AN ABSTRACT OF THE DISSERTATION OF

Dustin G. Gannon for the degree of Doctor of Philosophy in Botany and Plant Pathology presented on March 17, 2022.

Title: Plant-Pollinator Interactions in a Changing World: Cryptic Specialization, Pollinator Movement, and Landscape Genetics of Pollinator-Dependent Plants

Abstract approved: ____

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Plant-pollinator mutualisms are one of the most prevalent and economically important mutualisms in nature. Like many other ecological systems, plant-pollinator communities are threatened by anthropogenic activity, both directly (e.g., habitat conversion and fragmentation) and indirectly (e.g., climate change). While we are aware of many of the activities that adversely impact these systems, further research is needed before we can predict how stable plant-pollinator communities are in the face disturbances. In my dissertation, I focused on: **1)** tests for cryptic pollinator specialization in plants of the Heliconiaceae that could mislead our predictions about the stability of the plant-pollinator interactions involving cryptic specialists; and **2)** how pollinator foraging movements relate to landscape characteristics and whether those relationships are reflected in the genetic structure of plant populations.

One important component for predicting the stability of plant-pollinator communities is the degree of partner specialization in the community. Generalist strategies, where polli-

nators forage at the flowers of many different plant species, each of which may be visited and pollinated by many pollinator species, result in partner redundancy and reduced dependency on any one mutualistic partner. Empirical estimates of specialization are usually constructed using observations of pollinators foraging at flowers; however, results from recent experiments with *Heliconia tortuosa* (Heliconiaceae) demonstrated that pollen tube germination was enhanced following visits from hummingbird pollinators whose bills match the shape of the flower but not visits from mismatched pollinators. Thus, despite perceived partner redundancy, *H. tortuosa* may be susceptible to coextinction following local extinctions of morphologically specialized pollinator species due to a cryptic pollinator filter and therefore cryptic specialization. If the capacity for plants to cryptically filter floral visitors based on pollinator traits is widespread, this would have implications for the robustness of many plant-pollinator communities. I tested for this 'pollinator recognition' behavior in three additional *Heliconia* species, *H. hirsuta*, *H. rostrata*, and *H. wagneriana*, spread widely across the Heliconiaceae phylogeny. Furthermore, I conducted experiments with *H. tortuosa* to test hypotheses about the mechanism of pollinator recognition and test the reproducibility of the finding that pollen tube success increases following visits from morphologically matched pollinators compared to visits from mismatched pollinators.

While I found little evidence to support that *H. hirsuta*, *H. rostrata*, or *H. wagneriana* preferentially invest in reproduction following visits by morphologically matched hummingbirds, results from my experiments are consistent with previous findings that single visits to *H. tortuosa* by pollen-free hummingbirds promote pollen tube success, particularly if the visiting hummingbird's bill matches the shape of the flower. However, the mechanism remains equivocal as results from my experiments did not support any of the hypothesized mechanisms of recognition. Still, successful pollen tube germination and growth in *H. tortuosa* appears strongly dependent on visits from morphologically matched hummingbird species that have been shown to be sensitive to forest fragmentation.

Habitat fragmentation results in reductions of habitat amount in a given landscape as well as alterations to the structure of habitat within a landscape (e.g., continuous or patchy). Pollinators that are sensitive to fragmentation may abandon or fail to discover small or disconnected patches of habitat, reducing pollen flow through the landscape and potentially increasing population genetic divergence among plants persisting in isolated patches. This has implications for the genetic diversity, population persistence, and adaptive potential of plant populations in the face of environmental change. In Chapter 3, I explored how forest encroachment into alpine meadows in the Cascade Mountains, USA, could influence Rufous Hummingbird (*Selasphorus rufus*) pollinator movement patterns through the landscape. Using subcutaneous Passive Integrated Transponders and arrays of artificial hummingbird feeders equipped with Radio Frequency Identification data loggers, I recorded the identities of birds and the time at which they visited feeders placed throughout a mixed cover landscape in the H. J. Andrews Experimental Forest, OR, USA. Using data from four summers, I estimated the frequency of movement between food resources.

The vast majority of movements were made by females which tend to be less dominant in territorial and competitive interactions. This suggests that subordinate individuals could be important to maintaining plant population connectivity. While the uncertainty in the estimates is relatively high due to few recording stations, I would expect the frequency of movements between two locations to decrease with increasing amounts of forest between them based on the fitted spacial network model. Furthermore, placing one or both feeders under coniferous forest canopy decreased the frequency of movements between the two feeders. This indicates that hummingbird foraging searches may be focused on open habitat with limited exploration into and across forested areas and has implications for pollen flow through the landscape if forest encroachment continues to shrink and fragment meadow habitat.

In Chapter 4, I tested for landscape genetic signatures in a population of an ornithophilous plant species, *Aquilegia formosa* (Ranunculaceae), that are consistent with isolation by forest cover. While I found evidence consistent with the hypothesis that plants growing beneath taller woody vegetation are less frequently discovered and visited by pollinators, I found the opposite of the genetic signature I would predict under the hypothesis of isolation by forest cover. I proposed that this unexpected result may be due to the spatial arrangement of resources from a pollinator's perspective. In a patchy landscape, a pollinator must travel among patches of resources that are separated in space while, in a continuous landscape, a pollinator may more easily forage from one plant to the next. Because of this difference, I expect that foraging in a patchy landscape could promote more frequent long-distance pollen dispersal.

The results from Chapters 3 and 4, when considered together, suggest that: **1)** in the initial stages of meadow fragmentation, the frequency of long-distance pollen dispersal could increase due to the nature of foraging in a patchy landscape; **2)** subordinate hummingbirds may be particularly important to maintaining connectivity of plant populations since they are more likely to be forced out of areas with prime resources and instead forage in patchy, marginal habitat; and **3)** in the later stages of forest encroachment as more plants are overgrown by taller woody vegetation, fewer plants will be discovered and visited by hummingbird pollinators. These plants should contribute less pollen to the pollen pool, potentially resulting in a decrease in effective population sizes.

Plant-pollinator communities are threatened on many fronts in this time of global change. My work adds to the body of scientific literature that aims to understand how plant-pollinator interactions may be disrupted by global change. In particular, I provide data lending insight into pollinator foraging behavior in fragmented landscapes and the implications for pollinator dependent plants as well as data on cryptic pollinator specialization in plants (or lack thereof). Furthermore, my work provides examples of applications of useful statistical methods (namely, spatial network models) that I believe are underutilized in ecology and evolution.

Plant-Pollinator Interactions in a Changing World: Cryptic Specialization, Pollinator Movement, and Landscape Genetics of Pollinator-Dependent Plants

by

Dustin G. Gannon

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I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Dustin G. Gannon, Author

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CONTRIBUTION OF AUTHORS

- Chapter 2: Dustin Gannon led pollination experiments, data analysis, and the initial draft of the manuscript and assisted with experimental design. Adam Hadley designed experiments and assisted with data collection. Urs Kormann and F. Andrew Jones assisted with data collection and experimental design. Matt Betts designed experiments and assisted with data collection. All authors contributed to critical review and revision of previous versions of the manuscript.

- Chapter 3: Adam Hadley and Sarah Frey conceived of and designed the study and led data collection. Dustin Gannon assisted with data collection, analyzed the data, and wrote the original draft of the manuscript. All authors contributed to critical review and revision of previous versions of the manuscript.

- Chapter 4: F. Andrew Jones and Adam Hadley conceived of the research. Dustin Gannon designed the sampling effort, collected and analyzed the data, and wrote the original draft of the manuscript. Yanming Di assisted with data analysis. All authors contributed to critical review and revision of previous versions of the manuscript.

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Chapter 1: Introduction

The diversity of floral forms has captivated biologists and the public alike for centuries. This immense diversity is credited, at least in part, to coevolution between plants and their pollinators (Crane et al., 1995; van der Niet & Johnson, 2012). As sessile organisms, plants have evolved myriad floral forms that attract and enhance the transfer of male gametes (pollen) to intermediary agents (pollinators) as they forage at flowers on resources such as the sucrose-rich floral nectar or the protein-rich pollen itself. Because floral resources are limited, pollinators must visit many flowers in any given day to meet their energetic requirements, thus inadvertently delivering pollen to the stigmas of geographically distant conspecific plants. This results in sexual reproduction for plants and has led to one of the most widespread and economically important mutualisms in nature. An estimated 87.5% of angiosperm species (c.a. 308,000 species) are pollinated by animals (Ollerton et al., 2011), and Klein et al. (2007) estimated that 80.5% of the world's leading food crops are moderately to completely dependent on animal pollinators for reproduction. Rodger et al. (2021) estimated that one-third of the planet's flowering plant species would fail to produce any seeds in the absence of pollinators while half would suffer at least an 80% reduction in seed/fruit production.

Anthropogenic activity can disrupt plant-pollinator interactions either directly (e.g., habitat conversion and fragmentation) or indirectly (e.g., climate change), potentially destabilizing plant and pollinator communities (Kremen & Ricketts, 2000; Biesmeijer et al., 2006;

Potts et al., 2010; Cameron et al., 2011; Potts et al., 2016; Rodger et al., 2021; Zattara & Aizen, 2021). Human-related disturbances that have been identified to impact at least some plant-pollinator systems include habitat fragmentation and conversion (e.g. Hadley et al., 2014), introductions of non-native plant and pollinator species (e.g. Valido et al., 2019), spread of novel pathogens (e.g. Camp et al., 2019), pesticide use, and climate change (Memmott et al., 2007; Goulson et al., 2015). The robustness of plant-pollinator communities to disturbance likely depends on a multitude of factors and further research is needed before we can predict how stable plant-pollinator communities are in the face of these threats.

In my dissertation, I focused on: **1)** tests for cryptic pollinator specialization in plants of the Heliconiaceae that could mislead our predictions about the stability of the plant-pollinator interactions involving cryptic specialists (see Chapter 2); and **2)** how pollinator foraging movements relate to landscape characteristics and whether those relationships are reflected in the genetic structure of plant populations (Chapters 3 & 4).

1.0.1 The structure of plant-pollinator interactions

The structure of interactions within a community can be defined more precisely when the plant-pollinator community is represented as a bipartite network (also called a bipartite *graph*) with nodes representing species and edges representing interactions between species (Jordano, 1987; Bascompte et al., 2003). Most pollinators exploit multiple species of flowering plants throughout the year, or even within a single foraging bout (Jordano, 1987; Waser et al., 1996; Brosi, 2016). Similarly, many plants have evolved floral forms that attract or may be exploited by multiple pollinator species (e.g. Schiestl et al., 2018), while others appear highly specialized to one or few pollinators (Waser & Ollerton, 2006; Brosi, 2016; Armbruster, 2017). Thus, there exists a spectrum from specialized to generalized players in plant-pollinator species interaction networks. This yields highly connected (as opposed to interactions within sub-groups but not among sub-groups of the community; Jordano, 1987) and asymmetric interaction networks, where a specialized species i of one guild often depends strongly on a generalized species j of the other guild, but j depends only weakly on i (Bascompte et al., 2006).

Generalist strategies in plant-pollinator communities result in greater connectedness of the network, which is directly related to the amount of partner redundancy in the community. High partner redundancy can theoretically buffer a species from the negative effects of local extinction of one of its mutualistic partners if the remaining partners are sufficient to maintain the mutualism (Waser et al., 1996; Memmott et al., 2004; Okuyama & Holland, 2008; Thébault & Fontaine, 2010; Kaiser-Bunbury et al., 2010). Furthermore, remaining pollinators may compensate the loss of a pollinator species by shifting their foraging behaviors to exploit the untapped floral resources that would have otherwise been used by the extinct pollinators. This 'rewiring' of the network is predicted to confer additional robustness to species loss (Kaiser-Bunbury et al., 2010).

Indeed, few empirical studies have attempted to test the theoretical predictions that plant-pollinator communities are robust to local extinction of species; however, results from those that have indicate that our understanding of these complex systems is underdeveloped. For example, upon removing a generalist pollinator, Brosi and Briggs (2013) found that pollinators rewired but the quality of pollination services diminished as pollen loads became mixed, increasing heterospecific pollen transfer and decreasing seed set. Similarly, Biella et al. (2020) sequentially removed four common plant species from a plant-pollinator community and compared the response to predictions based on models of coextinction. In the field experiment, community disassembly, including coextinctions and the disappearance of network edges (interactions between plant-pollinator species pairs), was more rapid and pronounced than predicted by theoretical models.

One mechanism for the discrepancy between theoretical predictions and empirical data on local extinctions in a plant-pollinator community is that not all pollinators contribute equally to pollination success (Schemske & Horvitz, 1984; King et al., 2013; Ballantyne et al., 2015; Betts et al., 2015) and not all flowers provide equally high quality resources to pollinators (Heil, 2011). Thus, plant-pollinator communities may not always be able to absorb the loss of a species through rewiring. For example, recent results from experiments with *Heliconia tortuosa* demonstrated that pollen tube germination was enhanced following visits from morphologically matched pollinators, but not mismatched pollinators after standardizeing pollen quality on the stigma and the number of visits by different pollinator species (Betts et al., 2015). Betts et al. (2015) coined this behavior ‘pollinator recognition’ and posited that pollinator recognition may be adaptive if it allows plants to preferentially invest in reproduction following visits from high-quality pollinators (those more likely to carry high quality, outcrossed or unrelated pollen).

In Chapter 2, coauthors and I describe our tests for pollinator recognition in three *Heliconia* species, *H. hirsuta*, *H. rostrata*, and *H. wagneriana*, spread widely across the Heliconiaceae phylogeny (Iles et al., 2016). Furthermore, we describe our efforts to reproduce the experiments by Betts et al. (2015) with *H. tortuosa* and test hypotheses about the mechanism of pollinator recognition in *H. tortuosa*. This line of scientific inquiry is important for two reasons. First, if abundances of morphologically matched pollinators were to decline in a given community with *H. tortuosa*, rewiring of the remaining pollinator community would not compensate for the loss of pollination services to *H. tortuosa*. Thus, despite an apparently connected network with many generalist species, *H. tortuosa* may be susceptible to coextinction due to a cryptic pollinator filter and therefore cryptic specialization. If the capacity for plants to actively filter floral visitors based on morphological trait matching is widespread, this would have implications for the robustness of many plant-pollinator communities under climate and anthropogenic change (Kaiser-Bunbury et al., 2010; Thébault & Fontaine, 2010). Second, pollinator recognition based on trait matching broadens the conditions under which we would expect trait matching to be maintained through evolutionary time (see Appendix A) and could be one way in which trait matching evolves despite apparently diffuse plant-pollinator interaction networks.

While we found little evidence to support that *H. hirsuta*, *H. rostrata*, or *H. wagneriana* preferentially invest in reproduction following visits by morphologically matched hummingbirds, we corroborated the results of Betts et al. (2015) that single visits to *H. tortuosa* by pollen-free hummingbirds promote pollen tube success if the visiting hummingbird's bill matches the shape of the flower. However, our results did not support the hypothesis that nectar removal is the proximate cue to which plants respond. The mechanism remains equivocal, but successful pollen tube germination and growth in *H. tortuosa* appears strongly dependent on visits from morphologically matched hummingbird species (Hadley et al., 2014; Betts et al., 2015, Chapter 2). This is likely an example of a cryptic pollinator filter that increases the dependence of *H. tortuosa* on a small subset of the pollinator community that is sensitive to forest fragmentation (Hadley & Betts, 2009; Kormann et al., 2016). Thus, the plant-pollinator community is likely less robust to species extinction than we would predict based on interaction networks (Betts et al., 2015) and theoretical models (Thébault & Fontaine, 2010; Kaiser-Bunbury et al., 2010).

1.0.2 Pollinator movement and connectivity of plant populations

Pollinator behavior has implications for rewiring a community as well as gene flow within and among plant populations. Thus, the effects of inherently spatial processes, such as habitat fragmentation, on plant populations are partially dependent on how pollinators respond. Studies of bees and hummingbirds, two important groups of pollinators, have shown that the structure of habitats across landscapes can influence foraging behavior (Hadley & Betts, 2009; Kormann et al., 2016; Cranmer et al., 2012), thereby determining subsequent pollen flow (Townsend & Levey, 2005; Van Geert et al., 2010; Kormann et al., 2016) and ultimately seed set (Hadley et al., 2014). These demographic processes ultimately influence plant population genetic structure (Breed et al., 2015; Torres-Vanegas et al., 2019; Torres-Vanegas et al., 2020), which has implications for genetic diversity, population persistence, and adaptive potential in the face of environmental change (Funk et al., 2018).

In Chapter 3, coauthors and I explored how forest encroachment into alpine meadows in the Cascade Mountains, USA, which is posited to be linked to changes in climate (Taylor, 1995; Coop & Givnish, 2007; Miller & Halpern, 2009) and has been observed to fragment and isolate meadow habitat (Dailey, 2008), could influence hummingbird movement patterns through the landscape (Gannon et al., 2021). Rufous Hummingbirds (*Selasphorus rufus*) are a common pollinator species in these systems and alterations to their foraging behaviors could influence pollen flow through the landscape. Using subcutaneous Passive Integrated Transponders and arrays of artificial hummingbird feeders equipped with Radio Frequency Identification data loggers to record the identities of birds and the time at which they visited feeders, coauthors and I estimated the frequency of movement between food resources in a mixed cover landscape in the H. J. Andrews Experimental Forest (HJA), OR, USA. We inferred relationships between the frequency with which birds moved from one feeder to another and characteristics of the landscape, including the local conditions at the feeders as well as the features of the intervening landscape.

We recorded 20,877 separate relocations of tagged hummingbirds and 2,221 movements between recording stations. Interestingly, the vast majority of movements were made by females which tend to be less dominant in territorial and competitive interactions (Carpenter et al., 1993), suggesting that subordinate individuals could be important to maintaining plant population connectivity. While the uncertainty in our estimates is relatively high due to few recording stations, we would expect the frequency of movements between two locations would decrease with increasing amounts of forest between them based on our fitted model. Furthermore, placing one or both feeders under coniferous forest canopy decreased the frequency of movements between the two feeders. This indicates that hummingbird foraging searches may be focused on open habitat with limited exploration into and across forested areas. This has implications for pollen flow through the landscape if forest encroachment continues to shrink and fragment meadow habitat in the Cascade Mountains.

As an additional test of our hypothesis that forest encroachment could reduce connectivity of alpine meadows, Chapter 4 describes our tests of whether there are landscape genetic signatures in a population of a common ornithophilous plant species, *A. formosa*, that are consistent with isolation by forest cover. If forested areas between meadows impede pollen movement, we would expect that plants in an isolated meadow would be more related (i.e., show greater genetic similarity) than plants in a connected meadow. This prediction follows from our hypothesis that pollen immigration into the isolated meadow should be low, resulting in primarily local mating. However, if we compare pairs of plants from different meadows that are separated by some distance d, we would expect that the genetic similarity between plants would decay more rapidly as we increase the distance between them (d) when at least one of the plants in the comparison is growing in an isolated meadow. This prediction follows from our hypothesis that pollen emigration from isolated locations should be low.

Interestingly, we found exactly the opposite relationships. We proposed that this unexpected result may be due to the spatial arrangement of resources from a pollinator's perspective. In a patchy landscape, a pollinator must travel among patches of resources that are separated in space while, in a continuous landscape, a pollinator may more easily forage from one plant to the next. We expect foraging in a continuous landscape in this manner could result in a pollen dispersal kernel that has a thinner tail than foraging among patches, which could promote more frequent long-distance dispersal and therefore a fatter tail in the dispersal kernel (i.e., greater excess kurtosis). However, we also found evidence consistent with the hypothesis that plants growing beneath taller woody vegetation are less frequently discovered and visited by pollinators. This was evidenced by greater genetic similarity among neighbors that were growing beneath forest canopy compared to those growing in the open and a rapid spatial decay of similarity moving away from plants beneath the forest canopy. We interpreted this as evidence of reduced pollen flow into and out of locations under the canopy accompanied by increased proportions of seeds produced through selfing and/or near-neighbor mating.

The results from Chapters 3 and 4, when considered together, suggest that: **1)** in the initial stages of meadow fragmentation due to forest encroachment, the frequency of long-distance pollen dispersal could increase due to the nature of foraging in a patchy landscape, increasing the excess kurtosis of pollen dispersal kernels; **2)** subordinate hummingbirds may be particularly important to maintaining connectivity of plant populations since they are more likely to be forced out of areas with prime resources and instead forage in patchy, marginal habitat (Carpenter et al., 1993); and **3)** in the later stages of forest encroachment as more plants are overgrown by taller woody vegetation, fewer plants will be discovered and visited by hummingbird pollinators. These plants should contribute less pollen to the pollen pool, potentially resulting in a decrease in effective population sizes.

Indeed, plant-pollinator communities, like so many other ecological communities, are threatened on many fronts. Empirical work to test the theoretical predictions that they should be robust to disturbance is in early stages. Here, I focus on the implications of a recently described plant behavior that, if common among plant taxa, could mean that the true potential for coextinctions in plant-pollinator communities is greater than that predicted based on plant-pollinator species interaction data. I then focus on how habitat configuration within a landscape may influence a pollinator species' foraging patterns which may, in turn, influence gene flow among (sub)populations of the plants it pollinates. This work adds to the body of scientific literature that aims to understand how plant-pollinator interactions may be disrupted by global change.

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Chapter 2: Testing for pollinator recognition in multiple species of *Heliconia*

2.1 Abstract

Many plants have evolved floral traits that reduce visitation by a subset of floral visitors, promoting pollination by efficient pollinators. However, results from recent experiments with *Heliconia tortuosa* indicate that an additional filter may exist after pollinator visitation. Pollen tube germination was enhanced following visits from morphologically matched pollinators, but not mismatched pollinators after standardizing pollen quality and visitation rates. This plant behavior, 'pollinator recognition' (PR), is thought to promote outcrossing by conferring the capacity to preferentially invest in reproduction following visits by long-ranging hummingbirds with specialized bills. To date, PR has only been described in *H. tortuosa*, but it could have important ecological implications if common among plant species; if abundances of morphologically matched pollinators decline, visits by mismatched pollinators would not compensate for the loss of pollination services to plants with PR. We therefore tested for PR in four taxa spread widely across the Heliconiaceae.

Using aviary experiments that standardized pollen quality and minimized variation in pollen quantity, we replicated previous findings that visits by pollen-free long-billed hummingbirds increased pollen tube counts in *H. tortuosa* styles compared to short-billed hummingbirds and hand pollination alone. For *H. rostrata*, we found some evidence that pollen tubes counts were enhanced in flowers visited by pollen-free hummingbirds compared to hand pollination alone, but regardless of the bird's bill morphology. For *H. hirsuta* and

H. wagneriana, hummingbird visits decreased pollen tube counts compared to hand pollinations. Furthermore, we did not substantiate any specific mechanism for PR. Together, our results highlight the poorly understood complexities of pollination in the Heliconiaceae and that further work is needed to confirm PR in *Heliconia* and understand the mechanism underlying the consistent finding that pollen tube success in *H. tortuosa* appears dependent on visits by morphologically matched hummingbirds.

2.2 Introduction

Pollinator filters are floral traits that manipulate animal visitation patterns to promote conspecific pollen transfer or limit access to floral rewards (e.g., nectar) for poor pollinators. For example, nectar that is distasteful to some pollinators will deter them from visiting (Stevenson et al., 2017) and exploitation barriers, such as long corolla tubes that limit access to nectaries (Gill, 1987; Temeles et al., 2009), may make visitation unprofitable for some animals (Rodríguez-Gironés et al., 2015; Temeles et al., 2019). These filters reduce the richness of pollinator species (or groups within species) that visit a given flower species or group. However, previous work with *Heliconia tortuosa* (Heliconiaceae) documented a cryptic pollinator filter that could act at a stage after the pollinator visit to promote pollen germination and pollen tube growth (Betts et al., 2015).

In single-visit aviary experiments that controlled for variation in pollen deposition and visitation rates by different pollinator species (Betts et al., 2015), the average number of pollen tubes that germinated in a style (henceforth ‘pollen tube rate’) was nearly six times greater in flowers visited by hummingbirds with bill shapes that are morphologically matched to the flowers (i.e., long, decurved bills) than in flowers visited by hummingbirds with mismatched bill shapes. Betts et al. (2015) coined this behavior 'pollinator recognition'. Furthermore, in a separate experiment, manual nectar removal showed higher pollen tube rates than hand pollination alone. Since long-billed hummingbirds are able to extract more nectar than short-billed species (Stiles, 1975; Betts et al., 2015), Betts et al. (2015) reasoned that nectar extraction is the proximate cue used by *H. tortuosa* to distinguish long- versus short-billed hummingbirds.

Betts et al. (2015) speculated that pollinator recognition may be adaptive if it allows plants to invest in reproduction following visits from high-quality pollinators (those more likely to carry high-quality, outcrossed or unrelated pollen) and limit reproduction with the pollen deposited by poor pollinators (those more likely to carry low-quality pollen). Despite receiving visits from at least six hummingbird species, *H. tortuosa* specializes on long-billed hummingbirds that are highly mobile (Stiles & Wolf, 1970; Betts et al., 2015) compared to the short-billed hummingbirds which tend to defend territories and therefore move less. The mobile foraging behaviors of these birds may make them more likely to carry high-quality pollen from geographically and genetically distant sources (Wright, 1943), thus promoting outcrossing and genetic diversity in *H. tortuosa* populations.

