Food for thought: the ecological complexity of natural selection on resources in mutualisms

Investigations with theory and *Amianthium muscaetoxicum*

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Abstract

Resources mediate diverse species interactions in communities, including competition, predation, parasitism, and in many cases, mutualisms. In consumer-resource species interactions, the ecological resources in flux are governed by species traits that can evolve, thus shifting the dynamics of resource supply and the dynamics of the interactions they mediate. Hence, resources can be an important mediator of eco-evolutionary feedbacks in species interactions. Understanding such feedbacks requires building a mechanistic understanding of how natural selection operates on the resources that shape both species' population dynamics. Such a resource-focused perspective has yet to be strongly incorporated in the study of the evolutionary ecology of consumer-resource mutualisms such as plant-pollinator mutualisms mediated by the plant's production of floral nectar. Furthermore, selection on resources such as floral nectar may vary across space and through time within a single population if the consumer species, such as a pollinator, responds to spatial and temporal variation in resource. In this body of work, I applied a resource-focused perspective to plant-pollinator mutualisms, exploring the ecological drivers of selection, and possible sources of variation in phenotypic selection, on nectar traits. In Chapter one, I built a theoretical framework of hypothesized eco-evolutionary dynamics on nectar evolution in plant-pollinator mutualisms, finding that selection for higher nectar production is strongest when ecological factors such as pollinator behavior and resource availability for nectar production reduce the frequency of plant-pollinator interactions. In the following chapters, I used *Amianthium muscaetoxicum*, a self-incompatible Appalachian perennial, and its interactions with beetle pollinators as an empirical model for the resource-focused perspective on the evolutionary ecology of plant-pollinator interactions. In Chapter two, I found strong individual-level consistency and high among-individual variation in nectar traits, providing a firm basis for phenotypic selection to act on nectar trait variation. In Chapter three, I measured direct and net selection on nectar traits in the *Amianthium* population and further found that spatial variation in nectar traits among small plant neighborhoods affected plant seed set, revealing that pollinators responded to local among-individual variation in plant nectar traits in ways that reinforced the direction of individual-

scale selection. In Chapter 4, I found that the direction of selection on nectar traits remained largely consistent across high- and low-water environments, despite changes in mean fitness and water-induced plasticity in nectar trait distributions among water environments. My empirical work with *A. muscaetoxicum* reinforces my theoretical framework of resource evolution in consumer-resource mutualisms: nectar traits can experience strong, consistent directional selection, mediated in part by patterns of pollinator foraging behavior.

TABLE OF CONTENTS

Introduction: the evolutionary ecology of resource supply in mutualisms

Resource exploitation as a unifying perspective on species interactions

Interactions between consumers and their resources are a unifying framework for the population and community ecology of many species interactions: predation, parasitism, resource-based competition, and more recently, mutualism (Tilman 1980; Herre et al. 1999; Murdoch et al. 2003; Holland and DeAngelis 2009, 2010). Predators and parasites consume resources from their prey, directly suppressing prey abundance while enhancing their own (Lotka 1925; Volterra 1928; Holling 1959). Resource competitors indirectly reduce each other's population sizes by depleting shared resources (Macarthur and Levins 1967; Abrams 1980; Tilman 1982; McPeek 2022). Many mutualisms also involve one species providing a nutritional resource to another species in exchange for some form of fitness benefit (Herre et al. 1999; Holland et al. 2005; Holland and DeAngelis 2009, 2010; Bronstein 2015). Consumer-resource interactions connect all species in communities via direct and indirect exploitation of resources.

