffee farms can play in sustaining native pollinators and maintaining the population viability of native plants in degraded tropical landscapes.

As the second most valuable commodity in the world (Vandermeer 2003) coffee extends across 11 million ha of land, embedded in some of the world's richest centers of terrestrial biodiversity (Myers et al. 2000). In the neotropics, coffee is traditionally 'shade-grown' under a diverse canopy of overstory trees (Moguel and Toledo 1999) which supports millions of small-scale farmers who benefit from ecological services provided by the shade trees, such as nitrogen-fixation, soil erosion and pest control (Perfecto et al. 1996, Donald 2004, Williams-Guillen et al. 2008). Though shade-grown coffee has garnered interest as habitat for forest dwelling mammals and migratory songbirds (Greenberg et al. 1997, Klein et al. 2008, Van Bael et al. 2008), little is known

about the diversity of insect pollinators within shade coffee farms and their role in maintaining the genetic diversity of remnant trees.

Because most tropical plants are dependent on insect pollinators (Bawa 1990), depauperate insect faunas within agricultural landscapes may lead to reproductive declines and restricted gene flow among remnant forest trees (reviewed in Nason and Hamrick 1997, Dick et al. 2008). Though recent tropical studies suggest that pollen dispersal may not be as limited in fragmented habitats as expected (Dick et al. 2003, Sezen et al. 2005, 2007), these studies have examined large, self-compatible canopy trees that can be pollinated by ubiquitous Africanized honeybees, and thus represent systems where pollen-mediated gene flow may be maintained even if native pollinators are lost. Alternatively, self-incompatible reproductively specialized plants that rely only on native pollinators may be especially sensitive to pollinator loss in degraded landscapes (Aguilar et al. 2006).

Our analysis of reproductive isolation and gene flow focused on a buzz-pollinated understory tree, *Miconia affinis* (Melastomataceae), within a 1200 ha shade coffee and forest landscape mosaic in Chiapas, Mexico (Fig. 1). As a buzz-pollinated plant, *M. affinis* requires sonication for pollen removal, a mechanism that cannot be effected by Africanized honeybees. Mating system studies conducted on *M. affinis* trees across habitats revealed that *M. affinis* is self-incompatible, yielding no fruits without cross pollination and significantly higher fruit set with cross-buzz-pollination (bee movement simulated with a vibrating tuning fork)(Linear Mixed Effects Model, $p < 0.0001$, Fig. 2B). Thus *M. affinis* serves as a unique bio-indicator of native pollinator communities in coffee agroecosystems.

Across 59 *M. affinis* trees, the most common native bees visiting flowers were buzz-pollinating carpenter bees (*Xylocopa* spp), Meliponine bees (*Melipona beecheii* and *Trigona fulviventris*) and orchid bees (*Euglossa viridissima*). We found that a significantly greater number of buzz-pollinating native solitary and social bees visited *M. affinis* in forest habitats ($t_{23,36} = 4.36$, $p < 0.0001$ and $t_{23,36} = 3.04$, $p = 0.003$, respectively) and a significantly greater number of Africanized honeybees visited *M. affinis* in coffee habitats ($t_{23,36} = -5.62$, $p < 0.0001$)(Fig. 2A). Ambient fruit set was significantly higher in

forest than in the coffee habitats ($t_{29,59} = 16.86$, $p < .0001$, Fig. 2C), suggesting that *M. affinis* experiences high reproduction in forests due to increased native bee pollination.

Pollen dispersal was assessed via direct paternity analysis (Marshall et al. 1998) and indirect pollen dispersal estimation (Smouse et al. 2001) utilizing microsatellite genotypes of seeds collected from *M. affinis* trees (24 seeds from each tree, 24 scattered mother trees, 12 in each habitat). Based on paternity analysis, *M. affinis* pollen dispersal distances across habitat types ranged from 0.10m - to 1890m ($n = 375$ total events), with an average pollen dispersal distance of 419m (average probability of exclusion $P_e = 0.986$). Despite extensive gene flow across habitats, pollen dispersal distributions within forest and shade coffee habitats were significantly different (Kolmogorov-Smirnov Z test, $Z = 1.83$, $p = 0.002$), with longer pollen dispersal distances in shade coffee ($589\text{m} \pm 90.6\text{m}$) than in forest ($279\text{m} \pm 59.3\text{m}$) (Fig. 3). Mean pollen dispersal distances in both habitats were significantly greater than nearest neighbor distances, which averaged 11.2m ($\pm 3.81\text{m}$) for mothers in forest habitats and 37.6m ($\pm 11.1\text{m}$) in coffee habitats (Kolmogorov-Smirnov Z test, $Z = 0.83$, $p < 0.0001$, and $Z = 6.34$, $p < 0.0001$, respectively), indicating that pollinators frequently bypass nearby flowering plants, especially within coffee habitats where *M. affinis* populations are more scattered. Additionally, pollen dispersal exhibited a fat-tailed exponential distribution in forest and coffee habitats, indicating that long distance dispersal is highly likely for trees in both habitats.

In coffee habitats, 65.1% of seeds per mother tree were fathered by *M. affinis* forest trees, while in forest habitats 78.0% of seeds per mother tree were fathered by *M. affinis* forest trees (Fig. 3). Given the average landscape pollen dispersal distance, the proportion of seeds pollinated by *M. affinis* forest trees was not significantly different than expected in coffee habitats (expected 66%) (Wilcoxin Signed Ranks test, $Z = -0.784$, $p = 0.433$) but was significantly less than expected in forest habitats (expected 85.4%) (Wilcoxin Signed Ranks test, $Z = -2.43$, $p = 0.014$), revealing extensive pollen flow between habitats and demonstrating that trees within coffee farms can play a disproportionately large role in maintaining gene flow across the entire landscape.

In this study, we provide evidence for the conservation of native bee pollination and the maintenance of extensive pollen dispersal within shade coffee systems. Though native bee visitation was lower in coffee, *M. affinis* exhibited relatively high reproductive

success in both forest and coffee habitats. This indicates that native bees are foraging substantially within shade coffee farms, a benefit to cultivated coffee (Perfecto et al. 1996, Donald 2004, Klein et al. 2008) and to native plants. Furthermore, we documented frequent cross-habitat gene flow and some of the longest precisely recorded pollination distances for native neotropical bees. We found that pollen dispersal distances for *M. affinis* trees were greater where interplant distance was greatest (e.g., Dick et al. 2003); however, in this study, Africanized honeybees were not the source of extensive pollen-mediated gene flow. Rather, native bees mediate long distance pollen dispersal across the landscape, providing essential gene flow for native tropical trees.

ACKNOWLEDGEMENTS

This chapter was coauthored with Christopher Dick and is currently being submitted to journals. The project was supported by the Helen Olson Brower Fellowship at the University of Michigan (to S. Jha) and the National Science Foundation (award DEB 043665 to C. Dick). The authors would like to thank John Vandermeer, Ivette Perfecto, Beverly Rathcke and all lab members for help in the development and implementation of this project. The authors would also like to thank Gabriel Dominguez-Martinez, for his help scouting trees, and the farmers in *Nueva Alemania*, for their permission to conduct experiments on their land. All experiments conducted were in compliance with current laws governing biodiversity protection in Mexico.

TABLES, FIGURES AND LEGENDS

Figure 7.1 Study region in Chiapas, Mexico, where *M. affinis* individuals in forest and coffee habitats are labeled as green and brown squares, respectively. Black dashed lines outline the forest fragment and shade coffee habitats. Three focal trees are labeled with white numbers, located in coffee (1 & 3) and forest (2) habitats. Solid black lines radiating from the focal trees indicate 10 recorded pollen sources, as revealed by paternity analysis.

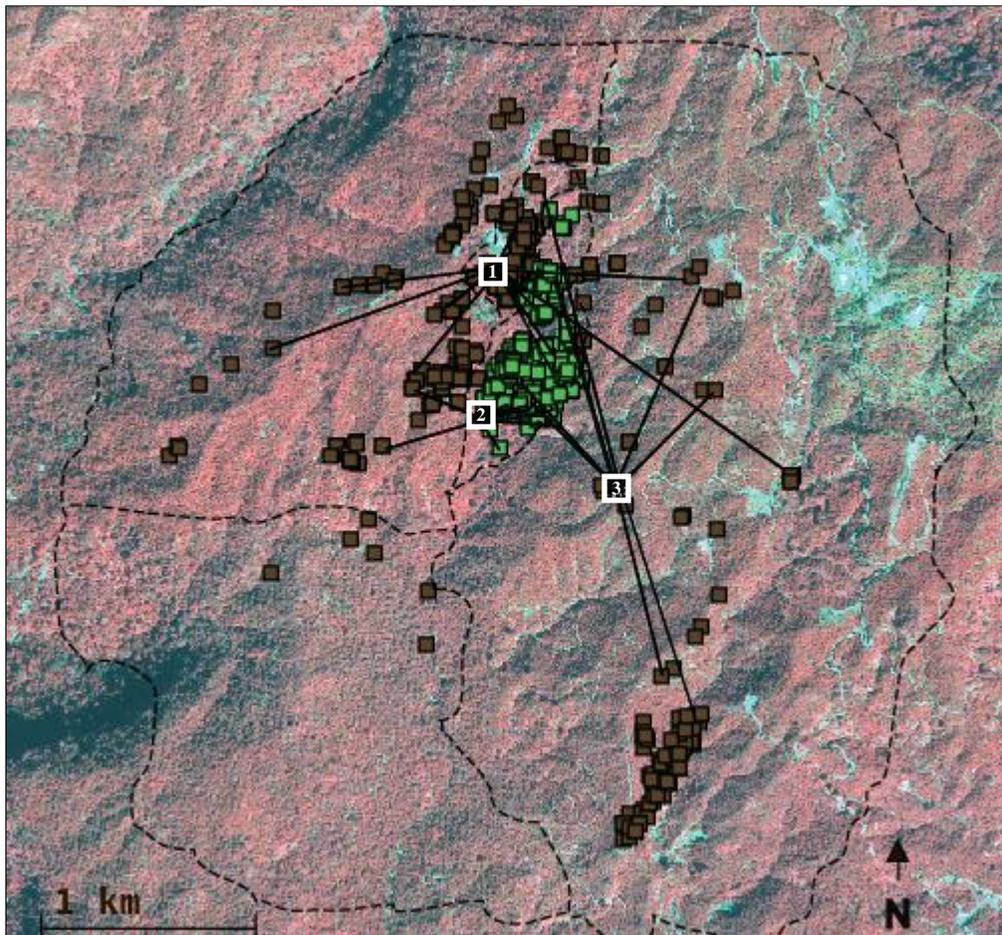


Figure 7.2 (A) Bee visitation to *M. affinis* in forest (green) and coffee (brown) habitats and photos (top to bottom) of the native solitary bee *Xylocopa tabaniformis*, *tabaniformis*, native social bee *Trigona fulviventris*, and exotic social bee *Apis mellifera scutellata*. (B) Photo of *M. affinis* inflorescence and pollination treatments for 3 flowers per inflorescence (control, self-pollinated, self-buzz-pollinated, cross pollinated, cross-buzz-pollinated, and open) in both habitats and (C) the open pollination treatment examined separately in forest and coffee habitats. *P < 0.05, **P < 0.01, ***P < 0.001

