Bee-Plant Interactions in Coffee Agroecosystems:
Management and Matrix Effects on Mutualistic and Antagonistic Relationships

by

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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
2021

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Dedication

To Noah, for everything.

Acknowledgements

While my name is the only one listed as the author of this dissertation – and I take full responsibility for its contents – in reality, the following pages represent the effort of a large number of people, without whom it never would have happened.

Thanks to my advisor, John Vandermeer, and honorary co-advisor, Ivette Perfecto, for helping me to be critical, creative, and committed both in my science and in general. My other committee members, Meg Duffy and Gina Baucom, both provided helpful feedback and encouragement along the way. Mark Hunter served on my committee until his retirement at the end of 2020, and I am particularly indebted to his keen insights and critical perspective, both of which have helped me to significantly improve this dissertation. Additional support, intellectual stimulation, and hard questions were generously given by Martin Burd, Phil Conner, Becky Irwin, and Stacy Philpott.

John and Ivette cultivate a wonderful community, and I have benefited tremendously from my association with Perfectameers past and present. Friends, collaborators, critics, support network: immense thanks to Chatura Vaidya, Lauren Schmitt, Kaleigh Fisher, Jonno Morris, Zach Hajian-Forooshani, Iris Rivera, Kristel Sanchez (honorarily), Nicholas Medina, Beatriz Otero Jimenez, Estelí Soto-Jimenez, Alexa White, Theresa Ong, Senay Yitbarek, Aaron Iverson, Dave Gonthier, Paul Glaum, Carolina Simao, Kevin Li, Mariana Valencia, Aldo de la Mora, Naim Edwards, Kim Williams-Guillén, Kate Ennis, fern MacDougal, and Sarah Barney.

The fieldwork upon which this dissertation is based would not have been possible without a great many people. Gustavo López Bautista worked tirelessly to make the experience of being at the finca as smooth as possible, and was also a good friend through long, rainy afternoons and bumpy car rides. Gabriel Dominguez provided invaluable support in the field, as well as updates on the latest *chisme*. Doña Cruz, Flor, Suly, and Tania kept me well-fed and well-organized and made the field station feel like a home-away-from-home. Miguel Crisóstomo and the *vivero* crew provided essential support in my efforts to grow firespike. Sara González was a great partner in adventure. In Tapachula, the staff of Hotel Cervantino, especially Melany, provided a much-needed home-away-from-home-away-from-home. Many thanks to the Peters family for allowing us to conduct research on their farm, and to the workers who maintain it.

In Ann Arbor, my path through the PhD was made easier by the EEB department’s marvelous staff: thanks to Kati Ellis, Gail Kuhnlein, Nathan Sadowsky, Michael Ennis, and John Megahan for your helpfulness, patience, and encouragement. I also benefitted tremendously from the opportunity to GSI with some inspiring professors – thanks to Deborah Goldberg, Annette Ostling, Fernanda Valdovinos, and Ben Winger.

Ann Arbor feels like home due in large part to all the wonderful friends I have made here. The Kingsley St/Traver Rd. family provided a warm welcome and sweet ready-made community as soon as I landed – with a trip to Babe Island on my very first day in Ann Arbor, I should have known it’d be good. The Existential High-Fivers, Peach St. crew, and many others made that community stronger with shared dinners, camping trips, bike rides, paddles, and more. And Babycat, whose life is fully coterminous with my graduate career, has been a constant source of laughter, purrs, scratches, and fur. Faraway friends, too many to mention, have also provided love and support and good times, and I thank all of you. Special shout-out to Mary Elston and Jess Gordon for being commiserators and co-conspirators in the Ph.D. process from afar.

My family has been a tremendous source of support and inspiration. My parents provided early exposure to the natural world, and endless encouragement to follow my interests. My mom in particular gave me a model for how to manage being a committed scientist, partner, parent, and friend. My brother has been there with me at every step. And I'm fortunate to have a tremendous set of aunts and uncles and grandparents who showed me different ways of being thoughtfully in the world. Finally, thank you to Noah for being my partner in all things. I could not have done this without your love, encouragement, and support.

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Abstract

Given the rapid pace of anthropogenic environmental change, understanding how such change influences biotic interactions and ecosystem functions is a key challenge for ecologists. My dissertation addresses this challenge by examining how land management practices in coffee agroecosystems affect multiple interactions between stingless bees and the plants upon which they feed, and explores the effects of the resulting restructuring of plant-bee interactions on the interacting species. To do this, I focus on two overarching questions:

1) How do farm management practices and landscape context affect stingless bee foraging patterns in coffee agroecosystems, and how do these foraging patterns in turn influence coffee pollination?

2) How is nectar robbing by stingless bees influenced by agricultural land use, what are the underlying drivers of land use-mediated changes to nectar robbing behavior, and to what extent does this behavior lead to adaptive pollen limitation in the plant firespike (*Odontonema cuspidatum*) by constraining floral display size?

In Chapter 1, I introduce a framework for understanding the multiple pathways by which anthropogenic environmental change can influence the frequency, outcome, or consequences of interspecific interactions without changing species composition.

In Chapter 2, I evaluate how the management of weedy herbaceous vegetation and canopy trees shaped the way coffee (*Coffea arabica*) interacts with neighboring plants for both pollination and abiotic resources. Co-flowering plants that share pollinators can interact with one another simultaneously for both pollination and abiotic resources, yet few studies have

considered the joint effects of both interaction types on plant reproduction or yield. In this study, I tackle this problem by examining coffee and non-coffee pollen deposition, pollen tube formation, initial fruit set, and final fruit set in coffee plants with different background floral environments, using structural equation models. I find that coffee competes with neighbors for pollination, but that this has little effect on yield because the crop is not pollen-limited. Interactions with neighbors for abiotic resources have a stronger effect, and I find evidence for both competition (with weeds and co-flowering canopy trees) and facilitation (with non-co-flowering canopy trees).

In Chapters 3-5, I examine nectar robbery (extracting nectar from a flower via an opening other than the corolla mouth) by stingless bees of firespike. In Chapter 3, I show that habitat-based heterogeneity in the intensity of nectar robbery is due to changes in floral traits and associated bee preferences, with plants growing in coffee fields producing more and more nectar-rich flowers and therefore experiencing more nectar robbery than plants growing in forest fragments. In Chapter 4, I use a reciprocal-translocation experiment to show that light availability drives differences in both floral traits and nectar-robbing behavior, and that light environment exerts clonal transgenerational effects on floral traits. In Chapter 5, I develop a novel conceptual framework to explain strong pollen limitation in firespike: conflicting selection on floral traits by pollinators and floral antagonists (nectar robbers). I develop this framework using data from field surveys and a field experiment, and explore its potential generality as a mechanism of pollen limitation.

Finally, in Chapter 6, I situate the studies presenting in Chapters 2-5 in the context of the framework developed in Chapter 1, using them to highlight directions for future work.

Chapter 1
Introduction

The earth is being transformed by human activity. The consequences of this transformation for the functioning of ecological communities and the wellbeing of both human and non-human life are profound (Cardinale et al., 2012; Ceballos et al., 2015; Oliver, Heard, et al., 2015; Oliver, Isaac, et al., 2015). Perhaps the most prominent consequence of anthropogenic environmental change on ecological communities is extinction; certainly, it is the one that has received the most attention from both academic researchers and popular media. This emphasis on extinction makes a great deal of sense, given the urgency of biodiversity loss in the midst of a staggering and ongoing mass extinction event (Cardinale et al., 2012; Ceballos et al., 2015). Yet, in focusing primarily on the presence or absence – or even the abundance – of species, we risk missing important effects of anthropogenic environmental change on ecological communities and ecosystem function that are not wrought by changes to species composition (Tylianakis et al., 2010; Valiente-Banuet et al., 2015a).

Recently, in association with surging interest in conceptualizing ecological communities as networks, we have witnessed increased attention to the effects of perturbations not just to species composition but also to the structure of interactions among individuals and populations (McCann, 2007; Tylianakis et al., 2010; McConkey and O'Farrill, 2015; Valiente-Banuet et al., 2015a). This work has demonstrated that significant alterations to interaction networks – including both who interacts with whom, and the outcome of those interactions for one or both partners – can occur without changes to species composition, via a number of mechanisms. In fact, alterations to interaction structure are likely to be more common – and more sensitive to environmental change – than changes to species composition (Valiente-Banuet et al., 2015a). Moreover, such changes to interaction structure have the potential to propagate across the community (Figueroa et al., 2020). And, with or without propagation, interaction shifts have the potential to dramatically alter ecosystem function (McConkey and O'Farrill, 2015). Thus, these phenomena are worthwhile of study in their own right (for their potential consequences on ecosystem function and the provision of ecosystem services), and also may presage changes in species composition.

A framework for understanding effects of environmental change on species interactions

The relative dearth of research on the effects of environmental change on species interactions stems, at least in part, from the difficulty of studying these effects, both empirically and theoretically (Tylianakis et al., 2008a). Detecting the presence/absence or even abundance of a species, while not always straightforward, is generally easier than determining which other species it is interacting with, which in turn is easier than determining the outcome of those interactions (Ballantyne et al., 2015). The complex multidimensionality of ecological communities, in which perturbations can simultaneously directly influence multiple components of the community, with each direct effect in turn initiating knock-on effects that can propagate across the network, makes this a particularly daunting task. And while recent advances in the application of network science to ecology have resulted in powerful new tools for understanding the effects of perturbations on community interaction structure (Kéfi et al., 2016; Delmas et al., 2019), the proximate effect of the perturbation in studies using these tools is generally a change in species composition (Tylianakis and Morris, 2017), and many of these tools rely on underlying knowledge or assumptions of how species interact with one another that may not hold true under environmental change scenarios (Schleuning et al., 2020). Clearly, a general framework for understanding and predicting the effects of environmental change on interactions would be useful both for crystallizing this phenomenon as a topic of study and for helping to mitigate damage to ecosystem function and the provision of ecosystem services. While I do not think we have the body of data yet to develop such a predictive framework, below I outline a taxonomy for the effects of environmental change on species interactions as a means of organizing the current state of our understanding more clearly (Figure 1.1).

We begin with the proximal effect of the environmental change agent on the ecosystem. I identify three possible categories of effect, noting that simultaneous changes to multiple categories are possible. These categories are 1) changes to landscape composition or configuration [e.g., conversion of a diversified farming landscape to industrial monoculture (Figueroa et al., 2020)], 2) changes to local the local biotic community apart from the species of interest (Smith et al., 2021), and 3) changes to the local physical environment or abiotic conditions [e.g. changes in temperature regime with climate change (van Beest et al., 2012), artificial light at night (Lewanzik and Voigt, 2014), or the presence of physical infrastructure, such as roads (Fitch and Vaidya, 2021)]. A key question is whether each of these effect types are equally likely to cause changes to species interactions.

Focusing in on the interaction(s) of interest, changes to an interaction must in turn stem either from changes to the density or traits of one or more of the interacting organisms. I use the term ‘trait’ here in its broad sense – as it is used in the literature on trait-mediated indirect interactions (Werner and Peacor, 2003a; Schmitz et al., 2004a; Irwin, 2012) – to include both more stable morphological characteristics and more flexible behavioral traits, while noting the potential utility of distinguishing between effects on morphology and behavior. As with the proximal ecosystem change, a complication here is that multiple traits and densities may be impacted simultaneously, and/or there may be internal causal links among traits or densities. For example, seed dispersal by flying foxes (*Pteropus tonganus*) depends strongly on high flying fox population density; factors that decrease flying fox density even moderately lead to changes in flying fox behavior (a decrease in aggressive intraspecific interactions) that cause a collapse in seed dispersal (McConkey and Drake, 2006). Thus, a change to the population density of one of the interacting species leads to a trait (behavior) change, which in turn leads to a dramatic change to the interaction outcome (that is, a large reduction in seed dispersal distances).

Despite these complications, this framework has utility in clarifying the multiple pathways by which environmental change may lead to interaction change without species loss. In this dissertation, I present two case studies of how land management practices at multiple scales in agricultural landscapes influence interactions between plants and bees. In the conclusion, I return to the framework outlined above, situating the case studies within this framework and pointing to persistent gaps in our understanding.

Why focus on bee-plant interactions?

Bee-plant interactions are an excellent system within which to study how anthropogenic environmental change impacts interactions for multiple reasons. First, these interactions are likely to be particularly sensitive to environmental change (Waser et al., 1996; Chamberlain et al., 2014). Second, disruption to these interactions has the potential to dramatically affect ecosystem function and the provision of ecosystem services, given bees' key role as pollinators (Heard, 1999; Delaplane and Mayer, 2000; Kremen et al., 2002). Finally, populations of many bees are in decline, making it particularly important to understand the changes in interaction structure that may presage population declines (Goulson et al., 2015; Reyes-González et al., 2020). Below, I explore each of these topics more fully.

Not all interactions are equally likely to have context-dependent outcomes, and those that are more context-dependent are also more likely to be impacted by anthropogenic environmental change. In a meta-analysis, Chamberlain et al. (2014) found that mutualistic interactions, such as pollination, are more context-dependent than predator-prey or competitive interactions. While not all plant-bee interactions involve pollination, the vast majority do. And many of those that do not, such as nectar robbery, are facultative, such that the same individual, or other individuals of the same species, may act as both pollinator and antagonist, depending on conditions (Irwin et al., 2010). Moreover, many bee species are generalists (Waser et al., 1996) – including those studied in this dissertation; generalists are more likely to interact with a larger number of potential food sources, and are these interactions are more likely to vary with ecological conditions (Gaiarsa et al., 2021).

Bees are ecologically and economically important, acting as essential pollinators of many plants, both wild and crop species (Heard, 1999; Delaplane and Mayer, 2000; Ollerton et al., 2011). As such, changes to the outcome or frequency of their interactions can have profound implications for the maintenance of wild plant populations (Biesmeijer et al., 2006; Carvalheiro et al., 2013) and crop yield (Kremen et al., 2002; Greenleaf and Kremen, 2006). Some bee populations are in decline, with trends of others uncertain (Cameron et al., 2011; Koh et al., 2016). Anthropogenic environmental change – including agricultural intensification – is implicated as a major driver of these declines (Goulson et al., 2015; Koh et al., 2016). These declines are occurring at the same time that the share of agricultural production from animal pollination-dependent crops is growing (Aizen et al., 2008, 2019), making it increasingly likely that agriculture will face a "pollinator shortage" in the future (Aizen et al., 2008). Better understanding how anthropogenic environmental change alters interactions can help inform management practices to stem population declines. In addition, widespread public concern over the implications of bee decline – principally of the managed honey bee (*Apis mellifera*), but more recently spilling over to wild bees – has, in the past decade, rendered bees conservation icons (Wilson et al. 2017; Hall and Martins 2020; Nicholls et al. 2020). Thus, efforts to better understand the impacts of anthropogenic environmental change on these charismatic organisms has the potential to raise awareness more generally about cryptic effects of anthropogenic environmental change. For all these reasons, improving our understanding how agricultural land management affects bee-plant interactions is of particular importance.

Why focus on coffee agroecosystems?

Agriculture occupies ~40% of the earth's terrestrial surface (FAO 2020), and agricultural conversion and intensification are major drivers of ongoing land use change worldwide, and particularly in the Global South (Song et al., 2018; Winkler et al., 2021). As such, agriculture is implicated as a key driver of biodiversity loss (Maxwell et al., 2016; Wagner, 2020). But the term 'agriculture' encompasses a dizzyingly diverse set of land use practices, and the effects of agriculture on ecological communities depends in complex ways on the type of agriculture being practiced and the biogeophysical conditions of the landscape. Or in not-so-complex ways: industrial agriculture – which, using the logic of industrial production, creates biologically simplified monoculture landscapes, and maintains these landscapes with synthetic inputs to manage soil fertility and the pests that attend monocultures in any biological system – by definition leads to dramatic reductions in biodiversity, with effects that often extend well beyond the field margin (Hallmann et al., 2017; Grab et al., 2019). The dominance of this kind of agriculture in the Global North, along with the preeminence in conservation narratives of ‘slash-and-burn’ agriculture decimating tropical forests, has contributed to a sense, common among ecologists and conservationists, that agricultural production and biodiversity conservation are antithetical (Phalan et al., 2011; Wilson, 2016).

Yet a growing body of work has pushed back against this notion, highlighting that diverse, high-functioning ecological communities can exist within, be supported by, and in turn support, highly productive agricultural landscapes (Perfecto and Vandermeer, 2010; Chappell and LaValle, 2011; Kremen et al., 2012). One of the key concepts to emerge from this work is that of the ‘agroecological matrix’ (Perfecto and Vandermeer, 2010). This framework recognizes that ecology happens not only in habitat fragments but also within farm fields in agricultural landscapes; agricultural and non-agricultural patches interpenetrate, woven together by the movement of organisms, matter, and energy. Management practices and social organization at multiple scales influence the quality of this matrix for human and non-human organisms and ecosystem function. The need to understand how distinct management practices interact with ecological and geophysical conditions within the agroecological matrix to generate positive or negative outcomes for agricultural production, human flourishing, and the maintenance of biodiversity is acute.

Coffee (*Coffea* spp., Rubiaceae) agroecosystems represent a particularly good system within which to investigate these questions. Coffee is one of the most highly-traded commodities in the world, and supports the livelihoods of some 20 million people worldwide, the majority of them smallholder farmers (Donald, 2004). Coffee cultivation is widespread across the tropics, including in many of the most biodiverse regions of the world. In addition, coffee cultivation practices vary dramatically, with coffee farms ranging from “sun coffee” near-monocultures to “rustic coffee” planted in the understory of near-intact forest (Moguel and Toledo, 1999), with dramatic implications for the ability of these farms to sustain biodiversity (Perfecto et al., 1996; De Beenhouwer et al., 2013). The potential to harmonize coffee cultivation with biodiversity conservation – through management practices summarized under the moniker “shade-grown coffee” – has received considerable academic and public attention (Perfecto et al., 1996; Moguel and Toledo, 1999; Lyon, 2006; Jha et al., 2014; Jimenez-Soto, 2020). Yet we still know relatively little about how farm management practices affect species interactions, particularly as relates to bee-plant interactions and non-crop plants [though see Jha and Dick (2010) for an exception]. This dissertation addresses this gap, reporting on a series of studies conducted in the Soconusco region of Chiapas, Mexico.

Outline of the dissertation

In Chapter 2, I explore how fine-scale variation in management of non-crop plants growing in coffee farms influences coffee pollination (primarily by bees), and evaluate the relative importance of interactions for pollination and interactions for abiotic resources in determining the effects of plant neighborhood at multiple scales and strata – herb layer, shrub layer, and canopy layer – on coffee yield. I show that effects of plant neighborhood on pollination are scale-dependent, but less stratum-dependent than expected. I find that coffee plants generally compete with neighbors for pollination, but that, at least on the farm where the study took place, this does not translate into reduced fertilization, since pollen deposition levels are uniformly very high. I find that interactions for abiotic resources are more important in determining plant neighborhood effects on coffee yield, with evidence for both competition and facilitation depending on stratum and neighbor identity.

Chapters 3-5 focus on interactions between the non-crop plant firespike (*Odontonema cuspidatum*, Acanthaceae) and nectar-robbing stingless bees (Apidae: Meliponini). In Chapter 3, I investigate the drivers and consequences of a pattern of difference in the intensity of nectar robbery of plants growing in coffee fields vs. forest fragments (plants growing in coffee experience higher levels of nectar robbery). I find that this is due primarily to habitat-based differences in traits – rather than densities – of the plant and nectar-robbing bees. Specifically, firespike plants growing in coffee produced more (and more rewarding) flowers, and bees showed a stronger preference for and higher fidelity to firespike in coffee fields vs. forest fragments.

In Chapter 4, I use a reciprocal translocation experiment to show that these trait differences are driven by differences in light availability between the two habitats. The higher light availability in the coffee fields leads to higher flower number and affects flower morphology, which in turn influences nectar robber behavior. But light also directly influences nectar robber behavior, increasing foraging activity on firespike. Together, Chapters 3 and 4 show that changes to the abiotic environment (i.e. light availability) via alterations to canopy tree density and identity alter the intensity of interaction between plant and floral antagonist.

In Chapter 5, I use the firespike nectar robbery system to develop a conceptual model that provides a novel mechanism that may give rise to pollen limitation in plant populations: adaptive response to conflicting pressures from antagonists and pollinators. I outline expectations for when this mechanism might operate, and use a combination of survey data and a field experiment to illustrate that it is likely operating in firespike. This work highlights the importance of considering interactions beyond the plant-pollinator dyad in developing hypotheses for the ubiquity of pollen limitation, and presents a mechanism that may operate commonly in populations where flowers experience antagonist damage.

In the concluding Chapter 6, I situate the findings reported in Chapters 2-5 within the framework presented above, and use these examples to point to general conclusions and highlight areas where further research is needed.

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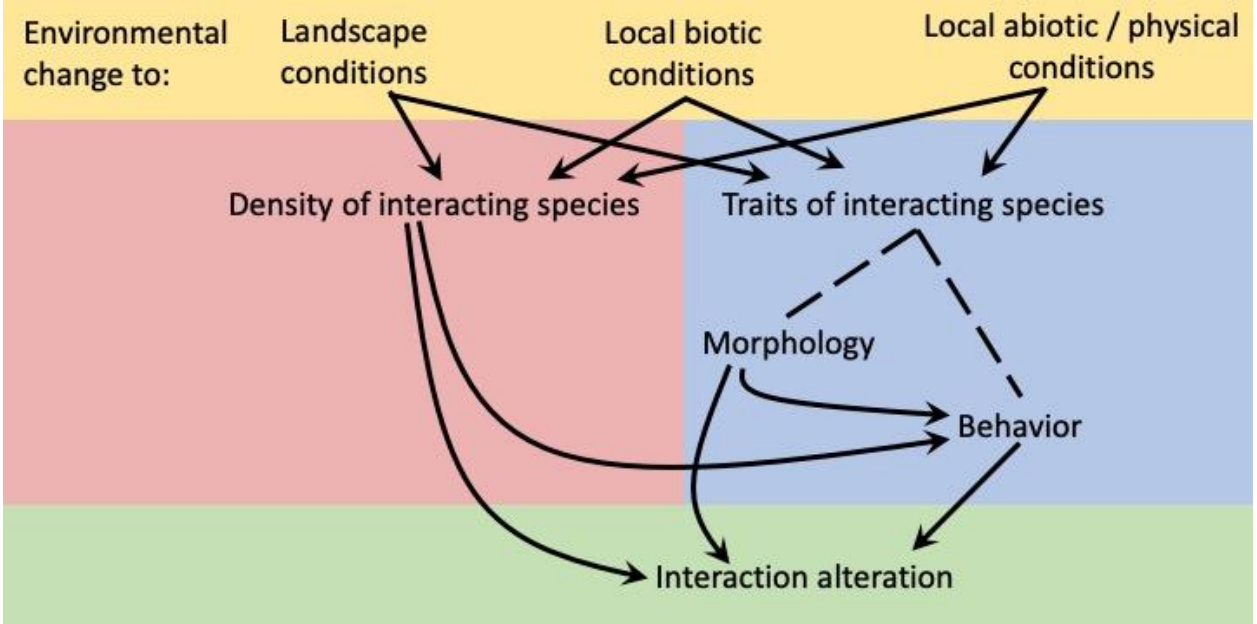
FIGURE

Figure 1.1. A conceptual framework for understanding how anthropogenic environmental change leads to the alteration of an interaction (interaction frequency or outcome) without species loss. Arrows indicate causal pathways; dashed lines distinguish morphological and behavioral traits.



Chapter 2
Integrating Interactions for Pollination and for Abiotic Resources to Understand Neighbor Effects on a Mass-blooming Crop in a Complex Agroforest[1]

ABSTRACT

Animal-pollinated plants interact with neighboring plants for both abiotic resources and pollination, with strong consequences for plant reproduction and crop yield. Yet few studies have compared the relative magnitude of these effects, particularly in agroecosystems. Moreover, in vertically-stratified communities, such as agroforestry systems, neighbor effects may be stratum-dependent. Understanding the net effects of neighbors on crop yield is of crucial importance in managing multifunctional agroecosystems that can simultaneously support production of multiple products and biodiversity. This study evaluated the effects of neighboring plants on pollen deposition, fertilization, and fruit set in *Coffea arabica* in a shaded organic coffee farm with high non-crop plant abundance and diversity in Chiapas, Mexico. We separately considered the effects of neighbors at two spatial scales and in three strata (herbs, shrubs, and canopy trees). We found evidence for competition for pollination with neighboring conspecifics and heterospecifics across scales and strata, primarily via reduction in pollination quantity. Pollen load influenced final fruit set, but the resulting effect of neighbor interactions for shared pollinators was weaker than effects mediated by interaction for abiotic resources. Effects of interactions for abiotic resources were heterogeneous across strata, with weak negative effects of

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herb-layer flower density but net positive effects of canopy trees on final fruit set. Our results indicate surprisingly weak effects of neighbor interactions on coffee yield. Promisingly, this suggests that coffee agroecosystems can be managed in ways that maintain high plant density and diversity, supporting diversity in both on-farm production and associated biotic communities.

INTRODUCTION

Organisms often interact with one another for access to multiple resources. But ecologists commonly focus on only a single interaction type at a time; studies integrating the effects of multiple interaction types are less common. Plants, for example, frequently compete with neighbors for light, water, and soil nutrients, often with profound effects on growth and population dynamics of wild plants (Goldberg and Barton, 1992; Gurevitch et al., 1992; Soliveres et al., 2015) and yields of crop plants (Njoroge, 1994; Oerke, 2006). At the same time, co-flowering plants interact via shared pollinators, with outcomes ranging from mutually detrimental to mutually beneficial (Rathcke, 1983a; Mitchell et al., 2009; Braun and Lortie, 2019). Moreover, there may be complex feedbacks between interaction types, since the availability of abiotic resources – influenced by neighbors – can impact floral traits and pollinator attraction (Carroll et al., 2001; Prado et al., 2019; Fitch and Vandermeer, 2020). Fully understanding the net effect of neighbors on plant fitness and, in agricultural systems, crop yield therefore requires integrating multiple interaction types. Yet, while interactions for shared abiotic resources and interactions for shared pollinators have each received significant attention in isolation, studies integrating them are far less common (Underwood et al., 2014).

Multiple features of interactions for shared pollinators compound the challenge of synthesizing neighbor effects across interaction types. First, co-flowering plants can affect both the quantity of pollination [influencing the number of visits a plant receives (Brown et al., 2002)] and the quality of pollination [increasing heterospecific pollen deposition and/or decreasing per-visit conspecific pollen deposition (Morales and Traveset, 2008)]; determining the relative importance of these is not straightforward (Ashman et al., 2020). Second, while interactions for shared abiotic resources generally occur only between immediate neighbors, interactions for pollination can occur over larger spatial scales (Mitchell et al., 2009; Braun and Lortie, 2019), with potentially contrasting effects of neighbors at different scales (Albor et al., 2019). As a result, it can be difficult to identify the effect of a particular neighbor or ascribe an observed effect to specific neighbors.

To date, most of the research on interactions for shared pollinators has focused on forbs in semi-natural meadow-type habitats (Braun and Lortie, 2019), where flowers tend to occur in a single stratum of vertical space. In habitats with greater stratification, neighbor effects on pollination may be stratum-specific, but this has received scant attention. Moreover, few studies have investigated interactions for shared pollinators in crop systems (Klein et al., 2008; Badillo-Montaño et al., 2019). The low diversity of conventional agroecosystems may ameliorate neighbor effects on pollination (Albor et al., 2019), but rising interest in agricultural practices that increase in-field plant diversity – including intercropping, hedgerows and wildflower strips, and reduced herbicide treatment – may increase the importance of neighborhood effects on crop pollination.

Coffee (*Coffea* spp.; Rubiaceae) is one crop whose management often supports high non-crop flowering plant diversity at multiple strata. The well-documented ability of coffee farms to support high biodiversity depends on management practices that maintain within-field non-crop plant diversity (Perfecto et al., 1996; De Beenhouwer et al., 2013), setting the stage for complex effects of plant neighborhood. But the crop's reproductive biology may reduce the impact of neighbor interactions for pollination in two ways. First, highland coffee (*Coffea arabica*) is self-compatible and generally not pollen limited (Prado et al., 2018). Thus, even if floral neighborhood influences pollination, its effects on yield may be small. Second, coffee is mass-blooming, with strong temporal synchrony in bloom time across plants within a region. High conspecific density may increase floral fidelity by pollinators (Duffy and Stout, 2011), reducing the effects of heterospecific neighbors.

Improved understanding of the integrated effects of neighbors on coffee yield can inform sound management practices that balance yield with biodiversity maintenance. This is particularly important as coffee farmers across the globe confront uncertainty from the compounded effects of climate change, emerging pests, and market volatility (Eakin et al., 2005). Coffee farms in our study region are addressing this uncertainty by diversifying income streams through the promotion of ecotourism and/or the cultivation of secondary crops, both of which necessitate within-field plant diversity.

In this study, we examined how plant neighborhood at two scales (2m x 2m and 2m x 20m) influences pollination and yield in coffee, asking whether co-flowering neighbors have a facilitative or competitive effect on pollen deposition and pollen tube formation (a measure of fertilization) in coffee. We then used path analysis to determine the relative importance of interactions for shared pollinators and interactions for abiotic resources to the net effect of neighboring plants on coffee yield. For both types of interactions, we asked whether the impact of neighbors is mediated by the strata in which they occur (herb, shrub, or canopy layer).