Resource exploitation in mutualisms can take many forms. Some mutualists directly exchange nutritional resources, as is the case in bacterial cross-feeding mutualisms and legume-rhizobia mutualisms. Other mutualisms involve the provisioning of a nutritional resource in exchange for an energy resource such as the movement of individuals or gametes (pollination mutualisms, seed dispersal mutualisms), or protection from another species such as a predator, herbivore or parasite (protection mutualisms, cleaning mutualisms). Plants are particularly adept at using resource production to hijack animal movement for their own benefit. Plants produce fleshy fruits or elaiosomes that encourage animal foraging and inadvertent movement of plant progeny in animal wastes (Moore and Dittel 2020). Many flowering plants produce nectar, oils, and waxes in addition to pollen, all of which can encourage animal consumers that inadvertently transport their gametes across the population (Simpson and Neff 1983; Willmer 2011). Some plants also provide nutritional secretions and food bodies that encourage insect colonization in exchange for inadvertent defense against the plant's herbivores (Bronstein 1998). The production of these various nutritional resources mediates the benefits to both mutualist partners.

Understanding the population dynamics of mutualisms requires that we understand the dynamics of

resource supply that supports both species' population growth. Theoretical ecologists have begun to address this challenge by explicitly incorporating the dynamics of the resources themselves in ecological models of mutualisms. Building resource-based population dynamics into plant-pollinator and seed-dispersal mutualisms reveals that many consumer-resource mutualisms are dynamically stable through time (Holland and DeAngelis 2010; Hale and Valdovinos 2021), counteracting a historical mindset that mutualisms were inherently unstable and on the verge of collapse. Theoretical work that allows mutualists to forage adaptively for resources predicts that adaptive foraging can further enhance the stability and diversity of diffuse consumer-resource mutualism networks (Valdovinos et al. 2013). Work that integrates mutualistic interactions with other kinds of consumer-resource interactions also shows that mutualists can increase species abundances and stabilize the community structure of broader food webs by increasing the abundance of the producer species on which the entire community depends (Hale et al. 2020). Integrating consumer-resource mutualisms into broader food webs grows our understanding of how different kinds of species interactions function in complex communities.

Consumer-resource dynamics shape the evolution of species interactions

In addition to being a unifying perspective for species ecology, consumer-resource dynamics may also be a unifying perspective for the evolution of species interactions. Theoreticians commonly model fitness as a species' per capita population growth rate, which is equivalent to the fitness of the individual with the mean phenotype in the population (Charlesworth 1994; Lande 2007). In antagonisms, predators gain higher fitness through traits that enhance prey consumption and prey gain higher fitness through traits that reduce predator attacks. As predator and prey evolve in response to each other and other environmental factors, predator-prey population dynamics will shift. Theory shows that these shifting population dynamics can further alter natural selection on species' traits because population sizes modulate the per capita costs and benefits of predator attack traits and prey defense traits (Roughgarden 1972; Slatkin 1980; Taper and Chase 1985; Abrams and Chen 2002; McPeek 2017a; McPeek et al. 2022). If predator abundance declines, the prey's benefit from maintaining high levels of defense also declines as predator attacks grow less frequent. Adding evolutionary dynamics into ecological models of species

interactions changes species' trait optima in coevolving communities of consumers and their resources (McPeek 2017a, 2019; McPeek et al. 2022). Empirical work in a variety of antagonistic systems bears out the role of evolutionary trait change in shaping the population dynamics of species, and vice versa how population dynamics shape trait evolution (Fussmann et al. 2007; Post and Palkovacs 2009; Walsh et al. 2012; Declerck et al. 2015). Thus, we cannot accurately interpret or predict patterns of trait evolution in any consumer-resource interaction without examining how ecology shapes selection on traits that mediate those interactions, and how evolution by natural selection feeds back on those ecological dynamics.

These vital eco-evolutionary dynamics have yet to be strongly incorporated in the study of consumer-resource mutualisms. To achieve this crucial advancement, we must develop a robust, mechanistic understanding of the operation of natural selection on the resources that shape both partners' population dynamics. The logic of abundance-based natural selection dynamics in mutualisms could follow the general logic of antagonisms with higher benefits at higher species abundances. If the resource species' benefit of providing resources for consumers declines when consumer abundance is low, this could lead to the population collapse of both partners, a hypothesized outcome from many ecological models of obligate mutualist population dynamics (Goh 1979), or a reduction and eventual end to partner reliance in the case of facultative mutualisms (Sachs and Simms 2006). However, if resource provisioning directly boosts consumer activity and consumer population growth, then we might expect the fitness benefits of resource provisioning to increase when consumer abundances are low, in opposition to patterns in antagonisms (Holland et al. 2004). Theoretical work based on mechanistic empirical evidence is needed to explicitly lay out how these feedbacks might operate in consumer-resource mutualisms.