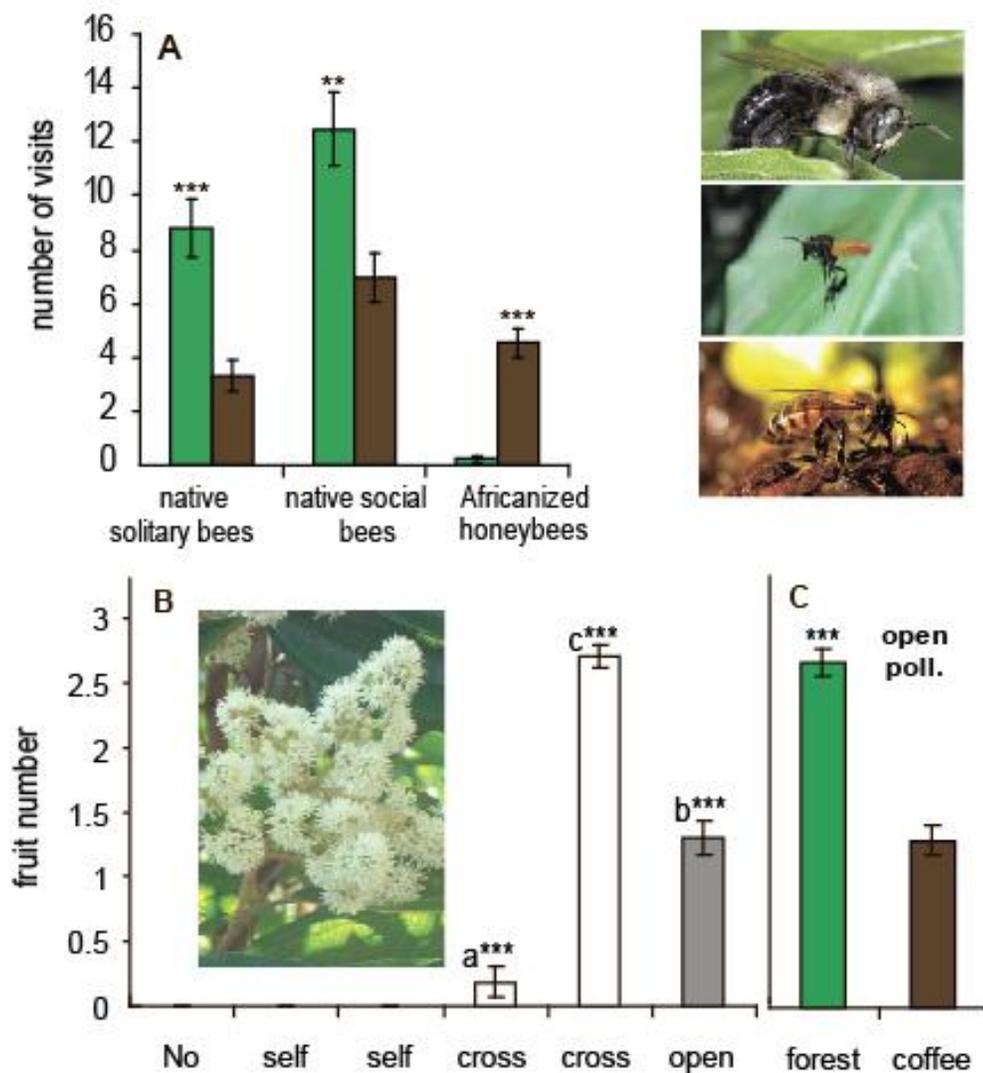
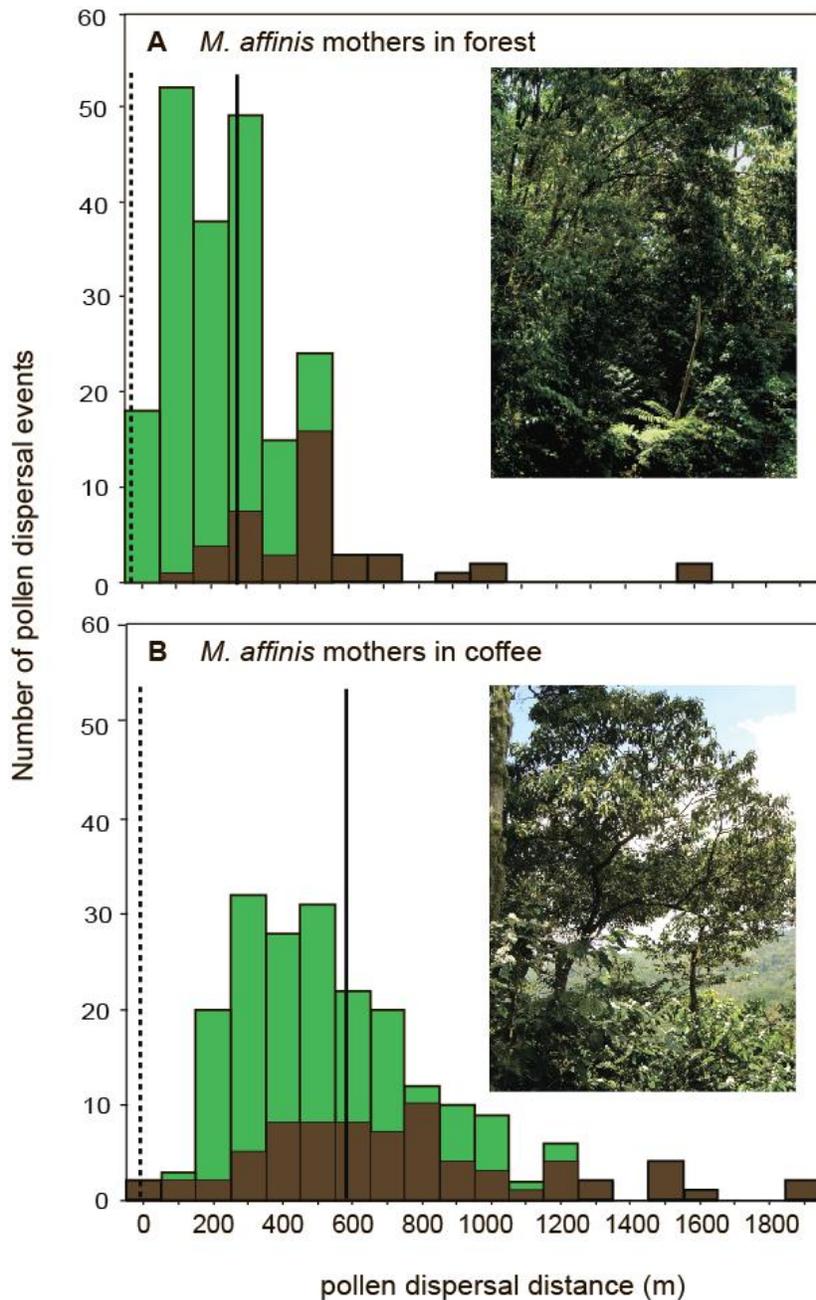


Figure 7.3 Pollen dispersal patterns for *M. affinis* mothers in (A) forest and (B) coffee habitats (photo of *M. affinis* mother in each habitat) in 100m binned distance categories. Green bars represent the number of pollen dispersal events from *M. affinis* fathers (pollen donors) in forest habitats and brown bars represent the number of pollen dispersal events from *M. affinis* fathers in coffee habitats. Dotted lines represent nearest neighbor-distances to mother trees in each habitat and the solid lines represent average pollen dispersal distances for mothers in each habitat.



APPENDIX

Materials and Methods

Study site

Shade coffee farms were clear-cut and burned in the late 1930's and immediately replanted with coffee bushes and canopy trees. Canopy trees include nitrogen-fixing leguminous trees (*Inga* spp.), fruit trees such as orange (*Citrus sinensis*) and mango (*Mangifera indica*), and other native species (mean of 157.21 ha⁻¹ overstory trees, and 14.67 ha⁻¹ tree species). Since the establishment of the coffee farms, managers have allowed for the colonization of understory trees, like *M. affinis*, because of their service in reducing soil erosion (Jha and Dick 2008). GPS coordinates were recorded and leaf tissue was sampled from all *M. affinis* trees in the 1200 ha landscape (445 individuals). DNA was extracted using the QIAGEN DNeasy kit (as described in Jha and Dick 2009).