We expected that co-flowering conspecific density would be positively related to coffee pollen load, while heterospecific flower density would reduce coffee pollen load, indicating competition for pollination with heterospecifics (see Figure 2.1 for a diagram of hypotheses). We predicted that co-flowering canopy trees (which are generally >3m above the height of coffee plants) would reduce pollination quantity, leading to lower total stigma pollen loads. Co-flowering forbs are less spatially separated from coffee flowers, so we anticipated more pollinator switching, leading to reductions in both pollination quantity and quality (decreased coffee pollen and increased heterospecific pollen). We expected coffee pollen load to positively influence pollen tube number, while heterospecific pollen would reduce pollen tube number via stigma clogging. We expected pollen tube number to independently influence both initial and final fruit set, thus linking pollen receipt to coffee yield. However, because *C. arabica* is not pollen-limited, we thought interactions for abiotic resources would be more important in determining the net effect of neighbors, with resource competition leading to negative effects of conspecific and heterospecific density.

MATERIALS AND METHODS

Study system

Research took place in Finca Irlanda (15.17358 -92.33827), a ca. 300-ha shaded organic coffee farm in SE Chiapas, Mexico. *Coffea arabica* mass blooms occur during the dry season and are generally initiated after a rain; individual plants generally bloom 2-4 times per year in the study region (Philpott et al., 2006). This research occurred during two mass-bloom events, one 19-24 Feb and another 6-10 Mar 2018.

The understory of Finca Irlanda consists of a spatially heterogeneous assemblage of herbaceous plants, most of which, like coffee, bloom during the dry season. Dominant herbs include Asteraceae spp., Commelinaceae spp., and grasses in the genus *Oplismenus*. Herbs are controlled by periodic cutting with machete. The frequency of cutting depends on labor availability and site accessibility; most sites are cut several times per year (G. Fitch personal observation), resulting in a mosaic of plants at varying stages of regrowth. Combined with underlying variation in species composition, this generates high spatial heterogeneity in floral abundance (Figure A.1). Shade trees represent another important source of floral resources; spatial variation in species composition of the shade layer across the farm further contributes to floral resource heterogeneity. Shade tree diversity is high, with >100 species present, but the majority (~60%) are *Inga* spp. (J. Vandermeer unpublished data). The farm does not use synthetic fertilizer, relying on compost made onsite to maintain soil fertility. Soil nutrient levels strongly influence coffee plant vigor on the farm (Gonthier et al., 2013).

Data collection

During the first mass bloom, we established 21 20 m x 2 m transects, following the orientation of coffee rows. Transects were chosen to represent the full range of floral densities present on the farm, and were >50 m apart (range: 54 m – 2250 m).

Within each transect, we selected three focal coffee plants. Focal plants had plentiful open flowers, were separated from one another by >1 m, and were distributed across the length of the transect. From each focal plant we collected the carpel from three haphazardly selected flowers from the outermost node of three branches; carpels were stored in 95% ethanol. For each focal plant, we measured its height; noted its location along the transect; and assessed canopy cover above its crown using CanopyApp 1.0.3 (University of New Hampshire, Durham, NH USA). We also selected a fourth branch from each focal plant to monitor fruit set. On the focal branch, we counted open flowers and buds that would open in that bloom event, starting with the outermost node, until we reached at least 30 open flowers (except in two cases where no available branch had ≥30 open flowers). Focal branches were marked with flagging tape.

To assess floral resource availability, we counted and identified to morphospecies all flowers within the transect at 1 m intervals. For the most abundant species, flower number was estimated by extrapolating from counts of a representative 2 m x 2 m section of the transect (for herbaceous species) or a fraction of the total area covered in flowers (for canopy species).

We hypothesized that proximity to an apiary would increase honey bee (*Apis mellifera*) visitation rates and pollen deposition, so we calculated the minimum distance between apiary and transect for each transect (range: 27–594 m).

During the second mass bloom, we resurveyed 12 transects. Transects for resurveying were chosen to represent one of the following three categories (four per category): high flower density (>25 flowers m^{-2}) during both mass blooms, high floral density during the first bloom/low floral density (≤25 flowers m^{-2}) in the second bloom, and low floral density during both mass blooms (no sites had low density in the first bloom/high in the second). We used the same protocol for resurveys, selecting flowers from the same focal plants, but different branches, for collecting flowers and assessing fruit set.

In June 2018, we resurveyed focal branches for initial fruit set. For each branch we counted the number of developing fruits on the same section of branch where we had tallied flower number. To calculate initial fruit set, we divided the number of developing fruits by the number of open flowers and large buds tallied in Feb-Mar.

Once coffee fruit began to ripen in Oct 2018, we surveyed all plants every 2-3 weeks. At each survey, ripe fruit on focal branches were counted, collected, and weighed. Collection continued until early Dec 2018, when no focal branches retained fruit. Final fruit set was calculated as for initial fruit set, using the total number of fruits harvested. We also calculated the proportion of fruits reaching maturation by dividing the number of harvested fruits by the number of developing fruits. Initial fruit set is strongly related to pollination levels, but plants – including coffee – frequently abort developing fruits that they cannot adequately provision (Stephenson, 1981; Bos et al., 2007), so final fruit set is the product of an interplay between pollination and resource availability, and is often substantially lower than initial fruit set (Bos et al., 2007). In coffee, micronutrient deficiency is a key driver of premature fruit abscission (DaMatta et al., 2007), with water stress also playing a role (Lin, 2009).

In the lab, coffee carpels were transferred to NaOH and left for 24h to soften. After softening, carpels were rinsed in water and transferred to a microscope slide, stained with a drop of decolorized aniline blue solution (Kearns and Inouye, 1993), and squashed. Using a UV fluorescent microscope, pollen tubes were counted at the base of the style, and the number of conspecific and heterospecific pollen grains were counted on one randomly chosen stigma lobe. Pollen grains were distinguished as coffee or non-coffee using a reference collection of pollen collected from flowers at the field site.

Data analysis

All analyses were conducted using R v.4.0.2 (R Core Team, 2020). To test whether there was spatial autocorrelation in any measure of pollination, yield, or neighborhood, we calculated Moran’s I autocorrelation coefficient for each variable and compared this to the null expectation of no autocorrelation, using the R package `ape` (Paradis and Schliep, 2019). In all cases, $p > 0.1$, indicating no spatial autocorrelation (Table A.1).

We compared measures of floral neighborhood, stigma pollen load, pollen tube number, and yield between mass bloom events for sites that were surveyed during both blooms, using paired t-tests (on plant-level means where we had >1 measurement per plant). A paired t-test was also used to compare initial and final fruit set. To test whether floral neighborhood density mediated the difference in pollen deposition between mass blooms, we analyzed how coffee pollen load differed between blooms for each neighborhood floral density comparison category (high/high, high/low, low/low) using ANOVA.

We used piecewise structural equation modeling (SEM) to examine the effect of neighborhood on conspecific and heterospecific stigma pollen load, pollen tube formation, initial fruit set, and final fruit set. We constructed two SEM models, one for the effect of the transect-scale neighborhood on pollination and another for the effect of local 2m x 2m neighborhood on pollination and yield, since different neighborhood scales may affect pollination differently, while interactions for abiotic resources occur only with immediate neighbors. We included coffee plant height (a proxy for age), distance to nearest apiary, and mass bloom event as additional predictors in the maximal model, but removed them if they did not improve model fit, as evaluated by AIC. Using the `lme()` function from package `nlme` (Pinheiro et al., 2020), we constructed linear mixed-effects models describing the hypothesized relationships between these variables. We combined these submodels in a SEM using the `psem()` function from `piecewiseSEM` (Lefcheck, 2016). All submodels included site as a random effect. Coffee pollen load was $\log_{10}$-transformed to achieve normality.

To evaluate SEM fit, we used Fisher's C and a chi-squared test comparing the hypothesized model to a null model. We examined tests of directed separation to determine if our initial model had omitted significant, biologically plausible relationships, and updated the model to include these where necessary (the only such relationship linked coffee pollen load to final fruit set).

RESULTS

We found very high levels of coffee pollen and low levels of heterospecific pollen on coffee stigma surfaces (Table A.2); 35% of stigmas had no heterospecific pollen. Coffee pollen load and pollen tube formation differed substantially among flowers within a single plant and among plants within a site (Figure A.2). Heterospecific and conspecific neighborhood floral density both varied across sites (Table A.2). Final fruit set was significantly smaller than initial fruit set ($t = 11.0$, $df = 81$, $p < 0.001$).

At sites that were sampled during both mass blooms, coffee flower density was significantly higher in the second mass bloom (Figure 2.2). This likely led to a dilution of pollinators, since, for plants that were sampled during both mass blooms, pollen loads were significantly higher in the first mass bloom (Figure 2.2). However, heterospecific floral density modified the difference in pollen load between mass blooms (Figure 2.2). In plants with similar neighborhood floral densities during both blooms, coffee pollen load was lower in the second mass bloom, indicating intraspecific competition for pollination. But for plants where heterospecific floral density was higher in the first mass bloom, coffee pollen load trended higher in the second mass bloom (Figure 2.2), indicating reduced interspecific competition for pollinator visits. Despite differences in pollen deposition, neither pollen tube number ($t = 0.30$, df

= 32, p = 0.76) nor initial (t = 0.73, df = 24, p = 0.47) or final fruit set (t = 1.48, df = 24, p = 0.15) differed between mass blooms.

Results from SEM, discussed below, are summarized in Figure 2.3 and Table 2.1. Both our 2m x 2m-scale model of neighborhood effects on pollination and yield and our transect-scale model of neighbor effects only on pollination provided good fit to the data (2m x 2m-scale: Fisher's C = 36.5, df = 40, p = 0.6; transect-scale: Fisher's C = 15.4, df = 12, p = 0.2).

Neighborhood floral density reduced coffee pollen load, indicating competition for pollination, though the strength of this effect was scale- and stratum-specific. At the transect scale, higher density of heterospecific flowers in both herb and canopy layers significantly reduced coffee pollen load, but there was no effect of coffee flower density (Figure 2.4A-C). By contrast, at the 2m x 2m scale, coffee flower density significantly decreased coffee pollen load; the effect of conspecific density trended negative at this scale, but was small and nonsignificant for both strata (Figure 2.4D-F). Neighborhood floral density did not significantly influence heterospecific pollen load, regardless of scale or stratum (Table 2.1). Distance to the nearest apiary did not influence pollen load (Table A.3).

Neither conspecific nor heterospecific pollen load predicted pollen tube formation, and pollen tube number was not correlated with initial fruit set (Figure 2.4G-H, Table 2.1), indicating that pollen receipt did not limit fertilization or yield. As expected, given that initial fruit set tends to reflect fertilization rather than resource availability, we found no effect of neighbors on initial fruit set. Focal plant height did not influence fruit set (Table A.3).

Initial fruit set strongly predicted final fruit set. Neither conspecific density nor flowering herb density influenced final fruit set (Figure 2.4J-K), indicating minimal effects of resource competition with neighbors in these strata. Canopy trees did influence final fruit set, but in complicated ways that suggest a role for both facilitation and competition. We observed a strong positive effect of canopy cover on final fruit set, with a weaker negative effect of canopy-layer flower density (Figure 2.4I,L). The positive relationship between canopy cover and final fruit set was not a result of shaded plants producing fewer flowers, since there was no relationship between focal plant flower number and canopy cover ($R^2 = 0.0$, $F_{1,99} = 0.34$, $p = 0.6$)

Unexpectedly, we found a positive effect of coffee pollen load on final fruit set (Figure 2.4M) and a negative effect of pollen tube number on final fruit set (Figure 2.4N). Per-fruit weight was strongly related to initial fruit set, but was not affected by any measure of floral neighborhood or pollination.

Via the influence of pollen load on final fruit set, competition for pollination ultimately affected yield, despite the lack of connection between pollen load and pollen tube formation. Since we found no evidence of competition for abiotic resources with either conspecifics or flowering forbs, neighbors in these two strata affected coffee yield only via competition for pollination. But these effects, along with the effects of competition for pollination with canopy trees, were small compared to the effects of interaction with canopy trees for abiotic resources. Considering all interaction pathways, there was a small net competitive effect of both conspecific density and flowering forb density on fruit set, but a net facilitative effect of canopy trees, via the positive influence of canopy cover.

DISCUSSION

This study provides one of the first estimates of the net effect of plant neighborhood on crop production in a complex agroforest. Our results demonstrate that interactions for both pollination and abiotic resources influence coffee yield, with interaction for abiotic resources having a greater effect.

Neighbor interactions for pollination

Coffee competed for pollination with co-flowering neighbors, both conspecific and heterospecific. Regardless of neighbor identity, this competitive effect was likely driven by reductions in pollinator visitation frequency, since neighborhood floral density reduced coffee pollen load without affecting heterospecific pollen load. At first glance, our finding that coffee experiences competition for pollination contrasts with the results of (Prado et al., 2021), who found no effect of floral neighborhood on the proportion of coffee pollen carried by foraging honey bees. But if neighbors primarily influenced pollination by reducing visit frequency, these findings are not in conflict. Rather, assuming bee pollen load or foraging rate do not scale linearly with coffee flower density, a constant proportion of coffee pollen in bees' pollen loads across floral densities would lead to reductions in coffee pollen deposition on flowers in denser neighborhoods. Overall, we found strikingly little heterospecific pollen on coffee stigmas, indicating high floral fidelity within a foraging bout.

The magnitude and significance of neighbor effects on pollen deposition depended on both the scale and stratum considered. At the smaller 2m x 2m scale, only coffee flower density significantly predicted pollen load, suggesting a classic dilution effect whereby, within a foraging bout, there is a maximum number of flowers a pollinator will visit regardless of local floral density; increases in floral density thus decrease per-flower visits. At the transect scale, by contrast, heterospecific floral densities were important predictors of coffee pollen load, suggesting a 'magnet effect' (Laverty, 1992) where high heterospecific floral densities draw foraging insects away from the focal plant. The lack of an effect of either herb- or canopy-layer flower density on heterospecific pollen load indicates that neighbors at all strata primarily affect pollination quantity rather than quality. This in turn suggests that, even in complex, multi-layered agroforestry systems, co-flowering neighbors can be considered in aggregate, rather than separating by stratum, in determining their likely effect on pollination of a focal plant.

Floral neighborhood, however, explained a small amount of the overall variance in coffee stigma pollen load, and even less of the variance in heterospecific pollen. Moreover, there was high variability in pollen load even among flowers from the same plant. *Coffea arabica* is capable of self-pollination, and it may be that flowers vary in the amount of pollen they produce, which in turn affects stigma pollen load. Understanding the degree of variability in pollen production both among flowers within a plant and among plants – and the drivers of this variability – requires further study.

Despite reducing stigma pollen load, co-flowering neighbors had no effect on fertilization, since stigma pollen load did not predict pollen tube number, and, in turn, pollen tube number did not predict initial fruit set. This is perhaps not surprising, given 1) the very high levels of coffee pollen found on all stigmas surveyed and 2) that coffee flowers generally contain only two ovules, making full pollination possible with little pollen.

While we found no link between pollen load and either pollen tube formation or initial fruit set, the direct, positive effect of coffee pollen load on final fruit set provides a link between neighbor effects on pollination and yield. This link suggests that coffee plants respond to information about pollen load in determining how to allocate limited resources for fruit maturation – a common phenomenon (Stephenson, 1981; Winsor et al., 1987), presumably because stigma pollen load reliably predicts offspring vigor (Mitchell, 1997). A commonly-invoked mechanism linking pollen load to offspring vigor is increased pollen tube competition

(Mulcahy, 1971; Niesenbaum and Casper, 1994). It is surprising, then, that we find a negative effect of pollen tube number on final fruit set. It may be that large numbers of pollen tubes clog the style, reducing fertilization and increasing the likelihood of fruit abortion (Young and Young, 1992); but see (Niesenbaum and Casper, 1994), while large pollen loads increase the likelihood of receiving particularly vigorous pollen grains that can rapidly fertilize ovaries (Mulcahy, 1971). However, our measures of fruit set did not include the particular flowers from which we collected data on pollen load and pollen tube formation. With high levels of variation in pollen load and pollen tube formation even among flowers within a single plant, we cannot assume that the flowers assessed for fruit set experienced equivalent pollination to the flowers we harvested. The positive correlation between coffee pollen load and final fruit set may alternatively reflect underlying differences in plant vigor, with plants that are able to put more resources towards developing fruit – leading to high fruit set – also producing more pollen per flower, which translates to high stigma pollen load via self-pollination. Further investigation is needed into the processes underlying fertilization and fruit development in coffee before we can conclusively determine what causes the correlation between final fruit set and pollen load and pollen tube formation.

Neighbor interactions for abiotic resources

Given the large difference between initial and final fruit set, we were surprised to find no evidence for competition for abiotic resource with neighboring conspecifics or flowering herbs. The lack of influence of conspecifics may reflect the relatively low planting density on this farm, which at ~2400 coffee plants ha^{-2} is only about half that recommended for optimal yield (DaMatta et al., 2007). Meanwhile, evidence for negative effects of herbaceous weeds on coffee yield are mixed, with some work indicating that weeds primarily affect coffee seedling growth and have little effect on mature plants (Ronchi and Silva, 2006). Given that we did not assess herbaceous plant density *per se* – which is uniformly high across the farm (G. Fitch personal observation) – but rather forb flower density, it may be that we missed the signal of competitive interactions with herbaceous plants. At the very least, our results suggest a weak effect of herbs on coffee yield.

The observed positive effect of canopy cover on final fruit set is consistent with several other studies finding higher coffee fruit set in shaded vs. unshaded farms (Lin, 2009; Prado et al., 2018), despite experimental evidence that shading reduces yield (Campanha et al., 2004). This may be due to beneficial effects of shade trees on soil moisture, soil nutrients, or both. Lin (2009) found that fruit abortion in coffee was negatively correlated with soil moisture levels, which in turn were positively correlated with canopy shading. At the same time, canopy trees are an important source of nutrients in low-intensity coffee cultivation via leaf litter production (Beer et al., 1997), and reduce nitrogen leaching (Tully et al., 2012). The canopy of Finca Irlanda is dominated by *Inga* spp. (Fabaceae), which are fast-growing N-fixers that are frequently pruned, potentially accelerating the transfer of nutrients from canopy trees to soil. Further research is needed to tease apart the relative importance of canopy trees' influence on soil moisture and soil nutrients for supporting high coffee yields.

The negative effect of canopy flower density on final fruit set likely represents competition for soil nutrients with a small number of tree species [especially *Roseodendron donnell-smithii* (Bignoniaceae), 58% of canopy-layer flowers in the 2m x 2m neighborhood, and *Schizolobium parahyba* (Fabaceae), 35%]. Like coffee, these species are mass-blooming, and produce large-seeded fruit which take several months to mature. Thus, resource needs – and consequently soil nutrient uptake rates – of these species are synchronous with those of coffee, leading to competitive effects despite the facilitative effect of canopy cover overall.

What is the relative importance of interactions for pollination vs. abiotic resources?

Pollination-mediated effects of neighbors on coffee yield were smaller than abiotic-resource-mediated effects. Our results suggest that while neighbors can be considered in aggregate to determine pollinator-mediated effects, the effect of interactions for abiotic resources differ substantially across strata. While we found weak evidence for resource competition with herb-layer neighbors, we found neighboring canopy trees to have a net facilitative effect on fruit set. The positive effect of canopy trees occurred despite evidence for competition with two co-flowering, mass-blooming canopy species. However, the positive effect of canopy trees on branch-level fruit set is unlikely to scale up to the economically-relevant farm scale, since the presence of canopy trees necessitates reduction in coffee plant density.

Management implications

Our results indicate that tradeoffs between coffee yield and non-crop flowering plant density and diversity are weaker than expected. This is heartening both from a conservation perspective and for the prospects of diversifying on-farm production streams. For example, previous work in the same region has shown that weedy forbs in the coffee understory are an important resource for maintaining bee populations across the year (Fisher et al., 2017). The density of managed honey bee colonies at the study site has increased dramatically in the past 20 years, from a single apiary with <100 colonies in 2004 (Jha and Vandermeer, 2009) to 6 apiaries with >500 colonies in 2018, as the farm has commercialized honey production. Supporting this density of managed honey bees, alongside wild pollinator populations, requires the maintenance of temporally-consistent high floral densities. Therefore, management practices that maintain high densities of flowering forbs in the landscape are essential to maintaining farm multifunctionality. Even in agroecosystems managed primarily for coffee, maintaining high non-crop plant diversity and abundance should not entail significant yield losses – a promising finding for efforts to promote win-win scenarios in managing lands simultaneously for agricultural production and biodiversity conservation.

ACKNOWLEDGEMENTS

Thanks to Fundación Peters for access to Finca Irlanda, and to the workers who maintain the farm. Gabriel Dominguez Martinez conducted substantial fieldwork, Gustavo López Bautista provided logistical support, and Gregg Sobocinski assisted with microscopy. Feedback from Chatura Vaidya, Zachary Hajian-Forooshani, Iris Rivera, Jonno Morris, Kristel Sánchez, and the Peach Street Writing Group improved this manuscript. Funding was provided by the Rackham Graduate School and Department of Ecology and Evolutionary Biology, University of Michigan.

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TABLES AND FIGURES

Table 2.1. Model output from structural equation models relating a) transect-scale floral neighborhood to stigma pollen load and b) 2m x 2m-scale neighborhood to stigma pollen load, pollen tube number, fruit set, and fruit weight. Boldface indicates a significant effect at $p < 0.05$.

Response	**Predictor**	**Std. estimate**	**Estimate ± s.e.**	**DF**	**Critical value**	**P**
a) Transect scale						
log(Coffee pollen load)	Coffee flower density	-0.08	-0.002±0.003	191	-0.51	0.6
	Herb-layer flower density	**-0.21**	**-0.004±0.002**	**191**	**-2.07**	**0.04**
	Canopy-layer flower density	**-0.17**	**-0.001±0.00**	**191**	**-2.12**	**0.04**
	Mass bloom event	**-**	**-**	**1**	**4.37**	**0.04**
Hetero-specific pollen load	Coffee flower density	-0.14	-0.06±0.05	191	-1.18	0.2
	Herb-layer flower density	-0.03	-0.01±0.03	191	-0.33	0.7
	Canopy-layer flower density	0.08	0.009±0.008	191	1.04	0.3
	Mass bloom event	-	-	1	1.41	0.2
b) 2m x 2m scale						
log(Coffee pollen load)	**Coffee flower density**	**-0.24**	**-0.03±0.01**	**193**	**-2.91**	**0.004**
	Herb-layer flower density	-0.07	-0.096±0.01	193	-0.91	0.4
	Canopy-layer flower density	-0.11	-0.004±0.003	193	-1.58	0.1
	Mass bloom event	-	-	1	3.27	0.07
Hetero-specific pollen load	Coffee flower density	-0.07	-0.17±0.22	193	-0.80	0.4
	Herb-layer flower density	0.08	0.22±0.20	193	1.10	0.3
	Canopy-layer flower density	0.11	0.08±0.05	193	1.49	0.1
	Mass bloom event	**-**	**-**	**1**	**4.35**	**0.04**
Pollen tube number	log(Coffee pollen load)	0.02	0.16±0.61	195	0.27	0.8
	Heterospecific pollen load	-0.03	-0.01±0.03	195	-0.41	0.7
Initial fruit set	log(Coffee pollen load)	-0.06	-0.02±0.02	194	-0.95	0.3

	Heterospecific pollen load	0.07	0.001±0.001	194	1.30	0.2
	Pollen tube number	0.05	0.002±0.002	196	0.79	0.4
Final fruit set	**log(Coffee pollen load)**	**0.12**	**0.03±0.01**	**189**	**2.82**	**0.005**
	Pollen tube number	**-0.10**	**-0.003±0.001**	**189**	**-2.62**	**0.009**
	Initial fruit set	**0.50**	**0.47±0.04**	**189**	**10.93**	**<0.001**
	Canopy cover	**0.23**	**0.002±0.00**	**189**	**3.62**	**<0.001**
	Coffee flower density	0.01	0.000±0.002	189	0.13	0.9
	Herb-layer flower density	-0.07	-0.003±0.002	189	-1.57	0.1
	Canopy-layer flower density	**-0.14**	**-0.002±0.00**	**189**	**-2.96**	**0.004**
	Mass bloom event	**-**	**-**	**1**	**5.29**	**0.02**
Fruit weight	Pollen tube number	0.03	0.001±0.003	190	0.48	0.7
	Initial fruit set	**0.32**	**0.49±0.10**	**190**	**4.92**	**<0.001**
	Canopy cover	0.05	0.000±0.001	190	0.57	0.6
	Coffee flower density	-0.04	-0.002±0.005	190	-0.50	0.6
	Herb-layer flower density	-0.04	-0.003±0.004	190	-0.61	0.5
	Canopy-layer flower density	0.09	0.002±0.001	190	1.30	0.2

Figure 2.1. Hypothesized effects of plant neighborhood on coffee pollination, fruit set, and fruit weight.

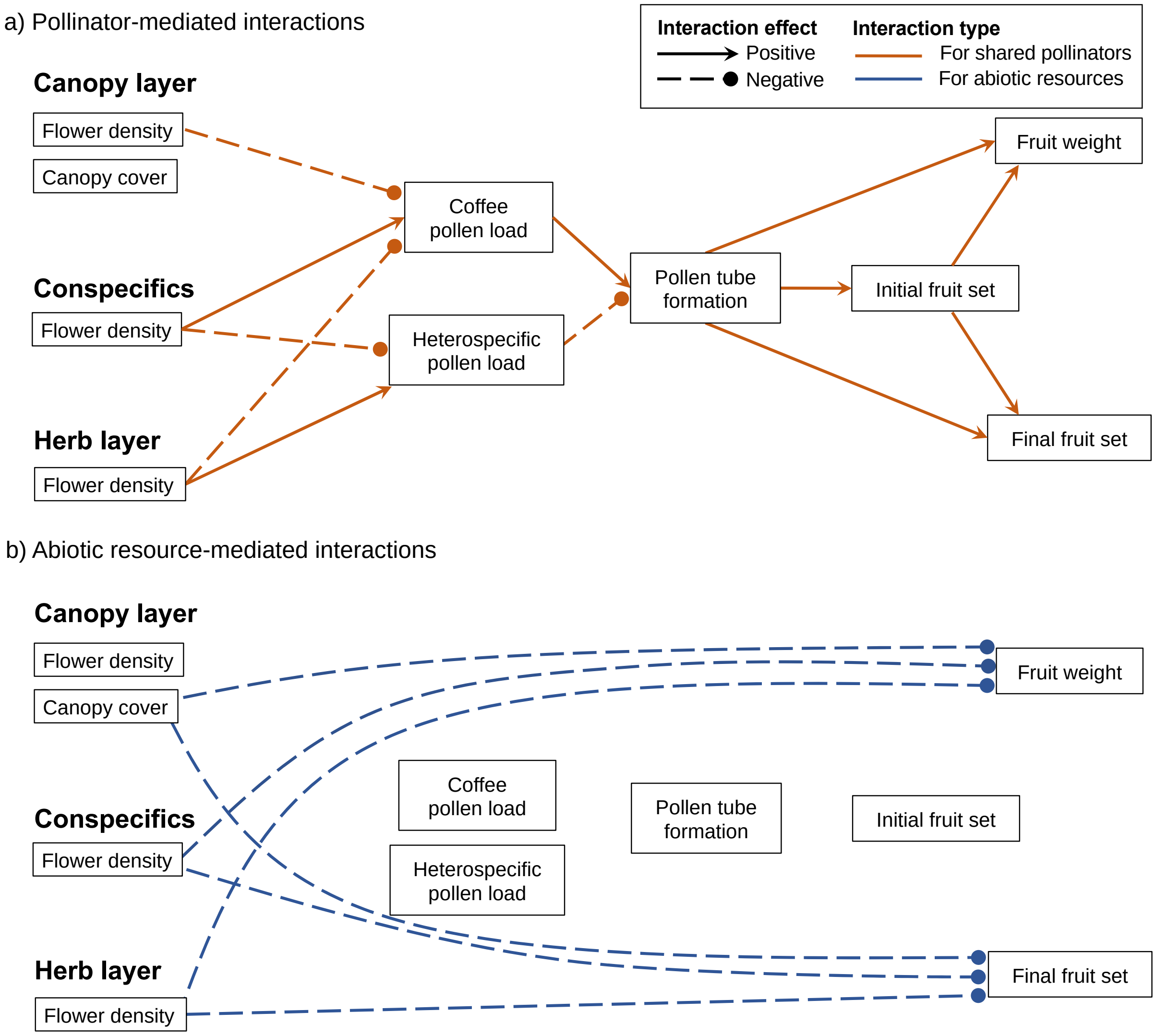


Figure 2.2. Comparison of coffee pollen load (top panel) and coffee flower density (bottom panel) between mass bloom events across high (>25 flowers m^{-2}; dark orange) and low (≤25 flowers m^{-2}; pale orange) heterospecific floral density neighborhoods. Significance codes (from paired t-tests): *p < 0.05, **p < 0.01. Numbers at bottom of top panel denote number of stigmas and, in parentheses, number of plants from which pollen load was recorded; all comparisons are of three sites each.