We postulated that pollinator recognition may occur in other plant taxa, particularly in relatively stable tropical systems with high pollinator diversity. This is based on reports from many *Heliconia* breeders and horticulturalists of failed hand-pollination attempts in the absence of hummingbirds (C. Black, *personal communication*). Furthermore, Pedersen and Kress (1999) reported that mean pollen tube counts were approximately four times higher in *H. paka* flowers that were visited by honey creepers (morphological match) than in flowers pollinated by hand (lack of morphological match). Because *H. paka* shares a common ancestor with *H. tortuosa* at the root of the Heliconiaceae phylogeny (Iles et al., 2016), we speculated that pollinator recognition, if heritable, could be a basal trait and therefore potentially present in more than one *Heliconia* species.

If the pollinator recognition behavior exists as a cryptic pollinator filter allowing plants to specialize on traplining hummingbirds with morphologically specialized bill shapes, we predict it should most likely be present in *Heliconia* species with long and/or curved corollas and are visited by many potential pollinator species with varying morphologies. Determining whether this is the case is important for two reasons. First, we agree with Betts et al. (2015) that filtering the short-billed, territorial hummingbirds could promote outcrossing and enhance the genetic diversity of pollen grains that reach the ovules, a hypothesis that is supported by landscape genetic studies of *H. tortuosa* populations in Costa Rica (Torres-Vanegas et al., 2019; Torres-Vanegas et al., 2020). Given the potential for fitness benefits, pollinator recognition could be present in many related taxa and could expand the conditions under which we expect morphological trait matching to evolve and be maintained (Appendix A). Indeed, hummingbird-*Heliconia* systems are often used as outstanding examples of morphological matching (e.g., Temeles & Kress, 2003).

Second, if the capacity for plants to actively filter floral visitors based on morphological trait matching is widespread, this would have implications for the robustness of plant-pollinator communities under climate and anthropogenic change (Kaiser-Bunbury et al., 2010; Thébault & Fontaine, 2010). Given local extinction or reduced densities of morphologically matched pollinators, mismatched pollinators may alter their foraging behaviors to exploit newly available resources (Brosi & Briggs, 2013; Temeles et al., 2016; Bezemer et al., 2019; Temeles & Bishop, 2019); however, visits from mismatched pollinators would not compensate for the pollination services lost to a plant with a pollinator recognition mechanism, even if mismatched visitors deposit pollen at the stigma. This could increase the likelihood of coextinctions (Rezende et al., 2007; Kaiser-Bunbury et al., 2010). Thus, we sought to test for pollinator recognition in three species distributed widely across the Heliconiaceae phylogeny (Iles et al., 2016) as a first step in assessing the potential for generality of this cryptic pollinator filter. Furthermore, because so little is known of this unusual plant behavior, we also sought to replicate the results of the original study for *H. tortuosa*, an exercise rarely undertaken in experimental ecology (Fraser et al., 2020).

2.3 Materials and Methods

2.3.1 Study species

Heliconiaceae is a monogeneric family consisting of an estimated 200-250 species which radiated rapidly c.a. 39-24 million years ago (Iles et al., 2016). *Heliconia* species are rhizomatous perennial herbs distributed widely throughout the Neotropics and on some South Pacific islands. Flowers are situated within showy bracts and composed of six tepals, five of which are fused to create a cylindrical perianth, the sixth peels back upon anthesis. A defining feature of the Heliconiaceae is a staminode (modified stamen) that partially covers the opening to the nectar chamber at the base of the perianth, which may need to be moved by a visiting animal when they extract the nectar reward (though the mechanics of this have not be studied in detail). Flowers of the Heliconiaceae last a single day from anthesis to dehiscence.

In addition to the native *H. tortuosa* which is common in the understory of forest fragments in Coto Brus, Costa Rica, we targeted species that were common in the living collection at the Organization of Tropical Studies Las Cruces Biological Station, Puntarenas Province, Coto Brus, Costa Rica, ($8^\circ\ 47'\ 7''$ N, $82^\circ\ 57'\ 32''$ W) or could be found in ornamental gardens in the area. We required that plants were setting seed when left unmanipulated, indicating that a viable pollen source existed in the area, since previous work on mating systems in Heliconia suggests that the hermaphroditic flowers of many species are self-incompatible to partially self-compatible, but largely not selfing (Kress, 1983a; Pedersen & Kress, 1999; Schleuning et al., 2011; Betts et al., 2015; Torres-Vanegas et al., 2019; Janeček et al., 2020). Furthermore, we required that wild, native hummingbirds could be seen visiting the flowers of each target species in camera trap data (Gannon et al., 2018) or during observation, indicating that wild-caught hummingbirds would visit and drink from the flowers inside aviaries despite the fact that many plant species in the collection are not native to Costa Rica. The plant species that met these criteria included *H. hirsuta*, which is native to South America and Trinidad (Bernal et al., 2016), *H. rostrata*, native to western South America (Bernal et al., 2016) but a common ornamental throughout the tropics, and *H. wagneriana*, native to Costa Rica and Panama (Stiles, 1979).

We selected two hummingbird species with different bill morphologies and foraging behaviors as “treatments” in order to accentuate differences in morphological matching to and nectar depletion from the range of flower shapes exemplified by the four *Heliconia* species (Figure 2.1). Green Hermit Hummingbirds (*Phaethornis guy*; GREH) are common traplining hummingbirds in the region with long ($\bar{x} = 41.90$ mm, $s_x = 1.52$ mm), moderately decurved bills ($\bar{k} = 0.022$ mm^{-1}, $s_k = 0.004$ mm^{-1}, $n = 27$ birds of mixed sex, where k_i is the curvature of the i^{th} bill measured as the inverse of the radius of the arc of the bill – see Temeles et al. (2009)). Rufous-tailed Hummingbirds (*Amazilia tzacatl*; RTAH) are common territorial hummingbirds with short ($\bar{x} = 21.60$ mm, $s_x = 1.55$ mm, $n = 14$ birds of mixed sex), slightly decurved bills ($\bar{k} = 0.016$ mm^{-1}, $s_k = 0.002$ mm^{-1}; Figure 2.1).

If morphologically matched floral visitors increase the numbers of successful pollen tubes for all *Heliconia* species, we would predict the following: 1) For *H. wagneriana* and *H. tortuosa*, we would predict greater numbers of pollen tubes in flowers visited by Green Hermit Hummingbirds compared to Rufous-Tailed hummingbirds due to long and curved flowers (Figure 2.1). 2) Because both *H. hirsuta* and *H. rostrata* have shorter, straighter flowers and both hummingbird bill shapes approximate the shape of the flowers well (Figure 2.1), we would not predict a large difference in the number of pollen tubes between flowers visited by Green Hermits and those visited by Rufous-Tailed Hummingbirds. We therefore used hand pollinations as a control treatment in all experiments since hand pollinations do not replicate the physical characteristics of a visit by a morphologically matched pollinator aside from pollen deposition. Hence, we would predict the fewest pollen tubes in flowers pollinated by hand for all plant species. Furthermore, this helped us control for potentially low genetic diversity in the pollen pool since the control flowers (hand pollination only) and the treatment flowers (hand pollination followed by a visit from a pollen-free hummingbird) both received pollen by hand from a donor in the same stock of plants used for all experiments.

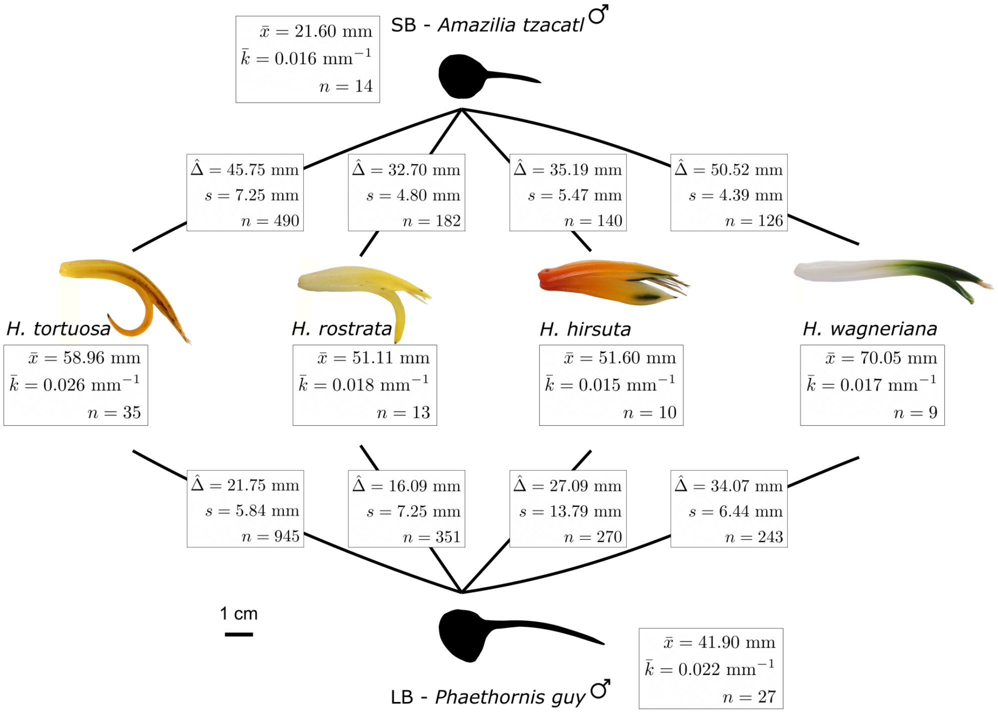

Figure 2.1: Morphological mismatch between the hummingbird and *Heliconia* species used in experiments. The average mismatch ($\hat{\Delta}$) between a plant and hummingbird species was measured as the Euclidean distance between a flower and a bird's bill in the $\mathbb{R}^2_+$ trait space, where one axis was the total length of a bill or flower (mm) and the other was the radius of the arc along the outside edge of the flower or bill (mm). We then computed the mean ($\hat{\Delta}$) and standard deviation (s) of the distances between each bill-flower pair, where n is the number of pairwise comparisons. Average bill or flower lengths ($\bar{x}$ mm) and curvatures ($\bar{k}$ mm^{-1}) (see Temeles et al., 2009) for each species are also included.

2.3.2 Aviary experiments

To test whether pollen germination and tube growth is dependent on interactions with morphologically matched floral visitors, we conducted 110 single-visit experiments ($n = 214$ flowers from 54 plants; see Table 2.1 for the number of replicates per treatment) with captive hummingbirds inside portable aviaries. The aviaries measured 2 meters tall and one meter on a side and could be quickly assembled around live plants (Appendix B). In these experiments, we used only virgin flowers that had been covered with mesh bags prior to anthesis in order to preclude pollination by free-ranging pollinators. Flowers were not emasculated, however, due to low numbers of pollen tubes in emasculated flowers in earlier experiments with plants in aviaries and in natural settings (M. G. Betts and A. S. Hadley, *unpublished data*).

We selected inflorescences based on the availability of two virgin flowers and erected the aviary around the whole plant. We then randomly assigned one of the flowers as a control flower that received hand-pollination but no visit from a bird (HP treatment). The remaining flower was hand-pollinated with pollen from the same donor flower, then allowed a visit by either a pollen-free short-billed hummingbird (SB treatment; $n = 14$ *A. tzacatl* individuals used in experiments) or a pollen free long-billed hummingbird (LB treatment; $n = 12$ *P. guy* individuals used in experiments). To ensure the birds were free of pollen before the visit to the focal flower, we cleaned them using a soft paint brush and damp cotton swab under 20x magnification prior to releasing them into the aviary. Thus, flowers were the experimental units and individual plants were treated as a blocking factor to account for potential dependence among measurements on flowers from the same plant. Where possible, plants received all treatments.

Table 2.1: Sample sizes for individual plants (grouping factor) and flowers (experimental units) for each species × treatment combination.

	HP*	**SB**	**LB**	**HP****	**BM**	**HPNE**	**NEHP**
H. hirsuta							
Plants	7	4	5	0	0	0	0
Flowers	11	5	7	0	0	0	0
H. rostrata							
Plants	19	13	10	13	7	15	5
Flowers	39	25	19	24	8	38	11
H. tortuosa							
Plants	10	8	6	10	7	10	4
Flowers	16	10	11	27	7	36	5
H. wagneriana							
Plants	12	6	11	11	7	8	4
Flowers	31	17	23	17	7	12	10

*Hand-pollination control group for aviary experiments.

**Hand-pollination control group for mechanism experiments.

By hand-pollinating all flowers, we were able to control for differences in the quality of pollen delivered by the different pollinator species. Indeed, we could not perfectly standardize the quantity of pollen grains at the stigmatic surface because the size of *Heliconia* pollen grains makes it impractical to quantify the number of grains in the field; however, we attempted to reduce variation in the quantity of pollen available to the flowers by having the same experimenter apply pollen in an even layer across the stigmatic surface with a toothpick under 20x magnification for every flower.

After the hummingbird visited the treatment flower (evidenced by bill insertion and a clear attempt to feed from the flower), we terminated the experiment and checked the stigma again to ensure that pollen was still present and in an even layer on the stigmatic surface before again covering the flowers with mesh bags. All flowers were collected the following day, the styles removed and preserved in formalin acetyl-acid, and scored for pollen tubes using epi-florescence microscopy (Kress, 1983a; Betts et al., 2015) (see Appendix B for more information). All aviary experiments were conducted during the 2018 and 2019 dry seasons (Feb-Mar).

2.3.3 Tests for a mechanism

We conducted additional experiments to test hypotheses of the mechanism of pollinator recognition. Betts et al. (2015) found increased pollen tube rates in flowers from which nectar was removed compared to hand pollination alone. As an independent test of whether nectar removal provides a cue to which plants respond, we manually extracted nectar from flowers of three of the four species (*H. hirsuta* did not produce flowers regularly enough to conduct the full suite of experiments) and compared pollen tube rates to control flowers that were hand-pollinated on the same day.

Alternatively, it is possible that the long-billed hummingbirds trigger a mechanical cue (Braam, 2005) when they insert their bills into the flower. To test whether we could induce an increase in pollen tube success rates using a mechanical stimulus, we moulded a pipette tip to match the curvature of the focal flower. We then inserted the pipette tip as a hummingbird would insert its bill but did not remove any nectar. Finally, because we were unable to perfectly replicate the timing of events in a natural pollinator visit in which nectar removal and pollen deposition happen concurrently, we conducted some experiments in which we hand pollinated before manually removing nectar and some in which we hand pollinated after removing nectar. Differences in these pollen tube rates may indicate the importance of the timing of pollen transfer and nectar removal or bill insertion (see Appendix B for more detail).

2.3.4 Statistical methods

We analyzed pollen tube count data from each plant species and each set of experiments separately (i.e., aviary experiments as one dataset and nectar removal experiments as a second dataset). We fit generalized linear mixed models (GLMMs) to our data using the R package `lme4` (Bates et al., 2015; Team, 2020) assuming pollen tube counts follow a Poisson distribution with a mean dependent on the treatment (treatments described above) and random intercepts for each plant (see Appendix B for full details). Since we scored pollen tubes in multiple flowers from the same plant, observations originating from the same plant could be correlated. We chose to analyze the data from each species separately such that the random intercepts for a plant of species i and those of species j are not assumed to be identically distributed (i.e., variance among plants may differ among species).

Below, we report maximum likelihood estimates of mean pollen tube counts per style (pollen tube rates) for a given treatment and plant species. Confidence intervals for the true pollen tube rates follow each estimate in square brackets. The estimates of the standard errors used to compute the confidence intervals include the variance of the random intercepts (computed as $\widehat{SE} = \sqrt{\hat{\sigma}^2_{\text{pred}} + \hat{\sigma}^2_{\text{plant}}}$ where $\hat{\sigma}^2_{\text{pred}}$ is the variance of the predicted value and $\hat{\sigma}^2_{\text{plant}}$ is the estimate of the variance of the random intercepts). We denote estimated pollen tube rates as $\hat{\lambda}$ with a subscript indicating the treatment and plant species. We use HP, SB, LB, HPNE, NEHP, and BM to indicate the treatment (Table 2.1). Treatment codes are as follows: HP identifies the hand-pollinated control flowers; SB indicates the treatment in which we hand pollinated flowers, then allowed a clean, Rufous-Tailed Hummingbird (short, straight bill) to visit; LB indicates the treatment in which we hand pollinated flowers, then allowed a pollen-free Green Hermit Hummingbird (long bill) to visit; HPNE identifies the treatment in which we hand pollinated the flowers then manually removed the nectar; NEHP identifies the treatment in which we hand pollinated the flowers after removing nectar; and BM identifies the treatment in which we inserted a pipette tip but did not attempt to remove nectar. We use the letters h, r, t, and w to identify *H. hirstuta*, *H. rostrata*, *H. tortuosa*, and *H. wagneriana* in the subscripts (respectively).

We additionally report differences between treatments (contrasts) on the log-link scale and use the notation $\hat{\beta}_{i,j}$ to denote the difference between treatment i and the control (hand pollination) for a given plant of species j. Exponentiating this value gives the fold change (or multiplier) in pollen tube rates between the control treatment and treatment i for species j. We consider contrast estimates of $|\hat{\beta}_{i,j}| \geq 0.5$, which reflect differences between treatments where the estimated pollen tube rate in one treatment is at least 1.5 times larger than the other, as potentially biologically interesting. All hypothesis tests for regression coefficients were likelihood-ratio tests in which the nested model included a constraint on one or more of the regression coefficients. We report results from all statistical tests conducted in Tables 2.2 and 2.3.

2.4 Results

2.4.1 Aviary experiments

When we compared pollen tube counts in hand-pollinated control flowers (HP) to those that were visited by a pollen-free, long-billed hummingbird, we found some evidence that a visit by a long-billed hummingbird increased pollen tube rates for *H. tortuosa* over hand pollination alone (Figure 2.2). Pollen tube rates were estimated to be 6.91 [1.78, 26.80] (estimate and 95% confidence interval) times greater on average following visits from long-billed hummingbirds compared to the control treatments with only hand pollination ($\hat{\lambda}_{HP,t} = 0.13\ [0.01,\ 1.45]$ tubes per style; $\hat{\lambda}_{LB,t} = 0.87\ [0.09,\ 8.79]$ tubes per style). While the estimated pollen tube rate in *H. tortuosa* flowers that were visited by pollen-free short-billed hummingbirds was nearly double the point estimate for hand pollination alone (1.88 times greater [0.39, 9.12]; $\hat{\lambda}_{SB,t} = 0.24\ [0.02,\ 2.77]$ tubes per style; Figure 2.2), the estimated effect of a visit by a short-billed hummingbird was considerably smaller than that of a visit by a long-billed hummingbird. The pollen tube rate in flowers visited by long-billed hummingbirds was estimated to be 3.32 [1.02, 13.23] times greater on average than the pollen tube rate in flowers visited by short-billed hummingbirds (Figures 2.1 & 2.2; Table 2.2). Note that the uncertainty in these estimates is high due to relatively small sample sizes and large variance among plants ($\hat{\sigma}^2_{\text{plant}} = 0.95$).

In *H. rostrata* flowers, there was some evidence that pollen tube rates in those visited by hummingbirds are on average greater than hand pollination alone regardless of the bird species used in experiments (Figure 2.2; Table 2.2). The point estimates for pollen tube rates were similar in flowers visited by long-billed hummingbirds and those visited by short-billed hummingbirds ($\hat{\lambda}_{SB,r} = 0.76\ [0.38,\ 1.53]$ tubes per style; $\hat{\lambda}_{LB,r} = 0.83\ [0.39,\ 1.75]$ tubes per style; Figure 2.2), but were approximately double the estimated rate in hand pollinated controls ($\hat{\lambda}_{HP,r} = 0.41\ [0.20,\ 0.85]$ tubes per style).

For *H. hirsuta*, we found very little evidence to suggest that single visits from cleaned hummingbirds enhance pollen tube success rates above hand pollination alone (Figure 2.2; Table 2.2). In fact, the point estimate of pollen tube rates in flowers visited by long-billed hummingbirds ($\hat{\lambda}_{LB,h} = 0.60\ [0.10,\ 3.60]$) was lower than the estimate of pollen tube rates in flowers from the hand pollination only treatments ($\hat{\lambda}_{HP,h} = 1.06\ [0.31,\ 3.58]$) as well as the flowers that were visited by short-billed birds ($\hat{\lambda}_{SB,h} = 1.19\ [0.23,\ 6.30]$) (Figure 2.2). We also found some evidence of reduced pollen tube rates in *H. wagneriana* flowers that were visited by clean birds, regardless of the bird's morphology (Figure 2.2; Table 2.2). The point estimate for the number of pollen tubes per style in *H. wagneriana* flowers visited by pollen-free Green Hermits was a little more than half that of hand pollinations (0.62 [0.36, 1.06] times the hand pollination rate for a given plant), while the estimate for flowers visited by pollen-free short-

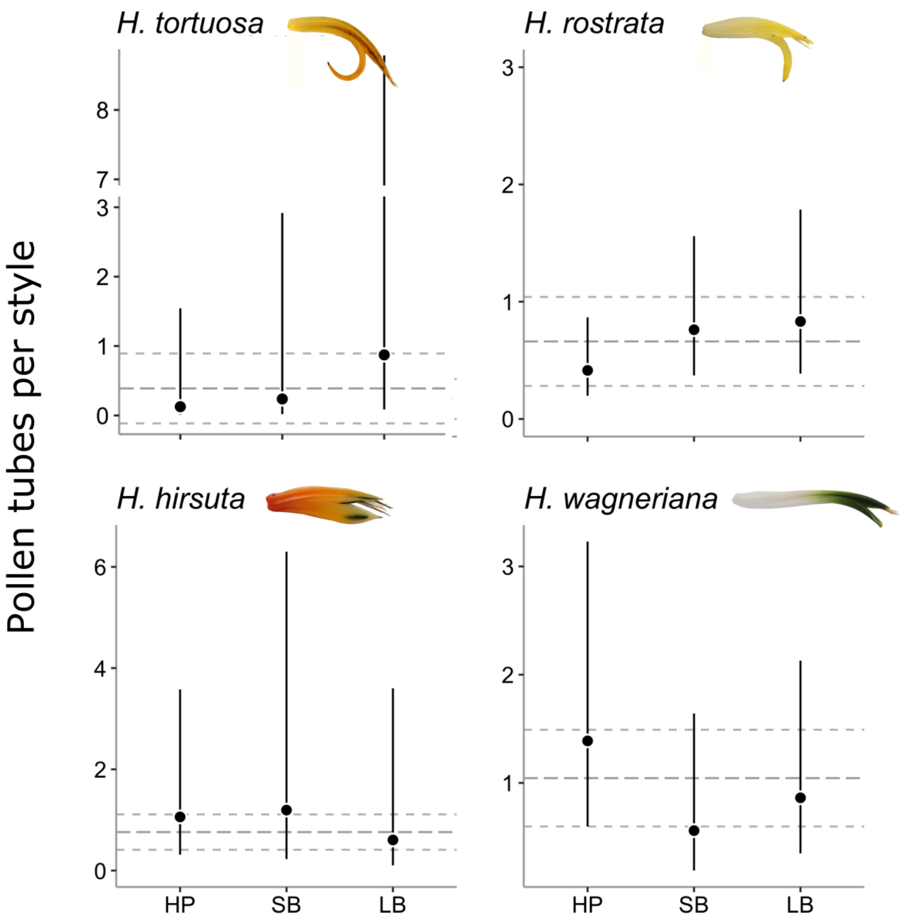

Figure 2.2: Pollen tube rates in flowers that received hand pollination only (HP) and those visited by a pollen-free hummingbird following hand pollination. Flowers were visited by either short-billed (SB) Rufous-tailed Hummingbirds (*Amazilia tzacatl*) or long-billed (LB) Green Hermit Hummingbirds (*Phaethornis guy*). Note that short-billed hummingbirds were consistently more morphologically mismatched with flowers than long-billed hummingbirds. Error bars show approximate 95% confidence intervals (estimate $\pm 2\widehat{SE}$). We estimated the standard error (on the link scale) as $\widehat{SE} = \sqrt{\hat{\sigma}^2_{\text{pred}} + \hat{\sigma}^2_{\text{plant}}}$ where $\hat{\sigma}^2_{\text{pred}}$ is the variance of the predicted value and $\hat{\sigma}^2_{\text{plant}}$ is the estimate of the variance of the random intercepts. The grey, horizontal dashed lines show estimates ($\pm SE$) of the pollen tube rates in flowers left open to free ranging pollinators.

Table 2.2: Summary of tests of contrasts between treatments from the aviary experiments. The estimates of the difference and the standard errors are on the log link scale, so exponentiating the estimated difference between treatments gives the multiplier on the response scale (number of pollen tubes). Chi-squared statistics were computed from likelihood ratio tests. P_{FDR} denotes the P-values adjusted for a false discovery rate of 0.05.