Mating and pollination system

We conducted mating system studies on 90 *M. affinis* trees across both forest and coffee habitats. We selected 3 flowers for each of six treatments on each of the 90 study trees. The inflorescences were bagged before flowering and only opened during the pollination treatment. The treatments included control flowers (not-pollinated), self-pollinated flowers (pollinated with a non-vibrating tuning fork, pollen donated from a flower in the same tree), self-buzz-pollinated flowers (pollinated with a vibrating tuning fork, pollen donated from a flower in the same tree), cross pollinated flowers (pollinated with a non-vibrating tuning fork, pollen donated from a flower on a tree >20m away) and cross-buzz-pollinated flowers (pollinated with a vibrating tuning fork, pollen donated from a flower on a tree >20m away). Pollinator observations consisted of 30 minute visual surveys of 4 inflorescences on each of 59 *M. affinis* trees located in both forest and coffee habitats. All counts were square root transformed before statistical analysis.

Pollen Dispersal Analyses

The genetic analysis was based on eight unlinked and highly polymorphic microsatellite loci with an overall exclusion probability of 0.9999 (Jha and Dick 2009). We used direct paternity analyses to calculate individual pollen dispersal distances by examining the multi-locus segregation probabilities, using the program CERVUS (Marshall et al. 1998). We used the critical delta values based on the paternity simulation to calculate paternity for 24 seeds collected from 24 mothers (12 mothers in coffee, 12 mothers in forest habitats, for a total of 576 seeds). We assigned paternity for 375 seeds with an exclusion probability of 99% or higher. To examine differences between actual parentage and expected parentage of maternal seed arrays, we compared the actual proportion of pollen donors from the forest with the proportion of forest trees within 419.6m (average pollen dispersal distance) of each mother tree. Square root and arcsine-square root transformed proportions and distances yielded the same statistical results as non-transformed data. Because the analyses do not assume normality, the reported statistics reflect tests conducted on non-transformed data.

We also conducted an indirect paternity analysis using the program TWOGENER (Smouse et al. 2001) within the program, POLLDISP (Robledo-Arnuncio et al. 2007) which uses genotypic data from the maternal seed arrays to estimate the differentiation of allelic frequencies (Φ_{FT}) among the pollen pools. We calculated greater differentiation in pollen allele pools within forest habitats ($\Phi_{FT} = 0.087$) than within coffee habitats ($\Phi_{FT} = 0.047$). Additionally, since maternal tree density was higher in the forest (0.70 trees/ha in forest, 0.01 trees/ha in the coffee), estimated average dispersal distances were much greater in coffee habitats (803.6m) than in forest habitats (101.5m). In both forest and coffee habitats, the exponential dispersal function best fit the data (coffee: $a=401.8$, $b=0.094$, $error=0.095$, forest: $a=47.35$, $b=0.057$, $error=0.223$) and a fat-tailed distribution can be inferred from the relatively low curve shape parameter ($b<1$) for both functions (Clark 1998).

Photos of bees in Figure 3 (top to bottom) were taken by Gary McDonald, Mike Reiskind, and James Nieh. Photos of *M. affinis* in Figures 3 and 4 were taken by S. Jha.

Chapter VII References

- Aguilar, R., L. Ashworth, L. Galetto, and M. A. Aizen. 2006. Plant reproductive susceptibility to habitat fragmentation: review and synthesis through a meta-analysis. *Ecology Letters* **9**:968-980.
- Bawa, K. S. 1990. *Reproductive Ecology of Tropical Forest Plants*. Unesco and Parthenon Press, Paris and USA.
- Clark, J. S. 1998. Why trees migrate so fast: Confronting theory with dispersal biology and the paleorecord. *American Naturalist* **152**:204-224.
- Dick, C. W., G. Etchelecu, and F. Austerlitz. 2003. Pollen dispersal of tropical trees (*Dinizia excelsa*: Fabaceae) by native insects and African honeybees in pristine and fragmented Amazonian rainforest. *Molecular Ecology* **12**:753-764.
- Dick, C. W., F. A. Jones, O. J. Hardy, and R. J. Petit. 2008. Spatial scales of seed and pollen-mediated gene flow in tropical forest trees. *Tropical Plant Biology* **1**:20-33.
- Donald, P. F. 2004. Biodiversity impacts of some agricultural commodity production systems. *Conservation Biology* **18**:17-37.
- Greenberg, R., P. Bichier, and J. Sterling. 1997. Bird populations in rustic and planted shade coffee plantations of eastern Chiapas, Mexico. *Biotropica* **29**:501-514.
- Jha, S. and C. W. Dick. 2008. Shade coffee farms promote genetic diversity of native trees. *Current Biology* **18**:1126-1128.
- Jha, S. and C. W. Dick. 2009. Microsatellite markers for *Miconia affinis* (Melastomataceae). *Molecular Ecology Resources* **9** 344-345.
- Klein, A. M., S. A. Cunningham, M. Bos, and I. Steffan-Dewenter. 2008. Advances in pollination ecology from tropical plantation crops. *Ecology* **89**:935-943.
- Marshall, T. C., J. Slate, L. E. B. Kruuk, and J. M. Pemberton. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology* **7**:639-655.
- Moguel, P. and V. M. Toledo. 1999. Biodiversity conservation in traditional coffee systems of Mexico. *Conservation Biology* **13**:11-21.
- Myers, N., R. A. Mittermeier, C. G. Mittermeier, G. A. B. da Fonseca, and J. Kent. 2000. Biodiversity hotspots for conservation priorities. *Nature* **403**:853-858.
- Nason, J. D. and J. L. Hamrick. 1997. Reproductive and genetic consequences of forest fragmentation: Two case studies of neotropical canopy trees. *Journal of Heredity* **88**:264-276.
- Perfecto, I., R. A. Rice, R. Greenberg, and M. E. VanderVoort. 1996. Shade coffee: A disappearing refuge for biodiversity. *Bioscience* **46**:598-608.
- Robledo-Arnuncio, J. J., F. Austerlitz, and P. E. Smouse. 2007. POLDISP: a software package for indirect estimation of contemporary pollen dispersal. *Molecular Ecology Notes* **7**:763-766.
- Sezen, U. U., R. L. Chazdon, and K. E. Holsinger. 2005. Genetic consequences of tropical second-growth forest regeneration. *Science* **307**:891-891.
- Sezen, U. U., R. L. Chazdon, and K. E. Holsinger. 2007. Multigenerational genetic analysis of tropical secondary regeneration in a canopy palm. *Ecology* **88**:3065-3075.

- Smouse, P. E., R. J. Dyer, R. D. Westfall, and V. L. Sork. 2001. Two-generation analysis of pollen flow across a landscape. I. Male gamete heterogeneity among females. *Evolution* **55**:260-271.
- Van Bael, S. A., S. M. Philpott, R. Greenberg, P. Bichier, N. A. Barber, K. A. Mooney, and D. S. Gruner. 2008. Birds as predators in tropical agroforestry systems. *Ecology* **89**:928-934.
- Vandermeer, J. H. 2003. *Tropical Agroecosystems*. CRC Press, Boca Raton, Florida.
- Williams-Guillen, K., I. Perfecto, and J. Vandermeer. 2008. Bats limit insects in a neotropical agroforestry system. *Science* **320**:70-70.

Chapter VIII: Conclusion

This dissertation research examines the impacts of land use on bee communities, bee foraging behavior, and pollen and seed dispersal processes for a reproductively specialized tropical tree. The integration of field-collected community data, regional geographic information and population-level genetic analyses provides a robust understanding of the ecology and genetics of a specialized tropical plant species in an understudied yet ubiquitous tropical agricultural system. By documenting native faunal biodiversity and revealing critical plant gene flow processes, this thesis provides mechanistic insight into the capacity of shade coffee agroecosystems to serve as biodiversity refuges and critical habitat corridors.

In chapters two and three, we found that Africanized honeybees and native bees exhibited strikingly different foraging patterns with coffee agroforestry systems. In the first study, we observed that Africanized honeybees exhibited only initial foraging preferences for low-shade coffee habitats, while native bees exhibited both initial and delayed recruitment-based foraging preferences for high-shade coffee habitats. In chapter three, we observed a significantly greater number of visitors to coffee flowers in high-shade coffee habitats than in low-shade coffee habitats for both native and exotic bees. We found that within high-shade coffee habitats, native solitary bees and native social bees exhibited a ‘dilution effect’ at the smallest spatial scale, while in low-shade coffee habitats, Africanized honeybees exhibited a ‘concentration effect’ at the largest spatial scale. This second study was the first to show that the foraging patterns of native bees and exotic honeybees contrast one another in response to floral resource level and that this response is mediated specifically by the vegetation management of the local habitat.

In chapter four, we utilized machine-learning regression tree modeling in order to reveal that, in general, native bees are more abundant and species rich in habitats with a greater number of flowering overstory tree species. We found that native solitary bees exhibited preferences for habitats with greater herb diversity, while native social bees exhibited preferences for greater overstory tree diversity and density. Bee communities differed in their response to habitat factors, with greater cavity-nesting bee abundance in habitats with greater overstory tree species richness, greater wood-nesting bee abundance in habitats with greater canopy cover, and greater soil-nesting bee abundance, with greater flowering overstory tree species. Overall, our results indicated that habitat heterogeneity was critical for maintaining diverse bee communities, and that within-farm habitat management was more predictive of native bee abundance than regional forest distribution.

In chapter five, we isolated eight microsatellite loci from *M. affinis* that were unlinked, polymorphic and did not exhibit null alleles. These microsatellites were used in chapters six and seven to examine the population genetics of *M. affinis* across coffee and forest landscapes. In chapter six, we conducted spatial genetic structure analyses in order to reveal historical seed dispersal patterns across the coffee and forest landscape. Using a Bayesian clustering approach, we classified all *M. affinis* individuals into 4 well-supported demes, which were overlapping and not aggregated by habitat or age class, providing strong support for recent colonization of *M. affinis* from multiple source populations. Despite recent colonization, *M. affinis* within the coffee matrix exhibited high allelic diversity that was not significantly different from levels found in the forest. Results from our genetic analysis indicated strong spatial genetic structure in the forest at the 100 and 200m distance class and no spatial genetic structure in the coffee agroecosystems. These results indicate strong seed dispersal limitation in the forest and extensive seed dispersal in coffee habitats, likely due to differences in seed disperser communities between habitats.