Unfortunately, little empirical work has explicitly examined selection on resources in mutualisms. In many consumer-resource mutualisms such as pollination, seed dispersal, and defense mutualisms, resource production directly improves the fitness of only the consumer species. The benefits to the resource-provider species are an indirect consequence of consumers foraging for resources such as floral nectar. This disparity may explain why most studies of phenotypic selection in plant-pollinator interactions, the most broadly studied category of mutualisms, have mainly focused on floral traits such as

floral display size, floral morphology, floral color, and floral scent e.g., (Johnston 1991; Benitez-Vieyra et al. 2006; Schiestl et al. 2011; Caruso et al. 2019; Brunet et al. 2021), and not on floral rewards. The very use of the term 'floral rewards' in the pollination literature diminishes nectar's role as a vital food resource for animal pollinators. Further, these other floral traits can all affect an animal's behavior to the benefit of plant reproduction, regardless of whether an animal receives a reward from the plant. In many cases, floral display traits can signal the presence, abundance, and quality of nectar (Armbruster et al. 2005; Wright and Schiestl 2009; Benitez-Vieyra et al. 2010; Knauer and Schiestl 2015). However, floral signals will not directly affect the fitness benefit for pollinators. Only resources impact consumer fitness, thereby affecting the population dynamics of consumers and changing selection on those resources.

Most work explicitly examining phenotypic selection on resources in mutualisms also comes from pollination mutualisms, though our knowledge in this area still pales in comparison to studies of selection on other floral traits that affect plant-pollinator interactions (Parachnowitsch et al. 2019). Floral nectar serves as an essential food source for many animal pollinators (Heinrich 1975; Nicolson and Fleming 2003; Nicolson 2011). Floral nectar traits also affect reproductive outcomes in a variety of plant species (Zimmerman 1983; Pyke 1991; Real and Rathcke 1991; Mitchell and Waser 1992; Irwin and Brody 1998; Brandenburg et al. 2012; Mackin et al. 2021). The idea that nectar traits could experience natural selection dates all the way back to Darwin (1859), who proposed floral nectar as a case study in the operation of natural selection in species interactions (*On the Origin of Species* first ed., pgs. 92-95). The first estimate of direct phenotypic selection on nectar traits (nectar volume) was conducted by Hodges (1995) in the hawkmoth-pollinated *Mirabilis multiflora*. Since then, several studies have measured selection gradients on nectar production rate (Mitchell et al. 1998), nectar volume and sugar concentration (Ferreiro et al. 2017; García et al. 2023; Powers et al. 2024), total nectar sugar content (Kulbaba and Worley 2012), amino acid composition (Gijbels et al. 2015), secondary metabolite content (Egan et al. 2022), floral signal-nectar reward accuracy (Benitez-Vieyra et al. 2010), and the pattern of nectar volume variation among flowers on an inflorescence (Zhao et al. 2016). Two studies have also measured standardized selection differentials on nectar volume (Dorey and Schiestl 2022) and nectar production

rate (Campbell and Powers 2015). To date, only three of these studies detected statistically significant selection on nectar traits under field conditions (Gijbels et al. 2015, amino acid composition; Zhao et al. 2016, spatially structured nectar variance among flowers; Egan et al. 2022, nectar secondary metabolites).