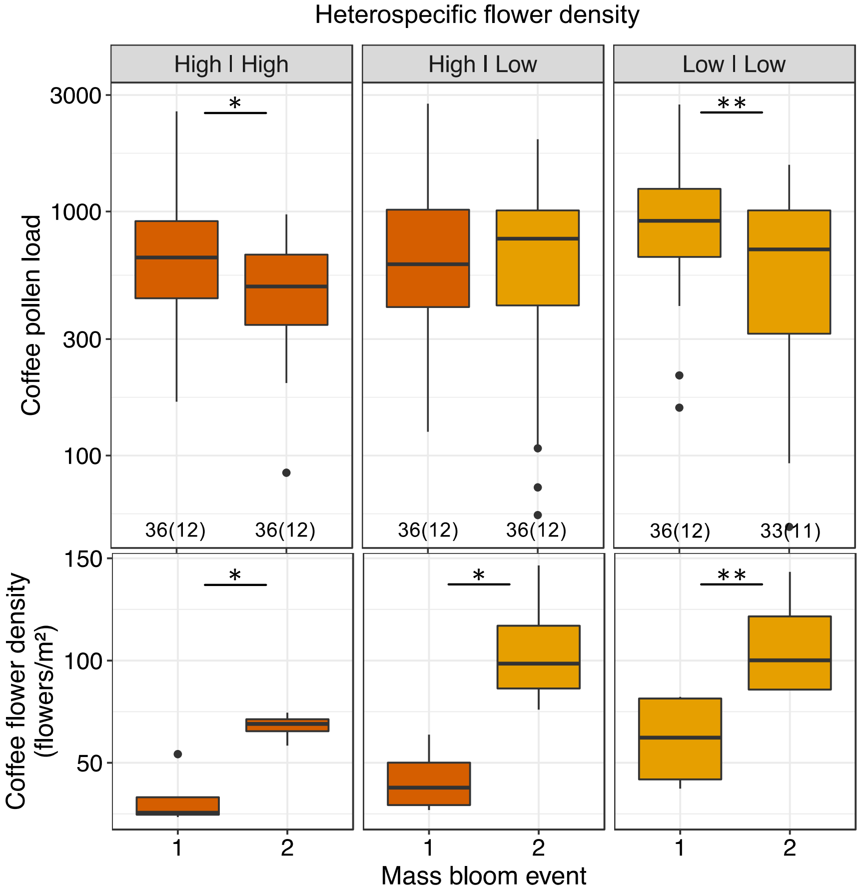


Figure 2.3. Results from SEM analysis of a) transect-scale neighborhood effects on pollination and b) 2m x 2m-scale neighborhood on pollination and yield. Only significant relationships ($p < 0.05$) are included. Coefficients represent standardized effect sizes; significance codes: *p < 0.05; **p < 0.01; ***p < 0.001. For visual clarity, mass bloom event, a covariate, has been omitted from the diagram (see Table 1 for full model output).

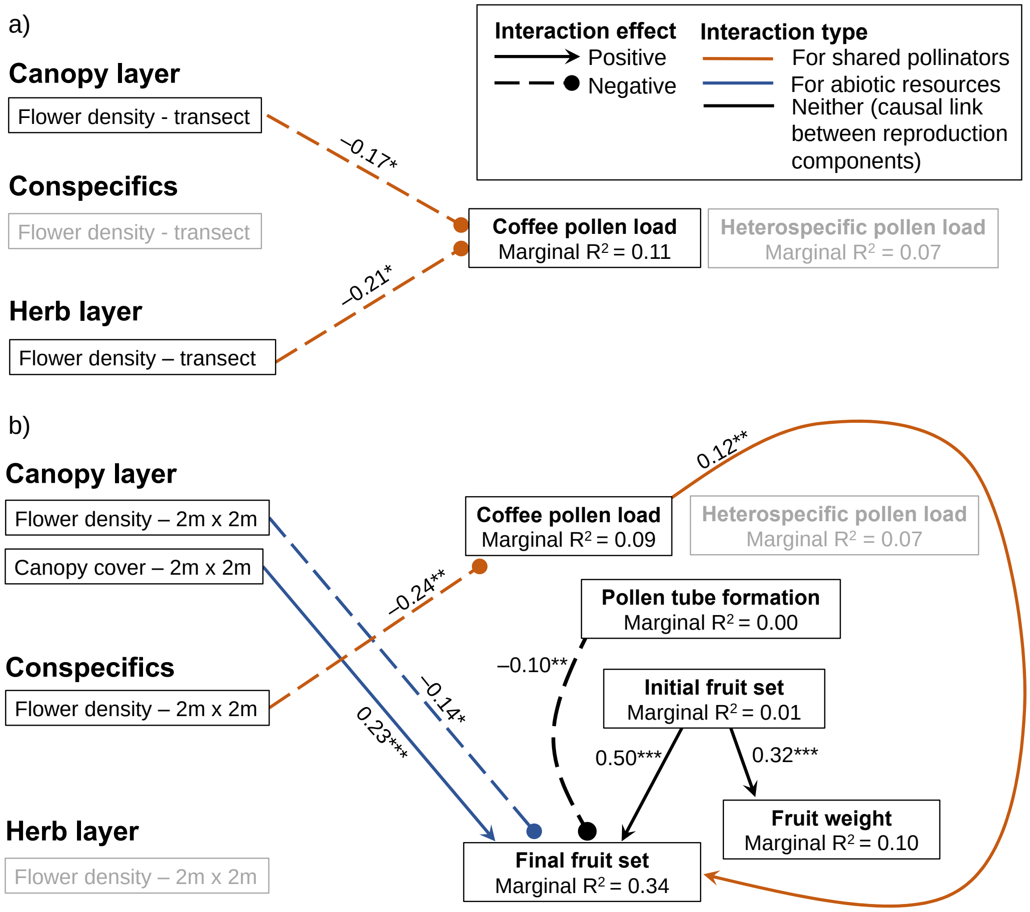
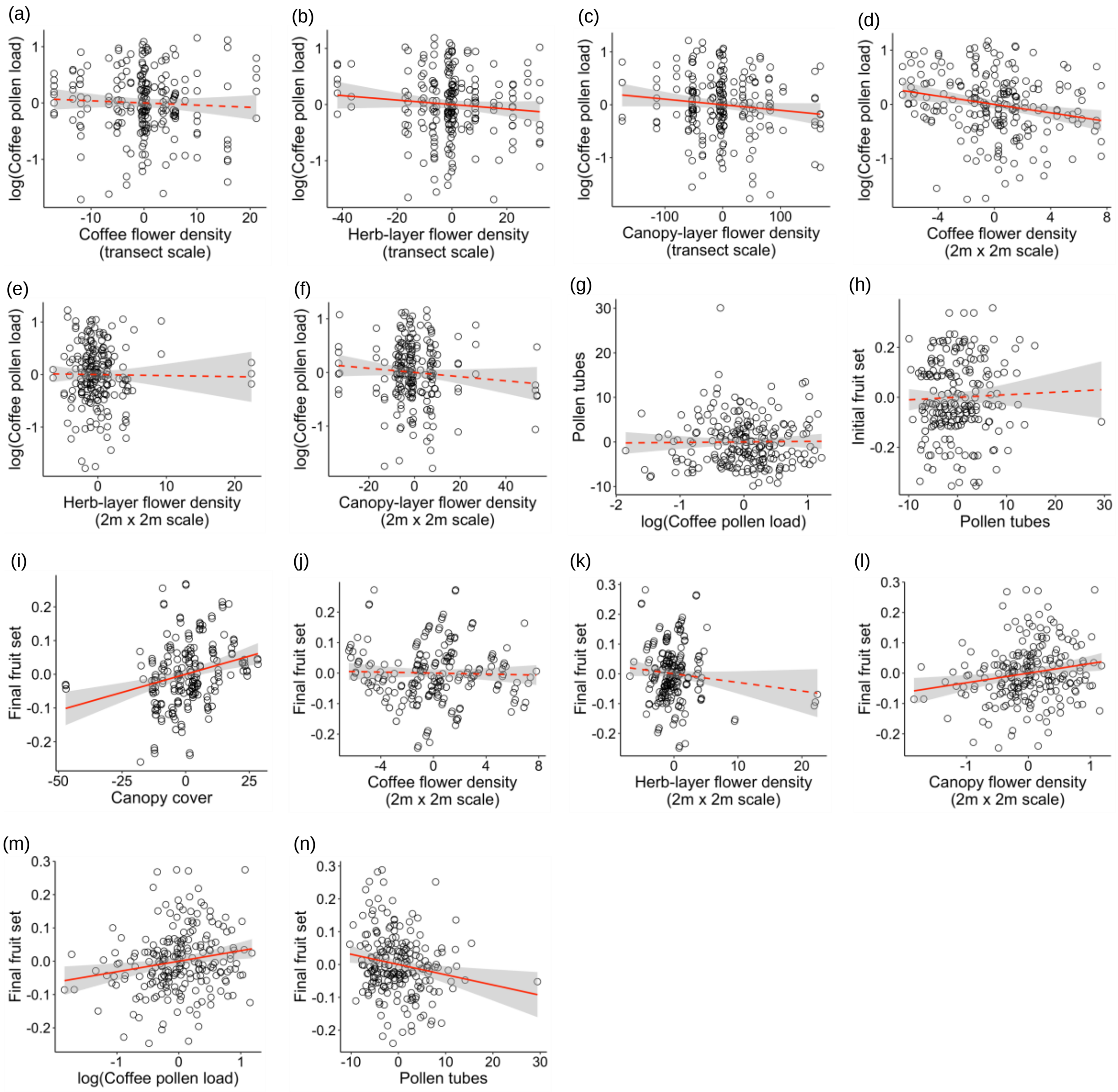


Figure 2.4. Partial residuals plots for selected relationships between neighborhood, pollination, and yield. Red line represents linear best-fit relationship, gray shaded area is 95% confidence interval. Dashed line: $p \geq 0.05$; solid line: $p < 0.05$.



Chapter 3
Changes in Partner Traits Drive Variation in Plant–Nectar Robber Interactions Across Habitats[†]

ABSTRACT

The frequency and outcome of biotic interactions commonly vary with environmental conditions, even without changes to community composition. Yet the drivers of such environmentally-mediated change in biotic interactions are poorly understood, limiting our ability to predict how environmental change will impact communities. Studying nectar robbery by stingless bees of *Odontonema cuspidatum* (Acanthaceae) in a coffee agroecosystem, we documented a temporally consistent difference in nectar robbing intensity between anthropogenic and seminatural habitats. Plants growing in coffee fields (anthropogenic habitat) experienced significantly more nectar robbery than plants growing in forest fragments (seminatural habitat). Using a combination of field surveys and manipulative experiments, we found that nectar robbery was higher in coffee fields primarily due to environmental effects on a) neighborhood floral context and b) *O. cuspidatum* floral traits. This led to both preferential foraging by nectar robbers in coffee fields, and to changes in foraging behavior on *O. cuspidatum* that increased robbery. Nectar robbery significantly reduced fruit set in *O. cuspidatum.* These results suggest that the effects of anthropogenic environmental change on species traits may be more important

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[†] This chapter was previously published as: Gordon Fitch and John Vandermeer (2021). Changes in partner traits drive variation in plant–nectar robber interactions across habitats. *Basic and Applied Ecology* 53: 1–11.

than its effect on species density in determining how interaction frequency and outcome are affected by such environmental change.

INTRODUCTION

Understanding the effects of anthropogenic environmental change on biotic communities and ecosystem function is a key challenge for ecologists. Anthropogenic environmental change is resulting in striking biodiversity loss at a global scale (Matzke et al., 2011; Dirzo et al., 2014), with consequences for ecosystem function and ecosystem service provisioning at smaller scales (Hooper et al., 2012). But while the bulk of research on the effects of anthropogenic environmental change has focused on changes to community composition (Tylianakis et al., 2008b; Valiente-Banuet et al., 2015b), there is growing appreciation that the structure of interaction networks within communities can be altered by environmental change even when community composition is unaffected (Figure 3.1; Poisot et al., 2015, 2017; Tylianakis et al., 2008; Tylianakis & Morris, 2017; Valiente-Banuet et al., 2015). Such interaction 'rewiring' may be mediated by changes to either the density (Figure 3.1, Pathways A-B) or traits (Figure 3.1, Pathways C-D) of one or more interacting species. These effects, moreover, may derive directly from altered environmental conditions (Figure 3.1, Pathways A, D), or be mediated by environmentally-driven changes to community context (Figure 3.1, Pathways B-C).

Given the multiple pathways by which environmental change can impact interactions, it is perhaps not surprising that both theoretical (Valiente-Banuet et al., 2015b) and empirical (Tylianakis et al., 2008b; Valiente-Banuet et al., 2015b) work suggest that changes in interaction structure are likely to occur more commonly, and more quickly, than species loss in response to environmental change. Yet, even where changes to the structure of interactions have been documented, we frequently lack understanding of the underlying drivers of these changes [Tylianakis et al. (2008), though see Fagundes et al. (2020) for an exception]. This limits our ability to predict how future environmental change will impact communities and ecosystems.

Nectar robbery (NR), in which a flower visitor extracts nectar from a flower via an opening other than the corolla mouth, is one interaction type that is likely to be strongly impacted by environmental context (Morris, 1996; Irwin and Maloof, 2002; Cuevas and Rosas-Guerrero, 2016), since it is generally a facultative behavior (Morris, 1996; Irwin et al., 2010; Richardson and Bronstein, 2012). The effects of NR on reproductive success of the robbed plant are variable; though negative, neutral, and positive effects have been reported (Maloof and Inouye, 2000; Burkle et al., 2007), negative effects, at least on components of female fitness, are most common (Irwin et al., 2001). The fitness outcome of NR for the robbed plant depends on the interaction of a number of factors, including plant mating system, the identity and foraging behavior of both robbers and legitimate pollinators, and the environmental (particularly floral) context in which the interaction occurs (Morris, 1996; Maloof and Inouye, 2000; Burkle et al., 2007).

Nectar-robbing intensity (NRI) – measured as the proportion of flowers that experience robbery – commonly varies both spatially (Morris, 1996; Irwin and Maloof, 2002; Cuevas and Rosas-Guerrero, 2016) and temporally (Navarro, 2000; Irwin and Maloof, 2002; Cuevas and Rosas-Guerrero, 2016). Multiple drivers of such variability have been postulated; these drivers are not mutually exclusive, and in some cases multiple drivers may be operating in tandem. Putative drivers include both direct responses of robber or plant to environmental conditions and responses mediated by the broader community. Direct responses may include variation in the density of robbers (Navarro, 2000; Irwin and Maloof, 2002) or the density, flower number, or nectar quality or quantity of the focal plant (Krupnick et al., 1999) due to environmental conditions. Community-mediated responses may include altered foraging behavior depending on the availability of alternative floral resources (Irwin and Maloof, 2002; Irwin et al., 2010) or density of other flower visitors (Roubik, 1982). Yet to date, there has been little work documenting which of these mechanisms operate in specific instances to generate variation in NRI. Without a mechanistic understanding of the ecological drivers of NR, it is difficult to predict the circumstances under which NR will occur and how it will be altered by environmental change.

In this study, we first assessed the intensity of NR by stingless bees of the shrub *Odontonema cuspidatum* (Nees) Kuntze (Acanthaceae) in a semi-natural habitat (forest fragments) and an anthropogenic one (coffee farm). We then evaluated the role of potential drivers of NR (Figure 3.1) in generating habitat-based spatial heterogeneity in NRI. We also evaluated whether NR influenced either the likelihood of individual flowers setting fruit or plant-level reproductive output across both habitats. Our aim was to understand the extent to which variation in the intensity and outcome of NR across habitats is driven by 1) changes in population density of robber or plant (Figure 3.1 Pathways A-B), 2) direct effects of environmental conditions (i.e. light availability) on one or more traits of either partner (Figure 3.1 Pathway D), or 3) indirect effects on robber or plant traits via changes in community context (Figure 3.1 Pathway C). We interpret the term ‘trait’ to include both physical characteristics and behaviors, consistent with the definition used in the literature on trait-mediated indirect effects (e.g. Werner & Peacor 2003, Schmitz et al. 2004, Utsumi et al. 2010). Specifically, the traits we focus on are, for the plant, flower number and floral nectar characteristics and, for the nectar robbers, foraging behavior.

MATERIALS AND METHODS

Study system

This research was conducted at Finca Irlanda, a shaded, organic coffee farm, approximately 300 ha in size, located in the Soconusco region of southeastern Chiapas, Mexico. The farm ranges from 900-1150 masl; above ~1000 masl it is comprised primarily of *Coffea arabica* plantations, with three small (<30 ha) forest fragments embedded within the farm. Forest fragments are characterized by a higher density and diversity of canopy trees in comparison to the coffee fields. As a result of the higher density of canopy trees in forest fragments, the amount of light reaching the ground is generally lower in the forest than the coffee farm (see Results). This difference in canopy cover represents a key environmental difference between these habitats.

Within this landscape, *O. cuspidatum*, a perennial shrub native to the region, grows both in areas under coffee cultivation and in forest fragments. In the study area, *O. cuspidatum* blooms primarily from June to August, in the early part of the rainy season. Slender red flowers, 2-2.5 cm long, are borne on indeterminate branching racemes; individual plants produce from 1 to approx. 30 racemes, and each raceme holds from approx. 10 to hundreds of flowers (G. Fitch unpublished data). *Odontonema cuspidatum* is self-fertile but requires animal pollination for fertilization, due to spatial separation of anthers and stigma (G. Fitch unpublished data). Hummingbirds, particularly the blue-tailed hummingbird (*Amazilia cyanura*), are the most frequent legitimate floral visitors (G. Fitch unpublished data); this, together with the flower's morphology, suggests that hummingbirds are the primary pollinators of *O. cuspidatum*. The flowers also attract a wide range of nectar-feeding insects, most of which engage exclusively in nectar robbery, extracting nectar from animal-made holes in the base of the corolla tube. Primary nectar robbers – i.e. those that make the hole themselves, hereafter 'PNR' – comprise two species of stingless bee in the genus *Trigona* (*T. fulviventris* and *T. nigerrima*; Hymenoptera: Apidae: Meliponini). Fertilized flowers produce explosively dehiscent capsules (Daniel, 1995).

Data collection

Spatiotemporal patterns of nectar robbing intensity

Within a 25ha area that included both coffee fields and forest fragments, we haphazardly selected 109 individual *O. cuspidatum* for inclusion in the study. This represents ~50% of all individuals found in the survey area. This 25ha section of the farm was selected because it included the principal forest fragments contained within the farm's boundaries, as well as high densities of *O. cuspidatum*. Because of the spatial arrangement of the forest fragments, each plant was within 500 m of a habitat edge. All plants were individually labeled and followed through both years of the study, except that thirty-three plants surveyed in 2017 either died or did not flower in 2018, and an additional 15 plants that flowered in 2018 but not 2017 were monitored in 2018 only. We recorded the GPS coordinates of each plant. Distance between plants included in the study ranged from 10-2200 m.

In 2017-2018, plants were surveyed for NR weekly for the duration of the flowering period. At each survey, all inflorescences with open flowers were surveyed. Flowers >1.5 cm long were checked for evidence of NR (characteristic hole at corolla base), and the number of robbed and unrobbed flowers on each inflorescence was recorded. The 1.5 cm cutoff was chosen because flowers of this length were generally within 2 days of opening, and prior to this stage flowers experienced minimal NR (G. Fitch unpublished data). In 2018 only, each monitored inflorescence was individually tagged.

Putative drivers of nectar robbing intensity

***Odontonema cuspidatum* density**

To determine the density of *O. cuspidatum* in each habitat, in June 2018 we counted the number of *O. cuspidatum* inflorescences, at any stage of development, within a 20 m radius of each of our target plants.

***Odontonema cuspidatum* floral traits**

Each time we surveyed plants for NR, we recorded the total number of flowers present on the plant, for our measure of per-observation flower number. At the end of the flowering period, the season-long total number of flowers produced was determined by counting all mature fruits and persistent ovaries (i.e. flowers that had not set fruit) on each plant.

On a subset of monitored plants (49 in 2017, 19 in 2018 with 7 included in both years), we assessed nectar volume and sugar content. Because standing nectar crop was minimal in unbagged flowers, to measure nectar content we covered 2 inflorescences/plant with a bag made of 0.5 cm tulle mesh to exclude floral visitors. During the flowering period, we checked bagged inflorescences for open flowers 2x/week. To assess nectar volume, we removed all nectar from a flower using a 75 μL microcapillary tube (Drummond Scientific, Broomall PA), then measured the height of the nectar in the tube using digital calipers (Thomas Scientific, Swedesboro NJ) and converted this measure to nectar volume. We used a pocket refractometer (Eclipse 45-81, Bellingham & Stanley, Tunbridge Wells UK) to assess the nectar sugar content of each sample.

From each plant, we assessed nectar characteristics of at least 4 flowers (range: 4-32 flowers, mean ± SE: 12.9 ± 0.3 flowers).

Primary nectar robber (PNR) density and foraging behavior

To determine PNR population density in each habitat, in 2018 we conducted surveys for PNR nests. Surveys were conducted along 30 m x 10 m transects oriented in one of the cardinal directions and centered on a target *O. cuspidatum* plant. We conducted surveys along 32 transects, 16 in coffee and 16 in forest. Within a habitat, target plants for surveys were selected at random, except once a plant was selected, all other plants falling within the transect were excluded from selection, so no transects overlapped. Collectively, these transects encompassed 44 monitored *O. cuspidatum* plants. The PNR species nest either in trees or in the ground at the base of trees (Fierro et al., 2012), so our nest search focused on trees ≥15 cm dbh (Hubbell and Johnson, 1977). On all such trees within each transect, we scanned the trunk and major limbs from 0-20m above the ground for evidence of nesting. Surveys were conducted between 0700-1100, when nest activity was highest. Both species of PNR of *O. cuspidatum* in the study area have prominent nests with high activity levels, so we are reasonably confident that we located all nests of these species within our transects.

To determine PNR forager density, in addition to nest density, in 2017 and 2018 we surveyed local PNR abundance for each focal *O. cuspidatum* plant. Surveys occurred during peak bee activity (0700-1100 h) on sunny days. Each survey consisted of two 10-min periods. The first focused on flowers from 2-5 inflorescences of the focal *O. cuspidatum*, and the second focused on flowers within 10 m of the focal plant, with a 10-min break in between surveys. Prior to beginning the survey, we counted the number of open flowers on focal inflorescences, and located all bee-attractive flowering plants in the 10 m neighborhood. During surveys, all insect flower visitors were caught using resealable plastic bags and held, one insect per bag, for the duration of the survey. At the end of each 10-min survey, captured insects were identified to species or morphospecies and then released. Insects were classified as primary nectar robbers, secondary nectar robbers, legitimate visitors, or unknown, based on prior observation of insect visitation to *O. cuspidatum*. These observations provided us with several measures of PNR forager abundance: total site-level abundance, abundance on focal *O. cuspidatum*, per-flower abundance on focal *O. cuspidatum*, abundance on non-focal flowers, and the proportional abundance on target plants (number of individuals caught on target plants divided by total number of individuals caught during that survey). We interpret the latter metrics as a measure of PNR foraging behavior, while we consider the others to be measures of PNR foraging behavior.

In 2018, we assessed within-plant PNR foraging behavior on 23 monitored plants, 13 in coffee and 10 in forest. On each plant, we monitored five foraging bouts by individual PNRs at focal *O. cuspidatum* plants, recording the species of PNR, the length of time spent on the inflorescence, and the number of flowers robbed. We then calculated the proportion of potentially robbable flowers that had been robbed.

Environmental conditions – Canopy cover

We measured canopy cover directly above the crown of each focal plant (N=109) in June 2017 (June 2018 for plants added in 2018) using CanopyApp 1.0.3 (University of New Hampshire, Durham, NH USA).

Community context – Floral resource availability

In 2017, floral surveys were conducted to determine floral resource availability in the neighborhood of 95 focal plants. For each plant, four 10 m x 2 m transects were established, each one beginning at the focal plant and extending 10 m in one of the cardinal directions. Along each transect, all blooms were counted and identified to species or genus. Surveys occurred once for each focal plant, near the peak bloom for each focal plant. In some cases, transects for two plants partially overlapped; in these cases, bloom tallies for the overlapping portions of the transects were included in the total for both plants' transect.

Effect of nectar robbery on reproduction

For monitored plants that had >20 flowers and had both robbed and unrobbed flowers (N=44), we evaluated the difference in probability of producing fruit and in seed set between robbed and unrobbed flowers. Flowers were marked with either red (robbed) or blue (unrobbed) nail polish on the pedicel. Robbed flowers were marked either on the day before they were to open or soon after opening, while unrobbed flowers were marked only as they were beginning to senesce, to ensure that robbing occurred before pollination and that flowers marked as unrobbed were not subsequently robbed. The number of marked flowers of each type (robbed and unrobbed) varied across plants depending on the availability of robbed and unrobbed flowers (robbed flowers: range = 1-26 flowers, mean ± SE = 4.7 ± 0.7 flowers; unrobbed flowers: range = 1-16 flowers, mean ± SE = 2.9 ± 0.4 flowers).

We assessed fruit set by counting the number of fruit and number of persistent ovaries on mature inflorescences. The fate of each marked robbed or unrobbed flower was recorded. Inflorescences that had been damaged by insect herbivores were excluded from further analyses. To measure seed set, up to 5 fruits (in 2017) or all undamaged fruits (in 2018) were collected from each inflorescence. These fruits, segregated by plant, were placed in small bags made of 0.5 cm mesh fabric and left in a drying oven at 50 ºC until all had dehisced, at which time seeds were counted.

Data analysis

All analyses were conducted in R v.3.5.1 (R Core Team, 2018). All models were checked for conformity to assumptions.

Influence of habitat on nectar robbing intensity

We evaluated the influence of habitat on NRI using GLMMs with habitat and year as fixed effects and plant and date as random effects. We separately considered three components of NRI: number of open flowers robbed, number of unopened flowers robbed, and total number of flowers robbed. In all cases, we included log(total number of flowers of that class) as an offset, and used a Poisson error distribution with log-link function. To check for spatial autocorrelation in NRI, we fit a parallel set of GLMs, and calculated Moran's I for the residuals of each of these models using package \`ape\` (Paradis and Schliep, 2019). We found no evidence for spatial autocorrelation in these data ($p > 0.1$ in all cases).

Influence of habitat on putative drivers of nectar robbing intensity

We tested for a significant effect of habitat on the following putative drivers of NRI: focal plant flower number, focal plant nectar volume and nectar sugar content, canopy cover, neighborhood floral density, PNR density at focal plants and in the 10-m neighborhood, and PNR nest density. We initially considered several floral neighborhood metrics: floral richness, total flower density, and *O. cuspidatum* flower density. These metrics were strongly correlated and total flower density was the best predictor of NRI, so for all analyses we used total floral density to assess effects of floral neighborhood. Focal plant flower number (assessed as the total number of flowers produced over the flowering period) and PNR nest density were fitted with a GLM with Poisson distribution and log-link function. Focal plant nectar volume and nectar sugar content, as well as canopy cover, were fitted using linear models. For other putative drivers, we had multiple measures per plant, and therefore used GLMMs with plant as a random effect and a Poisson distribution with log-link function. We calculated Moran's I for the residuals of all models to check for spatial autocorrelation. For drivers with significant spatial autocorrelation, we used the `spdep` package to calculate Moran eigenvectors (Bivand and Wong, 2018). These eigenvectors were included as additional predictors in an updated model. Where significant spatial autocorrelation was found, the estimates we provide for the effect of habitat on the relevant driver come from the model including Moran eigenvectors. To evaluate significant effects, all p-values were adjusted for multiple comparisons using the Bonferroni correction.

We also tested whether canopy cover (and therefore light availability) could account for differences in floral traits (flower number and flower nectar volume) between habitats. For flower number, we used a GLM with negative-binomial distribution, and for nectar volume we used a linear mixed-effects model with plant as a random effect.

Influence of putative drivers on nectar robbing intensity

The number of plant-year combinations for which we had observations of a particular putative driver varied substantially across drivers. This variation precluded analysis using structural equation modeling, because the combined dataset for all putative drivers contained too few observations for meaningful analysis given the number of drivers (Kline, 2015). Therefore, we initially assessed the relationship between NRI and each putative driver that differed significantly between habitats (that is, all the boldface variables listed in Table 3.1) separately, then included those drivers that had an effect on NRI at Bonferroni-corrected $p \leq 0.2$ in a combined model (see below). All models used the number of robbed flowers as the response variable, and included log(total number of flowers surveyed) as an offset; error distributions were either Poisson or negative binomial, depending on whether the data were overdispersed, and used a log-link function.

The relationship between flower visitation and both flower number and floral neighborhood is often nonlinear and unimodal (Rathcke, 1983b; Ghazoul, 2006). Beyond unimodality, we did not have *a priori* expectations for the relationship between these drivers and NR. Therefore, to assess their effect on NRI, we used general additive models (GAMs). To test for nonlinearity, we compared model versions with linear and smoothed relationships using AICc. In both cases, the nonlinear model indicated a unimodal relationship and improved fit over the linear model (for flower number, ΔAICc = 19; for floral neighborhood ΔAICc = 25). For all other drivers we assumed a linear response of NR; we used a GLM to evaluate the effect of canopy cover and floral neighborhood and GLMMs to evaluate the effect of all other drivers (since for these drivers we had multiple observations per plant).

These single-predictor models indicated that at the $p \leq 0.2$ level, the putative drivers that affected NRI were focal plant flower number, neighborhood floral density, and robber density at focal plants. To test whether these putative drivers had independent effects, we used a GAM with all three predictors; smooth terms were applied to flower number and floral neighborhood, while robber density was constrained to a linear function.