In chapter seven, we confirmed that *M. affinis* individuals are self-incompatible and reliant on buzz-pollination. We also found that honeybee visitation was significantly higher and ambient fruit set was significantly lower for *M. affinis* individuals in non-forested habitats. Pollen dispersal molecular analyses revealed extensive pollen

movement across the landscape, with some of the longest recorded dispersal events precisely recorded for native tropical bees. We documented pollen dispersal by native bees spanning 1800 m, with 43% of pollination events taking place across habitat types. These results provide strong evidence that shade coffee farms can serve as habitat corridors for native bees and can sustain native bee pollinator communities.

Synthesis

As agricultural areas continue to expand, especially in the tropics, it is critical that we understand how agricultural land management impacts pollinator communities and pollen dispersal processes for tropical trees. By focusing on a pantropically prevalent agricultural system, the findings from this research project provide global insight into the preservation of tropical tree populations in fragmented agricultural landscapes. As the second most valuable commodity in the world, coffee farming sustains the livelihoods of millions of people across the tropics. Results from this research provide information on how to most effectively manage native and exotic pollinators within coffee agroecosystems, where their services are essential for improved coffee yield.

This dissertation research also examines the reproductive and dispersal processes of a pantropically dominant plant family, the Melastomataceae, and one of the most diverse genera in the world (*Miconia* alone has 2200 pantropical species). The research reveals how native bee pollinators of *M. affinis* move within the forest and coffee landscape and whether pollen dispersal patterns are sufficiently extensive to maintain genetic diversity for native trees living within coffee farms and forest fragments. Though shade coffee farms have received attention for their ability to provide habitat for native and migratory birds (Perfecto et al. 1996, Greenberg et al. 1997, Donald 2004), till now little was known about the role of shade coffee farms in maintaining essential pollen and seed dispersal processes for native trees. This dissertation research revealed that both dispersal processes are maintained for *M. affinis* within shade coffee farms and between coffee farms and forest fragments. Though the preservation of tropical forest fragments is paramount, migration between forest fragments, across agroecological landscapes, is

also essential for metapopulation stability (Nee and May 1992, Hanski 1999, Perfecto and Vandermeer 2008). This dissertation reveals the potential role of shade coffee farms in providing a matrix permeable to native plant seed dispersers and pollinators.

By focusing on international collaboration, this research also strengthens understanding between scientists and managers in the United States and Mexico. Specifically, this dissertation research has provided us with the opportunity to communicate with local coffee farmers about land management, pollination services and erosion control within their farms. As worldwide tropical deforestation continues, it is urgent that we continue the dialogue between agriculturalists and ecologists in order to understand how to best maintain ecological processes required for crop production, native bee preservation, and the conservation of tropical plant populations.

Chapter VIII References

2005. R: A language and environment for statistical computing. R Development Core Team. R Development Core Team, R Foundation for Statistical Computing, URL <http://www.R-project.org>, Vienna, Austria.
- Abrams, P. A. 1982. Functional-Responses of Optimal Foragers. *American Naturalist* **120**:382-390.
- Abrams, P. A. 1991. Life-History and the Relationship between Food Availability and Foraging Effort. *Ecology* **72**:1242-1252.
- Ackerman, J. D. 1983. Diversity and seasonality of male euglossine bees (Hymenoptera, Apidae) in central Panama. *Ecology* **64**:274-283.
- Aguilar, R., L. Ashworth, L. Galetto, and M. A. Aizen. 2006. Plant reproductive susceptibility to habitat fragmentation: review and synthesis through a meta-analysis. *Ecology Letters* **9**:968-980.
- Aizen, M. A. and P. Feinsinger. 1994. Habitat fragmentation, native insect pollinators, and feral honeybees in Argentine chaco serrano. *Ecological Applications* **4**:378-392.
- Aldrich, P. R. and J. L. Hamrick. 1998. Reproductive dominance of pasture trees in a fragmented tropical forest mosaic. *Science* **281**:103-105.
- Aldrich, P. R., J. L. Hamrick, P. Chavarriaga, and G. Kochert. 1998. Microsatellite analysis of demographic genetic structure in fragmented populations of the tropical tree *Symphonia globulifera*. *Molecular Ecology* **7**:933-944.
- Allen-Wardell, G., P. Bernhardt, R. Bitner, A. Burquez, S. Buchmann, J. Cane, P. A. Cox, V. Dalton, P. Feinsinger, M. Ingram, D. Inouye, C. E. Jones, K. Kennedy, P. Kevan, H. Koopowitz, R. Medellin, S. Medellin-Morales, G. P. Nabhan, B. Pavlik, V. Tepedino, P. Torchio, and S. Walker. 1998. The potential consequences of pollinator declines on the conservation of biodiversity and stability of food crop yields. *Conservation Biology* **12**:8-17.
- Ashworth, L., R. Aguilar, L. Galetto, and M. A. Aizen. 2004. Why do pollination generalist and specialist plant species show similar reproductive susceptibility to habitat fragmentation? *Journal of Ecology* **92**:717-719.
- Asquith, N. M., J. Terborgh, A. E. Arnold, and C. M. Riveros. 1999. The fruits the agouti ate: *Hymenaea courbaril* seed fate when its disperser is absent. *Journal of Tropical Ecology* **15**:229-235.
- Banaszak, J. 1996. Ecological bases of conservation of wild bees. Pages 55-62 in A. Matheson, S. Buchmann, C. O'Toole, P. Westrich, and I. Williams, editors. *The Conservation of Bees*. Academic Press, London.
- Barron, A. B., H. Zhu, G. E. Robinson, and M. V. Srinivasan. 2005. Influence of flight time and flight environment on distance communication by dancing honey bees. *Insectes Sociaux* **52**:402-407.
- Bawa, K. S. 1990. *Reproductive Ecology of Tropical Forest Plants*. Unesco and Parthenon Press, Paris and USA.
- Bawa, K. S. 1992. *Mating Systems, Genetic Differentiation and Speciation in Tropical Rain-Forest Plants*. *Biotropica* **24**:250-255.

- Bawa, K. S., S. H. Bullock, D. R. Perry, R. E. Coville, and M. H. Grayum. 1985. Reproductive-Biology of Tropical Lowland Rain-Forest Trees .2. Pollination Systems. *American Journal of Botany* **72**:346-356.
- Bawa, K. S., W. J. Kress, N. M. Nadkarni, S. Lele, P. H. Raven, D. H. Janzen, A. E. Lugo, P. S. Ashton, and T. E. Lovejoy. 2004. Tropical ecosystems into the 21st century. *Science* **306**:227-228.
- Benton, T. G., J. A. Vickery, and J. D. Wilson. 2003. Farmland biodiversity: is habitat heterogeneity the key? *Trends in Ecology & Evolution* **18**:182-188.
- Biesmeijer, J. C., S. P. M. Roberts, M. Reemer, R. Ohlemuller, M. Edwards, T. Peeters, A. P. Schaffers, S. G. Potts, R. Kleukers, C. D. Thomas, J. Settele, and W. E. Kunin. 2006. Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science* **313**:351-354.
- Breiman, L., J. Freidman, R. Olshen, and C. Stone. 1984. *Classification and Regression Trees*. Wadsworth, Belmont, CA.
- Brosi, B. J. in press. The complex responses of social stingless bees (Apidae: Meliponini) to tropical deforestation. *Forest Ecology and Management* **in press**.
- Brosi, B. J., G. C. Daily, and P. R. Ehrlich. 2007. Bee community shifts with landscape context in a tropical countryside. *Ecological Applications* **17**:418-430.
- Brosi, B. J., G. C. Daily, T. M. Shih, F. Oviedo, and G. Duran. 2008. The effects of forest fragmentation on bee communities in tropical countryside. *Journal of Applied Ecology* **45**:773-783.
- Brosi, B. J., A. Smith-Pardo, and V. H. Gonzalez. 2006. A new wood-nesting Neocorynura (Hymenoptera : Halictidae : Augochlorini) from Costa Rica, with notes on its biology. *Zootaxa*:55-68.
- Brown, J. C. and C. Albrecht. 2001. The effect of tropical deforestation on stingless bees of the genus *Melipona* (Insecta: Hymenoptera: Apidae: Meliponini) in central Rondonia, Brazil. *Journal of Biogeography* **28**:623-634.
- Buchman, G. P., and Nabhan, S.L. 1996. *The Forgotten Pollinators*. Island Press/Shearwater Books.
- Buchmann, S. 1983. Buzz pollination in angiosperms. . Pages 73-113 *in* C. E. R. Jones, editor. *Little Handbook of experimental pollination biology* Van Nostrand, New York.
- Bullock, S. H. and J. A. Solis-Magallanes. 1990. Phenology of canopy trees of a tropical deciduous forest in Mexico. *Biotropica* **22**:22-35.
- Butz-Huryn, V. 1997. Ecological impacts of introduced honey bees. *Quarterly Review of Biology* **72**:275-297
- Cairns, C. E., R. Villanueva-Gutierrez, S. Koptur, and D. B. Bray. 2005. Bee populations, forest disturbance, and africanization in Mexico. *Biotropica* **37**:686-692.
- Cane, J. H. 2001. Habitat fragmentation and native bees: a premature verdict? *Conservation Ecology* **5**:art. no.-3.
- Cane, J. H., R. L. Minckley, L. J. Kervin, T. H. Roulston, and N. M. Williams. 2006. Complex responses within a desert bee guild (Hymenoptera : Apiformes) to urban habitat fragmentation. *Ecological Applications* **16**:632-644.
- Cardinale, B. J., J. J. Weis, A. E. Forbes, K. J. Tilmon, and A. R. Ives. 2006. Biodiversity as both a cause and consequence of resource availability: a study of reciprocal causality in a predator-prey system. *Journal of Animal Ecology* **75**:497-505.