Species	Contrast	Estimate	$\widehat{SE}$	χ^2_1	P	P_{FDR}
H. hirsuta	SB – HP	0.06	0.51	0.01	0.905	0.905
	LB – HP	-0.62	0.55	1.36	0.244	0.418
	LB – SB	-0.68	0.66	1.09	0.297	0.420
H. rostrata	SB – HP	0.61	0.34	3.15	0.076	0.154
	LB – HP	0.70	0.36	3.55	0.060	0.154
	LB – SB	0.09	0.36	0.06	0.806	0.880
H. tortuosa	SB – HP	0.63	0.79	0.66	0.418	0.502
	LB – HP	1.93	0.68	10.89	0.001	0.012
	LB – SB	1.30	0.65	4.86	0.028	0.112
H. wagneriana	SB – HP	-0.91	0.40	6.07	0.014	0.084
	LB – HP	-0.48	0.27	3.13	0.077	0.154
	LB – SB	0.43	0.44	1.01	0.315	0.420

billed hummingbirds is less than half (0.40 [0.18, 0.90] times the hand pollination rate for a given plant) that of hand pollination treatments ($\hat{\lambda}_{HP,w} = 1.39$ [0.60, 3.23] tubes per style; $\hat{\lambda}_{LB,w} = 0.86$ [0.35, 2.13] tubes per style; $\hat{\lambda}_{SB,w} = 0.56$ [0.19, 1.64] tubes per style; Figure 2.2).

2.4.2 Tests for a mechanism

When we experimentally removed nectar using pipette tips to test the hypothesis that differential nectar removal may be the mechanism for pollinator recognition, the estimated

Table 2.3: Summary of contrasts between treatments testing a hypothesized mechanism and the control (hand pollination only). The estimate of the difference is on the log link scale, so exponentiating the estimate gives the multiplier (how many times greater than the control) on the response scale (number of pollen tubes). Chi-squared statistics were computed from likelihood ratio tests. P_{FDR} denotes the P-values adjusted for a false discovery rate of 0.05.

Species	Contrast	Estimate	$\widehat{SE}$	χ^2_1	P	P_{FDR}
H. rostrata	BM – HP	-0.41	0.78	0.29	0.589	0.799
	HPNE – HP	-0.26	0.45	0.33	0.567	0.799
	NEHP – HP	-1.42	1.05	2.60	0.107	0.492
H. tortuosa	BM – HP	0.10	0.69	0.02	0.881	0.880
	HPNE – HP	0.17	0.45	0.14	0.710	0.799
	NEHP – HP	-0.47	1.06	0.22	0.641	0.799
H. wagneriana	BM – HP	0.20	0.45	0.20	0.657	0.799
	HPNE – HP	0.56	0.42	0.19	0.164	0.492
	NEHP – HP	-0.92	0.67	2.22	0.136	0.492

effect of nectar removal on pollen tube rates for *H. rostrata* and *H. tortuosa* was small ($\hat{\beta}_{HPNE,t} = 0.08$, $\widehat{SE} = 0.43$; $\hat{\beta}_{HPNE,r} = -0.26$, $\widehat{SE}_{HPNE,r} = 0.45$; Figure 2.3). For *H. wagneriana*, the estimated effect of nectar removal on pollen tube rates was positive and of a considerable magnitude ($\hat{\beta}_{HPNE,w} = 0.58$), but uncertainty in the estimate was high ($\widehat{SE}_{HPNE,w} = 0.42$; Figure 2.3).

Inserting a hummingbird bill mimic (i.e., pipette tip) into flowers as a mechanical signal without removing nectar also did not induce substantially higher pollen tube rates in any of the tested species (Tables 2.3; Figure 2.3). In all cases, hand pollinating flowers after removing nectar resulted in the fewest pollen tubes per style out of all treatments, but uncertainty in these differences was high (Table 2.3); Figure 2.3).

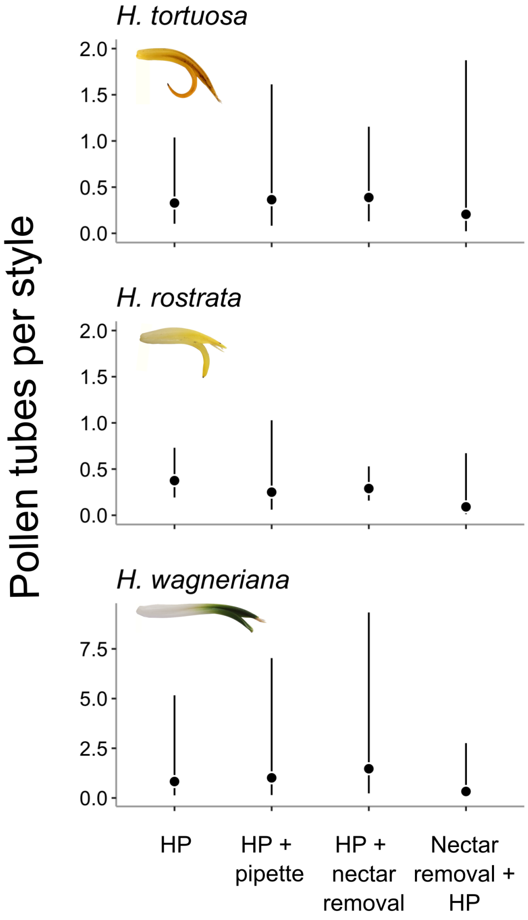

Figure 2.3: Results from experimental tests of the effect of nectar depletion on pollen tube rates. We used hand-pollination (HP) as a control treatment and compared pollen tube rates in flowers that received the control treatment to those in flowers that received outcross pollen by hand either before (HP + nectar extraction) or after (Nectar extraction + HP) manual removal of the nectar in the flower. As a test of whether pollen germination success could be boosted after the mechanical stimulus of a hummingbird inserting its bill to drink from the flower, we tested for an effect of pipette insertion without removing any nectar (HP + pipette). Error bars show 95% confidence intervals that include the variance of the random effects.

Table 2.4: Point estimates and approximate confidence intervals for pollen tube rates in each species for each treatment in the nectar removal experiments. HP denotes hand pollination, BM is hand pollination followed by the insertion of a pipette tip (bill mimic), HPNE is hand pollination followed by nectar removal, and NEHP denotes nectar removal followed by hand pollination. The standard errors used to compute confidence intervals were estimated as $\widehat{SE} = \sqrt{\hat{\sigma}^2_{\text{pred}} + \hat{\sigma}^2_{\text{plant}}}$ where $\hat{\sigma}^2_{\text{pred}}$ is the variance of the predicted value and $\hat{\sigma}^2_{\text{plant}}$ is the estimate of the variance among plants (random intercepts).

Species	Treatment	Estimate ($\hat{\lambda}$)	2.5%	97.5%
H. rostrata	HP	0.38	0.19	0.73
	BM	0.25	0.06	1.03
	HPNE	0.29	0.16	0.53
	NEHP	0.09	0.01	0.67
H. tortuosa	HP	0.32	0.10	1.11
	BM	0.36	0.07	1.77
	HPNE	0.39	0.12	1.25
	NEHP	0.21	0.02	1.96
H. wagneriana	HP	0.83	0.13	5.16
	BM	1.01	0.15	7.04
	HPNE	1.47	0.23	9.33
	NEHP	0.33	0.04	2.76

2.5 Discussion

We tested whether pollinator recognition, a plant behavior first described in *Heliconia tortuosa* in which plants may discriminate among floral visitors based on morphological matching to the flower, may be present in more than one species of *Heliconia*. Additionally, we tested the reproducibility of evidence supporting pollinator recognition *H. tortuosa*. Our results are consistent with the findings of Betts et al. (2015) that the number of successful pollen tubes in *H. tortuosa* flowers is, on average, greater in flowers visited by morphologically matched hummingbirds compared to those visited by mismatched birds after experimentally standardizeing pollen quality, visit frequency, and minimizing variation in pollen quantity. However, we did not find strong support for any of the hypothesized mechanisms of recognition and found different responses to our aviary treatments in each of the four *Heliconia* species we tested, highlighting the poorly understood complexities of pollination in *Heliconia* plants.

The estimate of the pollen tube rate in hand pollinated *H. tortuosa* flowers that were subsequently visited by pollen-free Green Hermit Hummingbirds was 1.1 pollen tubes per style in Betts et al. (2015) and 0.87 pollen tubes per style here. Similarly, the number of pollen tubes per hand-pollinated style was around 0.2 in Betts et al. (2015) and 0.13 here. Drawing on the two studies, the joint probability that both Betts et al. (2015) and we would find a difference between pollen tube rates in flowers visited by Green Hermit Hummingbirds and those visted by Rufous-tailed Hummingbirds at least as great as what was found in the respective studies is estimated to be $\hat{P} \approx 2.8 \times 10^{-5}$ when using unadjusted P-values to estimate the probability and $\hat{P} \approx 4.5 \times 10^{-4}$ using the FDR-adjusted P-values (Benjamini

& Hochberg, 1995). Clearly, there is evidence between the two studies that morphologically matched hummingbirds induce greater pollen tube counts than do morphologically matched hummingbirds after standardizeing pollen quality and visitation rates (at least under the conditions of our aviary experiments).

While our results from the aviary experiments are largely consistent with those of Betts et al. (2015), they are not consistent with those of Kress (1983b) who reported 2.2 pollen tubes per style in hand-pollinated flowers. We hesitate to speculate on the cause for this discrepancy, but we note a difference between our approaches; namely, Kress (1983b) emasculated flowers before pollinating by hand. This suggests that an alternative hypothesis for the mechanism underlying the difference in pollen tube rates between flowers visited by morphologically matched birds and those visited by mismatched birds is that matched hummingbirds reduce competition with self pollen at the stigmatic surface. We note that this could still have the same 'pollinator recognition' effect as the nectar removal hypothesis, just mediated by a different mechanism. Furthermore, while we believe this hypothesis should be tested in aviary experiments in the future, we have not found improved pollen tube rates in emasculated flowers compared to flowers that were hand pollinated but not emasculated in over 10 years of collective experience in this system (A. S. Hadley, *unpublished data*). Because our hand pollination treatments have consistently yielded low numbers of pollen tubes regardless of emasculation status, we do not expect that this is the cause of the difference between the pollen tube counts here and in Kress (1983b).

Indeed, for all species except *H.tortuosa*, our scope of inference may not extend to populations of plants in their native ranges because there may have been a lack of genetic diversity from which to draw for our experiments. Because many *Heliconia* species are reported to be self-incompatible to partially self-compatible (Kress, 1983b; Pedersen & Kress, 1999), and can be propagated using rhizomes, we expect this potential lack of genetic diversity could have dampened the variation in pollen tube counts, making it more difficult to detect differences between treatments. Thus, it should be noted that in generalizing our results to other populations of *H. rostrata*, *H. hirsuta*, or *H. wagneriana*, our treatment effects and pollen tube rates could be biased (likely towards zero). Below, we discuss the literature on the natural history of these plant species, but generalizations must be made with caution.

In forest fragments around Coto Brus (the population of *H. tortuosa* plants from which we sampled), mismatched hummingbirds account for c.a. 10% of honest visits (those in which the visitor contacts the reproductive organs of the flower) to *H. tortuosa* (K. Leimberger, *unpublished data*). Pollinator recognition in *H. tortuosa* (regardless of the mechanism) could therefore serve as an additional pollinator filter acting in conjunction with morphological barriers that often result in only imperfect resource partitioning by floral visitors (Feinsinger, 1976; Rodríguez-Gironés et al., 2015; Vlašánková et al., 2017; Metelmann et al., 2020). We posit this could increase outcrossing among populations in Coto Brus (Torres-Vanegas et al., 2019). However, the proportion of honest visits by mismatched pollinators likely increases in isolated fragments where morphologically matched hummingbirds are less common (Kormann et al., 2016; Hadley et al., 2018; Morrison & Mendenhall, 2020). Thus, the combination of fragmentation and pollinator recognition could result in pollen limitation even if the pollinator community rewires. Consistent with this prediction, *H. tortuosa* plants in isolated forest fragments show reduced seed sets compared to those in continuous forest (Hadley et al., 2014), presumably due to a paucity of morphologically matched hummingbirds.

In *H. rostrata* styles, we found some evidence that visits from clean hummingbirds to hand-pollinated flowers increased pollen tube rates irrespective of bird species identity. The estimated pollen tube rate in *H. rostrata* flowers that were visited by long-billed hummingbirds was nearly identical to that estimated for flowers visited by short-billed hummingbirds (Figure 2.1). However, given the relatively short, straight corolla of H. rostrata (Figure 2.1), both hummingbird species we used for experiments were able to achieve strong morphological matches and might not be expected to differ in their visitation characteristics (e.g., nectar consumption, hovering visits, etc.).

In Peru, seven hummingbird species of various sizes and with various bill shapes, including multiple hermits, have been observed visiting *H. rostrata*, but nothing is known about their pollination efficiencies (Dalsgaard et al., 2021). Based on our results showing increased pollen tube rates in bird-visited flowers compared to hand pollination, we posit that *H. rostrata* may not filter hummingbird communities to specialize on a subset of hummingbird pollinators since the bill shapes of the hummingbirds in the native ranges do not deviate drastically from the species used in our experiments (Dalsgaard et al., 2021). However, it is possible that *H. rostrata* could filter visits from animals with visit characteristics similar to our hand pollination treatments (if they exist). This idea is supported by data from Janeček et al. (2020) who recorded olive sunbirds (*Cyanomitra olivacea*) and Camaroon sunbirds (*Cyanomitra oritis*) visiting *H. rostrata* flowers in South Africa where it has been introduced. These authors found that *H. rostrata* flowers left open to visits from sunbirds had extremely low pollen tube rates, as did hand-pollinated flowers. Further work in the native range of *H. rostrata* is necessary to determine if *H. rostrata* cryptically filters floral visitors.

We did not detect strong differences among treatments in *H. hirsuta* flowers. Point estimates of the pollen tube rates in the aviary experiments were all within the range of estimates from flowers left open to free-ranging hummingbirds (grey lines in Figure 2.2). Interestingly, *H. hirsuta* flowers visited by long-billed hummingbirds following hand pollination showed the lowest pollen tube rate. Similarly, we found reduced pollen tube rates in *H. wagneriana* flowers that were visited by hummingbirds relative to hand-pollinations alone (Figure 2.2). While the mechanisms underlying these results in *H. wagneriana* and *H. hirsuta* flowers remain unclear, we identified one way in which these species differ from the others that could produce this result. Gannon et al. (2018) discovered that *H. wagneriana* plants have a mechanism for keeping the anthers protected within the perianth and then rapidly extending them as a hummingbird visits. This is thought to protect pollen from desiccation and/or increase pollen transfer to pollinators during the first visit. Once exposed, however, pollen grains desiccate relatively quickly, and often fail to adhere to the stigmatic surface. While *H. hirsuta* anthers protrude from the flower upon anthesis, pollen also appeared to dry quickly and often failed to cling to the stigmatic surface. This may make the pollen grains of *H. wagneriana* and *H. hirsuta* easy to dislodge. We checked that pollen was still present on the stigma after a bird visited, but the size of *Heliconia* pollen makes exact quantification in the field infeasible. Thus, it is possible that reduced outcross pollen loads after the birds visited resulted in reduced pollen tube counts relative to hand pollination alone.

Using camera traps, Gannon et al. (2018) found that c.a. 97% of the visits to open *H. wagneriana* flowers around Las Cruces were by traplining species with morphologically matched bill shapes. Similarly, Snow and Snow (1972) report only Green Hermit and Rufous-breasted Hermit (*Glaucis hirsutus*) visitors at *H. hirsuta* flowers in Trinidad (part of its native range), both of which have well-matched bills. Thus, even if we had found evidence of pollinator recognition for these two species, given reports of relatively specialized sets of floral visitors, it seems unlikely that any cryptic recognition mechanism would adaptively filter the community of hummingbirds.

2.5.1 The mechanism of pollinator recognition

Previous work demonstrated increased pollen tube rates with manual nectar extraction treatments compared to hand pollinations alone in *H. tortuosa* (Betts et al., 2015). Betts et al. (2015) hypothesized that, because birds with well-matched bill morphologies can drain the nectar chamber but those with mismatched bills often cannot (Betts et al., 2015; Temeles et al., 2019), nectar removal could provide a cue to which plants respond to promote successful pollen tube growth. We did not find an increase in pollen tube rates using manual nectar removal techniques, despite the use of identical nectar removal methods (aside from the experimenter), which does not support the nectar removal hypothesis (Betts et al., 2015). While we found slightly increased pollen tube rates in *H. wagneriana* flowers following manual nectar removal (Figure 2.3), this result is tenuous because it is in part driven by a few influential observations. Furthermore, the pollen tube counts in the flowers from which we removed nectar are approximately equal to the pollen tube counts in our hand pollinated flowers from the aviary experiments (Figures 2.2 & 2.3), indicating this difference in means may very well be due to chance.

Similarly, we found no evidence that the proximate cue for pollen tube germination and growth is a mechanical stimulus from a bird inserting its bill into the flower. However, pipette tips have different physical properties from bird bills and feathers and we expect hummingbirds are considerably more precise with bill placement than we could be with pipette tips. Thus, we cannot exclude a mechanical mechanism from the list of candidate mechanisms for pollinator recognition at this time.

In summary, the evidence that nectar removal provides the cue for pollinator recognition in *H. tortuosa* is equivocal and further experiments are necessary to verify nectar removal or establish a new mechanism. We refrain from speculating on additional mechanisms of recognition, but instead suggest the following experiments which may help future researchers to disentangle the apparent complexities of this system: 1) continued aviary experiments with *Heliconia* species in their native ranges with native floral visitors; 2) comparative studies of the mechanics of visits to *H. tortuosa* flowers by different hummingbird species using high-speed videography to identify potential mechanisms for higher pollen tube rates by morphologically matched species; 3) enhanced experimental techniques that can increase the consistency of manual nectar extraction and test alternative mechanisms linked to morphological matching (including, but not limited to, amount of pollen deposition/removal (Young & Young, 1992), vibrations from wing-beats (Veits et al., 2019), static charges of pollen and birds (Badger et al., 2015), and other aspects of visit mechanics).

2.6 Conclusions

Our results help to highlight the complexities of pollination and pollen germination success. Indeed, the presence of outcross pollen on the stigma does not always translate into pollen germination success (Young & Young, 1992). Despite our detailed manipulative experiments, we found great variability in the number of germinated pollen tubes depending on the floral visitor and plant species. We therefore add to calls for additional manipulative experiments to assess realized pollination network structure that take into account single-visit pollination efficiency, preferably efficiency in converting flower visits into seeds (King et al., 2013; Ballantyne et al., 2015). Furthermore, our results highlight the necessity and opportunity for further study of pollinator recognition (or lack-thereof) in *Heliconia*. Further research into the mechanism underlying increased pollen tube germination success in *H. tortuosa* following visits from morphologically matched hummingbirds and the generality of pollinator recognition in the Heliconiaceae is warranted. Notably, Pedersen and Kress (1999) report an approximate four-fold increase in pollen tube rates in *Heliconia paka* flowers that were visited by honeyeaters compared to those pollinated by hand. These results would be consistent with what we would predict for *H. paka* given a pollinator recognition mechanism. More generally, (Young & Young, 1992) report that hand-pollinated flowers had reduced reproductive output compared to open-pollinated flowers for 17 of 52 plant species from highly divergent lineages. We know of no follow-up experiments with these or related taxa, but we urge others to conduct similar experiments to those presented here to examine the potential for cryptic specialization in other pollination systems.

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All experimental methods involving hummingbirds were approved by the Oregon State University Animal Care and Use Committee (Animal Care and Use Permit 5020) and all international research guidelines and practices were followed.

Data from pollination experiments are available through Dryad. All R code necessary to reproduce the results can be found on a public Github repository (https://github.com/Dusty-Gannon/PR-in-Heliconia).

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Pairing automated mark-recapture and social network models to explore the effects of landscape configuration on hummingbird foraging patterns

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Chapter 3: Pairing automated mark-recapture and social network models to explore the effects of landscape configuration on hummingbird foraging patterns

3.1 Abstract

Landscape changes can alter pollinator movement and foraging patterns which can in turn influence demographic processes of plant populations. We leveraged social network models and four fixed arrays of five hummingbird feeders equipped with radio frequency identification (RFID) data loggers to study Rufous Hummingbird (Selasphorus rufus) foraging patterns in a heterogeneous landscape. Using a space-for-time approach, we asked whether forest encroachment on alpine meadows could restrict hummingbird foraging movements and impede resource discovery.

We fit social network models to data on 2,221 movements between feeders made by 29 hummingbirds. Movements were made primarily by females, likely due to male territoriality and early migration dates. Distance was the driving factor in determining the rate of movements among feeders. The posterior mean effects of forest landscape variables (local canopy cover and intervening forest cover) were negative, but with considerable uncertainty. Finally, we found strong reciprocity in hummingbird movements, indicative of frequent out and back movements between resources. Together, these findings suggest that reciprocal movements by female hummingbirds could help maintain bidirectional gene flow among nearby subpopulations of ornithophilous plants; however, if the distance among meadows increases with

further forest encroachment, this may limit foraging among progressively isolated meadows.

3.2 Introduction

In the Cascade Mountains of the western United States, rapid forest encroachment is shrinking and fragmenting alpine meadows that support diverse plant and pollinator communities (Dailey, 2008). Woody encroachment is known to have adverse impacts on herbaceous plant communities through changes to environmental conditions, such as light and soil characteristics (Hibbard et al., 2001; Haugo & Halpern, 2007; Eldridge et al., 2011; Celis et al., 2017); however, the decline of meadow communities could be expedited if increased tree and shrub cover limits pollinator movement through the landscape. Limited pollinator movement could result in reduced seed set and recruitment in subpopulations of outcrossing plants (e.g., Hadley et al., 2014; Breed et al., 2015).

Because of the long time scale over which forest encroachment occurs, we utilized a "space-for-time" approach in which we exchanged differences in landscape context at a fixed location over time for current differences across locations in space to infer how future changes in the landscape may influence functional connectivity. We collected data on movement rates by hummingbirds implanted with Passive Integrated Transponders (PIT tags; Bridge and Bonter (2011)) among feeders placed throughout a mixed-cover landscape. We then fit 'sender-receiver' models developed for social network data (Warner et al., 1979; Gill & Swartz, 2001; Li & Loken, 2002) to assess the functional connectivity of different locations across the landscape (Fletcher et al., 2011). These methods offer multiple benefits. First, our methods employ passive mark-recapture techniques which may yield large volumes of data with reduced labor cost (e.g., Bandivadekar et al., 2018). Second, sender-receiver models allow us to estimate the effects of landscape features on connectivity of fixed locations in a generalized regression framework and allow insight into movement patterns such as the reciprocity of movements which may elucidate source-sink dynamics (emigration out of high-density areas with minimal immigration) and directional gene flow.

We sought to test two hypotheses of how forest encroachment could reduce the connectivity of meadow plant populations by limiting foraging movements of Rufous Hummingbirds (Selasphorus rufus), common avian pollinators in the western United States. First, woody vegetation could act as a barrier to foraging movements if hummingbirds establish territories in open areas and limit foraging search patterns to open habitat with minimal exploration into forested areas (we refer to this hypothesis as the barrier hypothesis). If true, we predicted that movement rates should be reduced between feeders placed in meadows separated by closed canopy forest and also to feeders placed inside the forest. This barrier to movement could reduce effective population sizes by reducing visitation to plants that get overgrown by forest species as well as pollen flow among subpopulations.

Second, hummingbirds could avoid flying through closed canopy forest, but fly over the canopy to forage in disconnected meadows (Kreyer et al., 2004). This behavior could result in reduced capacity for birds to detect plants within forests but would not limit movements among meadows (resource discovery hypothesis). In this scenario, we predicted reduced movement rates to feeders placed inside the forest but not those placed in open habitat, regardless of the intervening landscape. Thus, hummingbirds could help maintain connectivity among subpopulations of ornithophilous plants, but the effective population size may still decline as some plants are overgrown by woody vegetation and visited less by pollinators.

3.3 Methods

We established four study sites in meadow complexes (clusters of meadows) located on summits that span the north-south extent of the H. J. Andrews Experimental Forest (AND), Oregon, USA (44.212° N, -122.256° E). The sites were selected such that we could establish arrays of five hummingbird feeders with one feeder in a central meadow and four satellite feeders ca. 250 m from the center (Figure 1d). The satellite feeders were placed with at least one under closed canopy coniferous forest (n=5), one in a meadow separated from the center feeder by closed canopy (n=4), and one in a meadow connected to the center feeder by open habitat (n=7). We established hummingbird feeders filled with 20% sugar water solution at the center meadow in each meadow complex (Figure 3.1) two weeks before trapping hummingbirds. Following the two-week habituation period, we placed Hall traps (North American Banding Council, 2001) around the center feeders and trapped for a period of 5 hours (05:00 – 10:00). We collected standard measurements on each trapped individual and banded each with a unique metal leg band. The final processing step was to implant the PIT tag under the loose skin between the shoulders (Figure 3.1). The full details of our procedure can be found in Appendix C.