Natural selection on nectar supply in pollination mutualisms

Floral nectar is the ideal target for beginning to build a resource-focused perspective into the theoretical and empirical study of mutualism evolution. A large body of empirical work demonstrates floral nectar's effects on pollinator behavior and plant reproductive success, and some work has directly connected reproductive outcomes to specific, nectar-mediated pollinator behaviors. Work in a small group of systems illustrates that nectar traits may experience direct phenotypic selection, although few of these estimates were statistically significant and even fewer were estimated in natural populations (Parachnowitsch et al. 2019). Nectar is a physiologically complex trait with potential fitness costs as well as benefits, which can complicate the translation of pollinator activity into plant reproductive success. More work is needed in a wider diversity of plant-pollinator systems to understand how phenotypic selection operates on nectar resources in natural plant populations.

The ecological diversity of plant-pollinator mutualisms further allows us to examine how species' natural history shape the evolution of nectar resources across populations and between species. Any biological or ecological factor that impacts the benefit-cost ratio of resource production could affect selection on resource traits. For example, we might expect selection on resource production will be stronger if one or both partners cannot perform vital functions without the involvement of their partner (i.e., they are obligate mutualists). Selection on nectar traits may thus be strongest in self-incompatible plant species that rely on pollinator-mediated transfer of gametes among plants. Additionally, different pollinator behaviors may impose selection for different nectar traits (Cruden et al. 1983). Most work on behavioral responses to nectar trait variation has focused on species that are pollinated by larger-bodied or social pollinator species such as hawkmoths, hummingbirds, and social bumblebees. Smaller-bodied, solitary pollinator species such as solitary bees, wasps, flies, and beetles require less nectar overall to meet their energy needs and may respond to nectar trait variation differently than the more commonly

studied species, potentially imposing different dynamics of selection on nectar traits. Abiotic environmental factors that impact the availability of raw materials for nectar production may further alter selection on nectar production (Parachnowitsch et al. 2019). Plant species growing in dryer environments may experience weaker selection on nectar traits if water resources are limiting for plant growth and reproduction (García et al. 2023), or they may experience stronger selection if high pollinator visitation substantially boosts plant reproduction. Expanding the taxonomic and ecological diversity of phenotypic selection studies in plant populations will help elucidate how local ecology shapes selection on nectar resources, and how the evolutionary response to selection will affect the quantity and quality of nectar resources available to pollinators over time.

The availability of resources in mutualisms can also vary across space and through time within a single population. Nectar resources can be patchily distributed across a plant population, creating a spatially variable resource landscape for pollinators (Klinkhamer et al. 2001; Leiss and Klinkhamer 2005; Leiss et al. 2009). Plant nectar production also responds plastically to environmental conditions such as temperature and water availability, shifting the size and quality of the resource pool through time (Villarreal and Freeman 1990; Boose 1997). Spatial structure and temporally plastic variation in a population's phenotypic distribution present challenges for examining and predicting the evolution of resources in mutualisms: a sustained, directional response to phenotypic selection requires consistent population-scale selective pressure over many generations. Eco-evolutionary models of consumer-resource dynamics typically assume such an environment, tracking the evolutionary trajectory of a spatially uniform population's mean phenotype across temporally consistent ecological conditions (Charlesworth 1994; Abrams and Chen 2002; Lande 2007; McPeek 2017a). If selection on resources is not consistent across space or through time, then selection dynamics and evolutionary responses to those dynamics may deviate markedly from the predictions of theoretical models. These dimensions of phenotypic resource variation and their consequences for phenotypic selection must be investigated in wild plant-pollinator mutualisms.