- Carvell, C., W. R. Meek, R. F. Pywell, and M. Nowakowski. 2004. The response of foraging bumblebees to successional change in newly created arable field margins. *Biological Conservation* **118**:327-339.
- Chacoff, N. P. and M. A. Aizen. 2006. Edge effects on flower-visiting insects in grapefruit plantations bordering premontane subtropical forest. *Journal of Applied Ecology* **43**:18-27.
- Challenger, A. 1998. Utilización y conservación de los ecosistemas terrestres de México. Pasado, presente y futuro. México. Conabio, IBUNAM y Agrupación Sierra Madre.
- Chao, A. 1987. Estimating the Population-Size for Capture Recapture Data with Unequal Catchability. *Biometrics* **43**:783-791.
- Chase, M. R., C. Moller, R. Kesseli, and K. S. Bawa. 1996. Distant gene flow in tropical trees. *Nature* **383**:398-399.
- Clark, J. S. 1998. Why trees migrate so fast: Confronting theory with dispersal biology and the paleorecord. *American Naturalist* **152**:204-224.
- Clavero, M. and E. Garcia-Berthou. 2005. Invasive species are a leading cause of animal extinctions. *Trends in Ecology & Evolution* **20**:110.
- Colwell, R. K. and J. A. Coddington. 1994. Estimating Terrestrial Biodiversity through Extrapolation. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **345**:101-118.
- Croat, T. C. 1978. The flora of Barro Colorado Island. Stanford University Press, Palo Alto, CA, USA.
- Daily, G. C. 1997. Nature's services: Societal dependence on natural ecosystems. Island Press, Washington, D.C., USA.
- De'ath, G. and K. E. Fabricius. 2000. Classification and regression trees: A powerful yet simple technique for ecological data analysis. *Ecology* **81**:3178-3192.
- De Marco, P. and F. M. Coelho. 2004. Services performed by the ecosystem: forest remnants influence agricultural cultures' pollination and production. *Biodiversity and Conservation* **13**:1245-1255.
- Dick, C. W. 2001. Genetic rescue of remnant tropical trees by an alien pollinator. *Proceedings of the Royal Society of London Series B-Biological Sciences* **268**:2391-2396.
- Dick, C. W., G. Etchelecu, and F. Austerlitz. 2003. Pollen dispersal of tropical trees (*Dinizia excelsa*: Fabaceae) by native insects and African honeybees in pristine and fragmented Amazonian rainforest. *Molecular Ecology* **12**:753-764.
- Dick, C. W., F. A. Jones, O. J. Hardy, and R. J. Petit. 2008. Spatial scales of seed and pollen-mediated gene flow in tropical forest trees. *Tropical Plant Biology* **1**:20-33.
- Donald, P. F. 2004. Biodiversity impacts of some agricultural commodity production systems. *Conservation Biology* **18**:17-37.
- Donaldson, J., I. Nanni, C. Zachariades, J. Kemper, and J. Thompson. 2002. Effects of habitat fragmentation on pollinator diversity and plant reproductive success in renosterveld shrublands of South Africa. *Conservation Biology Series (Cambridge)* **16**.
- Dornhaus, A. and L. Chittka. 1999. Insect behaviour - Evolutionary origins of bee dances. *Nature* **401**:38-38.

- Dornhaus, A. and L. Chittka. 2004. Why do honey bees dance? *Behavioral Ecology and Sociobiology* **55**:395-401.
- Dover, J. and J. Settele. 2009. The influences of landscape structure on butterfly distribution and movement: a review. *Journal of Insect Conservation* **13**:3-27.
- Ebeling, A., A. M. Klein, J. Schumacher, W. W. Weisser, and T. Tschamtk. 2008. How does plant richness affect pollinator richness and temporal stability of flower visits? *Oikos* **117**:1808-1815.
- Eickwort, G. C. and S. F. Sakagami. 1979. Classification of nest architecture of bees in the trip Augochlorini (Hymenoptera, Halictidae, Halictinae), with description of a Brazilian nest of *Rhinocorynura-inflaticeps*. *Biotropica* **11**:28-37.
- Engel, M. S. 2000. Classification of the bee tribe Augochlorini (Hymenoptera : Halictidae). *Bulletin of the American Museum of Natural History*:4-89.
- Evans, H. E. and M. J. West-Eberhard. 1970. *The Wasps*. University of Michigan Press, Ann Arbor.
- Faraway, J. 2005. *Extending the linear model with R: Generalized linear, mixed effects and nonparametric regression models*. Chapman and Hall/CRC, Boca Raton.
- Fauchald, P. 1999. Foraging in a hierarchical patch system. *American Naturalist* **153**:603-613.
- Fauchald, P. and T. Tveraa. 2006. Hierarchical patch dynamics and animal movement pattern. *Oecologia* **149**:383-395.
- Fenster, C. B., W. S. Armbruster, P. Wilson, M. R. Dudash, and J. D. Thomson. 2004. Pollination syndromes and floral specialization. *Annual Review of Ecology Evolution and Systematics* **35**:375-403.
- Foster, R. B. 1982. The seasonal rhythm of fruitfall on Barro Colorado Island. Pages 151-172 in E. G. J. Leigh, A. S. Rand, and D. M. Windsor, editors. *Ecology of a tropical forest: seasonal rhythms and long-term changes*. Smithsonian Institution Press, Washington, D.C., USA.
- Free, J. B. and I. H. Williams. 1983. Scent-marking of flowers by honeybees. *Journal of Apicultural Research* **22**:86-90.
- Geist, H. J. and E. F. Lambin. 2002. Proximate causes and underlying driving forces of tropical deforestation. *Bioscience* **52**:143-150.
- Glen, T. C. and N. A. Schable. 2005. Isolating microsatellite DNA loci. *Methods in Enzymology* **395**:202-222.
- Goulson, D. 2003. Effects of introduced bees on native ecosystems. *Annual Review of Ecology Evolution and Systematics* **34**:1-26.
- Greenberg, R., P. Bichier, A. C. Angon, C. MacVean, R. Perez, and E. Cano. 2000. The impact of avian insectivory on arthropods and leaf damage in some Guatemalan coffee plantations. *Ecology* **81**:1750-1755.
- Greenberg, R., P. Bichier, and J. Sterling. 1997. Bird populations in rustic and planted shade coffee plantations of eastern Chiapas, Mexico. *Biotropica* **29**:501-514.
- Greenleaf, S. S. and C. Kremen. 2006. Wild bee species increase tomato production and respond differently to surrounding land use in Northern California. *Biological Conservation* **133**:81-87.
- Greenleaf, S. S., N. M. Williams, R. Winfree, and C. Kremen. 2007. Bee foraging ranges and their relationship to body size. *Oecologia* **153**:589-596.

- Griffis-Kyle, K. L. and P. Beier. 2005. Migratory strategy and seasonal patterns of bird diversity in relation to forest habitat. *American Midland Naturalist* **153**:436-443.
- Guo, S. and E. Thompson. 1992. Performing the exact test of Hardy–Weinberg proportion from multiple alleles. *Biometrics*:361–372.
- Gustafson, E. J. and R. H. Gardner. 1996. The effect of landscape heterogeneity on the probability of patch colonization. *Ecology* **77**:94-107.
- Hanski, I. 1999. *Metapopulation ecology*. Oxford University Press, New York, NY.
- Hardy, O. J. and X. Vekemans. 2002. SPAGEDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes* **2**:618-620.
- Hegland, S. J. and L. Boeke. 2006. Relationships between the density and diversity of floral resources and flower visitor activity in a temperate grassland community. *Ecological Entomology* **31**:532-538.
- Heinrich, B. 1979a. Keeping a Cool Head - Honeybee Thermoregulation. *Science* **205**:1269-1271.
- Heinrich, B. 1979b. Resource Heterogeneity and Patterns of Movement in Foraging Bumblebees. *Oecologia* **40**:235-245.
- Heinrich, B. 1996. How the honey bee regulates its body temperature. *Bee World* **77**:130-137.
- 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.
- Hoehn, P., T. Tschardtke, J. M. Tylianakis, and I. Steffan-Dewenter. 2008. Functional group diversity of bee pollinators increases crop yield. *Proceedings of the Royal Society B-Biological Sciences* **275**:2283-2291.
- Hooper, D. U., F. S. Chapin, J. J. Ewel, A. Hector, P. Inchausti, S. Lavorel, J. H. Lawton, D. M. Lodge, M. Loreau, S. Naeem, B. Schmid, H. Setälä, A. J. Symstad, J. Vandermeer, and D. A. Wardle. 2005. Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. *Ecological Monographs* **75**:3-35.
- Hubbell, S. P. and L. K. Johnson. 1978. Comparative Foraging Behavior of 6 Stingless Bee Species Exploiting a Standardized Resource. *Ecology* **59**:1123-1136.
- Huelsenbeck, J. P., P. Andolfatto, and E. T. Huelsenbeck. in press. Structurama: bayesian inference of population structure. *Bioinformatics*.
- Jha, S. and C. W. Dick. 2008. Shade coffee farms promote genetic diversity of native trees. *Current Biology* **18**:1126-1128.
- Jha, S. and C. W. Dick. 2009. Microsatellite markers for *Miconia affinis* (Melastomataceae). *Molecular Ecology Resources* **9** 344-345.
- Jha, S. and C. W. Dick. in press. Microsatellite markers for *Miconia affinis* (Melastomataceae). *Molecular Ecology Resources*.
- Johnson, L. K. and S. P. Hubbell. 1974. Aggression and Competition among Stingless Bees - Field Studies. *Ecology* **55**:120-127.
- Johnson, R. 2007. CRS report for congress: recent honey bee colony declines (Government report prepared for members of committees of congress RL33938).in R. AiAE, Science and Industry Division, editor., Washington, DC.