Over the course of four summers (mid-June through July, 2014-2017), we captured and implanted 163 Rufous Hummingbirds with PIT tags. To automatically record the locations of individuals following the initial capture, we established the four arrays of hummingbird feeders as described above, equipped with Radio Frequency Identification (RFID) data loggers (Figure 3.1b&c). When a hummingbird visited a feeder, the PIT tag passed through a copper coil placed around the sole access point (Figure 3.1c), logging the time, date, and

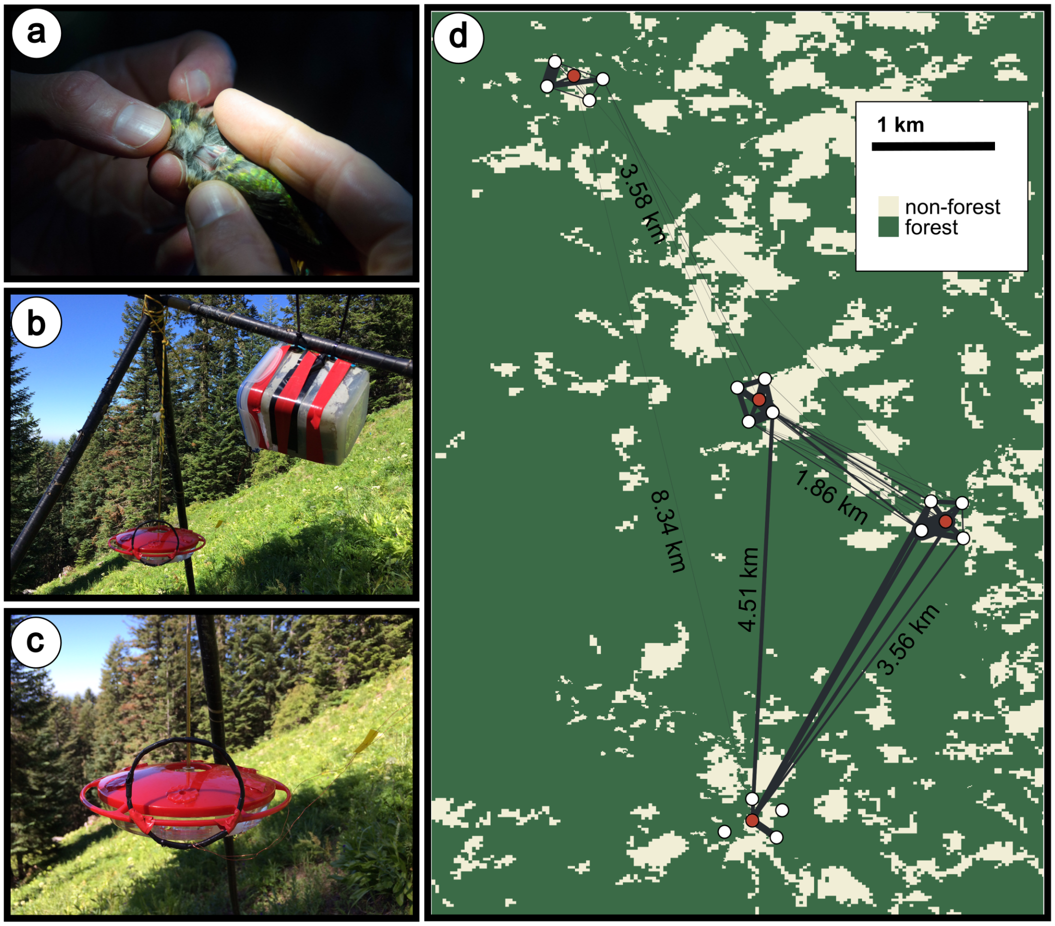

Figure 3.1: **a)** Recaptured Rufous-tailed Hummingbird (*Amazilia tzacatl*) with a PIT tag. **b)** RFID reader-feeder setup with datalogger. c) A copper coil (antenna) wrapped in electrical tape and attached to the hummingbird feeder triggers records from passing PIT tags. All but one port in the feeder were covered. **d)** Arrays of RFID-equipped feeders in the mixed-cover landscape. Capture locations are colored red. Lines connecting the readers are scaled to reflect the total number of movements detected among feeders.

individual identification code (further details on adjusting the methods in Bridge and Bonter (2011) for this system can be found in Appendix C). Feeders were maintained for 1.5-12 weeks per year for up to four years (the southernmost array was established in 2015 and the northernmost array was established in 2016; Figure 3.1d).

We extracted movement information from the relocation data by tallying occasions on which an individual was recorded at feeder i at time t and again at feeder j, $j \neq i$, at time t', $t' > t$, within the same day. We limited our focus to movement that occurred within the same day to gain insight into hummingbird movements that may be relevant to pollination. Additionally, we summed the movements over the summer to get multiple measurements of movement between two feeders (one per year) that can more reasonably be treated as independent given the regression parameters. While we lose information on inter-individual differences in foraging behaviors by summing movements over the year, our objective was to model functional connectivity informed by hummingbird movements. Whether a given number of movements is made by many birds each making few movements or by few birds making many movements is not important in this endeavor.

Briefly (but see Appendix C for full details), we treated each feeder as a node in a graph and modeled the edge weight (connectivity between two nodes) of directed edges (i.e., $\lambda_{ij} \neq \lambda_{ji}$) in the graph. We denote λ_{ij} as the weight of the edge connecting node i to j and assume that the number of movements between two feeders in a given year, $y_{ijk} \in \mathbb{N}$, where $k = 1, 2, \ldots, K$ indexes the year, was a draw from a Poisson distribution with rate parameter λ_{ijk}. Thus, in year k, when R_k feeders were maintained on the landscape, there were $n_k = R_k(R_k - 1)$ possible movements, yielding $N = \sum_{k=1}^{K} n_k$ total observations.

Within this framework, we fit a ‘sender-receiver’ regression model (Warner et al., 1979;

Gill & Swartz, 2001; Li & Loken, 2002) using the R package `rstan` (Carpenter et al., 2017; Team, 2020) that accounts for dependencies among movements that share a common origin, those that share a common destination, and dependence within a dyad (i.e., frequency of movements $i \to j$ and $j \to i$). Our covariates of interest included the effect of geographic distance between feeders i and j (β_{distance}), the amount of intervening forest in a 50-m belt transect between i and j (β_{forest}), and the average of two indicator variables (β_{cover}), one indicating whether feeder i was in the forest and one indicating whether feeder j was in the forest ($x_{ij,\text{cover}} \in \{0, 0.5, 1\}$). Strong negative effects of both placing a feeder under the canopy and the amount of intervening forest would support the barrier hypothesis while a negative effect of placing a feeder under the canopy combined with a negligible or positive effect of intervening forest would support the resource discovery hypothesis. For each year, we included offsets for the number of weeks a given pair of feeders was available to the birds and the cumulative number of birds that were implanted with PIT tags. Ninety-five percent credible intervals for parameters of interest are presented below in square brackets and in Table 3.1.

3.4 Results

Over the course of four summers (June-August), the four arrays of hummingbird feeders equipped with passive data loggers recorded 20,877 separate relocations (reads spaced by > 30s) of 63 Rufous Hummingbirds (13 males, 40 females, 10 of unknown sex), 51 of which were recorded on multiple days (12 males, 33 females, and 6 of unknown sex) and 8 over multiple years (1 male, 7 females). From these data, we extracted information on 2,221

Table 3.1: Posterior means and percentiles for the regression parameters of interest. β_{distance} is the effect of the distance between two feeders on the log-movement rate between them, β_{forest} is the effect of the proportion of forested area in the 50 m belt transect between two feeders, and β_{cover} is the effect of local canopy cover (feeders inside the forest or not) on log-movement rate.

Parameter	Posterior mean	2.5%	25%	50%	75%	97.5%
β_0 (Intercept)	-2.87	-4.58	-3.52	-2.91	-2.25	-0.97
β_{distance}	-1.46	-1.72	-1.54	-1.46	-1.37	-1.21
β_{forest}	-0.60	-1.92	-1.07	-0.59	-0.14	0.69
β_{cover}	-1.18	-3.08	-1.81	-1.18	-0.55	0.67

movements between feeders made by 29 hummingbirds (2 males, 27 females). Data on the birds that did not move among feeders can be found in Appendix C). Our final dataset included data on the number of times each of the n_k network connections was made in year k ($N = 1,060$ total edge measurements).

The majority of movements were among feeders within an array. Only 362 of the recorded movements (made by 8 birds, 1 male, 7 females) were between two feeder arrays (Figure 3.1d), indicating that home ranges did not often span an area that covered multiple meadow complexes. The fitted model predicts that the probability of at least one movement between two feeders per bird per week approaches zero when feeders are greater than 2 km apart (Table 3.1, Figure 3.2).

3.5 Discussion

We highlight the value of pairing an underutilized passive mark-recapture approach with social network models in the study of foraging patterns of small-bodied organisms (see also

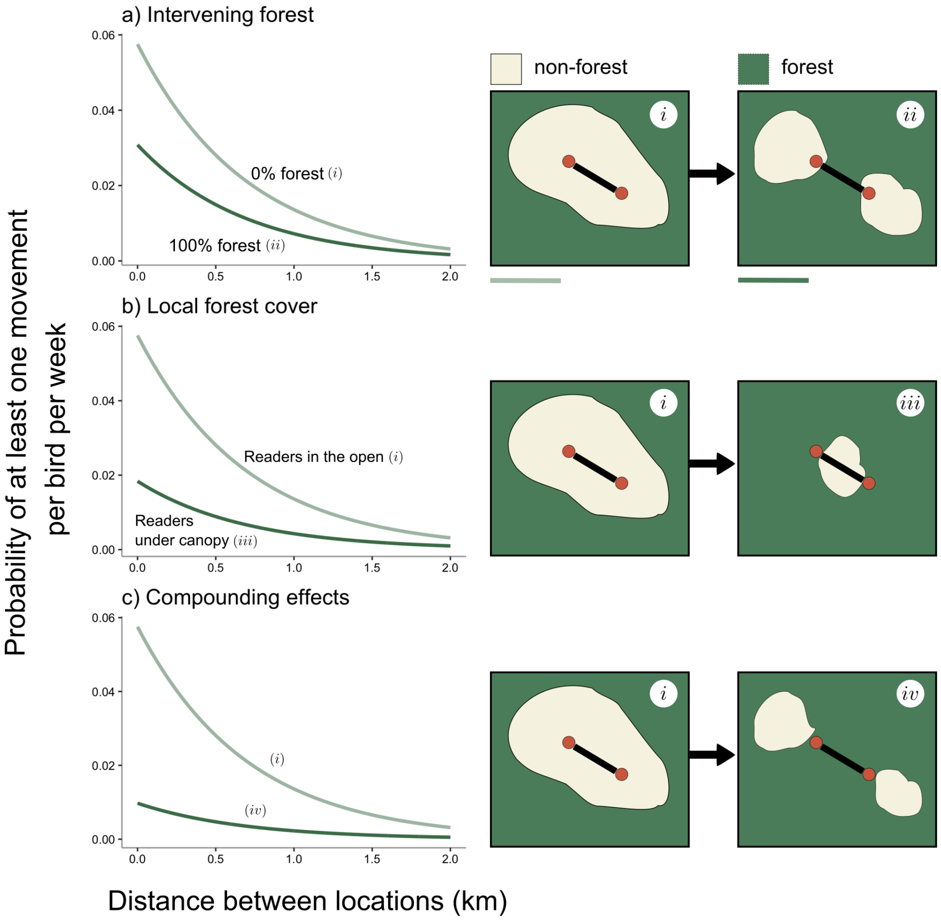

Figure 3.2: Predicted probability of movement between two food sources with increasing distance and changes in the surrounding landscape. The panels to the right illustrate the change in landscape configuration used to create the line plots. a) The probability of at least one movement per bird per week (y-axis) between two food sources decreases with increasing distance (x-axis) and is expected to be 45.12% lower if the two locations are isolated by intervening forest (ii). (i) depicts two food sources in open habitat with 0% intervening forest. (ii) depicts two food sources in open habitat with 100% intervening forest. b) (i) both sources in the open and (iii) both under coniferous forest canopy, holding intervening forest at 0%. c) (i) both food sources in the open and (iv) both sources grown over by woody vegetation and separated by 100% forest.

Bandivadekar et al. (2018) and Bailey et al. (2018)). We aimed to gain insight into the functional connectivity of high-elevation meadows in the Cascade Mountains in the face of forest encroachment by investigating foraging behaviors of a common pollinator, Rufous Hummingbirds, in the current landscape. While there remains uncertainty in our estimates of the effects of forest configuration due to a complicated model variance structure and relatively few recording arrays, our results are most consistent with the barrier hypothesis, that pollinator foraging may be limited to open habitat with minimal exploration into or across forested areas. The posterior probabilities that intervening forest and local forest cover have negative effects on hummingbird movement are $P\left(\beta_{forest} < 0\right) = 0.811$ and $P\left(\beta_{cover} < 0\right) = 0.897$, respectively. Furthermore, the effect sizes (Table 3.1) indicate that increased forest encroachment could substantially reduce the functional connectivity of the landscape (Figure 3.2).

Completely isolating two meadows by increasing the amount of forest in the intervening landscape from 0% to 100% is expected to reduce the background movement rate between them by 45.21% (Figure 3.2a). Similarly, movement between two food sources that are overgrown by woody plants is expected to be 69.27% less than if the two food sources are in the open, holding intervening habitat constant (Figure 3.2b). Movement rates would be expected to decline by 83.14% between plants at the edge of forest encroachment but in two isolated meadows such that both are overgrown and separated by woody vegetation (Figure 3.2c). Finally, while the high reciprocity in movement rates could help to maintain bidirectional gene flow among subpopulations of hummingbird-pollinated plants, hummingbirds rarely moved farther than 2 km (Figures 3.1d & 3.2). Further encroachment that increases the gaps among meadows could therefore reduce landscape connectivity.

Indeed, supplying artificially high volumes of sucrose at feeders could have reduced the incentive for birds to move among meadows since resource availability is known to influence home range sizes (e.g. van Beest et al., 2011); however, Rufous Hummingbirds (particularly males) are known to be highly territorial (Carpenter et al., 1993), and territoriality often increases at highly valuable resources (Justino et al., 2012; Ewald & Carpenter, 1978). Thus, it is likely that most birds did not have access to unlimited sugar supplies due to competition at feeders (see Appendix C for more exploration of this hypothesis). This and prior work in similar systems (Kormann et al., 2016) lead us to believe our results are largely representative of functional connectivity of the landscape.

Interestingly, all but three movements were by female hummingbirds, even though males were over-represented in the proportion of birds that were relocated following PIT tag implantation (15.1% males implanted, 20.6% of relocated birds were males). Females are known to be less successful in holding territories than males (Carpenter et al., 1993), so females may have been forced out of high resource areas, necessitating foraging over greater distances to fulfill energetic requirements. This highlights the potential importance of females in maintaining connectivity among meadows (Maruyama et al., 2016) since males are expected to spend more time defending territories, thus moving less, and also migrate early (Rousseau et al., 2020), narrowing the window during which they overlap with the flowering period of many plants. However, the relative importance of females over males to functional connectivity may be overstated in our data if experimentally high resource availability resulted in less than average male movement and territory size. Future experiments that focus on explicit measures of pollen flow and pollination efficiency of different pollinator sexes are necessary to test these ideas.

Data on animal movement are ever more available as technologies advance (Nathan et al., 2008; Cagnacci et al., 2010). Still, options for automated recording of small-bodied animals remain limited. We illustrate the potential for arrays of feeding stations (or natural forage) equipped with data loggers combined with social network models to provide insight into foraging movements and functional connectivity. From the fitted model, we obtained approximate estimates of foraging range sizes, information supporting frequent out-and-back movements, and tested for landscape resistance to movement. Data such as these may be useful for informing forecasts of the effects of landscape change on populations of plants and animals (Doherty & Driscoll, 2018; Urban et al., 2016). While considerable uncertainty remains, the posterior mean effect sizes of forest landscape variables suggest that further forest encroachment could substantially reduce pollen flow among ornithophilous plants by acting as a barrier and reducing resource discovery. Finally, if the distance among meadows increases with additional forest encroachment, this could limit foraging among progressively isolated meadows.

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All field work was approved by the H. J. Andrews LTER site coordinator. Animal procedures were approved by the Oregon State University Animal Care and Use Committee (permit numbers 4266 and 4665). Hummingbird banding occurred under the USGS banding permit 23521.

Data used in this study are available on the H. J. Andrews Long-Term Ecological Research Station database, DB code SA028 (http://andlter.forestry.oregonstate.edu/data/abstract.aspx?dbcode=SA028). R code necessary to reproduce the analyses presented here and in Appendix C can be found in the GitHub repository https://github.com/Dusty-Gannon/RUHU-movements.

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Chapter 4: Evidence that forest encroachment could increase fine scale spatial genetic structure but enhance gene flow among isolated locations of western columbine (*Aquilegia formosa*) in fragmented alpine meadows

4.1 Abstract

1. Woody plant encroachment into alpine meadows may reduce and fragment the habitat available to meadow plant communities. This could reduce population connectivity of alpine plant populations if seeds or pollen fail to disperse across forested areas, reducing effective population sizes, genetic diversity, and adaptive potential.

2. We genotyped 192 western columbine (*Aquilegia formosa*) individuals from a forest mosaic landscape in the Cascade Mountains, USA. We fit a spatial auto-regressive model to a subset of putative neutral loci to test for genetic signatures consistent with: ***(i)*** a model of isolation-by-resistance in which forest cover resists gene flow through a landscape; ***(ii)*** the hypothesis that forest cover may reduce resource discovery for pollinators by concealing herbaceous plants, thereby reducing gene flow to plants overgrown by taller forest species. Hypothesis ***(i)*** would be supported if neighboring plants in isolated meadows shared greater genetic similarity than neighboring plants in connected meadows, but plants in different isolated meadows showed greater divergence than plants in different but connected meadows. Hypothesis ***(ii)*** would be supported if neighboring plants growing underneath taller woody vegetation shared greater genetic similarity than neighboring plants growing in the open, but two plants growing

beneath different canopies showed greater divergence than two plants growing equally far apart in space but in the open.

3. We found support for the concealment hypothesis ***(ii)***, evidenced by a steeper spatial decay of genetic similarity when comparing plants growing under cover compared to two plants growing in the open. However, we found the opposite of what we would predict based on isolation-by-resistance ***(i)***. Genetic similarity decayed more slowly when moving away from an isolated plant compared to moving away from a well-connected plant. This indicates that gene flow in and out of isolated meadows may be relatively high.

4. *Synthesis*: Our results suggest that regional connectivity of *A. formosa* populations may be maintained despite meadow fragmentation from forest encroachment. Reduced connectivity of meadows may even enhance gene flow over small geographic distances. However, once plants are overgrown by taller forest species, they may become disconnected from the population, potentially resulting in decreased effective population sizes.

4.2 Introduction

Alpine meadows in the Cascade Mountains (Cascades) of the Pacific Northwest, USA, host diverse communities of plants and pollinators. However, forest encroachment into alpine meadows is shrinking and fragmenting these communities (Haugo & Halpern, 2007; Dailey, 2008) with potential implications for the genetic diversity and the persistence of herbaceous plant populations. The causes of forest encroachment are not fully understood, but are likely related to changes in climate and alterations to disturbance regimes. Thus, the current rate of climate change as well as the rate and scope of human alterations to landscapes could cause drastic changes to treeline dynamics in the Cascades and globally (Taylor, 1995; van Auken, 2000; Coop & Givnish, 2007; Miller & Halpern, 2009).

Known and immediate impacts of woody encroachment are altered light/shade environments for small-statured plants, changes to soil characteristics (Hibbard et al., 2001; Haugo & Halpern, 2007; Eldridge et al., 2011), changes in understory plant community structure (Celis et al., 2017), and altered disturbance regimes (Ratajczak et al., 2014). Investigated less, however, is the potential for forest encroachment to fragment and isolate herbaceous plant populations and restrict the movements of pollinators and seed dispersers among plant populations, thereby reducing gene flow across the landscape (but see Bagaria et al., 2017). Increased isolation and reduced movement of genes within and among meadow populations could expedite declines of meadow plant communities by reducing local effective population sizes, eroding genetic diversity, and lead to a loss of adaptive potential in the face of future climate and landscape change (Lowe et al., 2005; Aguilar et al., 2008).

The impact of woody encroachment on pollen-mediated gene flow for plants that rely on animal pollinators may depend on the degree to which pollinators restrict their movement patterns in response to increased woody plant cover in the landscape. If pollinators have difficulty dispersing across forested areas to small isolated populations or limit their search patterns to large open and connected habitats, (Hadley & Betts, 2009; Hadley et al., 2014; Breed et al., 2015) increasing forest cover could serve as a barrier to pollen-mediated gene flow through the landscape. While we cannot directly observe the effect forest encroachment on gene flow through the landscape, we can test whether there is evidence of an association between genetic dissimilarity within and among meadow populations and the current landscape configuration.

If woody plant cover serves as a barrier to pollen flow, we would expect that neighboring plants in an isolated meadow may be more genetically similar (i.e., higher coefficient of coancestry) than neighboring plants in a connected meadow due to reduced gene flow into and primarily local mating within isolated meadows (Figure 4.1a & 4.1b). However, two plants in separate, isolated meadows may be less genetically similar to one another than two plants growing the same distance apart but in connected meadows (Figure 4.1a & 4.1b). This prediction follows from the assumption of primarily local mating in isolated meadows, resulting in divergence due to drift (Fisher, 1930; Wright, 1931). However, corridors of open habitat that connect meadows should facilitate long-distance pollen flow and counteract the effect of drift. We refer to this idea below as the *isolation-by-resistance hypothesis* (note that these predictions are consistent with those of an isolation-by-distance metapopulation model (Hamrick & Nason, 1996) in which effective distance is determined by both distance and the resistance of the intervening landscape). Support for this hypothesis would be evidenced by a negative regression coefficient for the interaction between the geographic distance and the average degree of isolation covariates (Figure 4.1a) when regressing genetic similarity against the covariates. In other words, the spatial decay of relatedness should be steeper when comparing pairs of plants growing in isolated locations than when comparing pairs of plants growing in open areas with large continuous populations.

In addition to increasing the resistance to gene flow through a landscape, woody encroachment could reduce the capacity of pollinators to locate floral resources within a location as they get overgrown by taller woody vegetation (Gannon et al., 2021). Gannon et al. (2021) found some evidence that Rufous Hummingbird (*Selasphorous rufus*, a common avian pollinator in the Cascade Mountains) movements between two food sources were reduced when at least one of the resources was placed underneath forest canopy. This finding is consistent with the hypothesis that resource discovery by pollinators could be reduced by forest encroachment. This could ultimately reduce effective population sizes despite the maintenance of large census population sizes considering that plants at the edge of the invasion would no longer contribute to reproduction.

If taller woody vegetation impedes the ability of pollinators to locate floral resources, we would predict that two plants growing close together and under high canopy cover would be more likely to be related than two plants growing the same distance apart but in the open. This could occur if seeds produced through selfing (reproductive assurance, Eckert and Schaefer (e.g. 1998)) comprise a greater proportion of the seeds produced by plants under forest cover than those produced by plants in the open, since we would expect pollinator visits to be rare. As an additional consequence of rare pollinator visits, we would predict that pollen transfer between two plants at greater distances would be especially rare, resulting in less-than-average genetic similarity when comparing two plants growing beneath woody vegetation but far apart in space. However, we would not expect the decay in genetic similarity with distance to be so rapid in open habitats. We refer to this idea below as the *isolation by concealment hypothesis*. This hypothesis would be supported by a negative regression coefficient for the interaction between geographic distance and canopy cover covariates (Figure 4.1c & 4.1d).

To test our hypotheses, we sampled *Aquilegia formosa* (Ranunculaceae) plants, a common ornithophilous species in meadows of the Cascades across a mosaic of forest and meadow habitat. *A. formosa* was an appropriate study species for two reasons: First, Gannon et al. (2021) found some evidence that forest cover could serve as a barrier to Rufous Hummingbird movements as well as impede resource discovery, so testing whether this is supported by genetic data from an ornithophilous plant species was a logical next step. Second, there exist high-quality genomic resources for the *Aquilegia* genus (Filiault et al., 2018), allowing us to identify putative neutral polymorphic regions of the genome for our analysis.

We fit a modified version of a recently developed, spatially-explicit landscape genetics model that allowed us to leverage genetic distances between individuals to infer the relationships between landscape covariates and gene flow (see Hanks & Hooten, 2013; Peterson et al., 2019). We asked whether there are genetic signatures in populations of *A. formosa* consistent with 1) a model of isolation-by-resistance in which forest cover resists gene flow through a landscape (McRae, 2006) and 2) the hypothesis that forest cover may reduce resource discovery for pollinators by concealing herbaceous plants. Our statistical approach allowed us to explore nuanced relationships between landscape variables and genetic distances to test the isolation-by-resistance and the isolation by concealment hypotheses.