Based on GAM results for the relationship between NRI and neighborhood floral density, we classified neighborhood floral densities as low (less than the lower bound of the 95% confidence interval for the predicted maximum of the function relating NRI to floral density), moderate (within the 95% confidence interval), or high (higher than the upper bound of the 95% confidence interval).

Effects of nectar robbery and habitat on Odontonema cuspidatum *reproduction*

To determine the effect of NR on *O. cuspidatum* reproduction, we compared differences in fruit set between robbed and unrobbed flowers using a binomial GLMM with plant as a random effect. We additionally tested for an effect of habitat on fruit set, and of differences in the effect of NR on fruit set between habitats, by including habitat and a robbed status × habitat interaction term as fixed effects. To control for differences across plants in their ability to produce fruit, independent of the effects of NR, we only included plants for which we had data on the fate of both robbed and unrobbed flowers in a single year (N = 44).

To test the effect of NRI and habitat on measures of reproductive output, we examined fruit set, seed set, and seeds produced per plant. To model fruit set, we used a Poisson generalized linear mixed-effects model (GLMM) with number of fruits as the response variable, offset by log(total number of flowers produced). Fixed effects were habitat, NRI, and a habitat × NRI interaction term; plant was included as a random effect. Seed set and seeds per plant were assessed only in 2018, so for those metrics we used a negative binomial generalized linear model (GLM) with number of seeds as the response; the model for seed set additionally included log(total number of flowers) as an offset. Because there was no effect of the habitat × NRI interaction term in the model for fruit set, this predictor was omitted from our models of seed set and seeds per plant.

RESULTS

Spatiotemporal patterns in nectar robbing intensity (NRI)

Plants growing in forest fragments were robbed significantly less than those growing in coffee, consistent across years (proportion of flowers robbed in forest: 0.29 ± 0.02; in coffee: 0.42 ± 0.02; $z = -2.26$, $p = 0.02$). Across both habitats, NRI was significantly higher in 2018 than 2017 (2017: 0.32 ± 0.02; 2018: 0.46 ± 0.01; $z = 8.70$, $p < 0.001$) and, in both years, increased over the survey period ($ß = 0.19 \pm 0.02$, $z = 10.49$, $p < 0.001$).

Relationships between putative drivers and habitat

Environmental conditions and community context

Canopy cover over target *O. cuspidatum* was significantly higher in forest fragments than coffee (Table 3.1). Neighborhood floral density was significantly higher in coffee than in forest fragments (Table 3.1).

Partner densities

Density of *O. cuspidatum* did not differ between habitats (Table 3.1). Density of primary nectar robbers (PNRs), on the other hand, showed a complex response to habitat. There was no difference in nest density of the two PNR species between habitats (Table 3.1). Nevertheless, PNR density at monitored *O. cuspidatum* plants was higher in coffee than in forest (Table 3.1), suggesting greater forager density in coffee. However, PNR density at all other flowers within 10 m of focal plants (most of which received legitimate visits from PNRs) was equivalent across habitats (Table 3.1). Because of the high density of PNRs at target *O. cuspidatum*, total PNR density was still significantly higher in coffee than in forest (Table 3.1).

Partner traits

Odontonema cuspidatum flower number was significantly lower in forest (Table 3.1). Nectar volume and sweetness were both lower for plants growing in coffee, but these differences were not significant (Table 3.1). These changes in floral traits stem, at least in part, from reduced light availability in forest fragments, as canopy cover (significantly higher in forest fragments) had a negative effect on both total flower number (ß = –0.31±0.08, z = –3.72, $p < 0.001$) and nectar volume (ß = –0.19±0.08, t = –2.82, p = 0.03).

Primary nectar robber foraging behavior at *O. cuspidatum* plants differed between habitats. On a per-flower basis, PNRs spent more time per foraging bout on plants growing in coffee (Table 3.1).

Relationships between putative drivers and nectar robbery

Nectar robbery responded nonlinearly to both *O. cuspidatum* flower number and neighborhood floral density (Figure 3.2, Table 3.2). In both cases, NRI had a unimodal response, initially increasing, then decreasing as floral availability increased further (Figure 3.2). For *O. cuspidatum* flower number, the position of the predicted maximum was 645 blooms [95% confidence interval (CI): 467-850 blooms; Figure 3.2A]. This is larger than the mean flower number for individuals from either forest fragments (160 ± 26) or coffee fields (243 ± 37), indicating that for most of the surveyed plants, increasing flower number leads to increased NRI.

For neighborhood floral density, the predicted maximum was 1.8 blooms/m^2 (95% CI: 1.1–1.9 blooms/m^2; Figure 3.2B). Significantly, the neighborhoods of most forest-growing *O. cuspidatum* had low floral densities (i.e. below the lower bound of the 95% CI for the predicted maximum: 38 plants below, 11 within, 3 above). The floral neighborhoods of coffee-growing plants varied more in density (10 plants below the CI; 16 within; 23 above; Figure 3.2B), with a substantially higher percentage of coffee-growing plants found in floral neighborhoods within the 95% CI for peak NRI (33% versus 22%). Most (65%) of the plants whose neighborhood floral density fell within the 95% CI for predicted maximal NRI grew in coffee.

The other putative driver that significantly affected NRI was PNR density on target plants, which was higher for coffee-growing plants (Table 3.1). When these three significant drivers were combined into a single model, all three retained their significance level and showed a qualitatively similar effect on NRI as when they were considered independently (Table 3.2). Moreover, when habitat was added as a linear predictor to this model, it did not have a significant effect on NRI and did not improve model fit (Table 3.2).

Effects of nectar robbery and habitat on* Odontonema cuspidatum *reproduction

Nectar robbery significantly reduced the probability of a flower setting fruit, from 0.32 ± 0.04 (mean ± SE) for unrobbed flowers to 0.18 ± 0.03 for robbed flowers, representing a 43% decrease in fruit set ($z = 3.41$, $p < 0.001$). The effect of NR on probability of setting fruit was equivalent between habitats (robbed status × habitat interaction: $ß = 0.16 \pm 0.60$, $z = 0.27$, $p = 0.8$), as was the overall probability of setting fruit (coffee: 0.23 ± 0.03; forest: 0.23 ± 0.04; $z = –0.10$, $p = 0.9$).

Consistent with the negative effect of NR on the probability of individual flowers setting fruit, we found a significant negative relationship between NRI and fruit set at the plant level (Table 3.3). This effect was consistent across habitats, and fruit set did not differ between habitats (coffee: 0.12 ± 0.01; forest: 0.11 ± 0.01; Table 3.3). By contrast, neither seed set nor seeds per plant were influenced by NRI. Seed set was higher in forest-growing plants, though due to the smaller number of flowers produced by forest-growing plants, seeds per plant did not differ between habitats (Table 3.3).

DISCUSSION

Changes in the frequency or outcome of an interspecific interaction as a result of environmental change can be explained by effects on 1) the density of the interacting species and/or 2) the traits (including behavior) of the interacting species. These effects in turn can stem directly from altered abiotic conditions (e.g. changes to light or water availability influencing the density or traits of the relevant species), or be mediated by changes in the wider community (i.e., abiotic conditions influence the density or traits of other species in the community, which in turn impinge on the interaction in question) (Figure 3.1A; Poisot et al., 2015). In this study, we found that difference in nectar robbing intensity (NRI) of *O. cuspidatum* between a semi-natural and an anthropogenic habitat was primarily the result of partner trait differences, with a lesser contribution of density differences. From the plant's perspective, these trait differences are driven directly by differing environmental conditions between habitats, while on the robber's side, trait differences are primarily the result of altered biotic community context (Figure 3.1B). Specifically, we found that, together, 1) a nonlinear response of PNRs to the availability of alternative floral resources, 2) greater flower production for *O. cuspidatum* growing in coffee, and 3) higher density of foraging PNRs and greater preference for *O. cuspidatum* by PNRs in the coffee farm than in adjacent forest fragments can fully explain differences in NRI between habitats.

Of these three factors, floral neighborhood composition had the greatest influence on NRI (Table 3.2). The moderate floral densities found in the coffee fields likely attract more foraging PNRs than found in the low-floral-density forest fragments, leading to higher PNR density and therefore higher NRI for coffee-growing plants (Pathway B in Figure 3.1). Since PNR nest density was equivalent between habitats, this indicates preferential foraging by PNRs in coffee fields over forest fragments (the small size of forest fragments in the study area means that bees leaving a nest in the forest can readily move into coffee fields to forage). This finding highlights the importance of shade coffee as a habitat for stingless bees, a result consistent with other studies (Jha and Dick, 2010; Fisher et al., 2017). Moreover, it confirms the importance of floral context as a driver of spatiotemporal heterogeneity in nectar robbery, a relationship which has been hypothesized elsewhere (Irwin and Maloof, 2002), though not explicitly evaluated.

Differences between habitats in *O. cuspidatum* floral traits (flower number and perhaps nectar volume) contributed to higher NRI for plants growing in coffee (Pathway D in Figure 3.1). Across most of the observed range of floral display size, more flowers led to more NR. Plants growing in coffee produced more flowers on average [likely because higher light availability increased the amount of photosynthate that plants could allocate to flower production (Fitch and Vandermeer, 2020)], and therefore attracted more PNRs. In addition, PNR foraging behavior differed between habitats, with individual PNR visits lasting longer to flowers in coffee than those in forest. This may be due to differences in nectar rewards between habitats: per-flower nectar volume was 50% higher in coffee than in forest, though this difference was not significant.

Both flower-level and plant-level data indicate that NR significantly reduces fruit set in *O. cuspidatum*. The negative correlation between fruit set and NRI is striking, given that PNR attraction to *O. cuspidatum* is associated with floral traits – i.e. floral display size and nectar quantity – that are frequently positively correlated with both attractiveness to pollinators (Adler and Bronstein, 2004; Theis et al., 2014) and the availability of resources to allocate to reproduction (Bazzaz et al., 1987, 2000). In this population, negative effects of NR are sufficient to outweigh any benefits of increased pollination and/or resource availability, resulting in net reduction in fruit set. However, this effect does not translate to the number of seeds produced per plant, likely because NRI is positively related to flower number; even if a smaller proportion of flowers produces seeds, the net outcome is a consistent number of seeds across a range of NRIs. Thus, despite the difference in NRI intensity between habitats, there was ultimately no difference in reproductive output between plants growing in forest and those growing in coffee (Table 3.3).

These results suggest that spatial variation in NRI is due primarily to the effects of environmental conditions on the plant community – both on the availability of alternative floral resources and on traits mediating nectar robber attraction. However, whether these results can be generalized to other instances of nectar robbery remains to be seen. More generally, this study highlights the importance of trait differences, rather than or in addition to differences in density, as determinants of interaction frequency or outcome across environmental gradients. This is in line with a large body of research demonstrating that trait-mediated indirect interactions are often more important than density-mediated indirect interactions in determining the effect of one species on another species with which it does not directly interact (Werner and Peacor, 2003b;

Schmitz et al., 2004b). Further, this suggests that the importance of trait-mediated effects of anthropogenic environmental change – on species interactions and, ultimately, species persistence and ecosystem function – have been underappreciated. As this work highlights, detecting and understanding such trait-mediated effects may require fine-grained analysis of interacting organisms across environmental contexts. Thus, on the one hand we heartily endorse recent calls to use network approaches to better understand how environmental change can impact communities beyond just species loss (Tylianakis et al., 2008b; Poisot et al., 2015; Valiente-Banuet et al., 2015b; Poisot et al., 2017). On the other, we caution that the empirical data upon which networks are built often lack the level of detail needed to describe the effects of environmental change on biotic communities.

ACKNOWLEDGEMENTS

We are grateful to Walter and Bernd Peters for permission to conduct research at Finca Irlanda. We thank members of the Perfecto and Vandermeer lab groups for support in developing this project. In particular, thanks to Lauren M. Schmitt and Chatura Vaidya for helpful comments on this manuscript, and to Gabriel Dominguez Martinez, Gustavo López Bautista and Chatura Vaidya for support in the field. This study was funded by a Nancy W. Walls Award from the University of Michigan Department of Ecology and Evolutionary Biology and a University of Michigan International Institute Rackham International Research Award, both to Gordon Fitch.

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TABLES AND FIGURES

Table 3.1. Relationship between habitat and putative drivers of nectar robbery. Model results are from generalized linear models (canopy cover, neighborhood floral density, O. cuspidatum density, and PNR nest density) or generalized linear mixed-effects.

Putative driver	**Mean ± SE**		**Test statistic (t or z)**	**DF**	**p**	**%DE**
	Coffee	**Forest**				
Environmental conditions						
Canopy cover (%)†	**70.8 ± 2.6**	**90.5 ± 1.0**	**7.01**	**83**	**<0.001**	**51.0**
Community context						
Neighborhood floral density (blooms per transect)†	**73.7 ± 7.5**	**27.9 ± 4.5**	**–5.46**	**51**	**<0.001**	**25.4**
Partner densities						
O. cuspidatum density (inflorescences per survey)	18.8 ± 3.4	11.2 ± 1.9	–1.05	54	0.94	0.0
PNR nest density (nests per transect)	0.2 ± 0.1	0.1 ± 0.1	1.00	15	1.00	0.0
Total PNR density (individuals per observation)†	**1.9 ± 0.2**	**0.8 ± 0.2**	**–4.62**	**40**	**<0.001**	**31.4**
PNR density – target *O. cuspidatum* (individuals per observation)	**2.2 ± 0.3**	**0.8 ± 0.2**	**–4.10**	**46**	**<0.001**	**27.0**
PNR density – 10m neighborhood (individuals per observation)†	1.6 ± 0.3	1.0 ± 0.3	0.89	39	1.00	0.0
Partner traits						
***O. cuspidatum* flower number†**	**243.4 ± 37.1**	**160.2 ± 25.8**	**–9.22**	**156**	**<0.001**	**40.6**
O. cuspidatum nectar volume (µL)	6.6 ± 0.3	4.4 ± 0.4	–2.70	65	0.10	10.1
O. cuspidatum nectar sweetness (Brix)	25.4 ± 0.1	24.7 ± 0.3	–1.14	63	1.00	2.0
Per-flower visit duration (s)	**0.8 ± 0.1**	**0.4 ± 0.1**	**2.74**	**57**	**0.04**	**2.3**

†: includes spatial eigenvectors as additional predictors to account for spatial autocorrelation.

Table 3.2. Relative importance of the significant drivers of nectar robbing intensity (NRI). Results from a general additive model combining all of the putative drivers that significantly influenced NRI when considered independently. Boldface indicates a significant effect on NRI at $p < 0.05$. %DE: percent of the null deviance in NRI explained by each driver; PNR: primary nectar robber.

Driver	**% DE**	**ΔAICc for omitting this variable**	**p**
***O. cuspidatum* flower number**	**15.0**	**37.9**	**< 0.001**
Neighborhood floral density	**22.1**	**52.0**	**< 0.001**
PNR density – target plant	**15.2**	**31.3**	**< 0.001**
Habitat	0.0	–2.3	0.25

Table 3.3. Relationship between reproductive output and nectar robbing and habitat. DF: residual degrees of freedom; %DE: percent of the null deviance in the measure of reproductive output that is explained by each predictor; NRI: nectar robbery intensity. Boldface indicates statistical significance at $p < 0.05$. Significance codes: . p = 0.05; *** p<0.001.

Predictor	**Coefficient (ß ± SE)**	**Test statistic (t or z)**	**DF**	**%DE**
Fruit set (GLMM)				
NRI	**–0.63 ± 0.21*** **	**–2.94**	**123**	**8.0**
Habitat (Forest)	–0.34 ± 0.23	–1.45	123	0.8
NRI × Habitat	0.31 ± 0.31	1.00	123	0.0
Seed set (2018 only; GLM)				
NRI	–0.33 ± 0.36	–0.91	56	1.2
Habitat (Forest)	0.38 ± 0.20 **.**	1.92	56	5.5
Seeds per plant (2018 only; GLM)				
NRI	0.88 ± 0.52	1.71	56	2.7
Habitat (Forest)	–0.05 ± 0.30	–0.17	56	1.5

Figure 3.1. Pathways by which environmental change can result in changes to interactions without changing species composition. (A) Conceptual framework. Pathways are not mutually exclusive. Each line type represents one pathway. Bullet points indicate the aspects of each category assessed in this study. Pathways A-B: environmental conditions (e.g. insolation) directly affect the abundance (Pathway A) or one or more traits (Pathway B) of one or both of the interacting species. Pathways C-D: environmental conditions affect the traits or density of other species in the community, which in turn influences the abundance (Pathway C) or trait(s) (Pathway D) of one or more interacting species. (B) Schematic summary of results from this study, showing only pathways supported by the data.

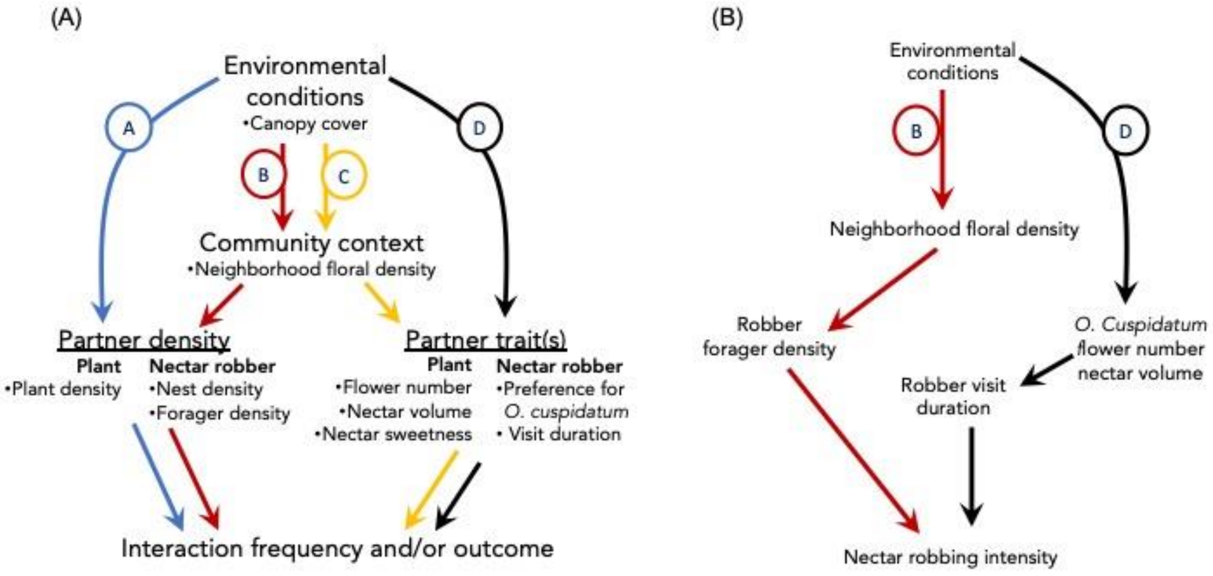
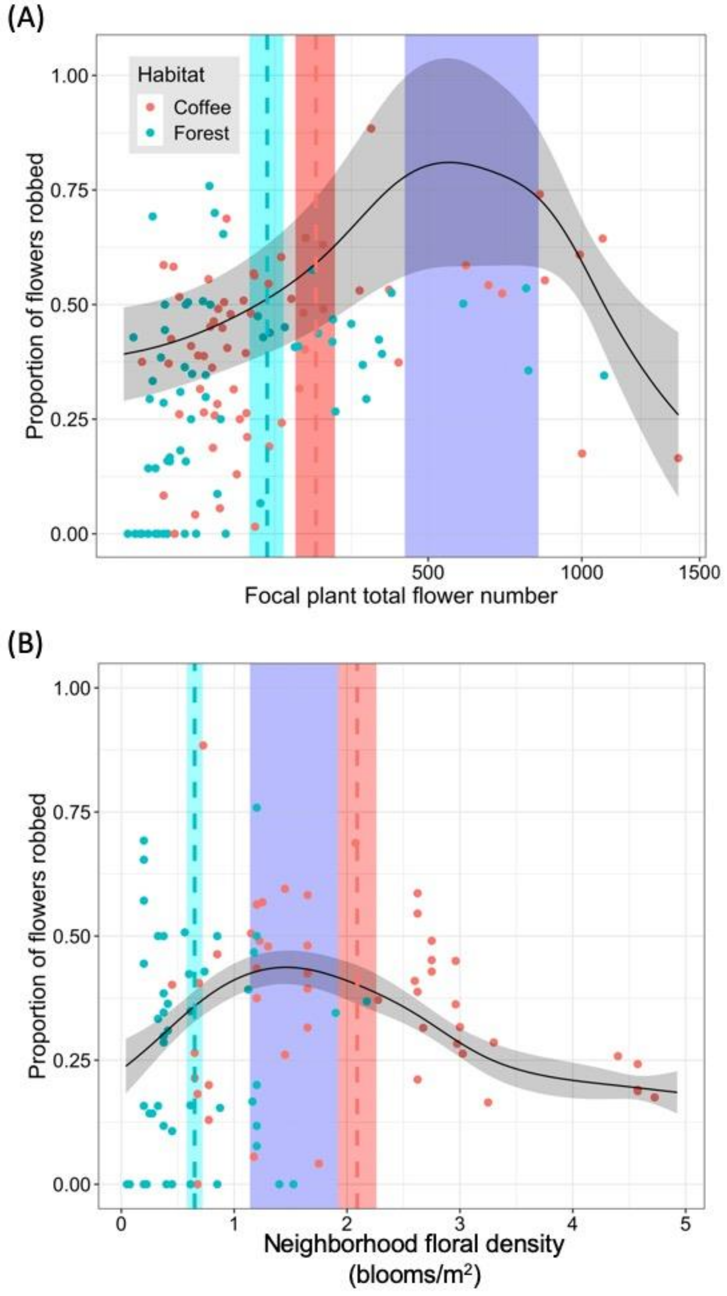


Figure 3.2. Relationship between nectar robbing intensity (NRI) of *O. cuspidatum* and (A) focal plant flower number and (B) floral neighborhood. In both panels, points represent individual plants, colored according to habitat. Black line represents best-fit from a general additive model; gray-shaded area represents standard error. Blue shaded area represents 95% confidence interval about (A) *O. cuspidatum* flower number or (B) the neighborhood floral density where maximum NRI is predicted. Dashed vertical lines and shaded area represent the mean ± standard error for (A) focal plant flower number and (B) neighborhood floral density in each habitat. 'Neighborhood floral density' refers to the mean number of blooms of all species per transect. In (A), the x-axis is square-root transformed to improve legibility.



Chapter 4

Light Availability Influences the Intensity of Nectar Robbery and Its Effects on Reproduction in a Tropical Shrub via Multiple Pathways[†]

ABSTRACT

Premise of the study: The multiple exogenous pathways by which light availability affects plant reproduction – e.g. via influence on attraction of mutualists and antagonists – remain surprisingly understudied. The light environment experienced by a parent can also have transgenerational effects on offspring via these same pathways.

Methods: We evaluated a) the influence of light availability on floral traits in *Odontonema cuspidatum*, b) the relative importance of the pathways by which light influences nectar robbery and reproductive output, and c) the role of parental light environment in mediating these relationships. We conducted a reciprocal translocation experiment using clonally propagated ramets and field surveys of naturally-occurring plants.

Main Results: Light availability influenced multiple floral traits, including flower number and nectar volume, which in turn influenced nectar robbery. But nectar robbery was also directly influenced by light availability, due to light effects on nectar robber foraging behavior or neighborhood floral context. Parental light environment mediated the link between light

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[†] This chapter was previously published as: Gordon Fitch and John H. Vandermeer (2020). Light availability influences the intensity of nectar robbery and its effects on reproduction in a tropical shrub via multiple pathways," *American Journal of Botany* 107(11): 1–10.

availability and nectar robber attraction, suggesting local adaptation to low-light environments in floral visitor attraction. However, we found no transgenerational effect on reproduction.

Conclusions: Our findings demonstrate that exogenous pathways by which light influences plants (particularly through effects on floral antagonists) can complicate the positive relationship between light availability and plant reproduction. Our results are among the first to document effects of light on floral antagonists and clonal transgenerational effects on flower visitor attraction traits.

INTRODUCTION

Light is a key resource for plants, providing the energy that is the basis for carbon assimilation; light availability therefore has strong effects on plant growth and reproduction. In low-light conditions, plant growth rates are commonly reduced (Coleman et al., 1994; Kilkenny and Galloway, 2008; Galloway and Etterson, 2009). Moreover, plants growing in low light may allocate a greater proportion of resources towards tissues that aid in light capture, rather than reproduction (McConnaughay and Coleman, 1999; Delerue et al., 2013). These two factors, operating independently or in tandem, can result in reduced reproductive output in low- as compared to high-light conditions (Figure 4.1F), whether through the production of fewer ovules (Mattila and Salonen, 1995; Kilkenny and Galloway, 2008; Cao et al., 2017) or reduced per-ovule provisioning levels (Niesenbaum, 1993; Kilkenny and Galloway, 2008).

Yet, beyond the endogenous pathways (that is, pathways involving only the effects of light availability on the plant in question) described above, there are multiple exogenous pathways (mediated by other organisms) by which light availability can influence reproductive output (Figure 4.1). For example, light may influence patterns of herbivory via effects on herbivore behavior (Suárez-Vidal et al., 2017) or on plant chemistry and palatability (Dudt and Shure, 1994; McDonald et al., 1999). Similarly, light availability can influence pollination by affecting either pollinator behavior (Figure 4.1B-C; Herrera 1995; Kilkenny and Galloway 2008) or floral traits that mediate pollinator attractiveness [including flower number (Cunningham, 1997; Kilkenny and Galloway, 2008; Cao et al., 2017) and flower size (Kilkenny and Galloway, 2008)].

Importantly, an individual plant's response to light availability may be mediated or constrained by the light environment in which its parent(s) grew (Galloway and Etterson, 2007, 2009; Heger, 2016). Such conditioning of offspring response to environmental stimuli by parental environment is known as a transgenerational effect. Transgenerational effects – also known as parental effects, and including maternal effects – are common in plants, and can reflect aspects of both the abiotic and biotic environments of parents (Roach and Wulff, 1987; Rossiter, 1996). Transgenerational effects have been primarily studied in sexually-reproducing plants, but there is mounting evidence for their importance in clonal plants as well (Latzel and Klimešová, 2010; Dong et al., 2017; Münzbergová and Hadincová, 2017; Dewan et al., 2018). However, the study of transgenerational effects in plants – whether in clonal or sexually reproducing populations – has focused largely on growth or defense traits, with very little attention paid to transgenerational effects on traits mediating floral attractiveness.

In addition to the effects of light environment on plant traits, light availability may also affect pollination via the influence of light on other plant-animal interactions. One interaction type that may be an important mediator of plant-pollinator interactions is nectar robbery (NR; Figure 4.1D-E). Nectar robbery refers to the extraction of nectar from a flower via an opening other than the corolla mouth (Irwin et al., 2010). The effect of this interaction on the plant can be negative, neutral, or positive (Maloof and Inouye, 2000; Burkle et al., 2007; Irwin et al., 2010). Since nectar robbers are frequently also pollinators of other plant species – and even of other flowers of the same species – they may respond to similar traits as pollinators (Irwin et al., 2010). Indeed, nectar robbers have been shown to prefer plants with more flowers, much like pollinators (Irwin, 2006; Gélvez-Zúñiga et al., 2018). Even if nectar robbers and pollinators use different cues to locate food sources – particularly likely when robbers and pollinators have different sensory biases, e.g. arthropod robbers and vertebrate pollinators (Schiestl and Johnson, 2013; Gegear et al., 2017) – light may simultaneously influence multiple plant traits, thereby affecting pollinator and nectar robber attraction in potentially complex ways. But the extent to which NR is influenced – whether directly or indirectly – by abiotic conditions has been scarcely evaluated. Aiming to fill this knowledge gap, the study reported here combined field surveys and a reciprocal translocation experiment using the polycarpic understory shrub *Odontonema cuspidatum* (Nees) Kuntze (Acanthaceae). In the study area (southeastern Chiapas, Mexico), *O. cuspidatum* experiences high levels of NR from stingless bees (Hymenoptera: Apidae: Meliponini); robbed flowers are significantly less likely than unrobbed flowers to produce fruit (Fitch and Vandermeer, 2021). The study, which took place in a coffee agroecosystem comprised of areas of coffee cultivation with a canopy tree cover of varying density and small forest fragments, addressed the following questions:

1) Does light availability affect flower number, flower morphology, or nectar rewards in *O. cuspidatum*?

2) Does light availability influence the intensity of NR by stingless bees, and, if so, is this due to direct effects of light on bee foraging behavior or mediated by floral traits?

3) What is the relative importance of endogenous effects, pollinator-mediated effects, and nectar robber-mediated effects of light availability on *O. cuspidatum* reproductive output?

4) Does parental light environment mediate the effect of offspring light environment during growth or flowering on pollination and NR (i.e. are there transgenerational effects of parental light environment on the link between light availability and pollination and/or NR)?

We expected that 1) light availability would influence multiple aspects of floral attraction traits, with higher light availability leading to the production of more flowers, larger flowers, and more floral nectar. We further predicted that 2) plants in high-light conditions would experience higher levels of NR, primarily due to the predicted effects of light availability on floral traits. Direct effects of light availability on flower visitor behavior are often due to increased activity levels associated with higher temperatures (Herrera, 1995; Kilkenny and Galloway, 2008). We therefore hypothesized that light availability would have little effect on bee foraging behavior, given that temperature may be relatively unimportant in regulating bee foraging in warm tropical environments (Willmer 1991; though see Figueiredo-Mecca et al. 2013; Aleixo et al. 2017).