Spatially patchy variation in nectar resources due to among-individual phenotypic variation creates

the potential for multilevel selection on nectar traits. Multilevel selection can alter the strength and directionality of selection on individual traits among groups with different trait compositions (Goodnight et al. 1992; Stevens et al. 1995; Aspi et al. 2003; Weinig et al. 2007; Formica et al. 2011; Cameron et al. 2021; Costello et al. 2023), which could cause net selection to differ at the population scale. Pollinators respond to spatial nectar variation, suggesting that they could act as an agent of multilevel selection on nectar traits. Many pollinators increase their foraging efforts when they encounter higher volumes of nectar resources, visiting proportionally more plants in high-resource patches (Zimmerman 1979, 1983; Pleasants 1981). Pollinators also tend to depart more quickly from plants and patches with lower nectar availability (Hodges and Wolf 1981; Pyke 1981; Pleasants 1989; Kadmon and Shmida 1992; Dreisig 1995). These behavioral patterns can occur at extremely fine spatial scales: plants that neighbor high-nectar producing individuals can experience enhanced pollinator visitation over plants that neighbor lower nectar-producing individuals (Klinkhamer et al. 2001; Leiss and Klinkhamer 2005). Since pollinators forage on patchily distributed resources, the spatial dimension of nectar trait variation may play an underappreciated role in shaping selection on nectar traits.

Dynamic plasticity of floral nectar in response to environmental conditions further suggests that the dynamics of selection on nectar traits may vary through time in accordance with environmental changes. Nectar volume production and sugar production can both be strongly influenced by water availability, with pronounced shifts in both trait means and variances (Zimmerman 1983; Villarreal and Freeman 1990; Wyatt et al. 1992; Boose 1997). Water-induced shifts in the distribution of nectar traits could affect selection by changing the phenotypic distribution exposed to a static selection function. Pollinator responses to temporal variation in resource availability could change the shape of the selection function if pollinators change their foraging behavior in response to changes in trait variance. For example, bumblebee pollinators visit fewer plants and travel longer distances between plants when neighboring plants display higher levels of among-plant nectar variance (Ott et al. 1985). This behavioral response suggests that plants could experience stronger selection in high-water conditions that expose among-individual variation in nectar production and weaker selection in low-water conditions where constrained

plant nectar production may lead to more uniformity in nectar offering. Additionally, water limitation can expose physiological costs of nectar production independent of the actions of pollinators, further altering the cost-benefit balance of selection (Pyke 1991; García et al. 2023). Experimental work in a small group of bee-pollinated species has begun to examine how nectar trait plasticity may alter selection on nectar and other floral traits (Dorey and Schiestl 2022; García et al. 2023; Powers et al. 2024). However, more work is needed to determine the effect of these environmentally induced trait changes on phenotypic selection in natural populations.

Synthesis: the ecological complexity of natural selection on resource supply in mutualisms

Resources mediate the ecology of many species interactions, including most mutualisms. Resources in mutualisms are also species traits that experience selection and evolve. The evolution of resource traits such as floral nectar quantity and quality will change the abundances of interacting consumers and their resources, further altering selection on resource traits. Exploring these eco-evolutionary feedbacks requires a clear understanding of the dynamics of selection acting on resource traits in mutualisms. Theoretical modeling of these processes can provide instructive predictions about the evolution of mutualisms in diverse ecological settings. However, this advancement must be paired with empirical work that explores the environmental context of selection in mutualisms.

Environmental context presents some complicating factors in natural populations. Resources in mutualisms are often patchily distributed in space and experience plasticity in response to environmental changes over time. Consumers may respond to spatial and temporal variation in resource availability, potentially impacting selection on resource traits. Pollinator responses to small-scale spatial variation could impose multilevel selection on nectar traits, which could reinforce or counteract the activity of individual-scale selection. Pollinator responses to plastic variation in resource production could further alter selection if plastic shifts in nectar traits impact how pollinators forage on variable resources within groups and across the entire population. Empirical work must examine these spatial and temporal dimensions of natural selection on resources in natural populations to determine their contributions to resource evolution in consumer-resource mutualisms.