4.3 Methods

4.3.1 Sampling leaf tissue

We collected leaves from alpine meadows in the H. J. Andrews Experimental Forest (HJA) of western Oregon, USA (44° 12′ N, 122° 15′ W). A mosaic of alpine meadow habitat in the HJA

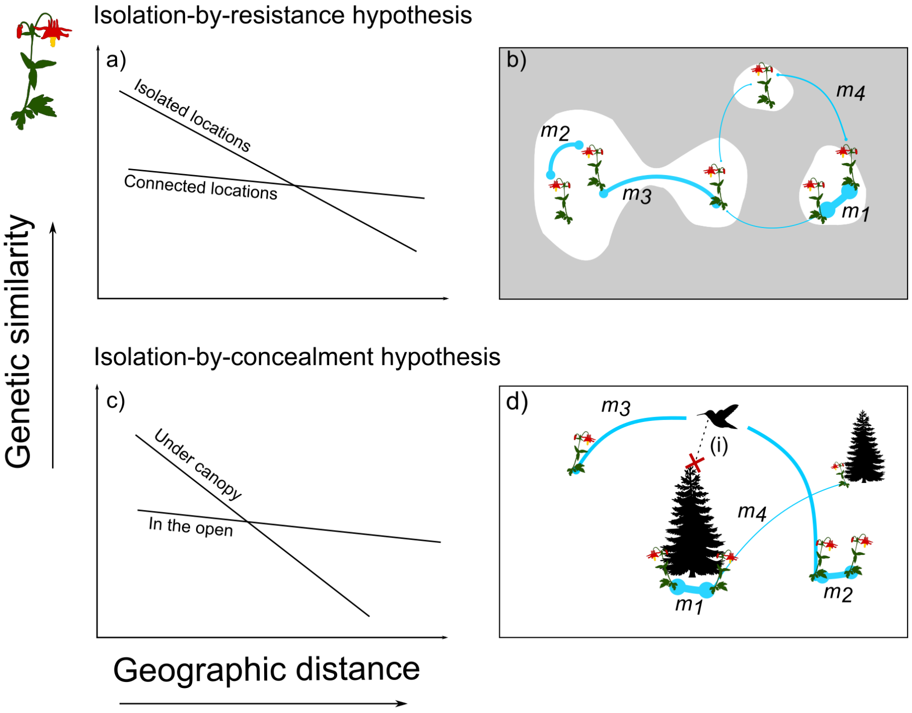

Figure 4.1: Predicted trends in genetic similarity with the *isolation-by-resistance* (**a**) and the *isolation by concealment* (**c**) hypotheses. **b)** A schematic of relative rates of pollen-mediated gene flow through a hypothetical landscape under the isolation-by-resistance hypothesis. White coloration represents open habitat and gray represents forested habitat. Neighboring plants in connected patches show reduced similarity (i.e., are less related) compared to neighboring plants in isolated locations ($m_2 < m_1$) due to low resistance to long-distance pollen movement through well-connected landscapes. Forested areas, however, resist pollen flow resulting in a greater effective distance between patches and therefore reduced genetic similarity. **d)** Mechanisms underlying the isolation by concealment hypothesis. If pollinators fly over the canopy to forage in disconnected meadows, plants that are overgrown by taller woody plants will be obscured and more frequently go undiscovered by pollinators (i). This could allow for greater rates of mating amongst nearby relatives and/or greater selfing rates underneath forest canopies compared to in the open ($m_1 > m_2$). However, at greater distances, the probability of pollen transfer between two “concealed” individuals should be much less than the probability of pollen transfer between two plants in the open ($m_4 < m_3 < m_2 < m_1$).

exists from c.a. 1,100 to 1,600 meters above sea level, nested within a matrix of old-growth coniferous forest, c.a. 60 year-old Douglas fir (*Pseudotsuga menziesii*) plantations, and shrub/scrub fields predominated by alder (*Alnus viridis*) and vine maple (*Acer circinatum*).

We sampled meadows from meadow complexes (clusters of meadows) on four peaks along Frizzel Ridge which we refer to as Lookout Mountain (LOM), M1, M2, and Carpenter Mountain (CM) (Figure 4.3). Within each complex, we stratified meadows based on a gradient in meadow size (m^2) and connectivity, measured as the proportion of forest cells within a 100 m radius around the meadow centroid (Bender et al., 2003). With little *a priori* information about biologically relevant scales, we selected a 100 m buffer because it gave us a greater range of values (6% – 100% forest cover) than did 500 or 1000 m buffers.

The classifications for meadow size (small or large) and connectivity (connected or isolated) were determined based on natural breaks in the data. We classified meadows larger than 1.26×10^3 m^2 as large meadows and those smaller than 1.26×10^3 m^2 as small. Those with less than 62% forest cover in a 100 m buffer around the meadow centroid we classified as connected and those with greater than 62% forest cover we classified as isolated. We then randomly selected two meadows per complex from each stratum (large-connected, large-isolated, small-connected, and small-isolated), with the constraint that the largest, most central meadow in a complex was always included. Since the meadow complexes are roughly centered around a large meadow near the summit of each high point on Frizzel Ridge, we believed that ensuring we sampled the central meadow was important for getting a representative sample of this naturally hierarchical collection of meadows. Notably, this stratification scheme was designed to ensure we sampled plants from meadows with a range of characteristics from each peak, but we did not use the strata in subsequent analyses since all analyses treated individual plants as the sampled unit.

From the selected meadows, we haphazardly collected leaves from a minimum of 5 plants per meadow. During leaf collection, we also quantified the number of conspecific individuals in a 5 meter radius around the focal plant and measured the percent canopy cover above the plant using the ‘Canopeo‘ application for Android mobile devices (Patrignani & Ochsner, 2015). We placed leaves in collection tubes, froze them in liquid nitrogen, and stored them at −80°C until DNA extraction.

4.3.2 Genotyping by sequencing

We randomly selected 6 meadows per complex from which to genotype plants, under the constraint that at least one meadow of each stratum (small-isolated, small-connected, large-isolated, and large-connected) was represented in each complex. We then selected 8 plants at random per meadow out of the total sampled in each meadow. In two meadows, CMX11C and M1X7, only 6 and 5 plants were found, respectively (Table 4.1). This left 5 wells open on a 96-well extraction plate, so we strategically included 5 plants from the meadow LOMCAGE since it is a small, isolated meadow that is relatively far from the central meadow in the LOM complex.

We randomly assigned samples to a well on a 96-well plate for DNA extraction. DNA extraction and library preparation was done according to the methods of Elshire et al. (2011), using the ApeKI restriction enzyme to fragment genomic DNA. Libraries were sequenced on an Ilumina HiSeq 3000 with 96 libraries per lane over two lanes. All lab work was performed by the Center for Quantitative Life Sciences, Corvallis, OR.

Following the Illumina run, we demultiplexed and aligned sequence data to the *Aquilegia coerulea* Goldsmith v3.1 reference genome (Filiault et al., 2018) using Stacks v2.0 (Catchen et al., 2013) and the Burrows-Wheeler Aligner algorithm (Li & Durbin, 2010) (respectively). The `process_radtags` program in Stacks demultiplexes data from HiSeq output files and filters out poor quality sequences based on the average Phred score (the Phred score of a base call is defined as $Q = -10\log_{10}(p)$, where p is the probability of calling an incorrect base) within a sliding window and thresholds defined by the user. We used default parameter specifications for read processing, running a sliding window with a width 15% of the total read length down the fragment. The program removes reads in which the average Phred score drops below 10 inside the window. Once reads were processed, filtered, and aligned to the *A. coerulea* genome, we used the `GStacks` program to build SNP models with aligned reads that had mapping quality scores greater than 20 (i.e., $P(\text{incorrect position}) = 0.01$).

We further filtered our dataset using the Populations module of Stacks v2.0 by requiring that SNPs must be present in at least 80% of individuals and have a minor allele frequency of at least 0.05. Finally, we removed SNPs with read depths above the 99th percentile in the read depth distribution (Knaus & Grunwald, 2017). Exceptionally high read counts may result from the spurious alignment of similar genetic sequences, repetitive regions, or multiple copies of genes, making SNPs with high read counts suspect as they may represent multiple true SNPs or spurious SNPs. We did not, however, remove SNPs with low coverage. While these filters are often applied in the population genetics literature due to high genotyping error rates at low depth, Stacks v2.0 uses a low-depth genotyping procedure that incorporates population level information on genotype frequencies and locus-specific error rates and avoids calling genotypes with high uncertainty (Maruki & Lynch, 2017). Our filtering process left us with a dataset containing 192 individuals genotyped at 11,878 SNP loci. A total of 7.76% of the genotypes were missing, so we imputed these remaining genotypes using the BEAGLE program with default parameters (Browning & Browning, 2016).

4.3.3 Landscape genetics analysis

In a heterogeneous landscape, features of the landscape may increase the resistance to movement of organisms or gametes, reducing migration rates among subpopulations. Mutation and genetic drift in the absence or reduction of gene flow results in genetic divergence among subpopulations, decreasing the genetic similarity between individuals sampled from different locations (i.e., isolation-by-distance) (Wright, 1938, 1943; McRae, 2006). We therefore used genetic dissimilarity (genetic distances) to infer the effects of landscape features on gene flow using the methods proposed by Hanks and Hooten (2013) – with some minor modifications.

Specifically, we modeled locations on the landscape where we sampled individual plants as nodes in a weighted, symmetric graph (network) with edge weights between the nodes denoted by $W_{ij} = W_{ji}$ for $i, j = 1, 2, \ldots, n$ when $i \neq j$. As in McRae (2006), the edge weights represent migration rates and therefore functional connectivity among locations on the landscape (see McRae (2006) for the connection between this model and classical population genetics theory). To test for effects of landscape features on migration and gene flow, we modeled the weight of the edge connecting locations i and j as a function of landscape variables measured between and at the locations i and j. We let $W_{ij} = \exp\{\mathbf{x}_{ij}^T \boldsymbol{\beta}\}$, where $\mathbf{x}_{ij}$ is a p vector of covariates measured for either the edge connecting the pair of individuals at i and j or the average of measurements taken at each location (e.g., $x_{ij,1} = \frac{1}{2}(z_{i,1} + z_{j,1})$, where

$z_{i,1}$ is the first explanatory variable measured at location i and $z_{j,1}$ is the first explanatory variable measured at location j). The vector $\boldsymbol{\beta}$ is a p vector of regression parameters to be estimated. Thus, a negative coefficient in the log-linear model for the edge weight implies that an increase in the explanatory variable of interest is related to decreased edge weights and therefore increased resistance to gene flow.

To provide this model with a likelihood function and therefore estimate the unknown parameters of interest, Hanks and Hooten (2013) used the results of McCullagh (2009), who demonstrated that linear contrasts on distance matrices can be modeled using the Wishart likelihood. Let $Z_1, Z_2, ..., Z_n$ be random variables observed on the nodes of the graph described above. If the conditional distribution of Z_i is taken to be

$$Z_i \mid Z_{j,j \neq i} \sim \mathcal{N}\left(\sum_j \frac{W_{ij} Z_j}{\sum_{j \neq i} W_{ij}}, \frac{\sigma^2}{\sum_{i \neq j} W_{ij}}\right) \tag{4.1}$$

such that the conditional mean of Z_i is a weighted average of the other nodes and the weights are determined by the edge weights in the graph, then the joint distribution of the $Z_i,\ i = 1, 2, ..., n$ is normal with mean vector $\mathbf{0}$ and covariance matrix

$$\boldsymbol{\Sigma} = (\mathbf{M} - \mathbf{W})^{-}, \tag{4.2}$$

where $\mathbf{W}$ is the edge weights matrix, $\mathbf{M}$ is an $n \times n$ diagonal matrix with $\sum_{i \neq j=1}^{n} W_{ij}$ in the i^{th} diagonal entry, and $(\mathbf{M} - \mathbf{W})^{-}$ represents the generalized inverse of $\mathbf{M} - \mathbf{W}$ (Besag, 1974). Now, assume K observations are collected on the whole graph, $\mathbf{z}_1, \mathbf{z}_2, ..., \mathbf{z}_K$, and concatenated into a $n \times K$ matrix $\mathbf{Z}$. Then, the random variable $\mathbf{S} = \mathbf{Z}\mathbf{Z}^T$ follows a Wishart distribution with degrees of freedom K and scale matrix $\mathbf{\Sigma}$. However, neither $\mathbf{Z}$ nor $\mathbf{S}$ are observed; only a measure of distance (or dissimilarity) among the nodes is observed.

The distance matrix that is said to be induced by $\mathbf{S}$ is defined as

$$\mathbf{D} = \text{diag}(\mathbf{S})\mathbf{1}^T - 2\mathbf{S} + \mathbf{1}\text{diag}(\mathbf{S})^T, \tag{4.3}$$

and contains pairwise Euclidean distances between each of the $\mathbf{z}_i$ in $\mathbb{R}^n$. The critical transformation is to introduce a new $n \times (n-1)$ matrix $\mathbf{L}$ with full column rank for which the set of constant vectors forms the kernel (i.e., $\{c \in \mathbb{R}, \mathbf{1} \in \mathbb{R}^n : c\mathbf{L}^T\mathbf{1} = \mathbf{0}\}$, where $\mathbf{1}$ is a vector of ones). We can then see that

$$\begin{aligned}
\mathbf{L}^T(-\mathbf{D})\mathbf{L} &= \mathbf{L}^T(-\text{diag}(\mathbf{S})\mathbf{1}^T + 2\mathbf{S} - \mathbf{1}\text{diag}(\mathbf{S})^T)\mathbf{L} \\
&= -\mathbf{L}^T\text{diag}(\mathbf{S})\mathbf{1}^T\mathbf{L} + \mathbf{L}^T 2\mathbf{S}\mathbf{L} - \mathbf{L}^T\mathbf{1}\text{diag}(\mathbf{S})^T\mathbf{L} \\
&= \mathbf{L}^T 2\mathbf{S}\mathbf{L},
\end{aligned}$$

and by the properties of Wishart random variables,

$$\mathbf{L}^T(-\mathbf{D})\mathbf{L} = \mathbf{L}^T 2\mathbf{S}\mathbf{L} \sim \mathcal{W}_K(\mathbf{L}^T 2\mathbf{\Sigma}\mathbf{L}). \tag{4.4}$$

Thus, we can relate the observed data $\mathbf{D}$ to the parameters of interest through the Wishart likelihood.

To fit this model to genetic data, let the vector $\mathbf{y}_i$ be the dosage vector of dimension K for the individual at location in the landscape i, where K is the number of loci at which plants were genotyped. In the case of biallelic SNP loci, $y_{ik} \in \{0, 1, 2\}$ counts the number of arbitrarily selected reference alleles individual i has at locus $k = 1, 2, ..., K$. We quantified the observed genetic distance (or dissimilarity) between two individuals i and j using the squared Euclidean distance, $D_{ij} = (\mathbf{y}_i - \mathbf{y}_j)^T(\mathbf{y}_i - \mathbf{y}_j)$. Let $\mathbf{L}$ be an $n \times (n-1)$ matrix of linear contrasts with full column rank such that $L_{ii} = 1$ and $L_{(i+1)i} = -1$ with zero elsewhere (though the choice of $\mathbf{L}$ is arbitrary under the constraint that $\mathbf{L}^T\mathbf{1} = \mathbf{0}$). Collecting the squared distances D_{ij} into an $n \times n$ distance matrix $\mathbf{D}$,

$$\mathbf{L}^T(-\mathbf{D})\mathbf{L} \sim \mathcal{W}_K(\mathbf{L}^T 2\mathbf{Q}^{-}\mathbf{L}), \tag{4.5}$$

where $\mathbf{Q} = \mathbf{M} - \mathbf{W}$.

We made three slight modifications to the model of Hanks and Hooten (2013): First, because generalized inverses are known to be numerically unstable (e.g., Söderström & Stewart, 1974), we let $\mathbf{Q}^* = \mathbf{M} - \rho\mathbf{W}$, where $\rho \in (0, 1)$ represents the degree of spatial autocorrelation in genotypes. This is equivalent to a conditional auto-regressive model, a class of models in which the ICAR model is a limiting case (i.e., $\rho \to 1$) (Besag, 1974; Peterson et al., 2019). With $0 < \rho < 1$, the matrix $\mathbf{Q}^*$ is positive definite which improves computational efficiency and stability. However, we put an informative prior on ρ, putting most prior weight towards the upper limit of 1 such that the model tends toward the limiting case. Second, we adjusted the log-linear model for the weights, $W_{ij} = \exp\{\mathbf{x}_{ij}^T\boldsymbol{\beta}\}$, to include geographic distance as an explanatory variable because we was interested in interactions between distance and landcape variables. Finally, we defined the degrees of freedom parameter as an unknown parameter to be estimated, κ. If all loci at which individuals are genotyped are mutually independent (i.e., at linkage equilibrium), then $\kappa = K$. Since this is unlikely in practice, we jointly estimated κ and the other parameters of interest (a similar approach is taken in Petkova et al., 2016). Putting these pieces together, the full model for the likelihood becomes

$$\mathbf{L}^T(-\mathbf{D})\mathbf{L} \sim \mathcal{W}_\kappa\left(\mathbf{L}^T 2(\mathbf{M} - \rho\mathbf{W})^{\mathbf{-1}}\mathbf{L}\right), \tag{4.6}$$

where $W_{ij} = \exp(\mathbf{x}_{ij}^T\boldsymbol{\beta})$.

The landscape variables of interest included: **1)** geographic distance between individuals i and j; **2)** the average density of conspecifics around the pair of focal plants; **3)** the average degree of isolation from other meadow habitat for the focal pair, measured as the average of the proportion of forested area in a 100 meter radius around each plant; and **4)** the average of the canopy cover above each plant in a focal pair. We allowed variables 2-4 to interact with geographic distance such that effects of the landscape features may diminish or switch signs with increasing distance. For example, my *a priori* prediction for the effect of local canopy cover on genetic similarity (inverse of genetic distance) was positive at small geographic distances but negative at greater geographic distances because pollinators may fail to locate plants under a forest canopy (Figure 4.1a & 4.1b). We therefore expected increased self-pollination and inbreeding at the local scale, resulting in increased genetic relatedness at small distances, but high resistance to gene flow at larger scales (isolation-by-concealment hypothesis).

We used the R package `rstan` (Carpenter et al., 2017; Team, 2020) to fit the model and checked chain convergence by checking that the $\hat{R}$ values were below 1.1 for all parameters being estimated (Gelman et al., 2013). The full model included all the covariates of interest described above as well as two indicator variables: one indicating whether a focal $\{i, j\}$ pair was found in the same meadow and one for whether the pair was found within the same complex to account for shared history, such as common ancestral founder populations and other demographic processes, both within meadows and on a given peak. Finally, the full model included varying intercepts for each meadow to allow for greater genetic similarity in some meadows compared to others (i.e., a meadow effect).

We used weakly informative Gaussian priors for regression coefficients (Gelman et al., 2013) and a Beta(5,1) prior for ρ that concentrated prior mass near one, thus approximating an ICAR model while maintaining a full rank precision matrix $\mathbf{Q}^* = \mathbf{M} - \rho\mathbf{W}$. We defined a shifted and scaled Beta prior for κ that supports positive density on the interval (n, K) and puts more prior weight towards the upper limit (K) since we took measures to minimize correlation among loci. Finally, we checked the model fit using graphical and numerical posterior predictive checks. All reported parameter estimates are the Bayes estimators ($\hat{\theta} = \mathbb{E}(\theta|\mathbf{D})$ for some parameter θ) followed by 95% posterior credible intervals in square brackets.

4.4 Results

Our SNP filtering process left us with 3,814 biallelic, putative neutral loci after removing 8,064 biallelic loci that mapped to coding regions within the *A. coerulea* genome. At putative neutral loci, individual heterozygosity, measured as the proportion of loci at which an individual is heterozygous, averaged 0.21 ($s = 0.02$, where s is the sample standard deviation) across sampled plants and did not appear to be related to landscape variables of interest (Figure D.2). When we defined meadows to be populations and computed basic population genetic statistics, all meadows showed some degree of heterozygote deficiency (i.e., $F_{IS} > 0$;

Table 4.1; Wright (1949)). The mean inbreeding coefficient across meadows was $\bar{F}_{IS} = 0.05$ ($s = 0.03$).

After fitting the landscape genetics model to our dataset of putative neutral loci, we found evidence of an overall negative relationship between geographic distance and genetic similarity ($\hat{\beta}_{\text{dist}} = -0.41\ [-0.50, -0.33]$; Figures 4.2 and 4.3), consistent with our expectation of isolation by distance (Wright, 1943). The estimated effects of the indicator variables indicating whether the two plants in a focal pair were sampled from the same meadow or same complex were also consistent with our predictions. Both estimates were positive, indicating shared histories in a meadow and on a peak ($\hat{\beta}_{\text{meadow}} = 1.36\ [1.28, 1.44]$; $\hat{\beta}_{\text{complex}} = 0.64\ [0.44, 0.83]$; Figure 4.2). Interestingly, average conspecific plant density around a pair of focal plants (number of conspecific individuals within 5 m of a focal plant) did not appear to be strongly related to genetic similarity of the focal pair ($\hat{\beta}_{\text{density}} = 0.00$ [-0.01, 0.01]; Figure 4.2).

Contrary to our predictions from the isolation by resistance hypothesis, we found evidence supporting less than average genetic similarity among neighboring plants (geographic distances close to zero) in isolated locations, fixing canopy cover at 0%. The main effect of isolation (mean proportion of forested area in a 100 m buffer around a focal pair) was estimated to be negative ($\hat{\beta}_{\text{isol}} = -0.97\ [-1.20,\ -0.76]$), while the interaction between isolation and distance was positive ($\hat{\beta}_{\text{dist}\times\text{isol}} = 0.53\ [0.44,\ 0.63]$), supporting low genetic similarity among neighboring plants in isolated locations and that genetic similarity between isolated plants does not further decay across increasing distances between plants (Figure 4.4). The rate of (exponential) decay in edge weight with increasing distance between two plants in connected locations (0% forest cover in a 100 m buffer around each of the plants) was esti-

Table 4.1: General population genetic summaries, treating the meadow as the population.

Meadow ID	**Sampling stratum**	**Individuals genotyped**	H_O	H_E	F_{IS}
CMX41A	LgC	8	0.202	0.215	0.058
CMM2	SmI	8	0.209	0.216	0.031
CMX11B	LgI	8	0.204	0.218	0.063
CMX41C	SmC	8	0.207	0.217	0.049
CMX43	LgI	8	0.205	0.219	0.06
CMX11C	SmI	6	0.214	0.218	0.02
LOMX4A	LgC	8	0.21	0.221	0.048
LOMX15E	LgI	8	0.212	0.214	0.011
LOMM14	SmC	8	0.213	0.22	0.031
LOMM27	SmC	8	0.211	0.221	0.045
LOMMWS	SmC	8	0.199	0.214	0.07
LOMCAGE	SmI	5	0.192	0.221	0.134
LOMW1	SmI	8	0.2	0.211	0.054
M1M3	LgC	8	0.2	0.211	0.052
M1X1A	LgC	8	0.21	0.218	0.039
M1X7	LgC	5	0.201	0.206	0.025
M1M5	LgI	8	0.2	0.21	0.047
M1X2B	SmC	8	0.211	0.217	0.027
M1LG1	SmI	8	0.217	0.226	0.041
M2X21A	LgC	8	0.189	0.211	0.103
M2X21B	LgC	8	0.195	0.221	0.117
M2X17	LgI	8	0.207	0.216	0.041
M2X21E	LgI	8	0.21	0.216	0.025
M2X48	SmC	8	0.206	0.214	0.036
M2M1	SmI	8	0.207	0.215	0.034

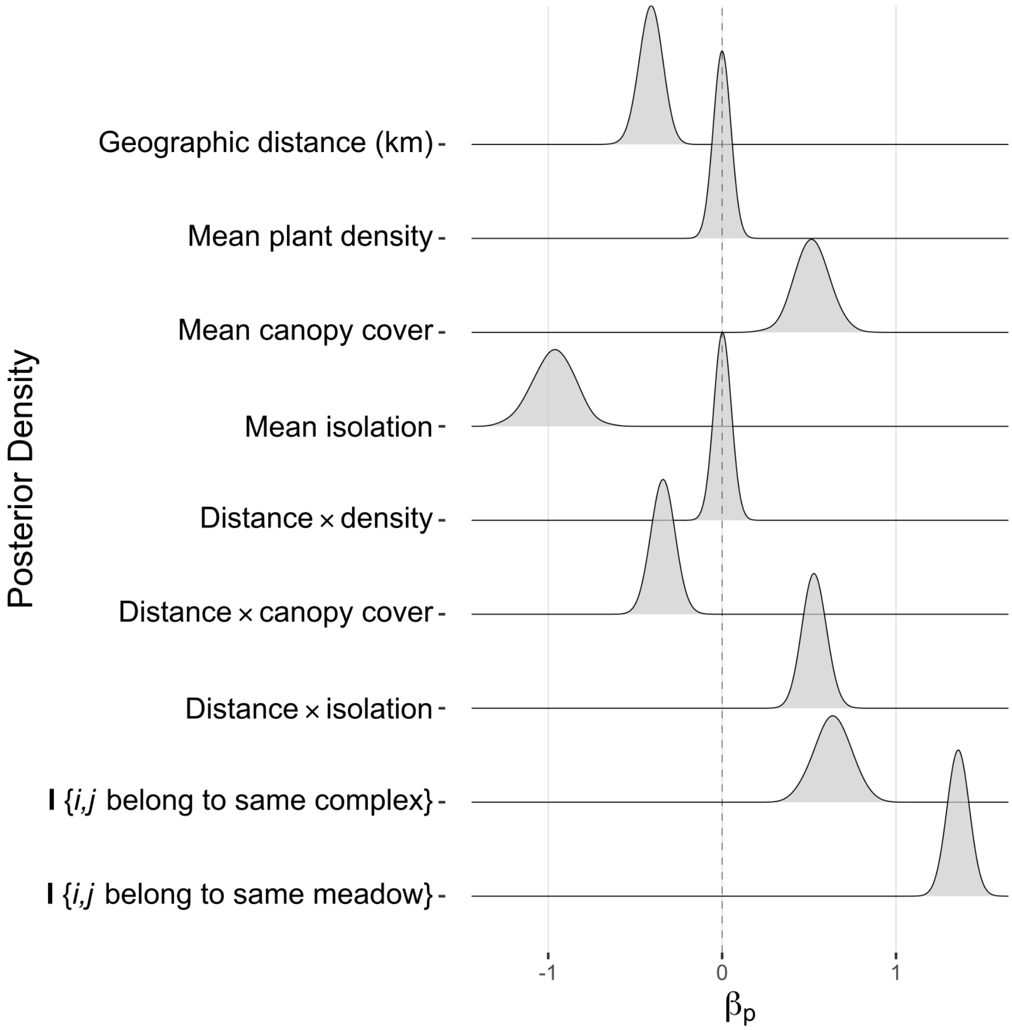

Figure 4.2: Posterior distributions of regression coefficients. The notation $\mathbf{I}\{\cdot\}$ denotes the indicator function returning 1 when the argument in brackets is satisfied and 0 otherwise.

mated at $-0.41d$ $[-0.50d,\ -0.33d]$, where d is the distance in kilometers. On the other hand, edge weight was estimated to increase slightly with increasing distance between two highly isolated plants (average of 95% forest cover in a 100 m buffer around each plant), at least at these relatively small spatial scales (exponential rate parameter $0.09d$ $[0.04d,\ 0.14d]$; Figure 4.4). This is the opposite of what we would predict based on the isolation-by-resistance hypothesis. We discuss possible explanations for this trend in the Discussion section below.