- Jones, F. A. and H. C. Muller-Landau. 2008. Measuring long-distance seed dispersal in complex natural environments: an evaluation and integration of classical and genetic methods. *Journal of Ecology* **96**:642-652.
- Karr, J. R. and K. E. Freemark. 1983. Habitat selection and environmental gradients - dynamics in the stable tropics. *Ecology* **64**:1481-1494.
- Kearns, C. A. and D. W. Inouye. 1993. *Techniques for Pollination Biologists*. University Press of Colorado, Niwot, Colorado, USA.
- Kearns, C. A., D. W. Inouye, and N. M. Waser. 1998. Endangered mutualisms: The conservation of plant-pollinator interactions. *Annual Review of Ecology and Systematics* **29**:83-112.
- Kerr, W. E. 1967. The history of the introduction of African bees to Brazil. . *South African Bee Journal* **39**:3-5.
- Kerr, W. E., S. D. Delrio, and M. D. Barrionuevo. 1982. The Southern Limits of the Distribution of the Africanized Honey Bee in South-America. *American Bee Journal* **122**:196-198.
- Kim, J., N. Williams, and C. Kremen. 2006. Effects of cultivation and proximity to natural habitat on ground-nesting native bees in California sunflower fields. *Journal of the Kansas Entomological Society* **79**:309-320.
- Kindlmann, P. and F. Burel. 2008. Connectivity measures: a review. *Landscape Ecology* **23**:879-890.
- Klein, A. M., S. A. Cunningham, M. Bos, and I. Steffan-Dewenter. 2008. Advances in pollination ecology from tropical plantation crops. *Ecology* **89**:935-943.
- Klein, A. M., I. Steffan-Dewenter, D. Buchori, and T. Tscharntke. 2002. Effects of land-use intensity in tropical agroforestry systems on coffee flower-visiting and trap-nesting bees and wasps. *Conservation Biology* **16**:1003-1014.
- Klein, A. M., I. Steffan-Dewenter, and T. Tscharntke. 2003a. Bee pollination and fruit set of *Coffea arabica* and *C-canephora* (Rubiaceae). *American Journal of Botany* **90**:153-157.
- Klein, A. M., I. Steffan-Dewenter, and T. Tscharntke. 2003b. Fruit set of highland coffee increases with the diversity of pollinating bees. *Proceedings of the Royal Society of London Series B-Biological Sciences* **270**:955-961.
- Klein, A. M., I. Steffan-Dewenter, and T. Tscharntke. 2003c. Pollination of *Coffea canephora* in relation to local and regional agroforestry management. *Journal of Applied Ecology* **40**:837-845.
- Klein, A. M., I. Steffan-Dewenter, and T. Tscharntke. 2004. Foraging trip duration and density of megachilid bees, eumenid wasps and pompilid wasps in tropical agroforestry systems. *Journal of Animal Ecology* **73**:517-525.
- Klein, A. M., I. Steffan-Dewenter, and T. Tscharntke. 2006. Rain forest promotes trophic interactions and diversity of trap-nesting hymenoptera in adjacent agroforestry. *Journal of Animal Ecology* **75**:315-323.
- Klein, A. M., B. E. Vaissiere, J. H. Cane, I. Steffan-Dewenter, S. A. Cunningham, C. Kremen, and T. Tscharntke. 2007. Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B-Biological Sciences* **274**:303-313.

- Krauss, J., T. Alfert, and I. Steffan-Dewenter. 2009. Habitat area but not habitat age determines wild bee richness in limestone quarries. *Journal of Applied Ecology* **46**:194-202.
- Kremen, C., R. Bugg, N. Nicola, S. SA, R. Thorp, and N. Williams. 2002a. Native bees, native plants and crop pollination in California. *Fremontia* **30**:41-49.
- Kremen, C., N. M. Williams, M. A. Aizen, B. Gemmill-Herren, G. LeBuhn, R. Minckley, L. Packer, S. G. Potts, T. Roulston, I. Steffan-Dewenter, D. P. Vazquez, R. Winfree, L. Adams, E. E. Crone, S. S. Greenleaf, T. H. Keitt, A. M. Klein, J. Regetz, and T. H. Ricketts. 2007. Pollination and other ecosystem services produced by mobile organisms: a conceptual framework for the effects of land-use change. *Ecology Letters* **10**:299-314.
- Kremen, C., N. M. Williams, R. L. Bugg, J. P. Fay, and R. W. Thorp. 2004. The area requirements of an ecosystem service: crop pollination by native bee communities in California. *Ecology Letters* **7**:1109-1119.
- Kremen, C., N. M. Williams, and R. W. Thorp. 2002b. 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.
- Kunin, W. E. 1993. Sex and the Single Mustard - Population-Density and Pollinator Behavior Effects on Seed-Set. *Ecology* **74**:2145-2160.
- Kunin, W. E. 1997. Population size and density effects in pollination: Pollinator foraging and plant reproductive success in experimental arrays of *Brassica kaber*. *Journal of Ecology* **85**:225-234.
- Lambin, E. F., H. J. Geist, and E. Lepers. 2003. Dynamics of land-use and land-cover change in tropical regions. *Annual Review of Environment and Resources* **28**:205-241.
- Larsen, T. H., N. Williams, and C. Kremen. 2005. Extinction order and altered community structure rapidly disrupt ecosystem functioning. *Ecology Letters* **8**:538-547.
- Laurance, W. F. 1998. A crisis in the making: responses of Amazonian forests to land use and climate change. *Trends in Ecology & Evolution* **13**:411-415.
- LeBuhn, G., S. Droege, N. Williams, R. Minckley, T. Griswold, C. Kremen, O. Messinger, J. Cane, T. Roulston, F. Parker, V. Tepedino, and S. Buchmann. 2005. A standardized method for monitoring bee populations- The Bee Inventory Plot. available online at <http://online.sfsu.edu/~beeplot/>.
- Levey, D. J., W. R. Silva, and M. Galetti. 2002. *Seed Dispersal and Frugivory: Ecology, Evolution and Conservation* CAB International, Wallingford, UK.
- Lin, B. B. 2007. Agroforestry management as an adaptive strategy against potential microclimate extremes in coffee agriculture. *Agricultural and Forest Meteorology* **144**:85-94.
- Lin, B. B. and P. L. Richards. 2007. Soil random roughness and depression storage on coffee farms of varying shade levels. *Agricultural Water Management* **92**:194-204.
- Lindauer, M. 1971. *Communication among social bees*. Harvard Univ. Press, Cambridge, MA.

- Loiselle, B. A., V. L. Sork, J. Nason, and C. Graham. 1995. Spatial Genetic-Structure of a Tropical Understory Shrub, *Psychotria Officinalis* (Rubiaceae). *American Journal of Botany* **82**:1420-1425.
- Lowe, A. J., D. Boshier, M. Ward, C. F. E. Bacles, and C. Navarro. 2005. Genetic resource impacts of habitat loss and degradation; reconciling empirical evidence and predicted theory for neotropical trees. *Heredity* **95**:255-273.
- Loyola, R. D. and R. P. Martins. 2008. Habitat structure components are effective predictors of trap-nesting Hymenoptera diversity. *Basic and Applied Ecology* **9**:735-742.
- Luck, G. W. and G. C. Daily. 2003. Tropical countryside bird assemblages: Richness, composition, and foraging differ by landscape context. *Ecological Applications* **13**:235-247.
- Macarthur, R. and J. W. Macarthur. 1961. On bird species-diversity. *Ecology* **42**:594-&.
- Mangel, M. and C. W. Clark. 1986. Towards a Unified Foraging Theory. *Ecology* **67**:1127-1138.
- Marshall, E. J. P. 2002. Field margins in northern Europe: Integrating agricultural, environmental and biodiversity functions. Pages 39-67 *in* Symposium on Field Boundary Habitats. Canadian Weed Science Soc, Soc Canadienne Malherbologie, Saskatoon, CANADA.
- Marshall, T. C., J. Slate, L. E. B. Kruuk, and J. M. Pemberton. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology* **7**:639-655.
- McIntosh, M. 1996. Nest-substrate preferences of the twig-nesters *Ceratina acantha*, *Ceratina nanula* (Apidae) and *Pemphredon lethifer* (Sphecidae). *Journal of the Kansas Entomological Society* **69**:216-231.
- Michener, C. D. 1979. Biogeography of the Bees. *Annals of the Missouri Botanical Garden* **66**:277-347.
- Michener, C. D. 2000. *Bees of the World*. Johns Hopkins University Press, Baltimore, MD.
- Moguel, P. and V. M. Toledo. 1999. Biodiversity conservation in traditional coffee systems of Mexico. *Conservation Biology* **13**:11-21.
- Morgan, J. N. and J. A. Sonquist. 1963. Problems in analysis of survey data, and a proposal. *Journal of the American Statistical Association* **58**:415-&.
- Murdoch, W. W., C. H. Peterson, and F. C. Evans. 1972. Diversity and Pattern in Plants and Insects. *Ecology* **53**:819-&.
- Myers, N., R. A. Mittermeier, C. G. Mittermeier, G. A. B. da Fonseca, and J. Kent. 2000. Biodiversity hotspots for conservation priorities. *Nature* **403**:853-858.
- Nagamitsu, T. and T. Inoue. 1997. Aggressive foraging of social bees as a mechanism of floral resource partitioning in an Asian tropical rainforest. *Oecologia* **110**:432-439.
- Nason, J. D. and J. L. Hamrick. 1997. Reproductive and genetic consequences of forest fragmentation: Two case studies of neotropical canopy trees. *Journal of Heredity* **88**:264-276.
- Nee, S. and R. M. May. 1992. Dynamics of metapopulations- habitat destruction and competitive coexistence. *Journal of Animal Ecology* **61**:37-40.