Given that NR has a negative impact on *O. cuspidatum* fruit set (Fitch and Vandermeer, 2021), and that pollen supplementation results in dramatic increases in fruit set and seed production (see Chapter 5), indicating that reproduction is pollen-limited, we expected that 3) the exogenous effects of light on *O. cuspidatum* reproductive output, mediated both by pollinators and nectar robbers, would be stronger than the endogenous effects. Finally, 4) we expected that strong direct effects of light during growth environment would overwhelm any effects of parental environment, and thus that transgenerational effects would be absent.

METHODS

Study system

Odontonema cuspidatum (Acanthaceae) is a polycarpic shrub endemic to southern Mesoamerica, where it occurs in the forest understory, particularly in light gaps and along watercourses; it is also commonly planted as an ornamental and for erosion control (Daniel, 1995, G. Fitch personal observation). Broken stems of *O. cuspidatum* readily root to become independent ramets (G. Fitch personal observation).

In the study area, *O. cuspidatum* blooms primarily during the rainy season, from June to August, bearing indeterminate branching racemes of tubular red flowers. Plants are self-fertile but not capable of autogamy (see Results). Flowers are primarily pollinated by hummingbirds (G. Fitch unpublished data), but are also attractive to a wide range of nectar-feeding insects. Many of these insects engage in nectar robbery, extracting nectar from perforations in the base of the corolla tube. Primary nectar robbers – i.e. those that make the perforation themselves – include two species of stingless bee in the genus *Trigona* (*T. fulviventris* and *T. nigerrima,* Hymenoptera: Apidae: Meliponini; Figure B.1). Other Hymenoptera, as well as several species of Lepidoptera, secondarily rob, using previously-made perforations. Flowers are commonly robbed before opening, generally once they are <1.5cm long and less than 2 days before opening; NR prior to opening generally does not impact blooming. Nectar robbery leads to a ~40% reduction in probability of setting fruit (Fitch and Vandermeer, 2021).

Fertilized flowers produce explosively dehiscent capsules containing up to four seeds. In the population under study, fruit set is quite low: on average <20% of flowers produce fruit (Fitch and Vandermeer, 2021).

Research was conducted at Finca Irlanda (15.17358 -92.33827), a shaded organic coffee farm in southeastern Chiapas, Mexico. The farm, approximately 300ha in extent and 900-1150masl, consists of coffee plantation with a diverse tree canopy, along with several forest fragments. On the farm, *O. cuspidatum* occurs both within areas of coffee cultivation and in forest fragments.

Odontonema cuspidatum ***mating system***

To determine whether *O. cuspidatum* was self-compatible and capable of autogamy, we selected 3 naturally-occurring plants in 2017 and another 3 plants in 2018 that were not otherwise part of the study. On each of these plants, we randomly assigned one inflorescence to each of the following treatments before flowering had begun: no pollination, ambient pollination, hand self-pollination, and hand cross-pollination. Inflorescences in the ambient pollination treatment were left open, while all others were covered with a pollinator exclosure bag made of 0.5cm mesh fabric. Inflorescences were monitored daily for open flowers. For the hand self- and cross-pollination treatments, *O. cuspidatum* pollen was applied to the stigma of all open flowers using a wire filament loop. For the self-pollination treatment, pollen was removed from the anthers of 4 flowers from an inflorescence that belonged to the same plant but was not part of the study. For the cross-pollination treatment, pollen was removed from the anthers of 4 flowers from 2 different *O. cuspidatum* plants that were not part of the study. Pollen from all contributing flowers was mixed before applying to treated flowers. Treated flowers were marked with nail polish on the pedicel. Flowers in the no pollination and ambient pollination treatment were manipulated and marked to control for handling effects. When fruits were mature, we recorded the number of fruits and of manipulated flowers, and collected all fruits. We placed fruits, segregated by inflorescence, in a drying oven until all had dehisced (approximately 24h), and then counted seeds, providing a measure of seeds per inflorescence.

Differences in fruit set and seeds per inflorescence across pollination treatments (no pollination, ambient pollination, hand pollination with self pollen, hand pollination with cross pollen) were evaluated using paired t-tests, with Bonferroni correction for multiple comparisons.

Field surveys

We haphazardly selected 109 *O. cuspidatum* individuals within the study area for inclusion in field surveys (hereafter, we refer to these plants as “naturally-occurring”). Each selected plant was individually marked with flagging tape at its base, and was monitored during the flowering period in 2017 and 2018. Among the 109 surveyed plants, 33 individuals surveyed in 2017 either died or did not flower in 2018; in 2018 we included an additional 15 plants that did not flower in 2017. We recorded the GPS coordinates of each plant, and determined the degree of canopy cover directly above the crown of the plant – our measure of light availability – using CanopyApp 1.0.3 (University of New Hampshire, Durham, New Hampshire, USA). Canopy cover ranged from 22%-98%. Distances between surveyed plants ranged from 10-2200m. The hummingbirds that serve as primary pollinators of *O. cuspidatum* have foraging ranges that span this distance, and move readily between forest fragments and areas of coffee production (Barney et al. unpublished manuscript), so all surveyed individuals represent a single population.

In 2017-2018, plants were surveyed for NR weekly during flowering. NR leaves visible perforations at the base of the corolla tube. At each survey, all flowers ≥1.5cm in length on inflorescences that contained at least one open flower were checked for evidence of robbery, and we recorded the number of robbed and unrobbed flowers per inflorescence. We tallied robbery for open and unopened flowers separately.

Beginning approximately two weeks after flowering ended on the earliest-flowering inflorescence, and continuing weekly until all inflorescences had matured, we assessed fruit set by counting the number of fruit and number of persistent ovaries (i.e. flowers that had not set fruit) on mature inflorescences. Inflorescences that had been damaged by insect herbivores [primarily *Chlysone* sp. (Nymphalidae: Lepidoptera) larvae; representing <5% of inflorescences] were not included in measures of fruit set, though we included counts from these inflorescences in plant-level flower number. To measure seed set, up to 5 fruits (in 2017) or all undamaged fruits (in 2018) were collected from each inflorescence. Collected fruits were placed in a drying oven until all had dehisced (approximately 24h), and then seeds were counted.

Floral traits

On a subset of 18 of the plants that were surveyed for nectar robbery, chosen to represent the overall gradient of canopy cover, we measured the following aspects of floral morphology: corolla tube length, corolla flare, corolla mouth width, and corolla base width. These traits were chosen because they are readily measurable in the field, and have been shown to influence flower visitor attraction in other species (e.g. Galen 1999; Rojas-Nossa et al. 2016; Gélvez-Zúñiga et al. 2018). On each plant, 5 open flowers were randomly selected for measurement. Measurements occurred between 21-29 June 2018, and were made using digital calipers (Thomas Scientific, Swedesboro, New Jersey, USA).

On another subset of monitored plants (49 in 2017, 19 in 2018 with 7 included in both years; see Figure B.2 for details of sampling scheme) – again chosen to represent the range of canopy cover seen across monitored plants – we assessed nectar volume and sugar content. Nectar sampling in 2018 was primarily to fill gaps in the range of canopy cover experienced by plants sampled in 2017, with repeat sampling of a subset of individuals to determine the degree of interannual variability in nectar traits within individuals, which was found to be low and showed no consistent temporal trend (G. Fitch unpublished data). Unbagged flowers consistently had no standing nectar crop, so we measured nectar production on flowers from which pollinators were excluded with mesh bags. We bagged two inflorescences/plant and checked bagged inflorescences for open flowers 2x/week. Nectar volume was measured by removing the nectar from a flower with a 75μL microcapillary tube (Drummond Scientific, Broomall, Pennsylvania, USA), and measuring the height of the nectar in the tube using digital calipers. To measure nectar sugar content, we used a pocket refractometer (Eclipse 45-81, Bellingham & Stanley, Tunbridge Wells, UK). Only plants for which we had measures of both nectar volume and nectar sugar content for ≥4 flowers were included in data analysis.

We used correlation between floral traits and light availability to assess the endogenous response of *O. cuspidatum* floral traits to light (Figure 4.1B). We did not investigate the physiology underlying these correlations, and only infer that these correlations are due to light impacts on photosynthate availability.

Reciprocal translocation experiment

See Figure 4.2 for a schematic diagram of the reciprocal transplant experiment design. In August 2017, we cut 12 stems (hereafter ramets) each from 12 plants, 6 growing in high light [canopy cover < 50%; high-light parental environment (PE)] and 6 in low light (canopy cover > 80%; low-light PE). Cut ramets were potted in 500cm^3 nursery sleeves filled with potting soil from the Finca Irlanda nursery. Half the ramets from each plant were placed in the Finca Irlanda nursery, where light availability was high [23% canopy cover; high-light growth environment (GE)]; the other half were placed together in a nearby forest fragment with dense canopy (95% canopy cover) and low light availability (low-light GE). Canopy cover at these sites fell within the range of canopy cover experienced by naturally-occurring plants. Ramets were left to grow for 10 months, until the onset of flowering. During the dry season, all ramets were given a soaking watering 1x/week, but were otherwise untended.

In June 2018, 38 of the potted ramets flowered and were placed in the field in arrays of 2 or 3 ramets prior to the onset of flowering (see Figure 4.2 for number of ramets in each PE-GE-FE combination). Arrays were located >10m and <100m from existing *O. cuspidatum* plants in bloom, and >10m from other arrays. Eighteen ramets were placed in low-light conditions [canopy cover >85%; low-light flowering environment (FE)] in a forest fragment to bloom, and 20 were placed in high-light conditions (canopy cover <35%; high-light FE) in an area of coffee cultivation. These ramets were monitored for NR, and assessed for fruit and seed set, as outlined above for naturally-occurring plants, with the difference that monitoring of potted ramets for NR occurred every other day rather than weekly. Several ramets were heavily damaged by *Chlosyne* sp. larvae during the course of the experiment and were excluded from analyses of season-long flower production and reproductive output. Five ramets from four different treatments were heavily damaged by *Chlosyne* sp. larvae during the course of the experiment and were excluded from analyses of season-long flower production and reproductive output. In all cases, damage occurred only after flowering was underway, so we included data on NR and per-observation flower number for all plants. We could identify no ecological correlate with *Chlosyne* sp. herbivory. Because of the small number of ramets that flowered in 2018, we were not able to assess floral traits, other than flower number, on ramets in the reciprocal translocation experiment. While 38 ramets spread over six treatments results in a small number of individuals per treatment, the fully-factorial design maximized statistical power by enabling us to group individuals across multiple treatments when considering the effect of any one environment.

Data analysis

All analyses were conducted in R v.3.6.1 (R Core Team, 2018). All models were checked for conformity to assumptions: linear models were checked for normality and heteroskedasticity; Poisson GLMs and GLMMs were checked for overdispersion.

To assess the effect of light availability on floral traits, we modeled each trait as a function of canopy cover using mixed-effects models, with plant as a random effect. Continuous traits were modeled using linear mixed models (LMMs), while discrete traits (i.e. flower number) were modeled with generalized linear mixed models (GLMMs) with Poisson error distribution, as implemented in the package `lme4` (Bates et al. 2015). To check for correlation among the measured floral traits, we determined Pearson's correlation coefficient for each trait pair; traits were largely uncorrelated (the highest correlation, between basal width and corolla mouth width, was 0.41; Table B.1).

To test for effects of light availability, flower number, and floral traits on nectar robbing intensity (NRI), we used the number of robbed flowers as the response variable, offset by log(total number of flowers assessed for NR) in order to effectively model the proportion of flowers robbed. To assess the effects of per-observation flower number, we used a Poisson GLMM with plant identity as a random effect; the response variable was per-observation measures of robbed and total flowers. For all other models we used season-long mean NRI as the response variable in negative-binomial GLMs. For season-long total flower number, we included year as an additional predictor to account for the fact that data came from two years. For flower morphology and nectar traits, we used plant-level mean trait values as the predictor variables.

We used three metrics of reproductive output to test for effects of light availability and NRI on reproductive output in naturally-occurring plants: fruit set, seed set, and seeds per plant. To model fruit set, we used a negative-binomial GLM with number of fruit as the response variable, offset by log(total number of flowers produced), which was determined as described under “Field surveys”, above. Canopy cover, season-long proportion of flowers robbed, and year were the predictors. Models for seed set and seeds per plant were similar to those for fruit set, except year was not included as a predictor, since we had data from only 2018. For both models, the predictor variable was the number of seeds collected; the number of fruits collected was additionally included as an offset in the model of seed set.

For the reciprocal translocation experiment, we evaluated the effect of parental environment (PE), growth environment (GE), and flowering environment (FE) on both flower number and NR. We evaluated the effect of each environment on two aspects of flower number: 1) the number of open flowers at each observation and 2) the season-long total number of flowers produced. In both cases, we used a GLMM with the three environments as fixed effects and ramet nested within replicate as a random effect; for the model assessing the effect of environment on number of flowers open at a time, date of observation was included as an additional random effect.

To assess the effect of PE, GE, and FE on NR of ramets in the reciprocal translocation experiment, we used per-observation measures of NRI, rather than a season-long measure. Because ramets in the reciprocal translocation experiment were monitored more frequently – such that we observed most of the flowers each ramet produced while they were open – our response variable was number of open robbed flowers, rather than all (open and unopened) robbed flowers. Our model for NRI of these ramets included log(total number of open flowers) as an offset and date and ramet nested within replicate as random effects. We began with a maximal model that included PE, GE, and FE, and all two- and three-way interactions between environments, as well as total flower number (including open flowers and closed flowers ≥1.5cm long). We then conducted stepwise simplification of the model, eliminating interaction terms in order of p-value and comparing model fits using AICc. AICc values for all models differed by >2, so we used the best model for inference.

RESULTS

Odontonema cuspidatum *mating system*

We conducted pollen manipulation on a total of 1713 flowers across the four treatments (ambient pollination, no pollination, supplemental self pollination, supplemental cross pollination). Pollination manipulations revealed that *O. cuspidatum* is self-fertile but incapable of autogamy. Flowers receiving no pollination never set fruit. Fruit set for inflorescences hand-pollinated with self pollen (mean ± SD = 0.54 ± 0.11) was not significantly different from that of inflorescences receiving cross pollination (0.57 ± 0.08; t = 0.45, df = 5, p = 0.7). While our sample size was small, power analysis using the effect size calculated from our data (d = 0.13) indicated that, to detect a difference between self- and cross-pollination at $p < 0.05$ and a power of 0.6, supplemental pollination treatments would need to be conducted on 226 plants. Seeds per inflorescence showed a greater difference between self- and cross-pollinated inflorescences (self: 162±49 seeds; cross: 206±53 seeds). Though this difference was again not significant (t = 0.88, df = 3, p = 0.4); the calculated effect size was higher (d = 0.43) and power analysis indicated that

23 plants would be needed to detect a significant difference in seeds per inflorescence between treatments. Inflorescences experiencing ambient pollination had significantly lower fruit set (0.12±0.02; t = 3.7, df = 5, p = 0.01) and fewer seeds per inflorescence (41 ± 21; t = 3.7, df = 3, p = 0.03) than inflorescences receiving supplemental pollen (self or cross; statistics reported for more conservative comparison with self pollen treatment).

Field surveys

Effects of light availability on floral traits

Of the floral morphology traits measured (corolla length, corolla flare, corolla mouth width, corolla base width), only corolla flare was affected by light availability, with plants growing in low light having significantly wider petals than those growing in high light (Table 4.1). Flower number was also significantly impacted by light availability: plants growing in low light produced fewer flowers overall – and fewer flowers at a time – than plants receiving more sunlight (Table 4.1). Per-flower nectar volume was also significantly lower in low-light plants, but nectar sweetness was not affected by light availability (Table 4.1).

Effects of light availability and floral traits on nectar robbery

Nectar robbing intensity (NRI) was not significantly related to light availability in naturally-occurring plants (GLM: ß = 0.05±0.04, z = 1.31, df = 166, p = 0.19). Of the measured floral traits, only flower number – measured as season-long total or as number of open flowers per observation – had an effect on NRI (Table 4.2). In both cases, flower number correlated positively with NRI.

Effects of light availability and nectar robbery on reproductive output

In naturally-occurring plants, neither fruit set nor seeds produced per plant were correlated with either light availability or NRI (Table 4.3). Seed set was not correlated with NRI, but was marginally negatively correlated with light availability (Table 4.3).

Reciprocal translocation experiment

Effect of light availability on floral traits

Of the three light environments considered [parental (PE), growth (GE), and flowering FE)], only GE had an effect on flower number (Table 4.4). Ramets in the low-light GE produced fewer total flowers and also had significantly fewer flowers ≥1.5cm on a per-observation basis, though the number of open flowers per observation was not affected by GE. The magnitude of the effect of shading on flower number is comparable to that seen in naturally-occurring plants (Fitch and Vandermeer, 2021).

Effects of light availability and floral traits on nectar robbing intensity

Flowering environment was the most important predictor of NRI, with ramets in the high-light FE experiencing higher NRI (Table 4.5). In addition to FE, the best model for NR of experimental ramets included flower number, PE, GE, and a PE × FE interaction (Table 4.5). Removing any single predictor resulted in a model with significantly poorer fit (ΔAICc > 2 in all cases), though there was no significant main effect of PE.

As in naturally-occurring plants, nectar-robbing intensity was positively correlated with total flower number (Table 4.5). Ramets grown in low light experienced higher NRI regardless of where they flowered, though this effect was relatively small and disappeared when flower number was removed from the model. Finally, ramets from a low-light PE and low-light FE experienced significantly more NR than ramets from a low-light PE and high-light FE, generating the significant PE × FE interaction included in the best model of NRI (Figure 4.3). There was no parallel relationship for experimental ramets from the high-light PE.

Reproductive output

Fruit set was significantly correlated with both GE and FE, though in contrasting manners. Fruit set was nearly three times higher in ramets from the high-light GE compared to the low-light GE (0.21 versus 0.07 fruits per flower; Table 4.4); the effect of FE was modest by comparison, but ramets in the high-light FE had a significantly lower fruit set than those in the low-light FE (0.16 versus 0.18 fruits per flower; Table 4.4). Parental environment had no effect on fruit set. Because of high levels of pre-dispersal seed predation on experimental ramets, we were unable to measure seed production on a sufficient number of ramets to draw conclusions about the effect of light availability on seed production.

DISCUSSION

While light availability is generally thought to positively influence plant reproduction by increasing the availability of resources to allocate to reproduction, the presence of multiple exogenous pathways linking light to plant reproduction (Figure 4.1) have the potential to complicate this direct link. In this study, in addition to increasing plant resources, light availability also influenced both pollination and nectar robbery (NR), via both direct and indirect pathways.

Light availability influenced multiple floral traits associated with pollinator attraction, in somewhat contrasting ways. Greater light availability was associated with higher flower number and increased nectar volume, but smaller corolla flare. Increases in flower number and nectar volume in plants receiving more light are consistent with the idea that light availability influences photosynthate production and therefore resources allocation to pollinator attraction and reproduction. It may be that plants growing in low light conditions compensate for reduced flower number and reward volume by increasing corolla flare to increase attractiveness to pollinators. However, while larger corolla flare increases pollinator attraction in many species (e.g. Conner and Rush 1996; Galen 1999; Mothershead and Marquis 2000), we do not know whether this is true for *O. cuspidatum*.

Light availability likewise influenced nectar robbing intensity (NRI). We hypothesized that this link would primarily be mediated by light availability's effects on floral traits. However, in the reciprocal translocation experiment, growth environment (GE) had only a small effect on NRI. Moreover, NRI was higher in plants from the low-light GE, contrary to our expectation. Thus, while flower number – which is influenced by light availability – has a modest effect on NRI, variation in NRI cannot be explained primarily by variation in floral traits. Instead, flowering environment (FE) was the most important predictor of NRI, with ramets flowering in high light experiencing more NR. Analogous patterns in insect pollinator visitation have, elsewhere, been invoked as evidence that higher light availability directly affects insect activity by increasing local temperature (Herrera, 1995; Kilkenny and Galloway, 2008). However, these studies were conducted in temperate regions, while the current study occurred in a warm tropical climate where low temperature is less likely to limit flower visitor behavior (Willmer, 1991). Another possibility is that low-light conditions affect foraging behavior not via temperature but by reducing visual acuity and sensitivity of foraging bees (Streinzer et al., 2016). In that case, plants growing in low-light conditions would experience reduced NR both because foraging efficiency would be lower than in high-light conditions and because the diurnal time frame in which foraging could occur would be narrowed.

Alternatively, the link between FE light conditions and NRI may be mediated by the community composition of co-flowering plants, which we have shown elsewhere to be an important driver of NRI (Fitch and Vandermeer, 2021). In this scenario, the low densities of co-flowering plants in low-light environments reduces local density of foraging nectar robbers, and therefore NR. Because our study design utilized pre-existing light availability regimes, with their concomitant floral communities, we are unable to disentangle the relative impact of light availability versus (light availability-influenced) co-flowering community composition on NRI. Nor did we directly measure pollinator or nectar robber visitation rates to either experimental or naturally-occurring plants. A more general concern with the reciprocal translocation experiment is that the relatively small number of experimental plants that flowered in 2018 limited our sample size. Thus, while the results from the reciprocal translocation study in many respects are consistent with findings from naturally-occurring plants, they should nevertheless be interpreted with caution, particularly where they conflict with results from surveys.

One unexpected finding from the reciprocal translocation study was that, once we accounted for the effect of flower number, NRI was actually higher for ramets from the low-light GE, regardless of FE. It may be that high light availability reduces the attractiveness of other traits, unmeasured in this study, that mediate nectar robber attraction (e.g. floral volatiles). In such a scenario, the positive effects of light availability on flower number and directly on nectar-robbing behavior would generally cancel out this hypothesized reduction in attractiveness. This is consistent with our findings from both naturally-occurring and experimental plants that GE light availability *per se* did not influence NRI.

Data from the reciprocal translocation experiment and field surveys support conflicting inferences regarding the importance of light availability on *O. cuspidatum* reproductive output. In the reciprocal translocation experiment, GE was the most important determinant of fruit set, with higher fruit set in ramets grown in high-light conditions. This suggests that reproductive output is limited primarily by photosynthate availability, i.e. that endogenous pathways linking light availability and reproductive output (Figure 4.1F) are more important than exogenous pathways (Figure 4.1B-E). Since *O. cuspidatum* primarily occurs in high-light microhabitats (e.g. light gaps, streambeds), a strong direct response to light availability is perhaps to be expected. But data from field surveys indicate that neither NRI nor light availability significantly influence reproductive output. In naturally-occurring plants, the effect of light availability on reproduction (via either endogenous or exogenous pathways) may be obscured by other factors (e.g. soil properties, biotic interactions, plant age or size) that were controlled in the reciprocal transplant experiment.

The modest negative relationship between FE light availability and fruit set in the reciprocal translocation experiment suggests that the pollinator-only-mediated pathways linking light availability and reproductive output (Figure 4.1B-C) are relatively unimportant in determining *O. cuspidatum* reproduction. In this population of *O. cuspidatum,* robbed flowers receive less pollination than unrobbed flowers, and as a result are significantly less likely to set fruit (Fitch and Vandermeer, 2021). Since NRI was positively correlated with light availability in the FE, pollinators avoiding robbed flowers will, all else being equal, pollinate more flowers on shaded plants. Interestingly, this suggests that light availability (and/or its impacts on the co-flowering community; see above) influences nectar robber preference for *O. cuspidatum* more than pollinator preference. This could also explain the lack of correlation between light availability and fruit set in naturally-occurring plants, as the contrasting impacts of, on the one hand, reductions in photosynthate availability and, on the other, increases in pollination as light availability decreases would negate one another.

Our finding of a significant effect of parental environment (PE) × FE interaction on NRI in experimental ramets suggests that clonal transgenerational effects (Latzel and Klimešová, 2010) influence plants' attractiveness to nectar robbers. Ramets sourced from parents growing in low-light conditions experienced significantly higher NRI when they flowered in low- versus high-light conditions, regardless of ramet GE. This suggests local adaptation of traits mediating nectar robber attraction to low light availability, conditioned by PE. Even when we controlled for the effects of NRI on fruit set – thereby recovering the effect of FE light availability on pollination – we found no evidence for a PE × FE interactive effect on fruit set. This is further evidence that nectar robbers are, surprisingly, more sensitive to *O. cuspidatum* floral traits than pollinators – at least to those traits that are affected by PE. Moreover, given the negative effect of NR on reproductive success in *O. cuspidatum*, this suggests that clonal transgenerational plasticity – at least in relation to pollination – is not adaptive in this population. Further work is needed to elucidate the specific traits influencing NRI that exhibit transgenerational effects. In addition, while we suspect that light influences floral traits primarily by increasing photosynthate availability, in the absence of physiological studies we cannot be certain of the causal pathway linking light availability and floral traits.

This study highlights how complex, interacting effects of light on interactions between plants and mutualist and antagonist partners can complicate the simple assumption that increases in light availability should increase plant reproductive success. Indeed, despite strong positive effects of light availability on plant growth and ovule production in *O. cuspidatum*, we found no effect of light availability on seed production. This was apparently due to strong effects of light availability on the nectar-robbing behavior of stingless bees, which in turn influenced pollination and seed production.

The effects of light availability on floral antagonists has received little attention to date; this study suggests that this oversight has limited our understanding of the often-complex relationship between light and plant reproduction. While we suspect that light availability commonly influences plant-floral antagonist interactions, further work in other plant-pollinator-floral antagonist systems is needed to evaluate the generalizability of our findings. In particular, future research that more precisely identifies the causal mechanism(s) by which light influences floral antagonists – focusing on a taxonomically diverse set of antagonists – will greatly advance our ability to predict the net effects of light on plant reproduction in such complex systems.

ACKNOWLEDGEMENTS

This research was made possible by the support of Walter Peters and Bernd Peters, owners of Finca Irlanda, where the study was conducted, as well as the work of the farm's residents. Particular thanks are due to Miguel Crisóstomo and his team in the farm's nursery. Gustavo Lôpez Bautista and Gabriel Dominguez Martinez provided invaluable support in the field. This manuscript benefited from thoughtful feedback given by members of the Perfecto-Vandermeer lab group (especially Ivette Perfecto, Lauren Schmitt, and Chatura Vaidya), Mark D. Hunter, Meghan A. Duffy, Nina Sletvold, and one anonymous reviewer. Funding was provided by the Rackham Graduate School and Department of Ecology and Evolutionary Biology, University of Michigan.

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TABLES AND FIGURES

Table 4.1. Effect of light availability on floral traits of naturally-occurring plants. Estimates derived from a GLMM with Poisson error distribution (for the 3 measures of flower number) or LMM (for all other traits). Light availability (measured as the inverse of canopy cover) and all traits except flower number were scaled to the mean to allow for comparison of effect sizes. Boldface indicates a significant effect (p<0.05) of light availability on that trait.

Trait	ß±SE	t or z	DF	p
Total flower number	**0.30±0.08**	**3.51**	**124**	**<0.001**
Open flowers per observation	**0.31±0.05**	**6.03**	**643**	**<0.001**
Flowers ≥1.5cm long per observation	**0.31±0.05**	**6.18**	**643**	**<0.001**
Corolla flare	**–0.27±0.12**	**–2.35**	**82**	**0.03**
Corolla mouth width	0.16±0.16	1.02	82	0.3
Corolla base width	0.07±0.14	0.50	82	0.6
Corolla length	–0.07±0.12	–0.60	82	0.5
Nectar volume	**0.18±0.08**	**2.16**	**475**	**0.03**
Nectar sweetness	0.04±0.13	0.29	336	0.8

Table 4.2. Effect of floral traits on nectar robbery in naturally-occurring plants. Model output from GLMMs with Poisson error distribution and plant as a random effect. In all models, response variable was season-long total number of robbed flowers, with log(total number of flowers) included as an offset in order to assess effect of floral traits on proportion of flowers experiencing nectar robbery.

Trait	ß±SE	z	DF	p
Total flower number	**0.14±0.02**	**5.48**	**125**	**<0.001**
Open flowers per observation	**0.08±0.03**	**2.47**	**644**	**0.01**
Flowers ≥1.5cm long per observation	0.02±0.03	0.47	645	0.6
Corolla flare	0.005±0.07	0.07	29	0.9
Corolla mouth width	0.10±0.07	1.45	29	0.2
Corolla base width	0.08±0.07	1.14	29	0.3
Corolla length	0.03±0.06	0.57	29	0.6
Nectar volume	0.03±0.05	0.49	65	0.6
Nectar sweetness	–0.06±0.06	–0.10	63	0.3

Table 4.3. Effects of nectar robbery and light availability on reproductive output in naturally-occurring plants, estimated using negative-binomial GLMs. Estimates for fruit set use two years of data; estimates for seed set and seeds per plant use a single year of data. Boldface indicates a significant effect (p < 0.05).