Consistent with the concealment hypothesis in which we posit that mating amongst relatives and/or selfing could increase if flowers become more difficult for pollinators to discover once overgrown by woody vegetation, we found evidence that genetic similarity between neighboring plants under high canopy cover was greater than between neighboring plants in the open ($\hat{\beta}_{\text{cover}} = 0.52$ [0.35, 0.70]). Furthermore, the rate of (exponential) decay in edge weight across space when comparing two plants under high (100%) canopy cover was steeper ($-0.70d$ $[-0.81d,\ -0.59d]$) than the decay across space when comparing two plants in the open ($-0.36d$ $[-0.43d,\ -0.28d]$), fixing isolation at an average of 10% forest surrounding the plants in the comparison (i.e., fixing the isolation covariate to 0.1).

4.5 Discussion

We found support for our concealment hypothesis that, once overgrown by taller, woody vegetation, *A. formosa* individuals may be discovered less frequently by pollinators (Gannon et al., 2021) and gene flow may be reduced between obscured plants that are far apart (> 2 km apart; Figure 4.4b) compared to plants in the open. The average amount of forest canopy cover immediately above a pair of focal plants was positively related to genetic similarity

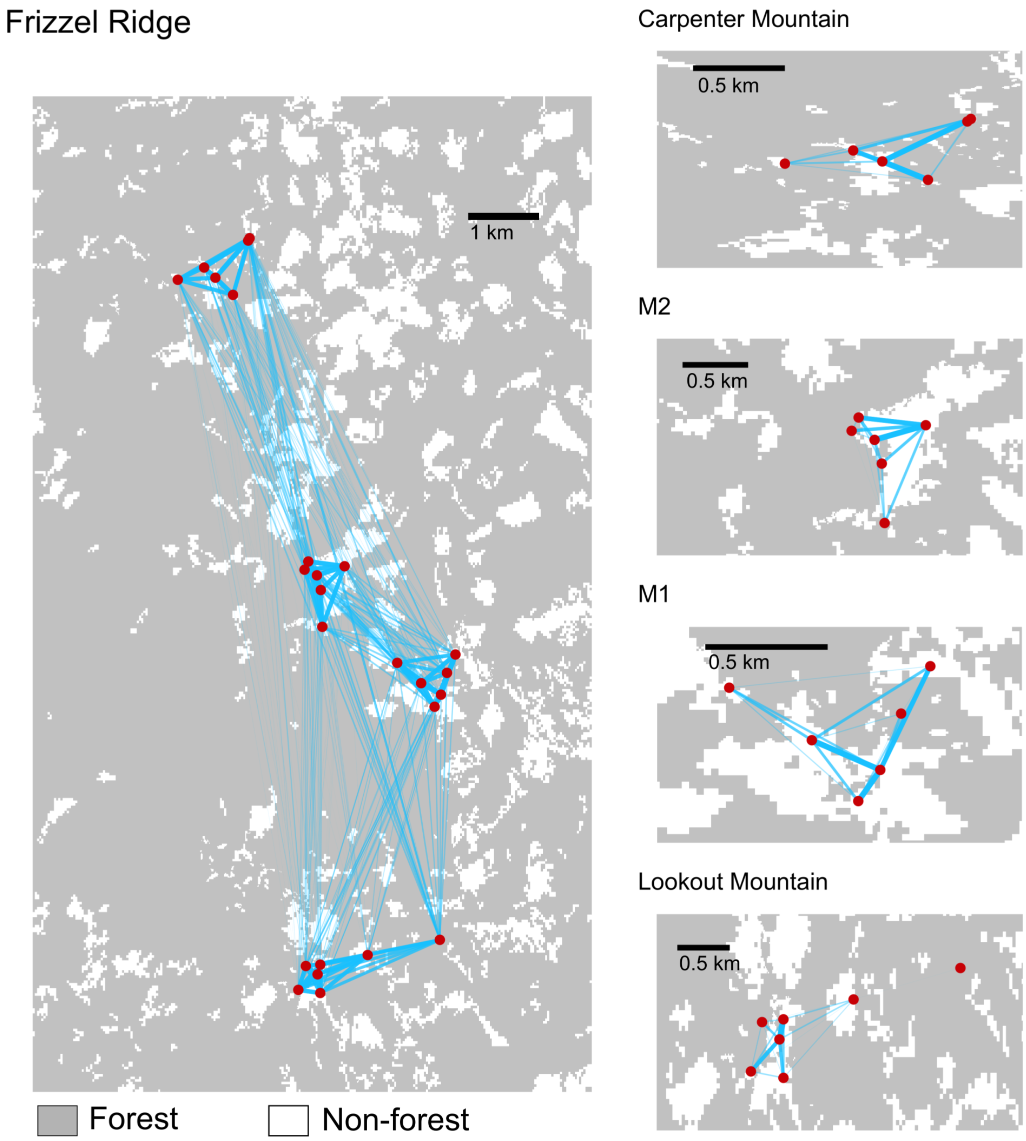

Figure 4.3: Predicted genetic network for western columbine sub-populations along Frizzel Ridge. The thickness of the line reflects the weight of the edge predicted based on explanatory variable measurements on the edge and posterior mean effects. Heavier weights reflect reduced genetic distances and therefore, presumably, greater gene flow. Note that differences in line thickness have been accentuated to facilitate visual inspection of the network.

when the pair of plants were close in space; however, the decay in genetic similarity across space was steeper when comparing plants under high canopy cover than when comparing plants in the open (Figures 4.2 & 4.4b). This supports our hypothesis that increasing canopy cover above a plant could increase the proportion of self-fertilized seeds and rate of near-neighbor mating which would result in relatively greater average relatedness among individuals at small spatial scales (as we observed here).

At a broad scale we found that isolation-by-distance appears to drive patterns in population genetic structure of *A. formosa* in the HJA (Figure 4.3). This was evidenced by positive relationships between the indicator variables indicating whether the focal pair of plants came from the same meadow and/or complex as well as a negative relationship between genetic similarity and geographic distance between plants (Figure 4.2). However, we found somewhat counter-intuitive results when examining genetic similarity at different spatial scales and in different landscape contexts. At small spatial scales (< 2 km; Figure 4.4) we found the opposite of the expected relationship between genetic similarity and the degree of isolation of the focal pair of plants (Figure 4.4a). Neighboring plants in open but isolated locations are expected to be less genetically similar than neighboring plants in locations connected to other open areas (Figures 4.4a & 4.4b). However, two plants in locations that are isolated from other open habitat and far apart from one another (>2 km; Figure 4.4d) are expected to be more genetically similar than two plants growing at the same distance apart but in locations that are connected to other non-forested areas. Thus, neighboring plants in isolated locations are less genetically similar to one another on average than neighboring plants in well-connected locations and similarity does not decay across space as it does in connected locations (Figure 4.4a). Consistent with these findings, we found some weak evidence that

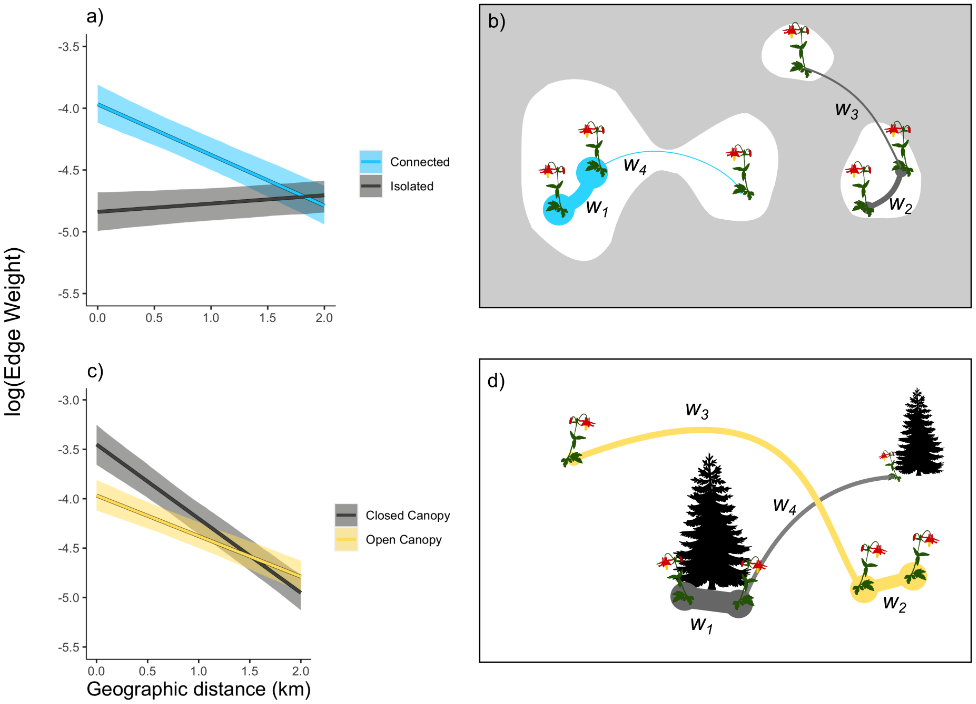

Figure 4.4: Interactions between landscape variables (canopy cover and isolation) and geographic distance and the relationship to edge weight (i.e., genetic similarity), with 95% credible intervals. **a)** Interaction plot showing the relationship between genetic similarity (edge weight) and geographic distance between two randomly selected plants when both plants are located in patches with high connectivity (blue line) and when the focal plants both exist in isolated locations. **b)** Schematic of genetic similarity (presumably a result of pollen-mediated gene flow) across two connected and two isolated patches. The thickness of the lines reflect the model-predicted edge weights ($w_1 > w_2 > w_3 > w_4$). Note that the direction of the interaction between isolation and distance is the opposite of what we would predict based on the isolation-by-resistance hypothesis. **c)** Interaction plot showing the relationship between genetic similarity (edge weight) and geographic distance between two randomly selected plants when both plants are in the open (yellow line) and when both plants are growing beneath closed canopy (grey line). **d)** Schematic of genetic similarity based on model-predicted edge weights. The model predicts an order of edge weights ($w_1 > w_2 > w_3 > w_4$) that is consistent with the isolation by concealment hypothesis.

observed heterozygosity (treating the meadow as the population) was slightly greater in isolated meadows compared to connected meadows (Figure D.1) and plants in isolated locations did not appear to be pollen limited (Figure D.3) or more inbred (Figure D.2). This is exactly the reverse of what we would predict based on the isolation-by-resistance hypothesis (Figure 4.1). As a potential explanation for these results, we propose the following:

Isolation, as measured here (the proportion of forested cells in a 100m buffer around a given plant), includes information on the amount of surrounding habitat available to conspecifics (Bender et al., 2003). Thus, an increase in this metric of isolation is accompanied by a decrease in the availability and/or quality of nearby habitat for conspecific individuals, and may be associated with gaps between patches of habitat. Thus, this metric captures and quantifies characteristics of what we would intuitively call a patchy or fragmented landscape (Bender et al., 2003). Foraging through a patchy landscape, pollinators must switch their behavior from foraging to dispersing when commuting to the next patch of habitat (we briefly switch our terminology to "patch" instead of "meadow" to broaden the scope of this discussion). Let T_c be a random variable with probability density function $f_{T_c}(t)$ and defined as the time a given pollen grain remains on a pollinator's body in a landscape of continuous habitat. Similarly, let T_p be the time a given pollen grain remains on a pollinator's body in a patchy landscape (with pdf $f_{T_p}(t)$). If the rate of pollen removal from a pollinator's body while foraging is greater than the rate of removal while in transit to another patch (assuming that frequent contact with stigmas and other flower organs during foraging increases rates of pollen removal from the pollinator's body), then the expected time a pollen grain remains on a pollinator's body (μ) is greater in a patchy landscape than in a continuous landscape (i.e., $\mu_p > \mu_c$; Figure 4.5). This is simply due to the fact that the pollinator must spend time in transit to the next patch, briefly reducing the rate of pollen removal until arriving in another patch to forage. Assuming a pollinator is moving away from the father plant during the time in question, this should also allow pollen to travel greater distances when the father is in a small, isolated patch.

Similarly, if gaps exist between patches and small patches cannot support a pollinator's food requirements, then the probability that a pollinator's previous visit was to a plant in the same patch may be lower for a plant growing in a small, isolated patch than one in a large or connected patch. Thus, on any given pollinator visit, it may be more likely that a plant in a small, isolated meadow receives pollen from a geographically and therefore genetically distant source than if the plant were growing in a large, connected meadow. This could result in high local genetic diversity and a flatter decay (or lack thereof) of genetic relatedness with increasing distance when comparing plants from isolated meadows, as we observed (Figure 4.4d).

Based on previous results in this system (Gannon et al., 2021), we extend this hypothesis further to propose that subordinate hummingbirds may be important in maintaining the connectivity of the isolated sub-populations of *A. formosa* (at least in the HJA, though there is no reason to believe this should not generalize to other systems). This is potentially supported by two separate lines of evidence. First, Gannon et al. (2021) found that the majority of movements among meadows were made by female hummingbirds which tend to be less dominant in competitive interactions than males (Carpenter et al., 1993). Second, we found here the unexpected result that the spatial decay of genetic similarity is lessened across moderately to extensively isolated locations, indicative of high rates of gene flow to and from isolated meadows. Combined with our simple model of how foraging in a patchy landscape

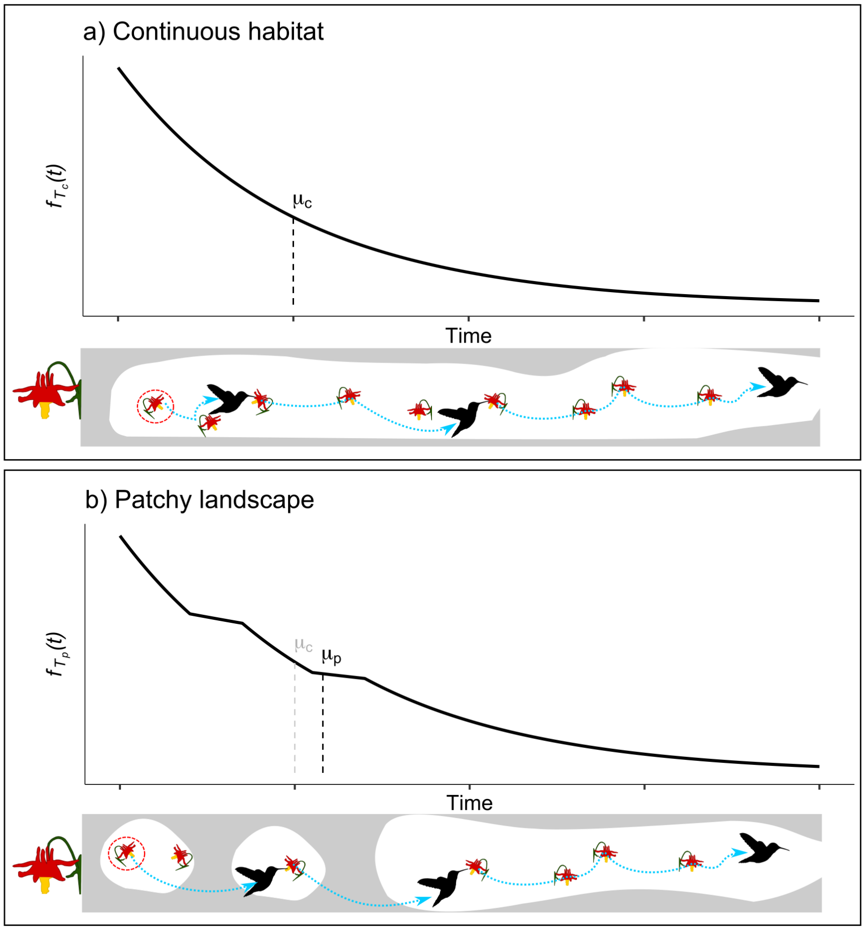

Figure 4.5: **a)** Hypothetical model of the distribution of times ($f_{T_c}(t)$) pollen grains remain on a pollinator's body before removal in a landscape of continuous habitat. The expected time a pollen grain spends on a pollinator's body ($\mathbb{E}(T_c) = \mu_c$) is marked with a dashed line. Beneath the distribution model is a schematic of hypothetical foraging behavior through the landscape, where T_c would track the time a randomly selected pollen grain from the plant inside the red circle remains on the pollinator's body. **b)** Hypothetical model of the distribution of times ($f_{T_p}(t)$) pollen grains remain on a pollinator's body in a patchy landscape, assuming the risk of removal is greater while foraging than while in transit between patches. The expected time a pollen grain spends on a pollinator's body is greater in the patchy landscape than in the landscape of continuous habitat ($\mathbb{E}(T_p) = \mu_p > \mu_c$).

could increase average pollen dispersal distances, these two lines of evidence suggest that birds that are unable to hold territories in prime, connected habitat could be important to maintaining pollen flow and plant population connectivity across isolated or marginal habitats. Indeed, many have suggested (Burd, 1995; Krauss et al., 2017; Wessinger, 2021) and some have found (Maruyama et al., 2016; Temeles et al., 2019; Torres-Vanegas et al., 2019; Torres-Vanegas et al., 2020) that non-territory holders carry more genetically diverse pollen loads, though more work is needed in this and other systems to test our hypothesis.

Indeed, there exist alternative explanations for the counter-intuitive result of relaxed spatial decay of genetic similarity across isolated locations. One possibility is that the genetic structure we observed throughout the connected meadow habitat reflects more recent landscape configuration while what we found in isolated locations reflects a more historical landscape with large, continuous open areas and large, panmictic populations of *A. formosa*. However, we find this explanation unlikely for two reasons. First, plants sampled from isolated locations would need to be primarily old individuals with genotypes reflective of demographic and selective processes on the landscape prior to forest encroachment; but, given the timescale of forest encroachment (over decades; Dailey, 2008) and the requirement of low seed production and establishment in the isolated locations for us to sample primarily old plants (seed set data in Appendix D suggest at least as high seed production in isolated locations as in connected locations), we do not expect this was the case. Furthermore, we expect that we would have observed the same pattern of a relaxed spatial decay of genetic similarity when comparing plants under high canopy cover if this alternative explanation were true.

Another alternative is that harsh conditions in marginal and isolated meadows increase the strength of inbreeding depression (Yang & Hodges, 2010), reducing the genetic similarity of nearby individuals in isolated locations compared to in well-connected habitat. However, we also find this explanation unlikely for two reasons. First, increasing the canopy cover above plants and therefore reducing light availability should enhance the growing stress placed on plants. Using the same argument, high canopy cover should also increase the strength of inbreeding depression. However, we did not find evidence consistent with this prediction. Genetic similarity among neighboring plants was greater under high canopy cover than among neighboring plants in the open (Figure 4.4c). Secondly, there is currently no evidence to suggest that isolated but open locations should pose more stressful growing conditions for plants. In fact, seed set could be limited under stressful conditions (Liu et al., 2013), but we found no evidence that seed set was limited in isolated locations (Figure D.3).

Our results highlight that the effects of woody encroachment and upward shifts of treeline on the persistence and connectivity of meadow plant populations can be multifaceted and complex. At a broad scale, isolation by distance appears to drive patterns in population genetic structure of *A. formosa* in the HJA (Figure 4.3), but our statistical approach allowed us to explore the relationship between genetic differentiation and landscape configuration at different spatial scales. At smaller spatial scales, we found evidence to support that gene flow may actually be enhanced among isolated locations. We also found evidence supporting that gene flow among plants growing under invading woody species may be low. Hence, woody encroachment could increase or decrease effective population sizes depending on whether gene flow among isolated but open locations is sufficient to compensate for reduced gene flow from plants that become overgrown by taller forest species. Finally, our results are consistent with the proposition that subordinate and non-territorial pollinators could be important for maintaining gene flow and population connectivity through marginal or degraded habitats (Maruyama et al., 2016; Temeles et al., 2019; Torres-Vanegas et al., 2019; Torres-Vanegas et al., 2020; Gannon et al., 2021), something that deserves experimental tests in the future.

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All raw data from this study will be available on the H. J. Andrews NSF LTER data portal (https://doi.org/10.6073/pasta/19c72b7b4ada35ce82b091b42b492b0b). All code necessary to reproduce the analyses will be made available on a public GitHub repository (https://github.com/Dusty-Gannon/HJA_Columbine_landscap_genetics).

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Chapter 5: General Conclusion

The overarching theme of my dissertation is the stability of plant-pollinator mutualisms in the face of global change. I focused on: **1)** tests for cryptic pollinator specialization in plants of the Heliconiaceae that could mislead our predictions about the stability of the plant-pollinator interactions involving cryptic specialists (see Chapter 2); and **2)** how pollinator foraging movements relate to landscape characteristics and whether those relationships are reflected in the genetic structure of plant populations (Chapters 3 & 4).

In Chapter 2, coauthors and I did not find evidence for pollinator recognition, where pollen tube success is enhanced following visits from morphologically matched pollinators, in *Heliconia hirsuta* or *H. wagneriana*. The data support the possibility of pollinator recognition in *H. rostrata*, but we cannot speculate on whether pollen tube counts would vary by pollinator species in the native range of *H. rostrata* based on the experiments we conducted. Thus, further research is necessary to determine how widespread pollinator recognition is (if it exists at all). Given the implications for coextinction of a plant species following local extirpation of its morphologically matched pollinator, further study of pollinator recognition is warranted.

In additional to testing for pollinator recognition in many taxa in their native ranges, further research is necessary to determine the mechanism underlying the evidence supporting pollinator recognition in *H. tortuosa*; namely, the increased pollen tube count following visits from pollen-free hummingbirds with morphologically matched bills but not those with mismatched bill shapes. The experiments I conducted for Chapter 2 did not support nectar removal as the mechanism for increased pollen tube counts as previous experiments did (Betts et al., 2015). Thus, the mechanism underlying the consistent increase in pollen tube counts following visits by morphologically matched birds (Chapter 2; Betts et al., 2015) is equivocal. We suggested a set of possible mechanisms to test in Chapter 2, including sonic vibrations due to hummingbird wing beats (Veits et al., 2019), static charges due to hummingbird flight (Badger et al., 2015), and stigma clogging with experimentally high pollen loads in hand pollination treatments (Young & Young, 1992) which could be 'unclogged' by morphologically matched pollinators during a visit.

During our aviary experiments with *H. tortuosa*, I noticed that Rufous-tailed Hummingbirds (the species with a mismatched bill shape) perched on the bract housing the flower while attempting to drink from the flower, while Green Hermit Hummingbirds (a morphologically matched species) hovered while visiting the flower. Thus, it is possible that the difference in pollen tube counts between these two treatments is due to differences in sonic vibrations during a hummingbird's visit to the flower, something to which other plants have been shown to respond (Veits et al., 2019). This hypothesis is supported by the observation that both species of hummingbirds hovered while visiting flowers of *H. rostrata*, the only other species showing some evidence of a response to visits by hummingbirds compared to hand pollination alone. Future work could test this idea using audio recordings of the wing beats of different hummingbird species (or arbitrary sonic vibrations at the same frequencies) played near the flower while pollinating the flower by hand.

Stigma clogging should also be tested in any future work on pollinator recognition. Tests could include treatments with varying quantities of pollen grains placed on the stigma. This work may require creating aliquots of different numbers of pollen grains under a microscope before pollination experiments, but it will be necessary to verify that the results from the aviary experiments in Chapter 2 and those in Betts et al. (2015) are not due to experimentally high pollen loads that are alleviated by morphologically matched hummingbirds. These alternative hypotheses for the mechanism should provide an interesting avenue for future research.