- Nieh, J. C. 2004. Recruitment communication in stingless bees (Hymenoptera, Apidae, Meliponini). *Apidologie* **35**:159-182.
- Ockinger, E. and H. G. Smith. 2007. Semi-natural grasslands as population sources for pollinating insects in agricultural landscapes. *Journal of Applied Ecology* **44**:50-59.
- Okazaki, K. 1992. Nesting habits of the small carpenter bee, *Ceratina dentipes*, in Hengchun Peninsula, Southern Taiwan. *Journal of the Kansas Entomological Society* **65**:190-195.
- Olden, J. D., J. J. Lawler, and N. L. Poff. 2008. Machine learning methods without tears: a primer for ecologists *The Quarterly Review of Biology* **83**:171-192.
- Oliver, I. and A. J. Beattie. 1996. Invertebrate morphospecies as surrogates for species: a case study. *Conservation Biology* **10**:99-109.
- Oosterhout, C., W. F. Hutchinson, D. P. M. Wills, and P. Shipley. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*:535-538.
- Pasquet, R. S., A. Peltier, M. B. Hufford, E. Oudin, J. Saulnier, L. Paul, J. T. Knudsen, H. R. Herren, and P. Gepts. 2008. Long-distance pollen flow assessment through evaluation of pollinator foraging range suggests transgene escape distances. *Proceedings of the National Academy of Sciences of the United States of America* **105**:13456-13461.
- Peakall, R. and P. E. Smouse. 2001. GENALEX (version 5.1): genetic analysis in Excel, Population Genetic Software for Teaching and Research, . Australian National University, Canberra, Australia.
- Perfecto, I., R. A. Rice, R. Greenberg, and M. E. VanderVoort. 1996. Shade coffee: A disappearing refuge for biodiversity. *Bioscience* **46**:598-608.
- Perfecto, I. and J. Vandermeer. 2008. Biodiversity conservation in tropical agroecosystems - A new conservation paradigm. Pages 173-200 *Year in Ecology and Conservation Biology 2008*. Blackwell Publishing, Oxford.
- Perfecto, I., J. H. Vandermeer, G. L. Bautista, G. I. Nunez, R. Greenberg, P. Bichier, and S. Langridge. 2004. Greater predation in shaded coffee farms: The role of resident neotropical birds. *Ecology* **85**:2677-2681.
- Philpott, S. M., W. J. Arendt, I. Armbrecht, P. Bichier, T. V. Diestch, C. Gordon, R. Greenberg, I. Perfecto, R. Reynoso-Santos, L. Soto-Pinto, C. Tejada-Cruz, G. Williams-Linera, J. Valenzuela, and J. M. Zolotoff. 2008a. Biodiversity Loss in Latin American Coffee Landscapes: Review of the Evidence on Ants, Birds, and Trees. *Conservation Biology* **22**:1093-1105.
- Philpott, S. M., B. Lin, S. Jha, and S. J. Brines. 2008b. A multi-scale assessment of hurricane impacts on agricultural landscapes based on landuse and topographic features. *Agriculture, Ecosystems and Environment* **128**:12-20.
- Plateaux-Quenu, C. 2008. Subsociality in halictine bees. *Insectes Sociaux* **55**:335-346.
- Potts, S. G., B. Vulliamy, A. Dafni, G. Ne'eman, and P. Willmer. 2003. Linking bees and flowers: How do floral communities structure pollinator communities? *Ecology* **84**:2628-2642.
- Potts, S. G., B. Vulliamy, S. Roberts, C. O'Toole, A. Dafni, G. Ne'Eman, and P. Willmer. 2005. Role of nesting resources in organising diverse bee communities in a Mediterranean landscape. *Ecological Entomology* **30**:78-85.

- Price, M. V. 1984. *Insect ecology*. 2nd edition. John Wiley and Sons, New York, New York, USA.
- Pritchard, J. K., M. Stephens, and P. J. Donnelly. 2000. Inference of population structure using multilocus genotype data. *Genetics* **155**:945-959.
- Raine, N. E., T. C. Ings, A. Dornhaus, N. Salefl, and L. Chittka. 2006. Adaptation, genetic drift, pleiotropy, and history in the evolution of bee foraging behavior. Pages 305-354 *Advances in the Study of Behavior*, Vol 36. Elsevier Academic Press Inc, San Diego.
- Ramirez-Marcial, N., M. Gonzalez-Espinosa, and G. Williams-Linera. 2001. Anthropogenic disturbance and tree diversity in Montane Rain Forests in Chiapas, Mexico. *Forest Ecology and Management* **154**:311-326.
- Rathcke, B. 1983. Competition and facilitation among plants for pollination. Pages 305–327 *in* L. Real, editor. *Pollination biology*. Academic Press, New York, New York, USA. .
- Raymond, M. and F. Rousset. 1995. GENEPOP version 1.2: a population genetics software for exact test and ecumenicism. *Journal of Heredity* **86**:248–249.
- Recknagel, F. 2001. Applications of machine learning to ecological modelling. *Ecological Modelling* **146**:303-310.
- Redford, K. H. 1992. The Empty Forest. *Bioscience* **42**:412-422.
- Rice, J., B. W. Anderson, and R. D. Ohmart. 1980. Seasonal habitat selection by birds in the lower Colorado river valley. *Ecology* **61**:1402-1411.
- Richter, M. R. 1990. Hunting Social Wasp Interactions - Influence of Prey Size, Arrival Order, and Wasp Species. *Ecology* **71**:1018-1030.
- Richter, M. R. 2000. Social wasp (Hymenoptera : Vespidae) foraging behavior. *Annual Review of Entomology* **45**:121-150.
- Richter, M. R. and V. L. Tisch. 1999. Resource choice of social wasps: influence of presence, size and species of resident wasps. *Insectes Sociaux* **46**:131-136.
- Ricketts, T. H. 2004. Tropical forest fragments enhance pollinator activity in nearby coffee crops. *Conservation Biology* **18**:1262-1271.
- Ricketts, T. H., G. C. Daily, P. R. Ehrlich, and C. D. Michener. 2004. Economic value of tropical forest to coffee production. *Proceedings of the National Academy of Sciences of the United States of America* **101**:12579-12582.
- Ricketts, T. H., J. Regetz, I. Steffan-Dewenter, S. A. Cunningham, C. Kremen, A. Bogdanski, B. Gemmill-Herren, S. S. Greenleaf, A. M. Klein, M. M. Mayfield, L. A. Morandin, A. Ochieng, and B. F. Viana. 2008. Landscape effects on crop pollination services: are there general patterns? *Ecology Letters* **11**:499-515.
- Rincon, M., D. W. Roubik, B. Finegan, D. Delgado, and N. Zamora. 1999. Understory bees and floral resources in logged and silviculturally treated Costa Rican rainforest plots. *Journal of the Kansas Entomological Society* **72**:379-393.
- Robledo-Arnuncio, J. J., F. Austerlitz, and P. E. Smouse. 2007. POLDISP: a software package for indirect estimation of contemporary pollen dispersal. *Molecular Ecology Notes* **7**:763-766.
- Root, R. B. and P. M. Kareiva. 1984. The Search for Resources by Cabbage Butterflies (Pieris-Rapae) - Ecological Consequences and Adaptive Significance of Markovian Movements in a Patchy Environment. *Ecology* **65**:147-165.

- Ross, K. and R. Matthews. 1991. *The Social Biology of Wasps*. Cornell University Press, Ithaca.
- Roubik, D. 1986. Sporadic food competition with the African honey bee: projected impact on neotropical social bees. *Journal of Tropical Ecology* **2**:97–111.
- Roubik, D. 1991. Aspects of Africanized honey bee ecology in tropical America. . Page 435 *in* F. D. Spivak M, Breed MD editor. *The “African” Honey Bee*. Westview, Boulder, CO
- Roubik, D. 1995. *Pollination of cultivated plants in the tropics*. Food and Agriculture Organization of the United Nations, Rome, Italy.
- Roubik, D. W. 1978. Competitive Interactions between Neotropical Pollinators and Africanized Honey Bees. *Science* **201**:1030-1032.
- Roubik, D. W. 1980. Foraging Behavior of Competing Africanized Honeybees and Stingless Bees. *Ecology* **61**:836-845.
- Roubik, D. W. 2002. Tropical agriculture - The value of bees to the coffee harvest. *Nature* **417**:708-708.
- Roubik, D. W. and H. Wolda. 2001. Do competing honey bees matter? Dynamics and abundance of native bees before and after honey bee invasion. *Population Ecology* **43**:53-62.
- Sakagami, S. and C. Michener. 1962. *The Nest Architecture of Sweat Bees* University of Kansas Press, Lawrence, Kansas, U.S.A.
- Sakagami, S. F. and Y. Maeta. 1977. Some presumably pre-social habits of Japanese *Ceratina* bees, with notes on various social types in Hymenoptera. *Insectes Sociaux* **24**:319-343.
- Sargent, R. D. 2003. Seasonal changes in pollen-packaging schedules in the protandrous plant *Chamerion angustifolium*. *Oecologia* **135**:221-226.
- Schaffer, W. M., D. B. Jensen, D. E. Hobbs, J. Gurevitch, J. R. Todd, and M. V. Schaffer. 1979. Competition, Foraging Energetics, and the Cost of Sociality in 3 Species of Bees. *Ecology* **60**:976-987.
- Schaffer, W. M., D. W. Zeh, S. L. Buchmann, S. Kleinhans, M. V. Schaffer, and J. Antrim. 1983. Competition for Nectar between Introduced Honey Bees and Native North-American Bees and Ants. *Ecology* **64**:564-577.
- Schmidt, J. O. and S. C. Thoenes. 1992. Criteria for nest site selection in honey-bees (Hymenoptera, Apidae) - preferences between pheromone attractants and cavity shapes. *Environmental Entomology* **21**:1130-1133.
- Schneider, S. S. and H. G. Hall. 1997. Diet selection and foraging distances of African and European-African hybrid honey bee colonies in Costa Rica. *Insectes Sociaux* **44**:171-187.
- Schneider, S. S., G. D. Hoffman, and D. R. Smith. 2004. The African honey bee: Factors contributing to a successful biological invasion. *Annual Review of Entomology* **49**:351-376.
- Schoener, T. W. 1971. Theory of Feeding Strategies. *Annual Review of Ecology and Systematics* **2**:369-404.
- Sekercioglu, C. H. 2006. Ecological significance of bird populations. Pages 15-51 *Handbook of the Birds of the World*.
- Sezen, U. U., R. L. Chazdon, and K. E. Holsinger. 2005. Genetic consequences of tropical second-growth forest regeneration. *Science* **307**:891-891.