Measure of reproductive output	Nectar robbery			Light availability			Year (2018)			DF
	ß±SE	z	p	ß±SE	z	p	ß±SE	z	p	
Fruit set – all plants	–0.07 ±0.19	–0.37	0.7	0.002 ±0.05	0.03	0.9	**–0.34 ±0.11**	**–3.06**	**0.002**	124
Seed set – all plants	–0.06 ±0.10	–0.54	0.6	–0.19 ±0.10	–1.91	0.06	—	—	—	55
Seeds per plant – all plants	0.23 ±0.15	1.54	0.1	0.14 ±0.15	0.93	0.4	—	—	—	55

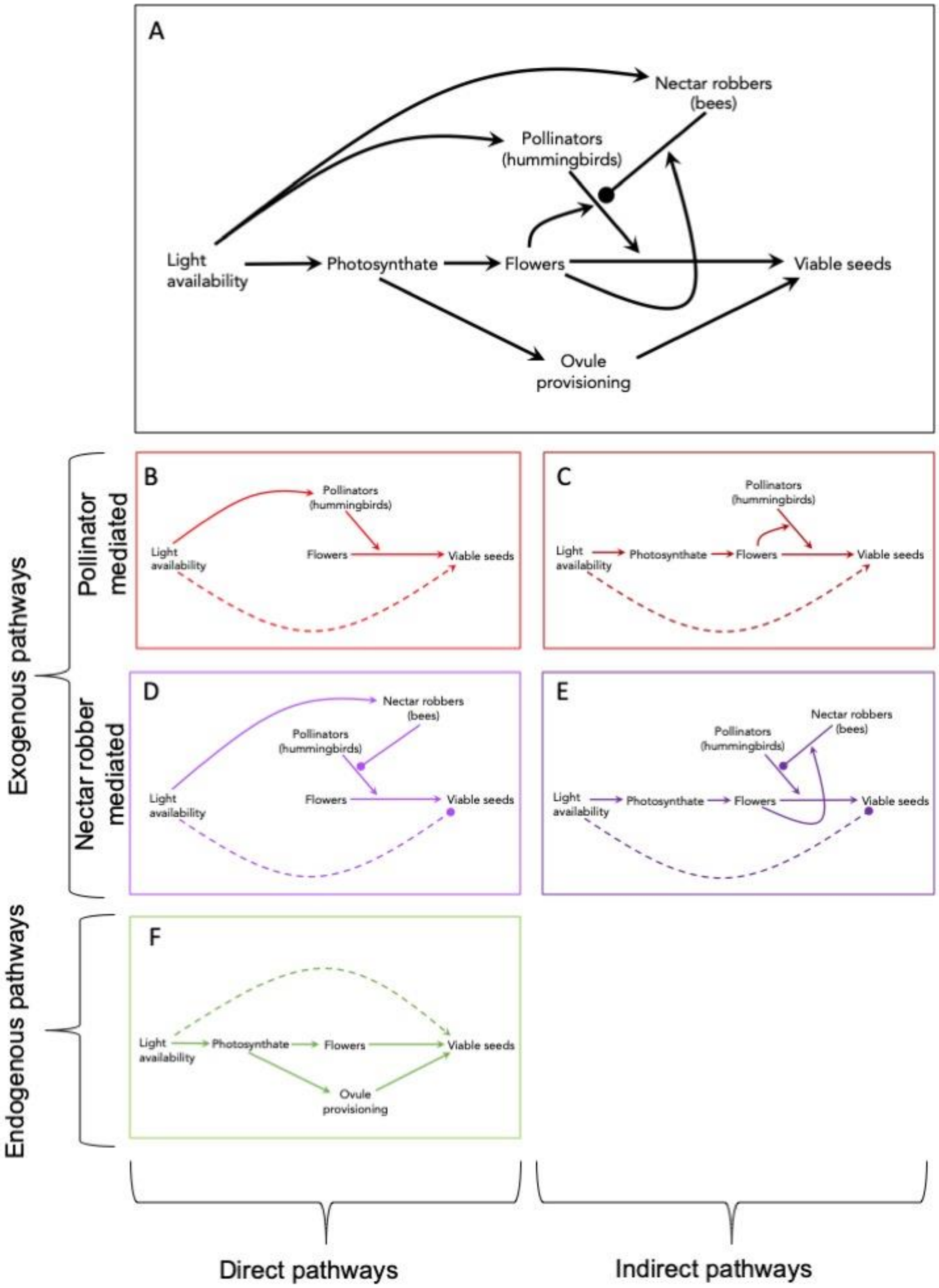
Table 4.4. Effect of light availability on floral traits and fruit set of experimental ramets. In all cases, ß estimates represent the effect of the high-light environment in comparison to the low-light environment. Boldface indicates a significant effect (p < 0.05).

Response variable	Parental environment			Growth environment			Flowering environment			DF
	ß±SE	z	p	ß±SE	z	p	ß±SE	z	p	
Total flowers	0.42 ±0.30	1.41	0.2	**1.01 ±0.27**	**3.71**	**<0.001**	0.26 ±0.31	0.86	0.4	32
Flowers ≥1.5cm, per observation	0.16 ±0.15	1.06	0.3	**0.34 ±0.16**	**2.18**	**0.03**	0.23 ±0.16	1.48	0.1	198
Open flowers, per observation	0.15 ±0.16	0.97	0.3	0.26 ±0.18	1.46	0.1	0.14 ±0.17	0.79	0.4	198
Fruit set	0.01 ±0.18	0.06	0.9	**1.44 ±0.35**	**4.33**	**<0.001**	**0.36 ±0.18**	**2.0**	**0.046**	31

Table 4.5. Best model for predicting robbery of ramets from reciprocal translocation experiment. For the different environments, ß-estimates indicate the effect of the low-light environment. Boldface indicates a significant effect (p < 0.05).

Predictor	ß±SE	z	p	ΔAICc for omitting variable
Total flower number	**0.04±0.02**	**2.43**	**0.02**	**3.7**
Parental environment	0.19±0.23	0.84	0.40	14.4
Growth environment	**0.67±0.29**	**2.35**	**0.02**	**2.9**
Flowering environment	**–3.02±0.58**	**–5.24**	**<0.001**	**21.3**
Parental environment × flowering environment	**2.23±0.62**	**3.61**	**<0.001**	**9.8**

Figure 4.1. Conceptual diagram illustrating the multiple pathways by which light availability can influence seed production, pollination, and nectar robbery in *Odontonema cuspidatum*. Arrows indicate positive effects, while filled circles indicate negative effects. In direct pathways, light availability modifies an interaction partner, while in indirect pathways, light availability modifies an interaction link. Dotted links indicate the predicted net effect of light availability on seed production for the illustrated pathway. Note that multiple pathways may operate in tandem. A. Complete path diagram. B-E. Exogenous pathways (i.e. pathways that involve nectar robbers and/or pollinators). B. Direct pollinator pathway: light directly affects pollinator behavior; pollinator behavior affects seed production. C. Indirect pollinator pathway: light affects pollinator behavior via effect on floral traits; pollinator behavior affects seed production. D. Direct nectar robber pathway: light directly affects nectar robber behavior; nectar robbery decreases pollination. E. Indirect nectar robber pathway: light affects nectar robber behavior via effect on floral traits; nectar robbery decreases pollination. F. Endogenous pathways (i.e. pathways that only involve light effects on plant). In C and E, the flow diagrams imply that light affects floral traits via changes to photosynthate availability, but in this study we did not investigate the physiological mechanisms underlying correlation between light availability and



floral traits. Note that some possible pathways (e.g. nectar robbery directly affects seed production) are omitted because they were eliminated as potential causal pathways in this study system (see text).

Figure 4.2. Schematic diagram of the experimental design for the reciprocal translocation experiment. Arrows indicate translocation; N denotes the number of plants included in the treatment.

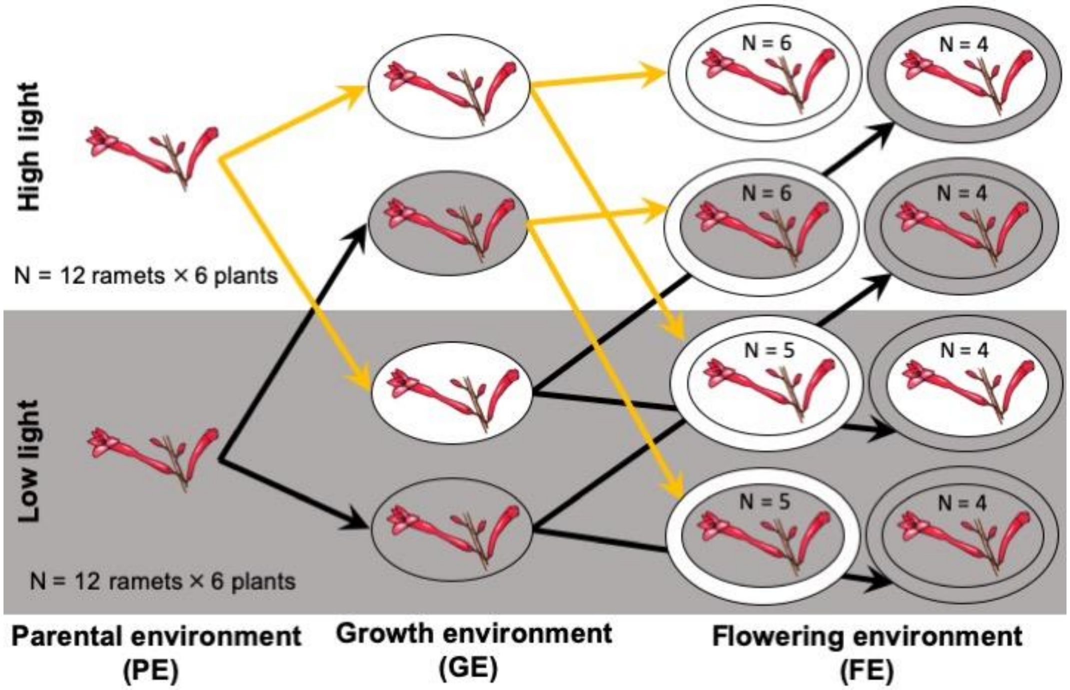
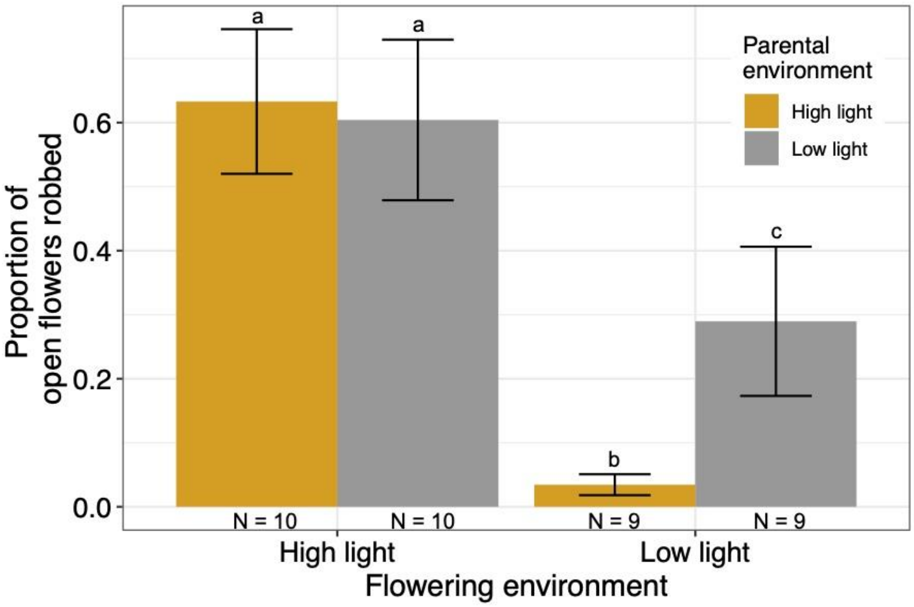


Figure 4.3. Effect of light availability at flowering time on nectar robbery, as mediated by light environment experienced by the parent plant. Error bars represent standard error; letters indicate significantly different levels of nectar robbery; N denotes the number of plants included in the treatment.


Proportion of open flowers robbed
0.0
0.2
0.4
0.6
a
a
b
c
N = 10
N = 10
N = 9
N = 9
High light
Low light
Flowering environment
Parental environment
High light
Low light

Chapter 5
Can Conflicting Selection from Pollinators and Antagonists Drive Adaptive Pollen Limitation? A Conceptual Model and Empirical Test[2]

ABSTRACT

Pollen limitation is widespread, despite predictions that it shouldn't be. We propose a novel mechanism generating pollen limitation: conflicting selection by pollinators and antagonists on pollinator attraction traits. We introduce a heuristic model demonstrating antagonist-induced adaptive pollen limitation, and present a field study illustrating its occurrence in a wild population.

For antagonist-induced adaptive pollen limitation to occur, four criteria must be met: 1) correlated attraction of pollinators and antagonists, 2) greater response by antagonists than pollinators to altered investment in attraction traits, 3) reduced investment in pollinator attraction leading to pollen limitation, but 4) maximizing fitness.

We surveyed nectar robbery and reproductive output for 109 *Odontonema cuspidatum* (Acanthaceae) plants in a pollen-limited population over two years and used experimental floral arrays to evaluate how flower number affects pollination and nectar robbery. Both pollinators and nectar robbers preferred larger floral displays, and nectar robbery reduced reproductive output, suggesting conflicting selection. Survey and experimental data agreed closely on the optimum flower number under antagonist-induced pollen limitation; this number was

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[2] Co-author is Vandermeer, J.

substantially overrepresented in the population. While criteria for antagonist-induced adaptive pollen limitation are restrictive, the necessary conditions may commonly obtain. Considering interactions beyond the plant-pollinator dyad illuminates previously overlooked mechanisms generating pollen limitation.

INTRODUCTION

Antagonists are commonly attracted by the same traits that plants use to attract pollinators (Adler and Bronstein 2004; Theis and Adler 2012; Ågren et al. 2013; Sletvold et al. 2015; Sun et al. 2016; Knauer and Schiestl 2017). Where both antagonists and pollinators favor particular trait values, this can lead to conflicting selection on these attraction traits (Sletvold et al. 2015; Sun et al. 2016; Ramos and Schiestl 2019). Depending on the relative strength of selection exerted by the antagonist and mutualist (Sletvold et al. 2015), as well as the degree of spatial and temporal variability in these pressures (Siepielski and Benkman 2010; Siepielski et al. 2013; Ågren et al. 2013), such conflicting selection can favor intermediate phenotypes, maintain within-population phenotypic diversity (Siepielski and Benkman 2010; Shumate et al. 2011), or lead to a spatial mosaic of populations with distinct dominant phenotypes (Ågren et al. 2013). Despite known examples of conflicting selection via correlated attraction of pollinators and antagonists affecting floral trait evolution, the potential role of such selective pressures in generating pollen limitation of plant reproduction (i.e., when seed production is limited by a lack of pollen receipt, rather than a lack of resources) has received little attention. Here, we outline a mechanism by which conflicting selection by pollinators and antagonists may give rise to adaptive pollen limitation (PL). We first present a conceptual model explaining the mechanism, then present data from field surveys and experimental manipulation that are consistent with the model.

Pollen limitation (PL) is ubiquitous in animal-pollinated plants (Burd 1994; Ashman et al. 2004; Knight et al. 2006). Haig and Westoby (1988) proposed a foundational framework for understanding PL. Their framework assumes that there is a trade-off between investment of resources in pollinator attraction and investment in ovule provisioning, and that the number of ovules fertilized is an increasing function of investment in pollinator attraction (in Figure 5.1, P represents effective pollination and R represents resources available for ovule provisioning, as a function of investment in pollinator attraction). Given these assumptions, optimum investment in pollinator attraction should occur at the intersection of the functions relating investment in pollinator attraction to 1) number or proportion of ovules fertilized and 2) number or proportion of ovules that could be provisioned (I_N* in Figure 5.1). At this intersection, plant reproductive output is simultaneously limited by resource availability and pollen receipt. This qualitative result holds for a wide range of functions specifying the pollination and resource curves, but the pollination function is generally modeled as an asymptotic function of allocation to pollinator attraction, while the resource function is modeled as a linear function (Haig and Westoby 1988; Ashman et al. 2004; Burd 2008). Within the Haig-Westoby framework, pollen limitation occurs when investment in pollinator attraction is less than the optimum (i.e. to the left of I_N*, Figure 5.1), leaving potentially provisionable ovules unfertilized.

Haig and Westoby's framework (1988) predicts that PL should be rare, given that it represents a suboptimal investment in pollinator attraction. Yet PL is common. Most explanations invoke variability in pollen receipt, which subsumes two dominant theories. The first suggests that pollen limitation arises from the failure of a plant population to respond to an altered pollination regime that results in decreased fertilization (Thomson 2001; Ashman et al. 2004; Vamosi et al. 2006). In this scenario, ecological change results in decreased pollen receipt compared to conditions in which the plant evolved. The plant, adapted to a prior equilibrium level of pollen receipt, is 'locked in' to producing more ovules than will be pollinated. The second theory posits that pollen limitation represents an adaptive response to stochastic variability across space or time in pollen receipt (Burd 1995*a*; Ashman et al. 2004). Burd (1995) showed theoretically that, in many cases – particularly when variance in pollen receipt across flowers within an individual is high – producing more ovules than are pollinated on average is adaptive, as it allows the plant to take advantage of rare instances of high levels of pollination.

While the two mechanisms outlined above likely generate many instances of PL, we believe the constrained focus on plant-pollinator interactions in existing theory on PL overlooks potential additional mechanisms that necessarily involve other types of biotic interactions. One such mechanism, which we call antagonist-induced adaptive PL, is an adaptive response by a plant to conflicting selection from pollinators and floral antagonists (e.g. florivores, nectar robbers) on pollinator attraction traits. Figure 5.1 provides a conceptual diagram, adapted from the Haig-Westoby framework, that depicts how antagonist-driven adaptive PL arises; below we outline this mechanism in greater detail.

The conceptual model

For antagonist-induced adaptive PL to occur, four criteria must be met:

1. Both pollinators and antagonists must be attracted by the same trait(s). This is illustrated in Figure 5.1A by the shape of the curves *P* and *A*, where *A*, hereafter referred to as the antagonist function, represents the relationship between investment in pollinator

attraction and proportional antagonist damage (ranging from 0, no damage, to 1, all flowers damaged); both P and A are increasing functions of investment in pollinator attraction. This establishes the potential for conflicting pressures on plant allocation to pollinator attraction.

2. The response of the antagonist to a change in investment in pollinator attraction must be equal to or greater than the response of the pollinator (see Discussion for an evaluation of how widespread we expect this to be). When this is the case, the maximum slope (and possibly the asymptotic value) of A will be greater than that of P. Therefore, the intersection of $1\text{-}A$ (the inverse of the antagonist function; i.e. the proportion of undamaged flowers) will intersect with P to the left of $I_N{*}$ (Figure 5.1B).
3. The resulting conflicting pressures should lead to reduced investment in the relevant attraction trait(s) and therefore a level of pollen receipt lower than that which would maximize viable seed production (i.e. PL).
4. Reduced investment in pollinator attraction should nevertheless result in higher fitness for plants, compared to those that allocate more to pollinator attraction.

There are two ways in which criteria 3 and 4 may be met. First, antagonist damage may have such a strong negative impact on the plant’s ability to reproduce that, in the presence of the antagonist, maximum reproductive success during that reproductive event is achieved at a level of investment in pollinator attraction below the Haig-Westoby optimum. Alternatively, for an iteroparous species (i.e. a species that reproduces multiple times), reduced allocation towards pollinator attraction may be advantageous if this increases the likelihood of surviving to reproduce again. In this case, what would be the optimum level of investment in pollinator attraction? While the answer to this question will depend in part on the precise nature of the trade-off between present and future reproduction, a first approximation of this optimum is indicated by the intersection point of curves P and $1\text{-}A$ (I_A*, Figure 5.1B). At this intersection, attraction of pollinators and antagonists is such that all flowers could, at least in theory, interact with either a pollinator or an antagonist but not both. Assuming that antagonist-damaged flowers are rarely successfully pollinated, this intersection point describes optimum investment in pollinator attraction. Why? On the one hand, investing more in pollinator attraction would result in losing flowers to antagonists that would otherwise attract pollinators (representing lost investment both of the additional resources allocated to pollinator attraction and of the resources spent on initial ovule formation for those flowers lost to antagonists). On the other hand, investing less in pollinator attraction than this optimum would result in flowers that were neither damaged nor pollinated, representing wasted allocation of resources to the formation of ovules that will not be fertilized. The degree to which this partitioning of flowers into exclusive "for pollinator" or "for antagonist" categories actually occurs is likely to depend on the mechanism by which antagonists affect seed production (i.e. by reducing pollinator attraction vs. reducing ovule viability; see Discussion for details).

The optimum investment in pollinator attraction in the presence of the antagonist (the antagonist-driven optimum) will be lower than the Haig-Westoby optimum predicted by the intersection of pollen limitation and resource limitation functions, so long as criterion 2 (above) is satisfied. This reduced investment in pollinator attraction will result in PL. Uniquely, in comparison to the other explanations of PL outlined above, this mechanism suggests that PL may be adaptive even in an environment of relatively predictable levels of pollen receipt (though it can also operate when pollen receipt varies unpredictably).

Empirical test of the conceptual model

We evaluated evidence for the idea that conflicting pressures from antagonists and pollinators lead to PL, using nectar robbery and pollination of the iteroparous shrub *Odontonema cuspidatum* (Acanthaceae) as a model system. *Odontonema cuspidatum* is likely pollinated primarily by hummingbirds (G. Fitch unpublished data; Meyer and Lavergne 2004), and, in the study region in Chiapas, Mexico (where it is native), is also heavily nectar-robbed, primarily by stingless bees of the genus *Trigona* (*T. fulviventris* and *T. nigerrima*; Meliponini: Apidae) (Fitch and Vandermeer 2021). Nectar robbery occurs when a flower visitor extracts nectar from the flower via an animal-made hole rather than the corolla opening.

In the study population, robbed flowers of *O. cuspidatum* are 41% less likely to set fruit than unrobbed flowers (Fitch and Vandermeer 2021). Moreover, at the plant level, nectar robbing intensity (i.e. the proportion of flowers that are robbed) increases as flower number increases, at least for floral displays of moderate size (Fitch and Vandermeer 2021). In other species, hummingbird visitation is likewise positively related to flower number (Schemske 1980; Rodriguez-Robles et al. 1992; but see Brody and Mitchell 1997). We therefore suggest that flower number is a key trait governing the attraction of both pollinators and antagonists (i.e. nectar robbers) to *O. cuspidatum* (satisfying criterion 1). We evaluated whether antagonist response to flower number is stronger than pollinator response (satisfying criterion 2), and determined the Haig-Westby and antagonist-induced optima, using a combination of field surveys and a manipulative experiment. Assuming a stronger response of antagonists to flower number, we predicted that naturally-occurring plants would have fewer flowers than predicted by the Haig-Westoby optimum (I_N*, Figure 5.1), and would be pollen-limited (satisfying criterion

3). Finally, we predicted that floral display sizes close to the antagonist-induced optimum (I_A*, Figure 5.1B), as predicted by data on pollinator and antagonist response to flower number, would be overrepresented among naturally-occurring plants (consistent with criterion 4). However, we hypothesized that the variable intensity of nectar robbery experienced by plants (Fitch and Vandermeer 2021) would maintain high within-population variability in floral display size (Siepielski and Benkman 2010).

MATERIALS AND METHODS

Study system

This study took place at Finca Irlanda, a large (ca. 300ha) shaded, certified organic coffee farm in southeast Chiapas, Mexico. In this area, *O. cuspidatum* occurs commonly as an understory plant. *Odontonema cuspidatum* is a sprawling shrub, 1.5-2.5 m in height. The plant flowers primarily from May-August, in the early part of the rainy season. Plants produce indeterminate, branching terminal racemes holding slender red flowers, 1.5-3.0 cm long. Individual inflorescences hold from ~10 to several hundred flowers, and plants produce from one to several dozen inflorescences per flowering period. Plants can live for at least five years, and generally produce flowers every year beginning in their second or third year (G. Fitch unpublished data).

Flowers of *O. cuspidatum* are visited by a wide range of nectar- and pollen-feeding animals. Legitimate visitors are primarily hummingbirds, with less frequent visits from butterflies and small solitary bees (G. Fitch unpublished data). While *Trigona* bees act as primary nectar robbers, a host of Hymenopterans secondarily rob *O. cuspidatum* flowers; these include several species of ants and wasps, as well as other species of stingless bees.

Evaluation of pollen limitation

We evaluated the strength of PL in the studied population of *O. cuspidatum* in 2016-2018, using a total of six *O. cuspidatum* individuals with ≥4 inflorescences. Four of these plants were growing within areas of coffee cultivation, under relatively high-light conditions, while two were growing in a forest fragment with reduced light availability. Total flower number for the manipulated plants ranged from 131 to 1286. Six plants is a small sample size from which to estimate PL, but logistical constraints (both the time involved in hand pollination of large numbers of flowers and the fact that there was only a limited number of plants available, and plants could not be both involved in this experiment and included in the monitoring study) made it difficult to include more plants, and the strength of PL meant that consistent, statistically significant effects were detected even with this small sample (see Results).

On each plant, one inflorescence was randomly assigned to each of the following treatments: ambient pollination and nectar robbery (unmanipulated), and hand cross-pollination (bagged to exclude nectar robbers; stigmas saturated with mixed pollen from 2 other *O. cuspidatum* individuals using a wire filament loop [see Fitch and Vandermeer (2020) for details]. All inflorescences were bagged during bud development, before any flowers were large enough to be nectar-robbed. Hand pollination occurred between 0600-0800 each day during the flowering period. All hand-pollinated flowers were marked on the pedicel with nail polish so that, in case we did not pollinate all flowers, we could distinguish between pollinated and unpollinated flowers. To control for the effects of handling and nail polish application, open flowers were similarly marked on inflorescences that were not hand-pollinated. Upon fruit maturation, we harvested inflorescences and determined fruit set (fruits per flower) and seeds per inflorescence for each inflorescence, including only marked ovaries in our calculations. While we did not quantify nectar robbery on the plants in the hand-pollination trials, nectar robbers were observed on all open inflorescences.

Inflorescence-level treatments like we used have been shown to overestimate PL, due to resource reallocation by plants (Zimmerman and Pyke 1988; Knight et al. 2006). However, there are several reasons to suspect that reallocation effects are small in this case. First, inflorescences of *O. cuspidatum* are terminal, and we selected inflorescences for manipulation that were spatially separated within the plant; terminal inflorescences and spatial separation among inflorescences both reduce the possibility of reallocation (Wesselingh 2007). Moreover, stems of *O. cuspidatum*, if separated from the parent plant, commonly root to become new individuals, indicating that individual stems draw from independent resource pools, which makes reallocation unlikely (Wesselingh 2007).

Nevertheless, to control for potential effects of inflorescence-level treatment, in 2019 we conducted full-plant hand pollination on 5 individual *O. cuspidatum* that had been propagated from stem cuttings in August 2017 and grown in plastic nursery sleeves filled with soil obtained on the farm. These propagated plants were grown in the farm nursery, which has light exposure similar to that experienced by the naturally-occurring plants growing in the area of coffee cultivation. In all cases, 2019 was the first year these plants had flowered; all plants produced either 1 or 2 inflorescences. Hand pollination was carried out using cross pollen as described for inflorescence-level treatments above, and fruit set and seeds per plant were compared with data from 5 individuals of similar condition (i.e. propagated in August 2017, first flowering in 2019, in high-light environment) that received ambient pollination.

To determine whether plants were pollen-limited, we examined whether hand cross-pollination had an effect on fruit set (i.e. fruits per flower), seeds per fruit, and seeds per inflorescence in naturally-occurring plants. We compared each measure for inflorescences that experienced hand cross-pollination and those receiving ambient pollination using paired t-tests. Since we found evidence for PL (see Results), we took the mean fruit set of hand-pollinated inflorescences to represent the proportion of flowers pollinated at the Haig-Westoby optimum for investment in pollinator attraction.

We assessed the effects of whole-plant hand cross-pollination on potted plants using a Mann-Whitney U test comparing fruit set in plants receiving hand pollination to those receiving ambient pollination.

Field surveys and estimation of optimal investment in pollinator attraction (I_N^* and I_A^*)

Survey methods are described in greater detail in Fitch and Vandermeer (2021). In brief, in May-July 2017-2018, we surveyed 109 individual *O. cuspidatum* plants (33 of these 109 were surveyed in 2017 only; 15 in 2018 only; 61 in both years) for nectar robbery every 5 days for the duration of the flowering period. At each survey, we checked all flowers >1.5 cm in length, whether open or not, for perforations at the corolla base – evidence of nectar robbery. Flowers >1.5 cm are generally either open or within 1-2 days of opening; flowers shorter 1.5 cm are unopened and are rarely robbed. Results for open and unopened flowers were tabulated separately, as were results for each inflorescence. At the conclusion of the flowering period, fruit set, seeds per fruit, and seeds per plant were assessed as described in Fitch and Vandermeer (2021).

To determine the theoretical fruit set that a plant would achieve in the absence of nectar robbery, we developed the following formula:

$$s_T = s_O + (r \times d)$$

where s_T represents fruit set in the absence of nectar robbery, s_O represents observed fruit set, r represents the proportion of flowers that were robbed, and d represents the population-level mean effect of nectar robbery on fruit set, derived from a subset of plants where we compared the fates of robbed and unrobbed flowers (Fitch and Vandermeer 2021):

$$d = \frac{fruit\ set\ for\ unrobbed\ flowers - fruit\ set\ for\ robbed\ flowers}{fruit\ set\ for\ robbed\ flowers}$$

In the population of *O. cuspidatum* under study, d = 0.70 in 2017 and 0.68 in 2018 (Fitch and Vandermeer 2021).

To test for a relationship between plant size, as measured by the number of stems, and flower number, we used a generalized linear model (GLM) with Poisson error distribution; number of flowers was the response variable, and number of stems the predictor.