In Chapter 3, coauthors and I found some evidence that forest encroachment into the alpine meadows of the central Cascade Mountains, USA (Haugo & Halpern, 2007; Dailey, 2008) could reduce the functional connectivity of the landscape by reducing the capacity for Rufous Hummingbirds to discover nectar resources. Based on the model we fit to hummingbird relocation data, the predicted probability that a hummingbird moves between two resources (a proxy for the probability of pollen-transfer between two flowers that are visited by hummingbirds) that are overgrown by woody vegetation is 69% less than if the two resources are in the open. When the intervening landscape is also forested, movement probability drops to 83% of the predicted probability for when the landscape is not forested and nectar resources are in the open. Taken together with the finding that most hummingbirds were not detected moving greater than 2 kilometers on any given day, forest encroachment that grows over and conceals existing nectar plants and/or increases the gaps among meadows could substantially reduce the functional connectivity of the landscape from the perspective of hummingbird movement and pollen flow among the plants they pollinate.

Our findings in Chapter 4 were also consistent with the hypothesis that forest cover could reduce gene flow among herbaceous plant species by concealing plants from pollinators. We found that neighboring plants growing under forest canopy shared greater genetic similarity than neighboring plants growing in the open, indicating higher rates of selfing or near-neighbor mating under forest canopy. Furthermore, plants growing under forest canopy but far apart in space (> 2 km) shared less genetic similarity than two plants the same distance apart but growing in the open. This is consistent with the hypothesis that plants that get overgrown by taller woody vegetation are discovered infrequently by pollinators, making pollen transfer between two plants that are far apart and concealed exceptionally unlikely.

Unexpectedly, plants growing in the open but that were generally more isolated, measured as the proportion of forested area in a 100 m radius around the focal plant, showed a flatter spatial decay of genetic similarity to surrounding plants than plants growing in open locations that were connected to other meadow habitat. This is indicative of a pollen dispersal kernel with a fatter tail (greater excess kurtosis) for isolated plants. I formalized a simple model built on pollinator foraging in continuous compared to fragmented habitat that could explain this result in the Discussion section of Chapter 4. Put simply, a pollinator foraging among plants in a patchy landscape must move among patches rather than foraging and depositing pollen at flowers more continuously. Assuming that the risk of removal for a given pollen grain is greater when the animal is foraging and reduced when the animal is commuting among patches, pollen grains may stand a greater chance of long-distance dispersal in a patchy landscape.

The results of Chapters 3 and 4 suggest promising trajectories for future research. In Chapter 3, we found that female hummingbirds, which tend to be less dominant in intraspecific competitive interactions (Carpenter et al., 1993), accounted for the vast majority of movements among artificial feeders. This, in combination with the finding that neighboring plants in isolated locations showed reduced genetic similarity compared to near neighbors in well-connected locations (Chapter 4) suggests to me that subordinate individuals of territorial pollinator species may be important to maintaining connectivity among subpopulations of plants that exist in marginal or edge habitat. While differences in foraging behaviors among species and the implications for pollen dispersal have been investigated in a few systems (Maruyama et al., 2016; Temeles et al., 2019; Torres-Vanegas et al., 2019), little is known about within-species differences in pollinator quality (but see Temeles et al., 2016).

Future work could utilize distinct pollen analogs (e.g., fluorescent powders that fluoresce different colors) applied directly to captive hummingbirds of different sexes (applying different colored powders to each sex). The birds could then be released to forage normally through the landscape. The range of dispersal distances for the unique pollen analogs could provide information on any differences in movement and pollen dispersal between the two sexes. Both sexual dimorphism in pollinator quality and the effect of pollinator territoriality on plant population genetics present intriguing avenues for future work.

Pollination ecology is a rich and interesting field of study with plenty of room for both theoretical and applied insights. While the future of efforts to conserve plant-pollinator communities is dependent on our understanding of complex and dynamic systems, I am confident that the importance of these communities to human food security and well-being will promote rapid scientific advances. I hope the work presented here will contribute to those advances through exposing readers to new and underutilized statistical techniques for modeling ecological processes using spatial networks and to the realization that cryptic pollinator filters may exist and mislead our predictions of plant-pollinator network stability.

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APPENDICES

Appendix A: A formal model for how pollinator recognition could stabilize a coevolutionary race and promote trait matching

Betts et al. (2015) hypothesized that pollinator recognition based on nectar removal, where pollen tube success is enhanced by complete nectar removal from the nectar chamber by a visiting pollinator, could promote trait matching. While empirical evidence for the mechanism of pollinator recognition is equivocal (Chapter 2), of all mechanisms tested so far, nectar removal has the most support (Betts et al., 2015, 2). Thus, I explore how pollinator recognition through nectar removal cues could influence floral evolution.

I formulate a model of coevolution between corolla length and hummingbird bill length in two infinite populations in which pollinator recognition by nectar removal has already evolved in the plants. First, I assume that any given pollen grain has probability p_m of being transferred to a hummingbird on a given visit, but that this probability varies as a function of the morphological mismatch (i.e., difference) between corolla and bill length. As theorized by Darwin (1862), I assume a model of coevolutionary escalation in which interacting partners benefit most from an interaction when their trait values (corolla length x or tongue length y) are more extreme than those of interacting partners. For example, a pollinator with a bill (or proboscis, tongue, etc.) shorter than the length of the corolla tube will be forced to push deep into the corolla to access nectar. This action should increase contact with anthers and stigmas of plants, promoting pollen transfer. Thus, the probability that a given pollen grain is transferred (either to the pollinator or from the pollinator's body to the stigma) during a visit to a plant with a longer than average corolla should be greater than to plants with shorter flowers (Nilsson, 1988; Alexandersson & Johnson, 2002; Pauw et al., 2009; Paudel et al., 2016). Increased pollen transfer should, in general, increase plant fecundity and therefore fitness (Minnaar et al., 2019).

Similarly, pollinators with longer tongues (for example) should have access to more nectar than pollinators with shorter tongues and gain a fitness advantage in the population (Darwin, 1862; Temeles et al., 2009; Pauw et al., 2009; Paudel et al., 2016). Directional evolution of trait values in either interacting population increases the strength of selection acting on the other. This increase in the strength of selection results in an evolutionary 'tit-for-tat' with each partner evolving longer and longer traits (Darwin, 1862; Nuismer, 2017).

A convenient expression with which to model tit-for-tat dynamics is a logistic curve constrained to the open (0,1) interval (e.g. Ridenhour & Nuismer, 2007). I assume that the probability that pollen is transferred from the pollinator to the plant is p_t and the probability that a hummingbird is successful in accessing the nectar reward is p_n. For tractability and because pollinator recognition should only directly affect the female component of fitness (number of ovules fertilized), I ignore the male component (pollen export to sire seeds of different plants). Given corolla length x and bill length y, these probabilities can be modeled as

$$p_t(x, y) = \frac{1}{1 + e^{-\beta(x-y)}} \tag{A.1a}$$

and

$$p_n(x, y) = \frac{1}{1 + e^{\alpha(x-y)}}, \tag{A.1b}$$

respectively. The parameters α and β ($\alpha, \beta \in \mathbb{R}^+$) control how sensitive the two probabilities are to the difference between corolla and bill length, with larger values resulting in greater sensitivity.

According to Darwin's model (formalized above), pollen transfer should increase as flower length exceeds bill length; however, if pollen germination success is dependent on complete nectar removal during the visit (Betts et al., 2015), then the fitness benefit a plant receives following an interaction with a pollinator with bill-length y will depend on the joint probability of pollen transfer, $p_t(x, y)$, and nectar removal, $p_n(x, y)$. Assuming these two processes are independent (for simplicity), the probability of 'successful' pollen receipt p_s, given x and y, is

$$p_s(x, y) = \left(\frac{1}{1 + e^{-\beta(x-y)}}\right)\left(\frac{1}{1 + e^{\alpha(x-y)}}\right). \tag{A.2}$$

To relate these functions of x and y to plant and pollinator fitness, I assume that hummingbirds gain a fitness benefit of magnitude N after draining the nectar chamber from a plant. Given x and y, this occurs with probability $p_n(x, y)$. Similarly, plants gain a fitness advantage for each pollen grain transferred from the pollinator to the stigma. Assuming that the pollinator population is saturated with pollen and each pollinator carries M pollen grains, the fitness benefit a plant with trait x receives after being visited by a pollinator with trait y is $Mp_s(x, y)$. Each pollen grain carries a fitness benefit of 1 unit and behaves independently of the others. While perhaps not true in reality (e.g., Sabban et al., 2012), this greatly simplifies the calculations.

Average fitness in the plant and pollinator populations is therefore

$$\bar{w_p} = \mathbb{E}(\eta_p + Mp_s(x, y)) = \int_{\mathcal{X}}\int_{\mathcal{Y}}(\eta_p + Mp_s(x, y))f_X(x)f_Y(y)dxdy \tag{A.3a}$$

and

$$\bar{w}_a = \mathbb{E}(\eta_a + Sp_n(x,y)) = \int_{\mathcal{X}}\int_{\mathcal{Y}} (\eta_a + Np_n(x,y)) f_X(x) f_Y(y)\, dx dy, \tag{A.3b}$$

respectively, where η_p and η_a provide a baseline fitness composed of components not related to pollination interactions and $f_X(x)$ and $f_Y(y)$ are continuous trait distributions for plants and pollinators (respectively). These expectations are not tractable, but approximate expectations can be found using Taylor expansions to approximate the functions $p_n(x,y)$ and $p_s(x,y)$.

Because $p_n(x,y)$ is monotonic along the axis of $x - y$, it makes sense to approximate this function with a first order Taylor expansion. However, I perform the expansion around $\alpha = 0$, thus implicitly making the assumption that fitness does not depend too strongly on interactions between plants and animals (Ridenhour & Nuismer, 2007; Nuismer et al., 2013; Nuismer, 2017). Denoting the first-order Taylor approximation for p_n as $\tilde{p}_n(\alpha)$,

$$\tilde{p}_n(\alpha) = p_n(0) + \alpha \cdot p_n'(0)$$

where $p_n'(0)$ is the first derivative of $p_n(\alpha)$ with respect to α and evaluated at zero. More explicitly,

$$\begin{aligned}
\tilde{p}_n(\alpha) &= \left.\frac{1}{1+e^{\alpha(x-y)}}\right|_{\alpha=0} + \alpha \cdot p_n'(0) \\
&= \frac{1}{2} + \alpha \cdot \left(\left.\frac{-(x-y)e^{\alpha(x-y)}}{(1+e^{\alpha(x-y)})^2}\right|_{\alpha=0} \right) \\
&= \frac{1}{2} - \frac{\alpha}{4}(x-y).
\end{aligned}$$

Thus, $\tilde{p}_n(\alpha)$ is a linear function in x and y for which the expectation can be easily computed.

However, because $p_s(x,y) = p_n(x,y)p_t(x,y)$ includes both α and β, and is concave in $x-y$, a second-order, multivariate Taylor expansion is appropriate. The second-order Taylor approximation for a bivariate, scalar function expanded around $\boldsymbol{\theta}_0$, which I denote $\tilde{p}_s(\boldsymbol{\theta})$, is

$$\tilde{p}_s(\boldsymbol{\theta}) = p_s(\boldsymbol{\theta}_0) + D(\boldsymbol{\theta}_0)\boldsymbol{\theta} + \frac{1}{2}\boldsymbol{\theta}^T\mathbf{H}(\boldsymbol{\theta}_0)\boldsymbol{\theta},$$

where $\boldsymbol{\theta}$ is the 2-dimensional vector of inputs with elements α and β, $D(\boldsymbol{\theta}_0)$ is a 2-dimensional row vector of partial derivatives evaluated at $\boldsymbol{\theta}_0$, and $\mathbf{H}(\boldsymbol{\theta}_0)$ is the Hessian matrix of second partial derivatives evaluated at the $\boldsymbol{\theta}_0$. Thus, defining $\boldsymbol{\theta}_0$ as the zero vector and letting $\Delta = (x-y)$,

$$D(\boldsymbol{\theta}_0) = \frac{1}{8}\begin{bmatrix} -\Delta & \Delta \end{bmatrix}$$

and

$$H_{11} = \left.\frac{\Delta^2(e^{2\alpha\Delta} - e^{\alpha\Delta})}{(1+e^{-\beta\Delta})(1+e^{\alpha\Delta})^3}\right|_{\alpha,\beta=0} = 0$$

$$H_{12} = H_{21} = \left.\frac{-\Delta^2 e^{(\alpha-\beta)\Delta}}{(1+e^{\alpha\Delta})^2(1+e^{-\beta\Delta})^2}\right|_{\alpha,\beta=0} = \frac{-\Delta^2}{16}$$

$$H_{22} = \left.\frac{\Delta^2(e^{-2\beta\Delta} - e^{-\beta\Delta})}{(1+e^{\alpha\Delta})(1+e^{-\beta\Delta})^3}\right|_{\alpha,\beta=0} = 0,$$

where H_{ij} is the element of $\mathbf{H}$ in the i^{th} row and j^{th} column. Thus,

$$\begin{aligned}\tilde{p}_s(\boldsymbol{\theta}) &= \frac{1}{4} + \frac{\beta-\alpha}{8}\Delta - \frac{\alpha\beta}{16}\Delta^2 \\ \tilde{p}_s(\boldsymbol{\theta}) &= \frac{1}{4} + \frac{\beta-\alpha}{8}(x-y) - \frac{\alpha\beta}{16}(x-y)^2.\end{aligned} \tag{A.4}$$

Using these approximate functions, $\tilde{p}_n$ and $\tilde{p}_s$, in place of p_n and p_s, an approximation to the desired expectation can be easily derived. First, assuming the traits X and Y are independent for simplicity and making use of the well-known identity that $\mathbb{E}(X^2) = \mathbb{E}(X)^2 + \mathrm{Var}(X)$, the average fitness for plants and pollinators is

$$\bar{w}_p \approx \eta_p + M\left(\frac{1}{4} + \frac{\beta-\alpha}{8}(\mu_x-\mu_y) - \frac{\alpha\beta}{16}(\mu_x-\mu_y)^2 - \frac{\alpha\beta}{16}(\sigma_x^2+\sigma_y^2)\right) \tag{A.5a}$$

$$\bar{w}_a \approx \eta_a + \frac{S}{2} - \frac{S\alpha}{4}(\mu_x-\mu_y), \tag{A.5b}$$

respectively.

Given expressions for (approximate) mean fitness as a function of mean trait values for each population, classical quantitative genetics approaches (Lande, 1976, 1979) can be used to calculate the change in the population mean phenotype over a generation, $\Delta\mu_x$ and $\Delta\mu_y$, and study the trajectory of coevolving traits (Kopp & Gavrilets, 2006; Nuismer et al., 2005; Nuismer, 2017). Assuming fixed genetic variances (G_x and G_y) across generations,

$$\Delta\mu_x = \frac{G_x}{\bar{w}_p}\frac{\partial\bar{w}_p}{\partial\mu_x} \tag{A.6a}$$

$$\Delta\mu_y = \frac{G_y}{\bar{w}_b}\frac{\partial\bar{w}_b}{\partial\mu_y}. \tag{A.6b}$$

Carrying out the differentiation and simplifying,

$$\Delta\mu_x \approx \frac{G_x M}{\bar{w}_p}\left(\frac{\beta - \alpha - \alpha\beta(\mu_x - \mu_y)}{8}\right) \tag{A.7a}$$

and

$$\Delta\mu_y \approx \frac{G_y S\alpha}{4\eta_a + 2S - 4S\alpha(\mu_x - \mu_y)}. \tag{A.7b}$$

An interesting result is evident by inspection of Eq. A.7: selection is directional for pollinators, meaning hummingbird bill lengths should evolve longer bills over time and selection will be strongest when $\mu_x > \mu_y$. However, selection for longer corollas will only occur as long as $\mu_x < \mu_y + \frac{\beta-\alpha}{\alpha\beta}$. Corolla evolution will reach equilibrium at $\mu_x = \mu_y + \frac{\beta-\alpha}{\alpha\beta}$ if μ_y is fixed. Thus, while plant and pollinator populations are engaged in tit-for-tat evolution towards longer corollas and bills, pollinator recognition constrains the evolutionary response of the plant population and instead promotes stronger trait matching between plant and pollinator populations than would otherwise occur (Figure A.1).

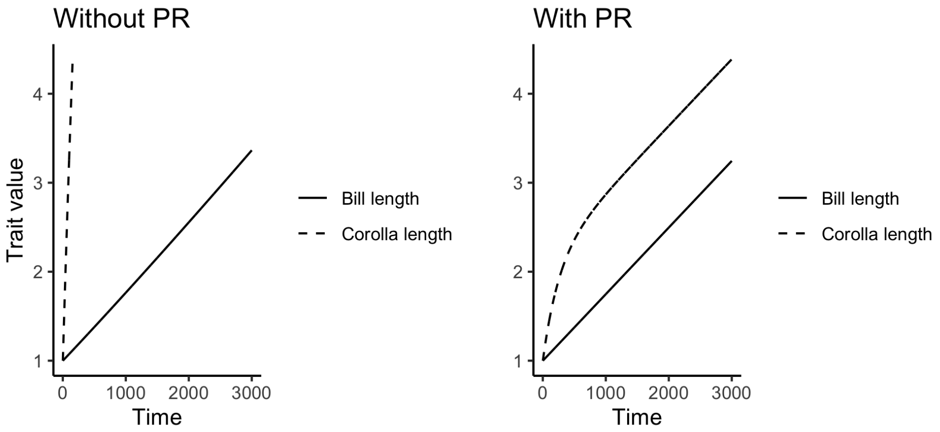

Figure A.1: Simulation of coevolution between a plant and a (hummingbird) pollinator population. **a)** Without pollinator recognition, traits may diverge quickly if the plant population has greater genetic variance or receives a greater fitness benefit for interacting with pollinators than does the pollinator population. **b)** With pollinator recognition, corolla evolution is stabilized and traits track one another through time, promoting greater trait matching.

Appendix B: Supplementary material for Chapter 2

B.1 Experimental methods

B.1.1 Aviary experiments

In identifying focal *Heliconia* species, we first identified species which were likely to have multiple individual plants (rather than multiple clones) in the Las Cruces Biological Station living collection and the surrounding area based on collection records and surveys of the area. Because *Heliconia* are self-incompatible to partially self-compatible (Kress, 1983; Pedersen & Kress, 1999; Betts et al., 2015; Janeček et al., 2020), we sought to maximize the genetic diversity of the pollen pool in order to limit the possibility of failing to detect a difference among pollination treatments simply due to a lack of compatible pollen. As an additional confirmation that compatible pollen was available in the area, we required that plants could be seen setting fruit. Indeed, these measures did not eliminate the possibility of non-detection due to poor quality pollen since we do not know the parentage of the plants used in the experiments, but this should not inflate the chances of erroneously detecting pollinator recognition since pollen quality was held constant across control flowers and treatment flowers.

Prior to anthesis, all flowers were covered with a mesh bag in order to preclude visits from free ranging pollinators. Each flower was hand pollinated by the same experimenter (author D.G. Gannon), using pollen sourced from plants located at least five meters away from the focal plant in order to reduce the chances of applying self or related pollen to the stigma (mean distance to donor ± 1 sd: $\bar{x} = 233\text{m} \pm 223\text{m}$). We gently separated styles from the stamens using forceps and cleaned all pollen from the stigma with a cotton swab under 20× magnification. We then adhered pollen to the stigmatic surface by scraping the pollen from an anther of the donor flower with a toothpick and touching the toothpick to the stigma of the focal flower. We checked that pollen adhered to the stigmatic surface and that pollen was dispersed across the stigma in a relatively even layer using a 20× hand lens. As mentioned in the main text, quantification of pollen grains on the stigma in the field is not feasible due to the size of the pollen grains, but we attempted to minimize variation in the quantity of pollen applied across treatments and replicates.

Aviary experiments began by locating a bagged inflorescence with two mature flowers. We hand-pollinated each flower using the methods described above, then assigned flowers to a treatment at random, one flower assigned as a control (hand-pollination only) and the other to be visited by a hummingbird (long-billed or short-billed). The control flower was covered with a red paper sleeve to block access. We erected small, portable aviaries (1m x 1m x 2m) to enclose focal plants (Figure B.1). Aviaries were constructed from sewn shade cloth, a one-inch PVC hoop at the top, and bamboo legs that could be embedded in the ground.

We used two common hummingbird species in our aviary experiments which represent guilds of short-billed and long-billed hummingbirds: Rufous-Tailed Hummingbirds (*Amazilia tzacatl*), and green hermit hummingbirds (*Phaethornis guy*), respectively (Figure 2.1). We captured hummingbirds using standard mist-netting procedures (OSU ACUP 5020), running nets from 0600 to 1000 hours. Males of focal bird species were placed in cloth bags for transport to the aviary, and all non-target species were immediately released. On some occasions, male hummingbirds were housed in a 2 m × 2 m × 1.5 m aviary for up to seven days to facilitate data collection (OSU ACUP 5020). This became necessary as capture rates decreased in the area of the experiments.

To ensure that hummingbirds did not contribute additional pollen to the stigmatic surface of the focal flower, we cleaned hummingbirds of all pollen under 20× magnification using damp cotton swabs and a photographer's brush before releasing them into the aviary. Hummingbirds were provided a perch inside the aviary and supplemental sugar water (20% sucrose by mass) if the hummingbird did not visit the focal flower after 30 minutes post-entry. At 60 minutes, if the hummingbird had not visited the focal flower, we terminated the experiment and the bird was either released or fed sugar water and moved to another experiment. We conducted all pollination experiments between the hours of 0600 and 1100, as *Heliconia* pollen viability and/or stigmatic receptivity may be in question later in the day (Dafni & Firmage, 2000; Hedhly et al., 2003; Schleuning et al., 2011). Flowers were labeled, covered with mesh bags to ensure no additional pollinator visits, and collected the following day after abscission.

B.1.2 Nectar removal experiments

To test whether nectar removal may provide a mechanism conferring pollinator recognition in *Heliconia*, we manually extracted nectar from flowers of three of the six focal species based on flower availability. We again included at least one control flower for each day and for each species on which conducted the experiments. We extracted nectar from flowers using a 20

Figure B.1: Portable aviary assembled around a live inflorescence.

μL micropipette tip bent to match the curvature of the flower. To the back of the pipette tip, we fit a length of 0.5 mm plastic tubing, connected to a 20 mL syringe with which we created suction. We measured the volume of liquid removed with a glass capillary tube, then dispensed it onto a temperature-calibrated hand refractometer to verify that the liquid was likely nectar produced by the flower. The minimum Brix index observed in an independent dataset of *Heliconia* nectar was c.a. 14.1% (K. Leimberger *unpublished data*). Thus, we recorded instances in which the sugar concentration measured below this level.

An alternative stimulus to which plants could respond is the mechanical action of a hummingbird inserting its bill into the perianth to access the nectar. To test whether we could induce pollen tube growth by the mechanical stimulus of inserting a morphologically matched object into the flower, we conducted experiments in which we simply inserted the pipette tip, then removed it without extracting any nectar. Nectar volumes moving up into the pipette tip due to capillary action were, except for in a few cases, not measurable. This experiment is therefore not confounded with the nectar removal experiments.

Indeed, our nectar removal treatments could not completely mimic the way in which birds interact with a flower, since pollinators deposit pollen at the same time nectar is removed. In our experiments, we could only complete these tasks in sequence. We therefore conducted some nectar removal experiments in which we hand pollinated the flower after removing nectar. These experiments allowed us to assess whether timing of nectar removal relative to pollen deposition could be important to pollen germination success.

B.2 Laboratory methods

We collected styles the following day (after abscission) and fixed them in formalin acetyl acid for at least 72 hours before transferring them to 70% ethanol for transport back to Oregon State University. We stained pollen tubes using aniline blue dye in a buffer of $KHPO_4$ preceded by four wash steps: 24 hours in dH_2O, 24 hours in 5 M NaOH to soften the tissues, followed by two 24-hour dH_2O washes (Kress, 1983; Betts et al., 2015). We mounted styles on microscope slides and scored pollen tubes by counting the maximum number of pollen tubes found in any given cross-section of the style using an epi-fluorescence microscope.

B.3 Trait (mis)matching

Upon capturing hummingbirds, we photographed their bills against a 0.25-inch gridded notebook. We then used `ImageJ` to measure the length of the bill along the outer (top) surface of the bill using the 'line segment' feature after setting the scale using the gridded paper. To measure curvature, we utilized the methods of Temeles et al. (2009) in which we related the outer edge of the bill to a circle (Figure B.2). The curvature of a circle is measured as the inverse of its radius. To estimate the radius of a circle with the same curvature as a bird's bill, we connected two points on the curved section of the bill, effectively drawing a chord on the circle (Figure B.2). We then measured the angle between the chord and the line tangent (labeled T) to the bill at one end of the chord, known as the *angle of declension* (θ in Figure B.2). Straight-forward trigonomentric identities then yield $\hat{r} = \frac{C/2}{\sin\theta}$, where C is the length of the chord (mm) and θ is the angle of declension (radians). Thus, we use $\hat{r}^{-1}$ as an estimate of the curvature of a bill or flower.