- Sezen, U. U., R. L. Chazdon, and K. E. Holsinger. 2007. Multigenerational genetic analysis of tropical secondary regeneration in a canopy palm. *Ecology* **88**:3065-3075.
- Shmida, A. and M. V. Wilson. 1985. Biological determinants of species-diversity. *Journal of Biogeography* **12**:1-20.
- Sih, A. and M. S. Baltus. 1987. Patch Size, Pollinator Behavior, and Pollinator Limitation in Catnip. *Ecology* **68**:1679-1690.
- Slaa, E. J. 2003. Foraging ecology of stingless bees: from individual behaviour to community ecology. Utrecht University, Department of Behavioural Biology., Utrecht, Germany.
- Smouse, P. E., R. J. Dyer, R. D. Westfall, and V. L. Sork. 2001. Two-generation analysis of pollen flow across a landscape. I. Male gamete heterogeneity among females. *Evolution* **55**:260-271.
- Sokal, R. R., Rohlf, FJ 1995. *Biometry: the principles and practice of statistics in biological research*. 3rd edition. WH Freeman, New York, N.Y.
- Soto-Pinto, L., Y. Romero-Alvarado, J. Caballero-Nieto, and G. S. Warnholtz. 2001. Woody plant diversity and structure of shade-grown-coffee plantations in Northern Chiapas, Mexico. *Revista de Biología Tropical* **49**:977-987.
- Spivak, M., D. J. C. Fletcher, and M. C. Breed. 1991. *The "African" Honey Bee*. Westview, Boulder, CO.
- Srinivasan, M. V., S. W. Zhang, M. Lehrer, and T. S. Collett. 1996. Honeybee navigation en route to the goal: Visual flight control and odometry. *Journal of Experimental Biology* **199**:237-244.
- Steffan-Dewenter, I., U. Munzenberg, C. Burger, C. Thies, and T. Tschardtke. 2002. Scale-dependent effects of landscape context on three pollinator guilds. *Ecology* **83**:1421-1432.
- Steffan-Dewenter, I., U. Munzenberg, and T. Tschardtke. 2001. Pollination, seed set and seed predation on a landscape scale. *Proceedings of the Royal Society of London Series B-Biological Sciences* **268**:1685-1690.
- Steffan-Dewenter, I., S. G. Potts, and L. Packer. 2005. Pollinator diversity and crop pollination services are at risk. *Trends in Ecology & Evolution* **20**:651-652.
- Steffan-Dewenter, I. and T. Tschardtke. 2001. Succession of bee communities on fallows. *Ecography* **24**:83-93.
- Steffan-Dewenter, I. and C. Westphal. 2008. The interplay of pollinator diversity, pollination services and landscape change. *Journal of Applied Ecology* **45**:737-741.
- Stein, B., L. Kutner, and J. Adams. 2000. *Precious heritage: The status of biodiversity in the United States*. Oxford University Press, New York.
- Stone, G. N. 1994. Activity Patterns of Females of the Solitary Bee *Anthophora plumipes* in Relation to Temperature, Nectar Supplies and Body-Size. *Ecological Entomology* **19**:177-189.
- Tautz, J., S. W. Zhang, J. Spaethe, A. Brockmann, A. Si, and M. Srinivasan. 2004. Honeybee odometry: Performance in varying natural terrain. *PLoS Biology* **2**:915-923.
- Tepedino, V. J. and N. L. Stanton. 1981. Diversity and Competition in Bee-Plant Communities on Short-Grass Prairie. *Oikos* **36**:35-44.

- Thomson, D. 2004. Competitive interactions between the invasive European honey bee and native bumble bees. *Ecology* **85**:458-470.
- Thomson, D. M. 2006. Detecting the effects of introduced species: a case study of competition between *Apis* and *Bombus*. *Oikos* **114**:407-418.
- Thorp, R. 1987. World overview of the interactions between honeybees and other flora and fauna. Pages 40–47 in J. Blyth, editor. *Beekeeping and Land Management* Department of Conservation and Land Management, Perth.
- Totland, O. and I. Matthews. 1998. Determinants of pollinator activity and flower preference in the early spring blooming *Crocus vernus*. *Acta Oecologica-International Journal of Ecology* **19**:155-165.
- Tylianakis, J. M., A. M. Klein, T. Lozada, and T. Tscharntke. 2006. Spatial scale of observation affects alpha, beta and gamma diversity of cavity-nesting bees and wasps across a tropical land-use gradient. *Journal of Biogeography* **33**:1295-1304.
- Tylianakis, J. M., A. M. Klein, and T. Tscharntke. 2005. Spatiotemporal variation in the diversity of hymenoptera across a tropical habitat gradient. *Ecology* **86**:3296-3302.
- Tylianakis, J. M., T. A. Rand, A. Kahmen, A. M. Klein, N. Buchmann, J. Perner, and T. Tscharntke. 2008. Resource heterogeneity moderates the biodiversity-function relationship in real world ecosystems. *PLoS Biology* **6**:947-956.
- Van Bael, S. A., S. M. Philpott, R. Greenberg, P. Bichier, N. A. Barber, K. A. Mooney, and D. S. Gruner. 2008. Birds as predators in tropical agroforestry systems. *Ecology* **89**:928-934.
- Vandermeer, J. H. 2003. *Tropical Agroecosystems*. CRC Press, Boca Raton, Florida.
- Veddeler, D., A. M. Klein, and T. Tscharntke. 2006. Contrasting responses of bee communities to coffee flowering at different spatial scales. *Oikos* **112**:594-601.
- Vekemans, X. and O. J. Hardy. 2004. New insights from fine-scale spatial genetic structure analyses in plant populations. *Molecular Ecology* **13**:921-935.
- Vergara, C. H. and E. I. Badano. 2009. Pollinator diversity increases fruit production in Mexican coffee plantations: The importance of rustic management systems. *Agriculture Ecosystems & Environment* **129**:117-123.
- Vitousek, P. M., C. M. Dantonio, L. L. Loope, and R. Westbrooks. 1996. Biological invasions as global environmental change. *American Scientist* **84**:468-478.
- Vonfrisch, K., A. M. Wenner, and D. L. Johnson. 1967. Honeybees - Do They Use Direction and Distance Information Provided by Their Dancers. *Science* **158**:1072-&.
- Wang, B. C., V. L. Sork, M. T. Leong, and T. B. Smith. 2007. Hunting of mammals reduces seed removal and dispersal of the afro-tropical tree *Antrocaryon klaineanum* (Anacardiaceae). *Biotropica* **39**:340-347.
- Wcislo, W. T., V. H. Gonzalez, and M. S. Engel. 2003. Nesting and social behavior of a wood-dwelling neotropical bee, *Augochlora isthmii* (Schwarz), and notes on a new species, *A-alexanderi* Engel (Hymenoptera : Halictidae). *Journal of the Kansas Entomological Society* **76**:588-602.
- Weising, K., H. Nybom, K. Wolff, and G. Kahl. 2005. *DNA Fingerprinting in Plants*. CRC Press, Boca raton.
- Westphal, C., R. Bommarco, G. Carre, E. Lamborn, N. Morison, T. Petanidou, S. G. Potts, S. P. M. Roberts, H. Szentgyorgyi, T. Tscheulin, B. E. Vaissiere, M.

- Woyciechowski, J. C. Biesmeijer, W. E. Kunin, J. Settele, and I. Steffan-Dewenter. 2008. Measuring bee diversity in different European habitats and biogeographical regions. *Ecological Monographs* **78**:653-671.
- Westphal, C., I. Steffan-Dewenter, and T. Tscharntke. 2003. Mass flowering crops enhance pollinator densities at a landscape scale. *Ecology Letters* **6**:961-965.
- Wilcove, D. S., D. Rothstein, J. Dubow, A. Phillips, and E. Losos. 1998. Quantifying threats to imperiled species in the United States. *Bioscience* **48**:607-615.
- Williams-Guillen, K., K. Goodall, and C. Taylor. *in prep*. Avian and bat seed dispersers of *Miconia affinis*.
- Williams-Guillen, K., I. Perfecto, and J. Vandermeer. 2008. Bats limit insects in a neotropical agroforestry system. *Science* **320**:70-70.
- Williams, C. 1964. Patterns in the balance of nature and related problems in quantitative ecology. Academic Press, London.
- Willmer, P. G. and G. N. Stone. 2004. Behavioral, ecological, and physiological determinants of the activity patterns of bees. *Advances in the Study of Behavior*:347-466.
- Winfree, R. 2008. Pollinator-Dependent Crops: An Increasingly Risky Business. *Current Biology* **18**:R968-R969.
- Winfree, R. and C. Kremen. 2009. Are ecosystem services stabilized by differences among species? A test using crop pollination. *Proceedings of the Royal Society B-Biological Sciences* **276**:229-237.
- Winfree, R., N. M. Williams, J. Dushoff, and C. Kremen. 2007. Native bees provide insurance against ongoing honey bee losses. *Ecology Letters* **10**:1105-1113.
- Winfree, R., N. M. Williams, H. Gaines, J. S. Ascher, and C. Kremen. 2008. Wild bee pollinators provide the majority of crop visitation across land-use gradients in New Jersey and Pennsylvania, USA. *Journal of Applied Ecology* **45**:793-802.
- Wolda, H. and D. W. Roubik. 1986. Nocturnal Bee Abundance and Seasonal Bee Activity in a Panamanian Forest. *Ecology* **67**:426-433.
- Wright, S. J. 2005. Tropical forests in a changing environment. *Trends in Ecology & Evolution* **20**:553-560.
- Wuellner, C. T. 1999. Nest site preference and success in a gregarious, ground-nesting bee *Dieunomia triangulifera*. *Ecological Entomology* **24**:471-479.
- Yamamura, K. 1999. Relation between plant density and arthropod density in cabbage. *Researches on Population Ecology* **41**:177-182.
- Yamamura, K. 2002. Biodiversity and stability of herbivore populations: influences of the spatial sparseness of food plants. *Population Ecology* **44**:33-40.