We evaluated the relationship between total flower number and 1) nectar robbery and 2) corrected fruit set. For each relationship, we fit four different functions (linear, quadratic, logistic, and Michaelis-Menten) to the data using the `nls` function. We used AICc scores to determine the best model. Once the best model was selected, we used the predicted best-fit curves in two ways. First, we used the fit of theoretical fruit set to floral display size to determine I_N*, the Haig-Westoby optimum flower number, based on the mean fruit set achieved by hand-pollinated inflorescences. Second, we overplotted the curves for theoretical fruit set and for the proportion of flowers that were not nectar-robbed (as in Figure 5.1B) to determine the I_A*, the antagonist-induced optimum flower number. In both cases, confidence intervals for I_N* and I_A* were generated by bootstrapping.

Because mean flower number differed between firespike plants growing in coffee fields and in forest fragments (Fitch and Vandermeer 2021), we conducted the same visual analysis to identify the antagonist-induced optimum, I_A*, separately for plants growing in each habitat. However, these analyses indicated that there was no significant difference between the antagonist-induced optimum floral display size estimated from the entire dataset vs. from each habitat separately, so hereafter we discuss only the results from the full dataset considered together.

We tested whether reproductive output was related to total flower number, using the following metrics of reproductive output: fruit set, seeds per fruit, and seeds per plant. In all cases we used GLMs with negative-binomial error distribution. For fruit set, number of fruit was the response variable, with log(total number of flowers) included as an offset. The ß-value of the offset is fixed at 1, allowing us effectively to model proportional data while avoiding the pitfalls of using proportions in regression analysis. For both seeds per fruit and seeds per plant, number of seeds was the response variable; for seeds per fruit we additionally included log(number of fruits) as an offset. Because we counted the number of seeds from all fruit only in 2018, we have data on seeds per plant only for that year, but models for fruit set and seeds per fruit incorporated data from 2017 and 2018, and for these models year was included as a covariate.

Experimental floral arrays – Is flower number the key attraction trait?

To test whether nectar robbers and pollinators responded specifically to flower number, rather than another correlated trait, we conducted an experiment using replicated experimental floral arrays. Each of the seven replicates consisted of one array each of 1, 3, 6, and 10 *O. cuspidatum* inflorescences. The inflorescences included in each replicate were cut from a single plant or adjacent plants, to control for the effects of variation in floral traits that might influence flower visitor attraction. Additionally, we harvested inflorescences of approximately the same size, though flower number nevertheless differed among inflorescences. Inflorescences, along with a 0.25-0.5 m length of stem, were cut from the plant the day before they were to be deployed and kept in water. The following morning, between 0600-0700 h, each array was placed in a 1 L clear plastic container filled with water and was set out in the field. Arrays within a replicate were placed ~10m apart; replicates were distributed within a 2 ha section of the farm, in areas under coffee production. Replicates were placed within 100 m of blooming *O. cuspidatum* plants and ≥100 m from one another. Each day, we deployed 1-3 replicates, depending on the availability of inflorescences.

Between 1300-1400h on the same day, we surveyed each array for nectar robbery and pollen removal. Nectar robbery was surveyed with the same methods as the field surveys, which are described above. We used pollen removal as a proxy for pollinator visitation, having previously determined that a single visit from a hummingbird was usually sufficient to remove the bulk of pollen from a flower's stamens. To check for pollen removal, a thin wire-filament loop was run over the surface of the stamen and checked for pollen. If the loop came away with more than trace amounts of pollen (i.e. >10 visible grains), we recorded the flower as unvisited by a pollinator. After checking for nectar robbery and pollen removal, we placed all inflorescences in a screened, pollinator- and nectar-robber free room, grouped by replicate, until the following morning.

Individual inflorescences continued to bloom, and newly opened flowers continued to contain nectar, for at least three days after cutting, so we continued to use inflorescences on three consecutive days, as long as the quality of the floral display did not visibly deteriorate.

Deteriorated inflorescences were replaced with fresh-cut inflorescences from the same plant as necessary. After three days of deployment, all inflorescences from a replicate were discarded. On consecutive days, inflorescences were used for the same replicate, but randomly assigned to array. Data collection for this experiment lasted from Jun 27 – Jul 18 2018.

To investigate how inflorescence number affected 1) flower number, 2) nectar robbery, and 3) pollen removal in experimental arrays, we used nonlinear mixed models, implemented with the `nlme` function in R package `nlme` (Pinheiro et al. 2019). For all response variables, we compared the same four functions as for naturally-occurring plants, and selected the best model as described above. For nectar robbery, our response variable was number of flowers robbed, and for pollen removal it was number of flowers with pollen removed. For nectar robbery and pollen removal we constructed two models each, one with flower number included as an offset in order to evaluate how the proportion of flowers robbed or visited by pollinators was influenced by inflorescence number, and one without the offset to evaluate how the number of flowers robbed or visited was influenced by inflorescence number. For all models, the number of inflorescences (i.e. array size) was included as a fixed effect; replicate was included as a random effect.

RESULTS

Evaluation of pollen limitation

We hand cross-pollinated 382 flowers on six inflorescences on six different naturally-occurring plants. For inflorescence-level treatments, hand pollination resulted in fruit set nearly 5x higher than that seen in inflorescences experiencing ambient pollination (ambient mean ± SE: 0.12 ± 0.02, hand-pollinated: 0.57 ± 0.04; t = 5.22, df = 5, p = 0.003). Similarly, hand pollination resulted in a doubling of seeds per fruit (ambient: 1.5 ± 0.5, hand-pollinated: 3.4 ± 0.1; t = 4.41, df = 3, p = 0.02) and a 5-fold increase in seeds per inflorescence (ambient: 40.9 ± 20.6, hand-pollinated: 206.0 ± 53.3; t = 4.35, df = 3, p = 0.02). These results indicate that the population of *O. cuspidatum* under study experiences strong PL, and that fruit set at the Haig-Westoby optimum (I_N*) should be approximately 0.6 (i.e. the fruit set achieved in hand-pollinated inflorescences). Plant-level results from propagated plants were qualitatively similar, with fruit set again 5x higher for hand-pollinated plants, but with substantially reduced fruit set overall, compared to naturally-occurring plants (ambient: 0.04 ± 0.01, hand-pollinated: 0.22 ± 0.01; W = 0, p = 0.01), likely due to the severe resource limitation experienced by these plants. Consumption of developing fruit by *Chlosyne janais* (Nymphalidae) larvae on several plants precluded comparison of seed production for whole-plant pollen manipulation.

Field surveys and estimation of optimal investment in pollinator attraction ($\mathbf{I_N}$** and *$\mathbf{I_A}$**)*

Total flower number on monitored plants ranged from 18-1402 flowers. Median flower number was 100 flowers, while the mode was 41 flowers (Figure 5.2). Flower number was not significantly correlated with plant size, as estimated by the number of discrete stems emerging from the soil (ß = 0.03 ± 0.02, z = 1.45, p = 0.15), though flower number is influenced by resource availability (see Chapter 4).

Nectar robbery showed a positive response to *O. cuspidatum* flower number (Figure 5.3A); Fruit set showed no correlation with *O. cuspidatum* flower number (ß = 0.00, z = –1.57, df = 126, p = 0.1). However, when fruit set data were corrected for the negative effect of nectar robbery following equation 1, expected fruit set in the absence of nectar robbery (hereafter referred to as theoretical pollination) showed a positive, saturating response to flower number

(Figure 5.3B). Thus, both nectar robbers and pollinators appear to respond to floral display size such that per-flower nectar robbery and pollination are higher for larger floral displays, satisfying criterion 1 of the conceptual model.

For both proportional nectar robbery and proportional theoretical pollination, the relationship with flower number was best represented by a Michaelis-Menten function (Table 5.1), though in the case of theoretical pollination, the Michaelis-Menten was barely distinguishable from the logistic. Both the half-saturation constant (K) and the asymptote (V_m) were lower for theoretical pollination than nectar robbery (K = 26 ± 7 vs. 41 ± 11; $V_m = 0.69 \pm 0.04$ vs. 0.86 ± 0.07; Figure 5.3B), indicating a stronger response by nectar robbers than pollinators to floral display size and satisfying criterion 2.

When the Michaelis-Menten fits for 1) theoretical antagonist-free pollination and 2) the inverse of nectar robbing intensity (i.e. the proportion of flowers not robbed) as a function of flower number are plotted together, their curves intersect at a flower number of 62 flowers (95% CI: 44-88 flowers) (Figure 5.3C). According to the Michaelis-Menten fit for pollination in the absence of nectar robbery, this flower number results in only 48% (95% CI: 42-54%) of flowers setting fruit, 30% less than the maximum fruit set predicted by V_m (Figure 5.3C) and >15% less than the mean fruit set for hand cross-pollinated inflorescences (57 ± 4%; see above). Twenty-seven percent of surveyed plants had a flower number that fell within the bounds of the 95% CI for the predicted optimum flower number, despite this range representing only 3% of the total observed range in flower number (Figure 5.2, blue-shaded region). By contrast, the 95% CI for the flower number needed to achieve fruit set matching that seen in hand-pollinated plants [i.e. the optimum according to the Haig-Westoby framework (Haig and Westoby 1988; Burd 2008); 0.57 ± 0.04] was 88-342 flowers (predicted optimum 146 flowers). Despite the 95% CI for the

Haig-Westoby optimum encompassing fully 18% of the observed range in total flower number, only 20% of plants had a total flower number within this range (Figure 5.2, gray-shaded region). For plants within the 95% CI for the antagonist-driven optimal total flower number (44-88 total flowers), mean±s.e. per-observation flower number was 13±1 flowers.

As with fruit set (see above), seeds per fruit was not significantly affected by flower number ($ß = –0.03 \pm 0.02$, $z = –1.37$, $df = 87$, $p = 0.17$). Per-plant seed production, however, was positively correlated with flower number ($ß = 0.74 \pm 0.10$, $z = 7.37$, $df = 77$, $p < 0.001$).

Experimental arrays – Is flower number the key attraction trait?

Arrays of different sizes differed significantly in the number of open flowers, with flower number increasing linearly with inflorescence number (Table 5.1; Figure 5.4A). Both nectar robbery and pollen removal increased with inflorescence number, providing further indication that criterion 1 is satisfied by this system. As with naturally-occurring plants, this response was better represented by a saturating function than linear, logistic, or quadratic functions (Table 5.1). And as in naturally-occurring plants, both the half-saturation constant (K) and asymptote (V_m) were higher for nectar robbery than pollen removal (nectar robbery: $K = 0.67 \pm 0.39$, $V_m = 0.76 \pm 0.10$; pollen removal: $K = 0.44 \pm 0.29$, $V_m = 0.51 \pm 0.08$; Figure 5.4B), again satisfying criterion 2. When the function for nectar robbery is inverted, the intersection point for the inverse nectar robbery function and pollen removal function is at 2.4 inflorescences, though the 95% CI is quite wide (Figure 5.4C). According to the linear function relating number of open flowers to array size, 2.4 inflorescences corresponds to 10 open flowers. This closely matches the mean per-observation flower number for naturally-occurring plants within the 95% CI for the antagonist-driven optimal total flower number (13±1 flowers).

When the number (rather than proportion) of flowers experiencing nectar robbery is considered, the relationship between inflorescence number and nectar robbery is best described by a linear function (Table 5.1, Figure 5.4A). For the number of flowers experiencing pollen removal, on the other hand, a logistic function provides the best fit (Table 5.1, Figure 5.4A). This indicates that increasing flower number beyond ~6 inflorescences results in minimal increase in the number of flowers pollinated, while continuing to attract more nectar robbers.

DISCUSSION

Results from both our field surveys and experimental manipulations suggest that pollen limitation (PL) in *O. cuspidatum* is the result of adaptive response to conflicting pressures on flower number exerted by hummingbird pollinators and bee nectar robbers. While researchers have previously noted conflicting selection pressures on floral traits exerted by pollinators and nectar robbers (Gélvez-Zúñiga et al. 2018), this study is among the first to point out the potential causal link with PL.

For PL to arise as an adaptive response to conflicting pressures from pollinators and antagonists, four criteria must be met. The first criterion requires that the same trait(s) mediate attraction of both pollinators and antagonists. This criterion was met in both naturally-occurring plant and experimental arrays. In naturally-occurring plants, *O. cuspidatum* attractiveness to both pollinators and nectar robbers was mediated by flower number, with both the proportion of flowers robbed and the theoretical proportion of flowers that would be pollinated in the absence of nectar robbery showing positive, asymptotic responses to flower number. Moreover, a similar pattern of positive, asymptotic response to flower number of both nectar robbery and pollen removal emerges from the data from our experimental arrays. This confirms that flower number – rather than another, correlated, trait – mediated both pollinator and nectar robber attraction.

The second criterion is that the antagonists' response to an increase in allocation to pollinator attraction must be stronger than the pollinators' response. Data from both surveys and experimental arrays indicate that this occurs in our study system: levels of nectar robbery are consistently higher than levels of pollination for a given floral display size.

The third criterion is that conflicting pressures lead to reduced investment in the relevant trait(s), lowering pollinator attraction and leading to PL. Again, our data confirm that this occurs in *O. cuspidatum*. The antagonist-induced optimum flower number for this population of *O. cuspidatum*, as predicted by field survey data, is less than half the predicted Haig-Westoby optimum (Figure 5.3C). At the antagonist-induced optimum, fruit set is >15% lower than the levels seen in hand cross-pollinated *O. cuspidatum* plants, indicating that plants at the antagonist-induced optimum experience PL. These results are closely matched by those from our experimental arrays; estimates of the antagonist-driven optimum flower number from the two datasets are remarkably similar (i.e. 10 vs. 13 open flowers at a time).

The fourth and final criterion to be satisfied is that plants reducing investment in pollinator attraction must nevertheless experience higher fitness than those investing at the Haig-Westoby optimum. We cannot conclusively determine whether this is occurring in the study population of *O. cuspidatum*, given that we do not have information on lifetime fitness; the evidence we do have is mixed in its support for this criterion. The distribution of total flower number in the population supports the idea that a flower number close to the antagonist-induced optimum is selected for. Total flower numbers within the range of the antagonist-driven optimum were dramatically overrepresented in naturally-occurring plants (Figure 5.2). This does not appear to be solely the result of resource availability dictating allocation to flower production, as flower number was not significantly correlated with plant size [although light availability does influence flower production, presumably via its influence on the availability of photosynthate to allocate to reproduction (Fitch & Vandermeer 2020)].

Despite apparent convergence on the antagonist-driven optimum flower number, the population exhibits high variability in this trait. This maintenance of trait variation is consistent with our hypothesis that high levels of variability in nectar robbing intensity – both across individuals and between years within individuals – should maintain trait variation. Indeed, the overrepresentation of plants with a very large number of flowers (Figure 5.2) suggests the possible existence of an alternative strategy. Very large floral displays may represent a form of antagonist satiation; there is some indication that levels of nectar robbing intensity decline for plants with very large floral displays (in Figure 5.3B, note two points in lower right), though these same plants also experienced relatively low levels of pollination, drawing into question the utility of antagonist satiation in this case.

We found a positive, linear correlation between flower number and seed production, suggesting that, despite higher levels of nectar robbery, short-term fitness is maximized by maximizing flower number. But in this population, a large flower number in one year is strongly correlated with reduced flower production the following year (Fitch and Vandermeer unpublished data). Costs of reproduction such as this are common in plants (Obeso 2002). Such costs suggest a potential trade-off between short-term and lifetime fitness maximization, increasing the likelihood that a smaller number of flowers, minimizing the loss of flowers to nectar robbers, may maximize lifetime fitness. Moreover, in the experimental arrays, the number of flowers experiencing pollen removal showed a saturating response to inflorescence number, indicating minimal benefit of floral displays larger than ~6 inflorescences (26±1 open flowers at a time). Thus, the linear relationship between total flower number and seed production may reflect benefits of a longer flowering period rather than of producing more total flowers. This explanation is not in contradiction with the idea that the antagonist-induced optimum flower number maximizes fitness.

This study suggests that conflicting selection between antagonists and pollinators is an important driver of PL in *O. cuspidatum*. But how widespread is antagonist-induced adaptive PL likely to be? According to our conceptual model, the conditions under which antagonists would drive PL are rather restrictive. Below, we discuss three primary restrictions. Despite these restrictions, we suspect that the necessary conditions for antagonist-induced adaptive PL occur widely, and that antagonist-induced adaptive PL may be common.

One restrictive requirement of the model is that antagonists must respond at least as strongly as pollinators to an incremental increase to investment in pollinator attraction. At first glance, greater sensitivity of the antagonist than the pollinator to pollinator attraction traits seems unlikely. Yet such outsize response from antagonists might be expected when the antagonist occurs at substantially higher densities than the pollinator. This is fairly likely to occur if the pollinator is a vertebrate and the antagonist is an insect, or even if the pollinator is a solitary insect while the antagonist is a social insect with the potential for rapid recruitment of large numbers of foragers to a food resource. At least for nectar robbery, these are common (though certainly not universal) scenarios: social Hymenoptera comprise a plurality of documented nectar robbers (Irwin et al. 2010), while the pollinators of flowers that experience heavy nectar robbery are often birds (e.g. Irwin 2006; Rojas-Nossa et al. 2016; Gélvez-Zúñiga et al. 2018). At somewhat longer timescales, if insects whose larvae consume floral tissues use pollinator-attracting floral traits to locate oviposition sites [as is the case for e.g. some Lepidoptera (Irwin et al. 2003; Adler and Bronstein 2004)], the effect of antagonist attraction is likely to outweigh that of pollinator attraction, since a single visit by an ovipositing adult will generally result in multiple larvae that will then consume the plant.

A second restriction of the model is that most individual flowers should be either ‘for pollinators’ or ‘for antagonists’ – that is, flowers that are visited by a pollinator should produce seeds, flowers attacked by an antagonist should not, and there should be little overlap in flowers visited both by pollinators and antagonists. The degree to which this occurs will likely depend on the mechanism by which antagonist activity reduces plant reproduction. If the antagonist reduces the attractiveness of flowers to pollinators, but does not directly damage reproductive organs, then the fate of a flower (pollinated or damaged) is essentially determined by the identity of its first visitor (assuming relatively high single-visit pollination efficiency). If the first visitor pollinates the flower, future interactions with an antagonist will not impact seed production. Alternatively, if an antagonist visits first, it is less that the flower will be subsequently visited by pollinators, and more likely that it will go unpollinated. It is common for floral antagonists – including nectar robbers and florivores – to affect reproduction by reducing pollinator attraction (Lohman et al. 1996; Irwin and Brody 2000; Castro et al. 2008; Sõber et al. 2010; Varma et al. 2020), including in our study system (Fitch and Vandermeer 2020).

On the other hand, if antagonists directly damage reproductive organs, the important question is not who visits the flower first, but whether or not the flower interacts with an antagonist at any point. In this case, there is likely to be substantial overlap in ‘for pollinators’ and ‘for antagonists’ flowers, unless antagonists avoid flowers that have been previously visited by pollinators. While antagonist avoidance of pollinated flowers is possible (particularly for antagonists seeking nectar rewards), its occurrence has not been widely documented. We therefore suggest that antagonist-induced adaptive PL is more likely to occur where the antagonist reduces fitness by deterring pollinators, rather than damaging reproductive organs.

We note, however, that the partitioning of flowers as either ‘for pollinators’ or ‘for antagonists’ does not need be complete for antagonist-induced PL to operate. In our study system, nectar robbery significantly reduces pollination, but approximately 18% of robbed flowers still set fruit (vs. 32% of unrobbed flowers; Fitch and Vandermeer 2021). This indicates that, while pollinators prefer unrobbed flowers, they do pollinate robbed flowers, leading to some overlap in the flowers that are ‘for pollinators’ and ‘for antagonists.’ Nevertheless, antagonist-induced adaptive PL appears to occur in this system. Further study, both empirical and theoretical, will be necessary to determine how much non-overlap in ‘for pollinator’ and ‘for antagonist’ categories is needed for antagonist-induced adaptive PL to arise.

Finally, antagonist-induced adaptive PL is likely to be more common in iteroparous than semelparous plant species. Reducing investment in traits that attract both pollinators and antagonists will be adaptive for semelparous species only if the negative effect of attracting additional antagonists outweighs the positive effect of attracting additional pollinators (this is also true for iteroparous species). In iteroparous species, on the other hand, reducing short-term investment in reproduction increases the likelihood of survival and future reproduction, so the negative effect of attracting antagonists need not fully outweigh the positive effect of attracting pollinators for reduced investment in pollinator attraction to be adaptive. Much of the existing theoretical work on PL has focused, at least implicitly, on semelparous species. This is due, in part, to challenges associated with estimating PL for iteroparous species, since experimental manipulation of pollen receipt in iteroparous species can overestimate PL if reproductive output is not measured for all reproductive events (Zimmerman and Pyke 1988; Knight et al. 2006). However, that PL is difficult to experimentally assess in iteroparous species does not mean that it does not occur. The current study suggests that a more explicit focus on iteroparous species may illuminate additional mechanisms that give rise to PL.

Existing explanations of PL have focused exclusively on the plant-pollinator interaction. This work highlights how expanding our field of view to consider other impinging interaction types can illuminate heretofore overlooked mechanisms. The present study should be considered a proof-of-concept: we show data consistent with the hypothesis that antagonist-induced PL may occur and be adaptive. Yet we are not able to conclusively rule out a role for other mechanisms – particularly plant response to stochastic variability in pollen receipt – that may be leading to PL in *O. cuspidatum*.

More generally, we do not know how widely antagonist-induced adaptive PL occurs. In the present scenario, the attractiveness trait under apparent conflicting selection was flower number. We know that pollinators and antagonists can exert conflicting selection on other traits, including flower morphology (Irwin et al. 2003; Ågren et al. 2013; Sletvold et al. 2015; Gélvez-Zúñiga et al. 2018), nectar rewards (Adler and Bronstein 2004), flower scents (Theis and Adler 2012), and flower phenology (Sletvold et al. 2015); can antagonist-induced adaptive PL also arise in these cases? Must the antagonist strictly attack floral tissues, or could a more generalized herbivore, still cueing to pollinator attraction traits (e.g. Irwin et al. 2003), exert the same pressures? Might a qualitatively similar pattern emerge in a system with two pollinators, where a high-quality pollinator competes for access to flowers with a poorer pollinator (e.g. Burd 1995*b*)? The answers to all these questions await further study; we hope that their pursuit can further motivate discussion regarding the ecological and evolutionary drivers of PL.

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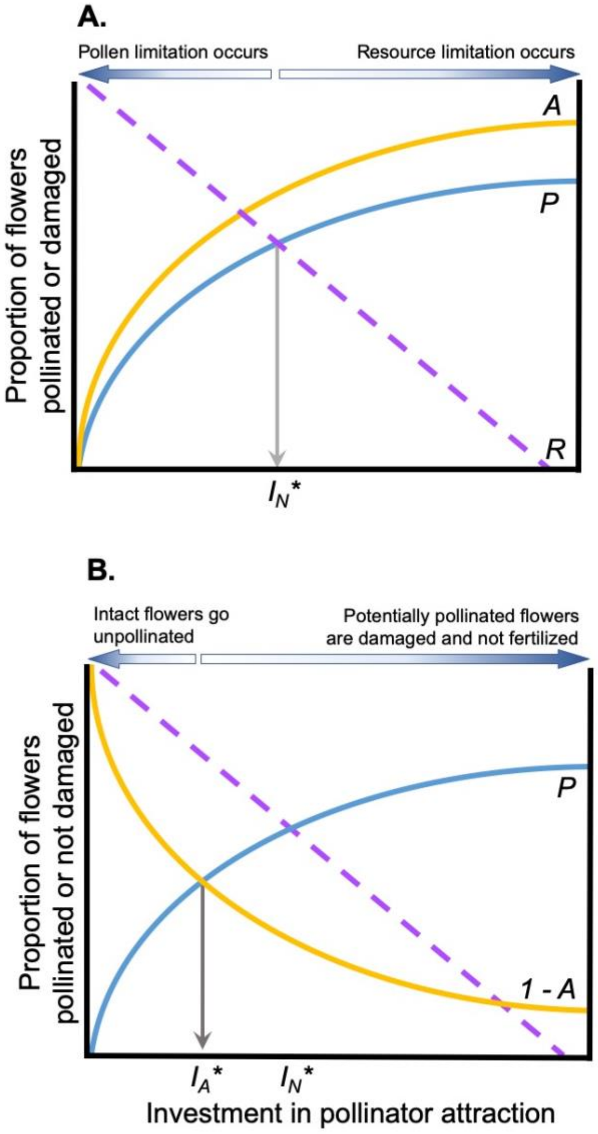
TABLE AND FIGURES

Table 5.1. Model comparison for the relationships between inflorescence number and flower number, nectar robbery, and pollen removal in A) naturally-occurring plants and B) experimental arrays of *O. cuspidatum*. 'Theoretical pollination' refers to observed fruit set corrected for the negative effect of nectar robbery on pollination, following equation 1. Boldface indicates the best model for that variable, determined by AICc score. ΔAICc indicates difference in AICc score from the best model for that variable.

Model	**Pearson's *r***	**AICc**	**ΔAICc**
A) Naturally-occurring plants			
Nectar robbery – proportion			
Michaelis-Menten	**0.43**	**39.26**	**0.00**
Logistic	0.43	41.54	2.28
Quadratic	0.41	116.32	77.06
Linear	0.22	198.86	159.60
Theoretical pollination – proportion			
Michaelis-Menten	**0.39**	**–33.71**	**0.00**
Logistic	0.40	–32.54	1.17
Quadratic	0.36	80.28	114.00
Linear	0.16	163.06	196.78
B) Experimental arrays			
Flower number			
Michaelis-Menten	0.80	413.50	2.28
Logistic	0.80	425.34	4.12
Quadratic	0.80	413.49	2.27
Linear	**0.80**	**411.22**	**0.00**
Nectar robbery – proportion			
Michaelis-Menten	**0.55**	**31.99**	**0.00**
Logistic	0.56	34.02	2.03
Quadratic	0.31	45.83	13.84
Linear	0.45	59.77	27.78

Pollen removal – proportion			
Michaelis-Menten	**0.68**	**–3.06**	**0.00**
Logistic	0.33	7.98	11.04
Quadratic	0.36	10.69	13.74
Linear	0.48	28.85	31.90
Nectar robbery - number			
Michaelis-Menten	0.85	386.42	3.20
Logistic	0.85	385.60	2.39
Quadratic	0.85	383.40	0.19
Linear	**0.85**	**383.22**	**0.00**
Pollen removal - number			
Michaelis-Menten	0.73	364.93	8.05
Logistic	**0.83**	**356.89**	**0.00**
Quadratic	0.83	358.00	1.11
Linear	0.76	361.02	4.13

Figure 5.1. Conceptual diagram illustrating how investment in pollinator attraction is affected by the presence of a floral antagonist, and may lead to pollen limitation.



a) According to the Haig-Westoby framework, optimum investment in pollinator attraction (I_N*), is determined by the intersection of the pollination function (P; solid pale blue line) and the resource availability function (R; dashed purple line). Pollen limitation occurs when investment in pollinator attraction is <I_N*; where investment is >I_N*, reproduction is resource-limited. The shapes of the functions illustrated here assume 1) a strict tradeoff between allocation to pollinator attraction and allocation to ovule provisioning and 2) diminishing returns on increasing investment in pollinator attraction, but model predictions are not sensitive to the precise shape of these functions. The antagonist function (A; solid orange line) indicates that attraction of pollinators and antagonists is correlated. b) Re-plotting panel a) with the antagonist function inverted (1-A), showing the proportion of flowers not damaged by the antagonist. Note that if the strength of antagonist response to investment in pollinator attraction is greater than pollinator response, I_A* < I_N* and pollen limitation will occur. In the text, I_N* is referred to as the "Haig-Westoby optimum", while I_A* is referred to as the "antagonist-induced optimum".

Figure 5.2. Frequency distribution of total flower number for surveyed plants. Dashed line represents optimum flower number under antagonist-induced adaptive PL; blue-shaded area represents 95% confidence interval (CI) for the antagonist-induced optimum (corresponding to area between dashed lines in figure 3C). Dotted line and gray-shaded area represent optimum and 95% CI, respectively, predicted from the Haig-Westoby framework.