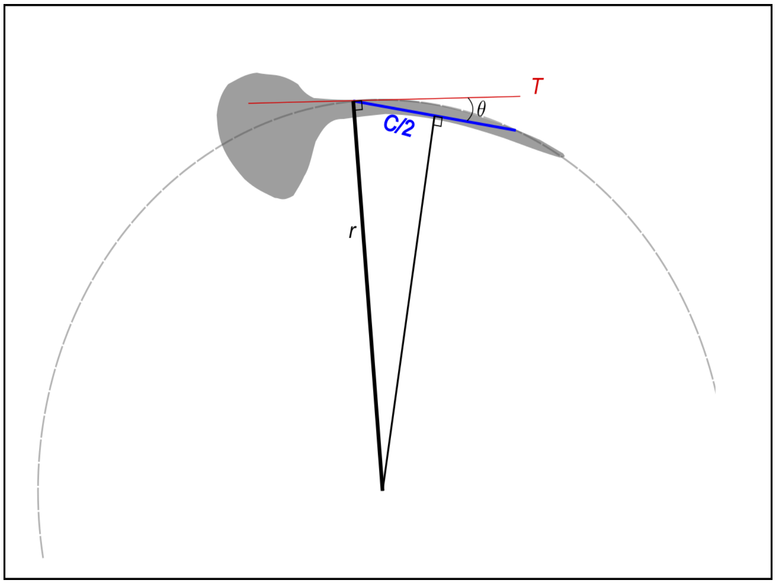

Figure B.2: Relating the arc of the top of a bird's bill to a circle as in Temeles et al. (2009)
.

B.4 Statistical methods

We analyzed data from each plant species separately since we did not necessarily expect effects of our treatments to be consistent across species. Furthermore, this allowed us to include random intercepts for each plant (accounting for potential dependence among styles from the same plant) without assuming plant effects are identically distributed across species.

Let $y_{ijk} \in \{0, 1, 2, ...\}$ be the number of pollen tubes scored in the k^{th} flower from the j^{th} plant of a given species, and let i index the treatment ($i = 1, ..., g$). We assume that $y_{ijk} \overset{iid}{\sim} \text{Poisson}(\lambda_{ij})$ for $k = 1, ..., K_{ij}$. The model for the experiment can be written as

$$\log(\lambda_{ij}) = \mu + \alpha_i + \beta_j$$

where

- μ is the overall mean log-pollen tube rate
- α_i, $\sum_{i=1}^{g} \alpha_i = 0$, is the average deviation from the mean for flowers that received treatment i
- $\beta_1, \beta_2, ..., \beta_J \sim \mathcal{N}(0, \tau^2)$, are the average deviations from the mean for flowers from plant $j = \{1, 2, ..., J\}$

For ease of defining the model in the R package `lme4` (Bates et al., 2015), we reparameterized the model to follow a regression parameterization (reference level for the aviary experiments was hand pollination for all models). The model therefore reads

$$\log \boldsymbol{\lambda} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\boldsymbol{\gamma},$$

where $\mathbf{X}$ is a matrix of indicator variables indicating the treatment and $\mathbf{Z}$ is a matrix of indicator variables indicating the plant individual for the n^{th} observation, $n = 1, ..., N$.

Appendix C: Supplementary material for Chapter 3

C.1 Sender-Receiver model description

C.1.1 Background

We compiled records of bird relocations from four years in which we maintained RFID-equipped hummingbird feeders and extracted movement information by tallying occasions on which an individual was recorded at feeder i at time t and again at feeder j, $j \neq i$, at time t', $t' > t$, within the same day. We chose to limit our focus to movement that occurred within the same day to gain insight into hummingbird movements that may be relevant to pollination. Additionally, we chose to sum the movements over the year in order to get multiple measurements of movement between two feeders (one per year) that can more reasonably be treated as exchangeable observations. If instead we sum over shorter periods of time, yielding multiple measurements in a given year, measurements within the same year are unlikely to be exchangeable with measurements of a different year, requiring a more complicated model. Instead, we effectively average movements over the flowering period of the primary nectar-producing plants. While we lose some information on differences between sexes, ages, and inter-individual differences in foraging behaviors by summing movements over the year, our objective was to model functional connectivity informed by hummingbird movements. Whether a given number of movements is made by many birds each making few movements or by few birds making many movements is not important in this endeavor.

Because we only consider movements during the flowering period, this approach should reflect the potential for pollen flow among locations in the landscape.

We treated each feeder as a node in a graph (network) and modeled the edge weight (degree of connectivity between two nodes) of each edge in the graph. We denote λ_{ij} as the weight of the directed edge (in directed graphs, $\lambda_{ij} \neq \lambda_{ji}$) connecting node i to j and assume that the observed number of movements between two feeders in a given year, y_{ijk}, where $k = 1, 2, \ldots, K$ indexes the year, was a random draw from a Poisson distribution with rate parameter λ_{ij}. Thus, in year k, when r_k out of the total R feeders were positioned and maintained on the landscape, there was a total of $n_k = r_k\,(r_k - 1)$ possible movements (because λ_{ii} is not defined), yielding $N = \sum_{k=1}^{K} n_k$ total observations.

C.1.2 Model description

Let $y_{ijk} \in \mathbb{N}$ be the number of movements detected between readers i and j by hummingbirds in year k, where $i \neq j = 1, 2, ..., R$, and R is the total number of feeders used (20 in total). Following Hoff (2005), we model the number of movements in a bilinear model:

$$\log(\lambda_{ijk}) = \mathbf{x}_{ij}^{T}\boldsymbol{\beta} + u_i + w_j + \gamma_{ij} + \log(b_k) + \log(d_k)$$

where $\boldsymbol{\beta}$ is a vector of dyad specific effects with $\mathbf{x}_{ij}$ the vector of dyad-specific regressors for dyad $\{i, j\}$. The effects u_i and w_i are the average effects of reader i as a “sender” (movements originating at feeder i) and “receiver” (movements ending at feeder i), respectively, and the term γ_{ij} is an average effect on movement for the pair of feeders $\{i, j\}$. Finally, b_k and d_k

are offsets for the cumulative number of birds that had been implanted with RFID tags in year k and the number of days the readers were maintained in year k (respectively).

We assume there are multiple levels of dependence in these data. For example, movements coming from another location to feeder i may be correlated, and those leaving feeder i may also be correlated. Finally, movements within a dyad ($\{i, j\}$ pair) may be correlated. To induce dependence among observations that involve reader i, we assume

$$\begin{aligned} \begin{bmatrix} u_i \\ w_i \end{bmatrix} &\sim \mathcal{N}\left(\mathbf{0},\ \boldsymbol{\Sigma}_{uw}\right), \\ \boldsymbol{\Sigma}_{uw} &= \begin{bmatrix} \sigma_u^2 & \sigma_{uw} \\ \sigma_{uw} & \sigma_w^2 \end{bmatrix}, \end{aligned} \tag{C.1}$$

such that observations that have a common sender or receiver may be correlated. Importantly, this allows for the potential that "good senders" may be "poor receivers", so negative correlation is a possibility. While unlikely in our landscape scale study, this can allow for source-sink dynamics in the movements (Pulliam, 1988). Finally, let

$$\begin{aligned} \begin{bmatrix} \gamma_{ij} \\ \gamma_{ji} \end{bmatrix} &\sim \mathcal{N}(\mathbf{0},\ \boldsymbol{\Sigma}_{\gamma}), \\ \boldsymbol{\Sigma}_{\gamma} &= \begin{bmatrix} \sigma_\gamma^2 & \rho\sigma_\gamma^2 \\ \rho\sigma_\gamma^2 & \sigma_\gamma^2 \end{bmatrix}, \end{aligned} \tag{C.2}$$

where ρ is the correlation between the rate of movement from $i \rightarrow j$ and $j \rightarrow i$. These differ from standard random effects models because they allow for negative correlation of the observations within a dyad. For our purposes, this flexibility may help elucidate *traplining* foraging behaviors in which birds forage in a regular circuit among food sources (Feinsinger, 1976). This could result in negative correlations within dyads if circuits tend to be directional (e.g., birds visit a circuit of meadows in a clockwise fashion).

C.1.3 Priors

We use weakly informative, $\mathcal{N}(0,1)$ priors for all regression coefficients. These priors reflect the prior assumption that, with probability 0.95, the effect of an increase of 1 unit in any of the covariates will not result in more than a ~7-fold increase/decrease in the movement rate. They therefore constrain the HMC sampler to biologically reasonable parameter space, while remaining conservative because evidence in the data needs to be strong to pull the posterior away from zero.

For the variance components, we utilize a decomposition of the covariance matrices $\boldsymbol{\Sigma}_\gamma$ and $\boldsymbol{\Sigma}_{uw}$ in order to simplify prior specification and improve computational efficiency (Stan Core Team, 2021). In particular, let $\boldsymbol{\Omega}$ be a correlation matrix and $\mathbf{L}$ be the lower triangular Cholesky factor of $\boldsymbol{\Omega}$ such that $\mathbf{L}\mathbf{L}' = \boldsymbol{\Omega}$. We put a *Cholesky LKJ correlation* prior (Lewandowski et al., 2009) on the matrices $\mathbf{L}_\gamma$ and $\mathbf{L}_{uw}$ (subscript notation following from above). For a $K \times K$ lower triangular Cholesky factor $\mathbf{L}$, the density $\pi(\mathbf{L} \mid \eta)$ for the prior is

$$\pi(\mathbf{L} \mid \eta) \propto \prod_{k=2}^{K} \mathbf{L}_{kk}^{K-k+2\eta-2}. \tag{C.3}$$

If $\eta = 1$, then the density is uniform over all correlation matrices of order K. We let $\eta = 5$ for both $\mathbf{L}_\gamma$ and $\mathbf{L}_{uw}$, which forms a peak at the identity matrix (no correlation). This peak gets sharper as $\eta \to \infty$.

By putting a prior on the (Cholesky factor of the) correlation matrix, this allows separate prior specifications for the scale parameters for the multivariate normal distribution, the vectors $\begin{bmatrix} u_i & w_i \end{bmatrix}^T$, $i = 1, 2, ..., R$, and $\begin{bmatrix} \gamma_{ij} & \gamma_{ji} \end{bmatrix}^T$, $i = 1, 2, ..., R$. For the scale parameters, we chose half-Normal(0, 2) priors in order to maintain some flexibility while also constraining the parameter space to reasonable values (e.g., a log-effect size of 100 for a given feeder's random effect is not very reasonable). The summarized movement data and code for fitting this model can be found in the github repository https://github.com/Dusty-Gannon/RUHU-movements. The raw data are publicly available on the H. J. Andrews Data portal, database code SA028 (https://doi.org/10.6073/pasta/3c5fbd4600faff33c6b9e252b095a5da).

C.2 Supplementary analyses

C.2.1 Summaries of feeder use

It is possible that, due to high sucrose supplies, individual hummingbirds assumed defense of certain feeders and limited their movements away from the feeders. To check this possibility (though we do not believe this check can be considered a perfect assessment), we quantified the average number of hummingbirds using feeders in a given year and compared this to the total number of visits per feeder-day (number of days a feeder was present on the landscape). Based on prior research on territoriality in hummingbirds, if feeders present extremely valuable resources, we might expect increased territoriality at feeders compared to natural flower patches (Ewald & Carpenter, 1978; Kodric-Brown & Brown, 1978; Justino et al., 2012).

With increased territoriality, we would expect the number of hummingbirds visiting a feeder to be largely decoupled from the total number of visits to that feeder (Figure C.1 a & b). Furthermore, we would expect that individual richness (number of birds recorded at a feeder) would be drastically greater than a diversity metric that weights richness by the evenness of visitation frequencies across individuals, which should tend towards 1 as a single dominant individual assumes control over the feeder. We therefore computed the Shannon-Weiner diversity of birds (diversity of individuals) for each feeder, $D_i = \exp\left\{-\sum_{b=1}^{B_i} p_{ib}\log(p_{ib})\right\}$, $i = 1, 2, ..., R$ indexes the feeder, $b = 1, 2, ..., B_i$ indexes the bird recorded visiting feeder i, and p_{ib} is the proportion of visits to feeder i that can be attributed to bird b.

Figure C.1: Summaries of feeder use over the four years of study. **a)** Visual representation of the average number of birds using each feeder over the years. **b)** Visual representation of the average number of separate visits to each feeder over the years. **c)** Visual representation of the Shannon-Weiner diversity of visits (diversity of individuals of the same species, *Selasphorus rufus*) to each feeder over the years. **d)** Average number of birds using the different feeder types ("treatments"), averaged over years for each feeder, then averaged over feeders belonging to a specific type. **e)** Average number of separate visits (records of birds spaced by at least 30s) per feeder per day to different feeder types, averaged over years for feeders then over feeders in a given type. **f)** Average Shannon-Weiner diversity across feeder types, averaged over years and feeders. **g)** Relationship between individual bird richness and diversity of individuals at each feeder.

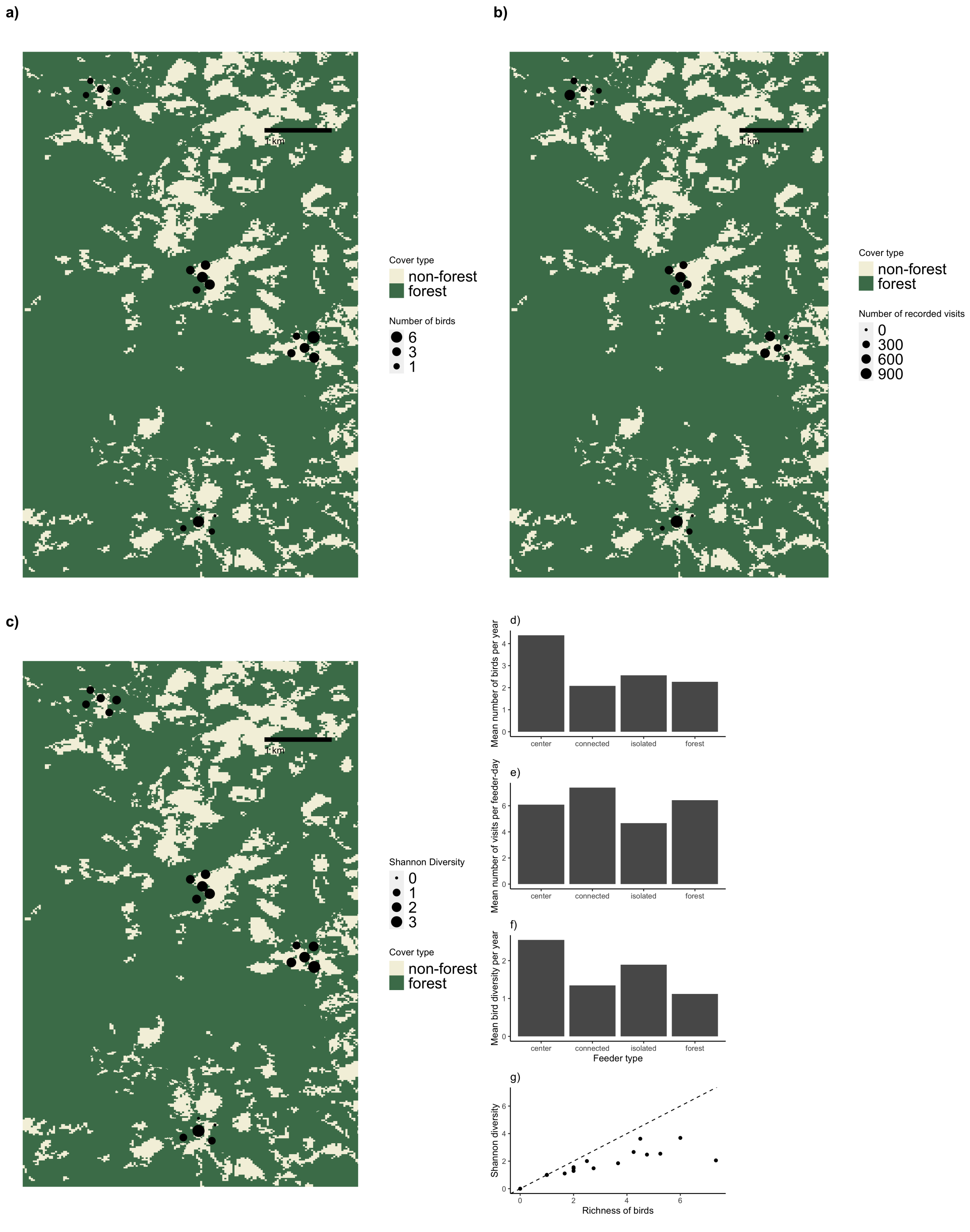

Figure C.1: (Continued)

In some cases, the number of visits to a feeder relative to others deviates from the number of birds using that feeder relative to others (Figures C.1 a & b). The Shannon-Weiner diversity also diverges from individual bird richness, indicating that one or two individuals compose the majority of visits to any one feeder. These results, unsurprisingly, support the idea that feeders present a coveted (nearly unlimited) resource. However, the deviations from the maximum value in the Shannon-Weiner diversities support that territoriality may have maintained movement through the landscape by all but the most dominant hummingbirds.

C.2.2 Movements following competitive interactions

If competition among hummingbirds maintains movement through the landscape, we might expect that birds would be more likely to move to another feeder following a visit to a busy feeder. To test this idea, we quantified feeder use during ten-minute intervals over the four-year study period by tallying the number of birds recorded at a feeder in each ten-minute window. We then fit a generalized linear mixed model to the data on whether or not a bird moved from feeder i at time t to j at time t', $t' > t$, using the number of birds that visited feeder i in the ten-minute window into which time t falls. We additionally included a random effect for the bird to account for inter-individual differences in movement propensity among birds.

We found little evidence that, on the whole, birds are more likely to move to another food resource following competitive interactions. The estimated effect of the number of birds at a feeder in a ten-minute window on the log-odds of movement was estimated to be $\hat{\beta} = -0.15$ ($SE_{\hat{\beta}} = 0.255,\ z = -0.578,\ P(Z > |z|) = 0.563$). Birds appeared to have highly variable

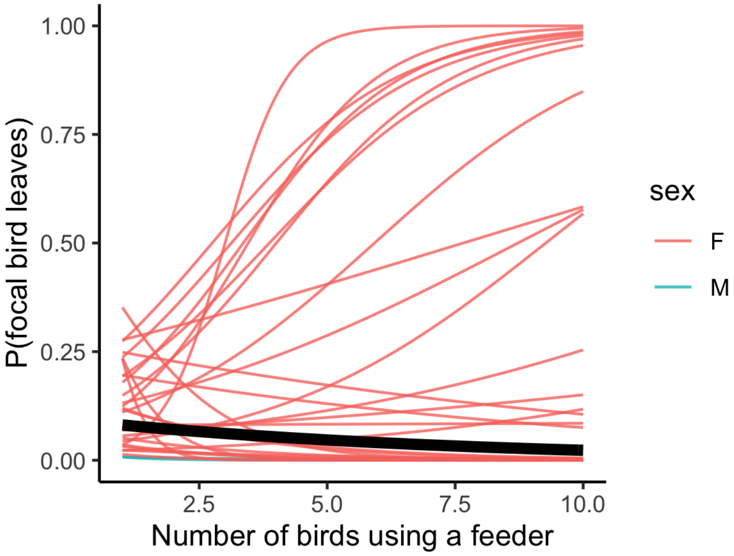

Figure C.2: Estimated probability that a hummingbird was recorded moving to another feeder j as a function of the intensity of use (measured as number of birds recorded at a feeder in a ten minute window) at feeder i before moving from i to j. Each line represents the estimated curve for a different individual. The black line is the average effect of the intensity of feeder use on the probability of movement.

responses ($\hat{\sigma}^2_{\beta_1} = 1.08$; Figure C.2), supporting a hierarchy in competitive dominance in which some birds were more competitive at feeders and forced other birds away from feeders. However, we caution readers to the fact that we do not have detailed information on the occasions on which birds were actually engaged in competitive interactions with other birds and can only speculate that the number of birds at a feeder could be related to competition at the feeder.

Table C.1: Data on birds with long tenures on the landscape but were not recorded moving among feeders in any given day.

Bird ID	Sex	Year	Feeder	Feeder type	Proportion of visits
0EB27598596F0001	F	2017	M2_C	Center	0.318
25B27598596F0001	M	2016	CM_C	Center	0.840
3E727598596F0001	F	2017	LOM_C	Center	1.000
4FB27598596F0001	M	2016	LOM_C	Center	0.019
4FB27598596F0001	M	2016	M1_NE	Forest	0.010
82727598596F0001	F	2017	M2_C	Center	0.011
9CEBC199F0870001	U	2015	LOM_C	Center	0.662
9CF27598596F0001	F	2017	M2_C	Center	0.517
B3F27598596F0001	F	2016	LOM_C	Center	0.185
B3F27598596F0001	F	2016	M1_NE	Forest	0.010

C.2.3 Data summaries of birds that did not move

Of the 63 Rufous Hummingbirds that were relocated, 35 were not recorded moving among feeders. The majority of these birds (31 of 35) were only recorded on a few occasions. Of the 4 (1 male, 3 females) that had longer tenures in the system, all appear to have established territories around a center feeder, composing the most visits to their preferred feeders of any other bird, though generally not the majority of all visits (Table C.1). Two of these birds (1 male, 1 female) were recorded at multiple feeders in the same year, but were not included in the movement data because they were not recorded moving among feeders in the same day (Table C.1).

Appendix D: Supplementary material for Chapter 4

D.1 Supplementary figures

In a separate study, we placed reusable tea bags around arbitrarily selected senescing flowers of the nearest plant to the 5 meter mark from the centroid of seven meadows in each cardinal direction. We counted the seeds collected in each bag, averaging over the number of seed pods enclosed by the bags on a given plant (Figure D.3).

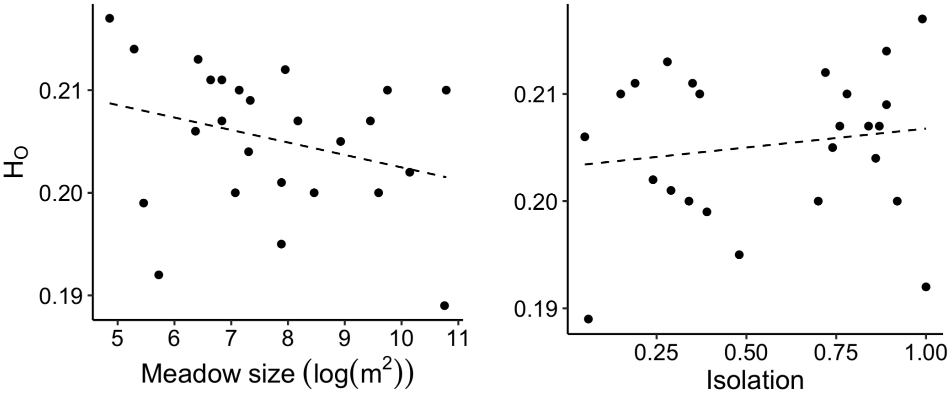

Figure D.1: Observed heterozygosity treating each meadow as the population plotted against meadow size and a measure of meadow isolation (proportion of forested cells in a 100 m buffer around the meadow centroid).

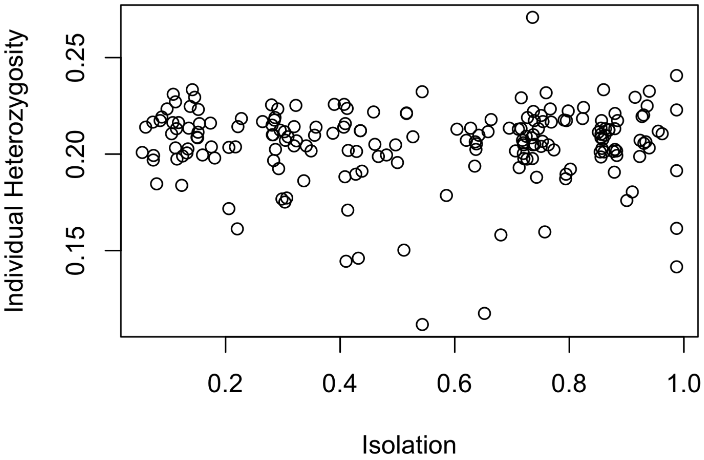

Figure D.2: Individual heterozygosity (i.e., proportions of heterozygous genotyped sites) plotted against isolation of the individual (proportion of forested cells in a 100 m radius around the plant).

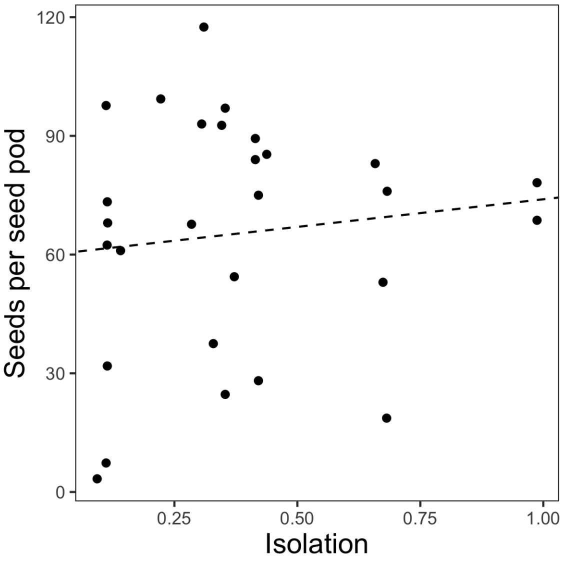

Figure D.3: Number of seeds per seed pod for 28 plants from 7 meadows following the 2017 flowering season plotted against isolation of the individual (proportion of forested cells in a 100 m radius around the plant).