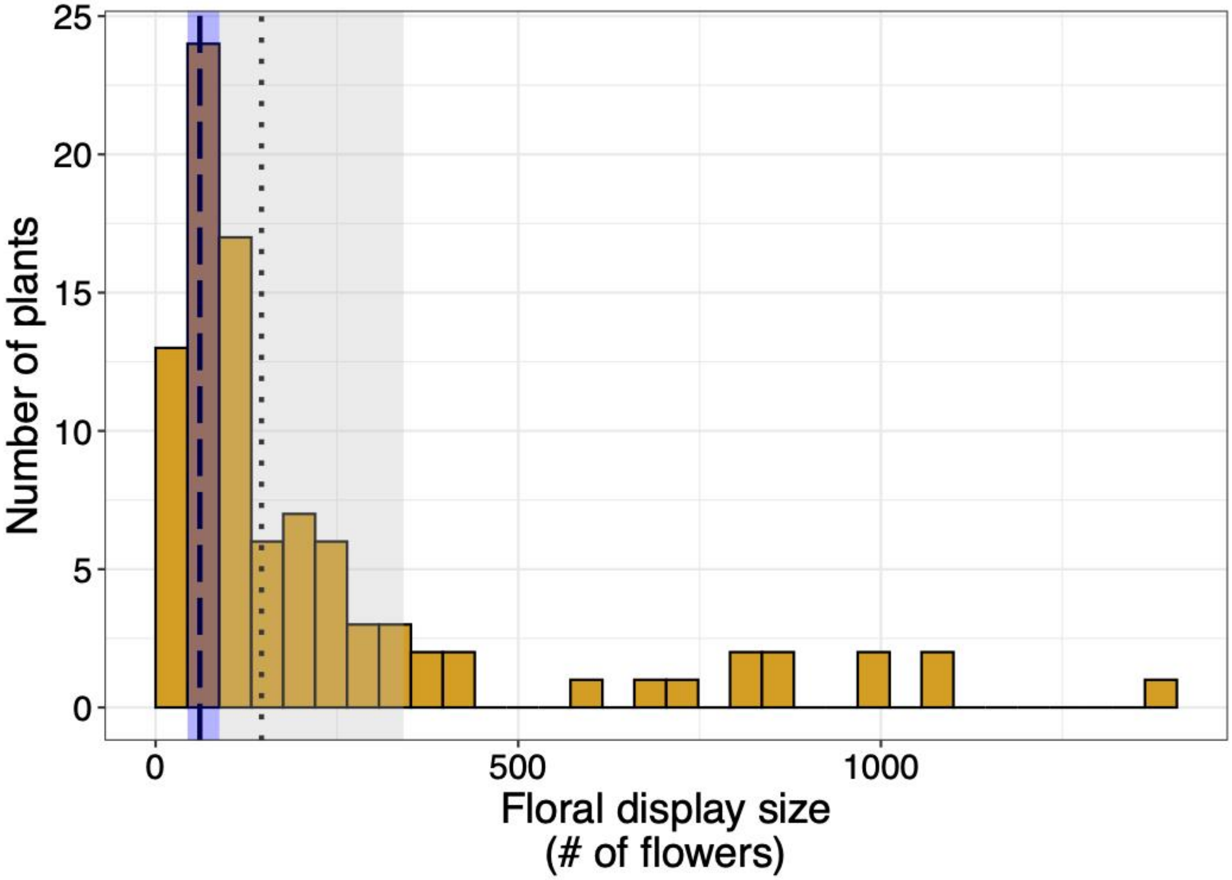
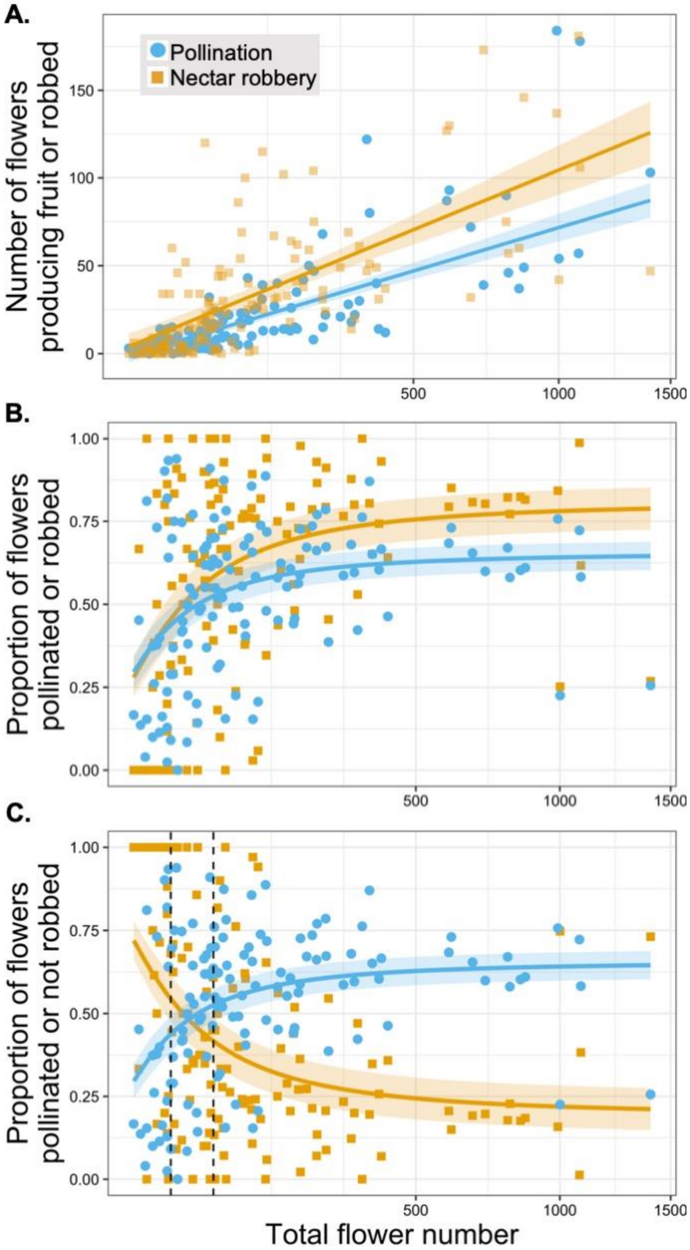
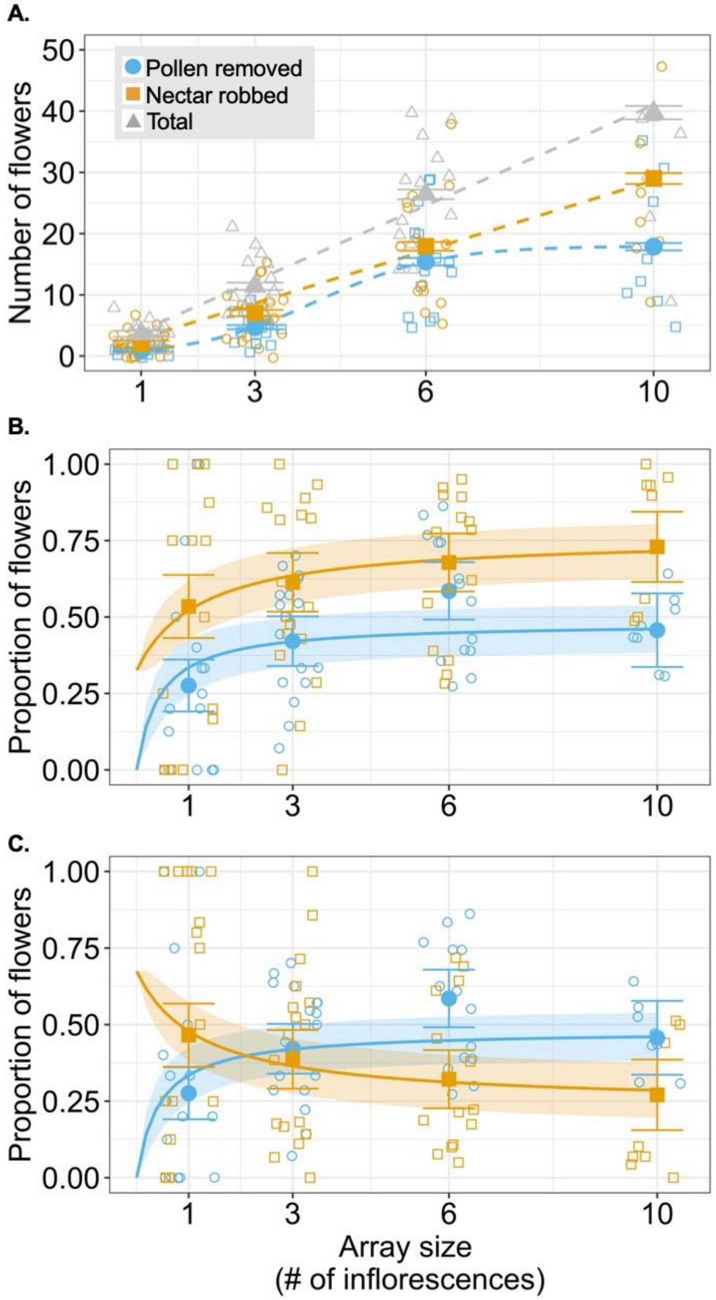


Figure 5.3. Relationship between flower number and pollination and nectar robbery in naturally-occurring plants.



In **A)**, blue points represent the total number of fruits produced and orange points represent total number of flowers robbed per plant. In both **B**) and **C**), blue points represent theoretical pollination in the absence of nectar robbery (see text). In **B**), orange points represent the proportion of flowers experiencing nectar robbery; in **C**), orange points represent the proportion of flowers that were not robbed. In **A**), solid lines represent linear best-fit line; in **B-C**), they represent the Michaelis-Menten function fit to each dataset; shaded areas represent 95% confidence intervals. In **C**), the dashed lines indicate the predicted optimum range of flower number, assuming conflicting selection on flower number from pollinators and nectar robbers. Note that proportion of flowers robbed in **B-C**) uses the total number of flowers surveyed as the denominator, rather than the flower number as determined at the end of the flowering season; the former is generally a smaller number, since we did not necessarily survey every flower produced by each plant.

Figure 5.4. A) Number and B-C) proportion of flowers in experimental arrays experiencing pollen removal and nectar robbery. Filled points with error bars represent means ± SE for each array size; open points show individual observations, jittered on x-axis for clarity. Lines represent the fit of the best model from among four candidates (linear, quadratic, logistic, Michaelis-Menten). In **B-C**), shaded area indicates 95% confidence intervals.



Chapter 6
Conclusion

Changes to environmental conditions can have profound impacts on the outcome of biotic interactions, even without changes to species composition (Tylianakis et al., 2008; Valiente-Banuet et al., 2015). Yet our understanding of such effects of environmental change is still in its infancy, with a good deal of uncertainty as to the predictability of the effect of a given change on a given interaction embedded in a given community. In the preceding chapters of this dissertation, I laid out a framework for classifying these effects, and presented two case studies for how such effects operate in agroecosystems.

In the first case study, presented in Chapter 2, I examined the effect of fine-scale (within-farm) variation in bloom density of neighboring co-flowering plants on pollination of coffee. I then evaluated the relative influence of neighbor effects on pollination and of neighbor interactions for shared abiotic resources on coffee yield. The variation in bloom density was generated by multiple farm management practices, including shade tree planting decisions and manual weed control. Within the schema introduced in Chapter 1, these management decisions represent an alteration to the biotic context within which the coffee–pollinator interaction occurs, via changes in the co-flowering plant community (Figure 6.1A). This altered biotic context in turn influenced pollinator behavioral traits, with lower density of co-flowering plants (in areas where weeds had been recently cut and lacking co-flowering canopy trees) leading to increased deposition of coffee pollen (Figure 6.1A, blue arrow). This was presumably due to increased pollinator visit frequency rather than a change in visit quality, since there was no effect of neighborhood flower density on non-coffee pollen deposition. Thus, we see that increased intensity of weed management results in increased frequency of coffee–pollinator interaction, at least at short timescales [over larger spatiotemporal scales, it is likely that weeds are an important source of food to sustain bee populations (Fisher et al., 2017), so sustained intensive management of weeds would likely lead to reduced bee populations and, by extension, reduction in pollination services to coffee]. However, this ultimately has little effect on the outcome of this coffee–pollinator interaction, at least from the plants' perspective, because the effects of neighbor interactions for abiotic resources – which are relatively insensitive to the weed management practices employed on the farm where the study took place – outweigh the modest effects of interaction for pollination on coffee fruit set (Figure 6.1A, green arrow).

In the second case study, presented in Chapters 3-5, I examined nectar-robbing of firespike (*Odontonema cuspidatum*) by stingless bees, comparing the frequency and outcome of these interactions between coffee fields and forest fragments. In this case, differences in abiotic conditions – specifically light availability – between habitats led to changes in the traits of both partners (Figure 6.1B, blue arrows). Higher light availability in coffee fields led to larger floral displays in firespike growing there (Chapter 4), and stingless bees preferentially robbed plants with larger floral displays (Chapters 3 & 5). Independently, higher light availability also led to increased foraging activity by stingless bees in coffee fields (with foraging levels likely additionally affected by higher availability of non-firespike floral resources in coffee fields compared to forests; Chapter 3); this served to reinforce the dynamic of higher nectar robbing intensity in coffee fields compared to forest fragments. Given the negative effect of nectar robbing on pollination of firespike, the increased levels of nectar robbery on plants growing in coffee fields results in decreased fruit set for those plants (Figure 6.1B, green arrow). However, because of greater flower production in plants growing in coffee, this reduction in fruit set does not translate into reduced seed production.

Implications

What can we learn about the effects of environmental change on species interactions through case studies such as these? Is it possible to generalize the findings presented here to other locations, other organisms, or other drivers of environmental change? In full honesty, I believe the answer to these questions must remain a tentative ‘We don’t really know.’ Below, I flesh out some of the complications involved in attempting to generalize from the present studies.

In both these case studies, the mediator between environmental change and interaction change is species traits, rather than species density. In general, should we expect changes to traits to be more common, or more impactful, than changes to density? The answer is not entirely clear, in part because the distinction between changes to density and changes to traits is somewhat fuzzy. For example, individual behavior is often contingent on conspecific density (McConkey and Drake, 2006; Hammill et al., 2015; Tollrian et al., 2015), so a change in density may result in a change in behavioral traits which then leads to a change to the interaction. Whether the cause of the interaction shift is judged as a change in density or in traits depends on a matter of perspective and emphasis. That said, given the sensitivity of behavior – and, in plants, of morphology – to environmental conditions, environmental change is likely to result in trait changes more commonly than it does in density changes.

The consequences of interaction shifts, in terms of measured impact on reproduction or yield of the plants involved, were modest in the systems presented here. In the first case, this was because the magnitude of change in interaction frequency was insufficient to impact its outcome (i.e. even the lowest levels of pollen receipt experienced by coffee in high-floral-density neighborhoods resulted in sufficient pollination). In the second, this was because of a countervailing direct effect of the environmental change on the fitness of the plant involved (i.e., the increased light availability that led to increased nectar robbery also allowed plants to allocate more resources towards reproduction, counterbalancing the negative effect of nectar robbery on reproduction). These results highlight the importance of considering interaction shifts not in isolation, but in their broader ecological context, in order to accurately assess their impact. While I found that the broader context attenuated the effects of the interaction shift in the systems I studied, I suspect that it will be just as common to find that the broader context amplifies the effects of the interaction shift.

Additionally, in both of the studied interactions, the effect of environmental conditions was primarily on interaction frequency, rather than the outcome of the interaction. It seems likely that environmental changes that result in a change in interaction outcome would be more likely to have fitness consequences, though this assertion has not been rigorously tested. Moreover, whether particular pathways from environmental change to interaction shift are more likely to result in changes to interaction outcome (rather than frequency), and are more like to have fitness consequences, remains to be seen.

In these studies, I did not investigate the fitness consequences of interaction shifts for the bees involved in the interaction. This focus on consequences for a single species is the norm in studies examining how environmental change drives interaction change (McConkey and Drake, 2006; Lewanzik and Voigt, 2014), highlighting a gap in our current understanding. While I suspect that, in this case, the fitness effects of the studied interactions shifts on the interacting bees were minimal, it would be instructive, if logistically complicated, to simultaneously measure effects of interaction shifts on the fitness of both interaction partners.

Despite the challenges inherent in studying such a multidimensional phenomenon, it remains crucially important that we improve our understanding – including predictive understanding – of the effects of environmental change on species interactions. While empirical approaches using large datasets and theoretical approaches such as the simulation of ecological networks have the potential to provide insight into general patterns and processes, the limited state of our understanding means that we must also continue to examine particular instances of environmental change affecting interactions, in order to better equip our models to encompass the complex multidimensionality of the effects of anthropogenic environmental change on ecological communities.

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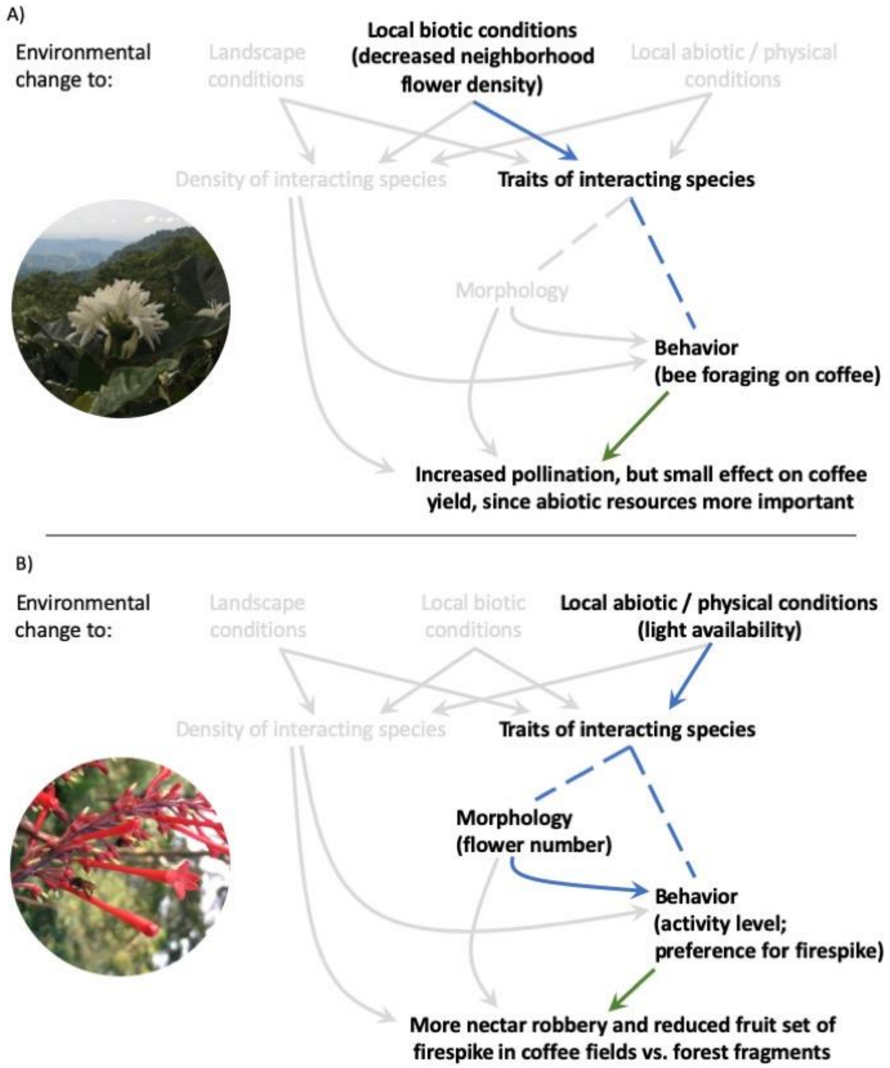
FIGURE

Figure 6.1. Paths from environmental change to interaction outcome for two bee-plant interactions. A) Weed and shade tree management practices influence neighbor interactions for pollination and abiotic resources in coffee (Chapter 2). B) Abiotic conditions influence the intensity and impact of nectar robbery by stingless bees of firespike (Chapters 3-5). Blue arrows indicate the effect of the environmental change on interacting species; green arrows indicate how species traits and /or densities influence interaction outcome.



Appendices

Appendix A. Supplementary tables and figures for Chapter 2

Table A.1. Assessment of spatial autocorrelation in coffee pollination, fruit set, and floral neighborhood measures. A p-value < 0.05 indicates significant spatial autocorrelation.

Variable	Moran's I		p
	Observed	Expected ± s.d.	
Coffee pollen load	–0.04	–0.05 ± 0.06	0.91
Heterospecific pollen load	–0.10	–0.05 ± 0.06	0.42
Pollen tubes	–0.13	–0.05 ± 0.06	0.18
Initial fruit set	–0.09	–0.05 ± 0.07	0.57
Final fruit set	–0.08	–0.05 ± 0.06	0.64
Coffee flower density	0.05	–0.05 ± 0.07	0.13
Herb layer flower density	–0.03	–0.05 ± 0.06	0.69
Canopy layer flower density	–0.04	–0.05 ± 0.05	0.92

Table A.2. Summary statistics for coffee pollination and neighborhood variables.

Variable	**Mean±s.e.**	**Median**	**Range**	**N**
Coffee pollen load (# of grains)	867±40	762	74–3777	293
Heterospecific pollen load (# of grains)	8±1	3	0–106	293
Pollen tubes	10±0.4	9	0–43	279
Initial fruit set (fruits flower^{-1})	0.49±0.02	0.49	0.10–0.94	128
Final fruit set (fruits flower^{-1})	0.29±0.02	0.25	0.02–0.80	113
Fruit weight (g fruit^{-1})	1.6±0.03	1.6	0.8–2.3	113
Coffee flowers m^{-2} (transect scale)	63±6	63	18–147	33
Herb layer flowers m^{-2} (transect scale)	23±6	6	0–153	33
Canopy layer flowers m^{-2} (transect scale)	43±22	0	0–534	33
Floral species richness (transect scale)	5±0.5	5	1–13	33
Canopy cover (%)	47.2±2.2	47.4	0.4–91.9	121
Distance to managed honey bee colony (m)	241.1±27.3	224.9	26.7–594.5	21

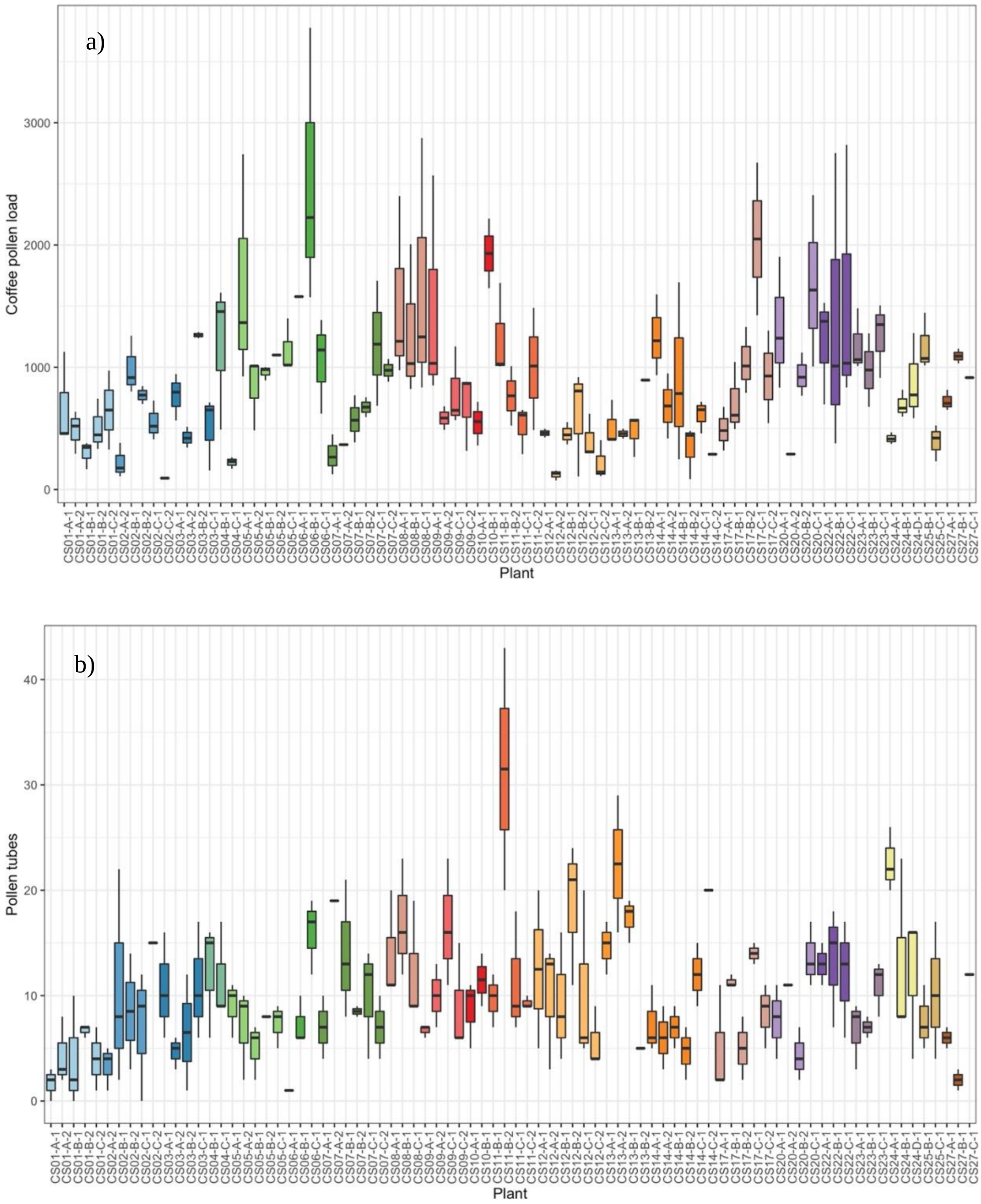
Table A.3. Structural equation model results, including nonsignificant covariates (distance to apiary and focal coffee plant height).

Response	Predictor	Std. Estimate	Estimate±s.e.	DF	Critical value	P
a) Transect scale						
log(Coffee pollen load)	Coffee flower density	-0.10	-0.002±0.004	191	-0.58	0.61
	Herb-layer flower density	-0.21	-0.004±0.002	191	-2.06	0.04
	Canopy-layer flower density	-0.17	-0.001±0.001	191	-2.11	0.04
	Distance to apiary	-0.03	0.000±0.001	19	-0.27	0.79
	Mass bloom event	-	-	1	3.42	0.06
Heterospecific pollen load	Coffee flower density	-0.07	-0.029±0.058	191	-0.49	0.62
	Herb-layer flower density	-0.02	-0.007±0.033	191	-0.22	0.83
	Canopy-layer flower density	0.09	0.010±0.009	191	1.19	0.24
	Distance to apiary	0.10	0.009±0.009	19	1.00	0.33
	Mass bloom event	-	-	1	2.23	0.14
b) 2m x 2m scale						
log(Coffee pollen load)	Coffee flower density	-0.25	-0.034±0.012	193	-2.99	0.003
	Herb-layer flower density	-0.06	-0.009±0.011	193	-0.89	0.37
	Canopy-layer flower density	-0.12	-0.005±0.003	193	-1.64	0.10
	Distance to apiary	-0.08	0.000±0.001	19	-0.64	0.53
	Mass bloom event	-	-	1	3.03	0.08
Heterospecific pollen load	Coffee flower density	-0.03	-0.083±0.223	193	-0.38	0.71
	Herb-layer flower density	0.09	0.240±0.201	193	1.20	0.23
	Canopy-layer flower density	0.13	0.095±0.053	193	1.80	0.07
	Distance to apiary	0.12	0.010±0.007	19	1.44	0.17
	Mass bloom event	-	-	1	4.73	0.03
Pollen tube number	log(Coffee pollen load)	0.02	0.164±0.615	195	0.27	0.79
	Heterospecific pollen load	-0.03	-0.012±0.030	195	-0.41	0.68

Initial fruit set	log(Coffee pollen load)	-0.05	-0.015±0.019	193	-0.82	0.41
	Heterospecific pollen load	0.08	0.001±0.001	193	1.37	0.17
	Pollen tube number	0.05	0.002±0.002	193	0.81	0.42
	Focal plant height	-0.12	0.000±0000	193	-1.51	0.13
Final Fruit set	log(Coffee pollen load)	0.13	0.035±0.012	188	2.91	0.004
	Pollen tube number	-0.10	-0.003±0.001	188	-2.63	0.009
	Initial fruit set	0.49	0.468±0.043	188	10.86	<0.001
	Canopy cover	0.23	0.002±0.001	188	3.64	<0.001
	Coffee flower density	0.00	0.000±0.002	188	0.08	0.93
	Herb-layer flower density	-0.08	-0.003±0.002	188	-1.61	0.11
	Canopy-layer flower density	-0.15	-0.002±0.001	188	-3.06	0.003
	Focal plant height	-0.07	0.000±0.000	188	-1.30	0.20
	Mass bloom event	-	-	1	5.40	0.00
Fruit weight	Pollen tube number	0.02	0.001±0.003	189	0.40	0.69
	Initial fruit set	0.29	0.450±0.092	189	4.92	<0.001
	Canopy cover	0.06	0.001±0.001	189	0.75	0.46
	Coffee flower density	-0.07	-0.004±0.004	189	-0.97	0.33
	Herb-layer flower density	-0.05	-0.003±0.004	189	-0.74	0.46
	Canopy-layer flower density	0.06	0.001±0.001	189	0.94	0.35
	Mass bloom event	-	-	1	0.76	0.38

Figure A.1. Images from the study area, illustrating the range of floral neighborhood conditions. A) Low heterospecific flower density, high conspecific flower density, low shade; B) high heterospecific flower density, low conspecific flower density, moderate shade.

Figure A.2. Within-plant and within-site variability in a) coffee pollen load and b) pollen tube number. Each box represents a single plant; fill color indicates site, with all plants from a given site grouped together.



Appendix B. Supplementary table and figures for Chapter 4

Table B.1. Correlation among *Odontonema cuspidatum* floral trait values (Pearson's r).

	Corolla mouth width	Corolla base width	Corolla flare	Corolla length
Corolla mouth width	1.0	0.41	0.17	0.3
Corolla base width		1.0	–0.08	–0.15
Corolla flare			1.0	0.38
Corolla length				1.0

Figure B.1. Photograph showing *Odontonema cuspidatum* inflorescence being nectar-robbed by the stingless bee *Trigona fulviventris* (black circle), and with perforation from previous robbing (white circle).

Figure B.2. Flow diagram of sampling scheme for naturally-occurring *O. cuspidatum* plants. Numbers indicate the number of plants in each category.