In this body of work, I asked the following question: how do local ecological factors shape natural selection on resources in consumer-resource mutualisms? I addressed this question through a combination of theoretical work on the evolution of nectar production in plant-pollinator mutualisms and empirical tests in the beetle-pollinated perennial, *Amianthium muscaetoxicum* (fly poison, Melanthiaceae). In Chapter 1: The evolution of resource provisioning in pollination mutualisms, I developed and analyzed a resource-explicit model of the evolution of nectar production in plant-pollinator mutualisms (McPeek et al. 2021). I modeled the relationship between plants and pollinators as a consumer-resource interaction where pollinator population growth responded to the size of the plant's resource pool and plant population growth balanced the pollinator-mediated benefits of resource supply with the physiological costs of resource production. I found that plants experienced the highest benefits, and thus evolved to produce more nectar when pollinator interactions were rarer, either due to behavioral differences in foraging effort or reductions in the pollinator's population size. This result suggested a population-scale benefit of mutualism for the species providing the resource: individual plants that produce more nectar indirectly enhance the growth of the plant population through time via positive effects of nectar on pollinator abundance. My comparative approach to modeling how ecological variation shapes nectar trait evolution highlighted the need to examine selection on nectar traits in a wider variety of plant systems that engage with different pollinator species.

My subsequent chapters explored nectar trait variation and the spatial and temporal dimensions of that variation that could affect selection on nectar traits in a wild population of the self-incompatible perennial, *Amianthium muscaetoxicum*. I chose *Amianthium* as the subject for this work for several reasons. First, *Amianthium* is a self-incompatible wildflower, which led me to hypothesize that the species could experience strong pollinator-mediated benefits of high nectar production. Second, *Amianthium* is pollinated by nectar-feeding beetles, a little explored but globally important group of pollinators, thus affording the opportunity to explore selection mediated by a different kind of nectar consumer with potentially different behavioral patterns. Third, the large understory population at Mountain Lake Biological Station provided large numbers of plants for wild studies of phenotypic selection and

experimental manipulations of ecological agents hypothesized to impact selection on nectar traits.

Chapter 2: Patterns of within- and among- plant variation in nectar production affect beetle foraging in *Amianthium muscaetoxicum*, characterized nectar production dynamics and population-scale phenotypic variation in nectar traits in this species and related those traits to the foraging behaviors of its beetle pollinators. I found strong individual-level consistency in nectar production per flower and an order of magnitude of among-plant variation in both nectar volume and nectar sugar concentration at the population scale. While both nectar trait components affected the time beetles spent interacting with flowers, nectar volume affected beetle behavior more strongly than did sugar concentration. Chapter 2 thus established the basis for pollinator behavior-mediated phenotypic selection on nectar traits in the wild population. This work extended my theoretical work in Chapter 1 by exploring evolved variation in nectar traits in a different group of plant-pollinator interactions. Theoretical work in Chapter 1 predicted that plants should experience stronger benefits of producing more nectar to maximize interactions with less active nectar consumers (pollinators with lower nectar foraging rates). *Amianthium* beetle pollinators are small-bodied pollinators that appear to fill their energy budget in short feeding bouts on just a few flowers, fitting the description of a low-activity consumer. *Amianthium,* on average, produces fairly large volumes of nectar in all its flowers, suggesting the plant may have evolved under a selection regime with high potential benefits of high nectar production.

Chapters 3: Nectar traits of neighbors shape pollinator interactions in *Amianthium muscaetoxicum*: and 4: Selection on nectar traits is robust to environmental variation in the pollinator-dependent *Amianthium muscaetoxicum* directly tested natural selection on the highly variable nectar traits in this wild *Amianthium* population. Both chapters examined the possibility for spatial and temporal variation in phenotypic selection mediated by the behavior of foraging pollinators. Chapter 3 examined how the nectar traits of neighboring plants impacted pollinator foraging behavior and served as a potential cause of multilevel selection on nectar traits. I found a patch-level effect of higher mean nectar production on pollinator visitation, but no evidence that this behavioral pattern translated into group selection on nectar traits. Instead, I detected a neighborhood-level effect of the focal individual's total sugar production

relative to its neighbors via a plant's seed set fitness measure and via behavioral tests with one of the plant's primary beetle pollinators. This pattern of neighbor effects may again be related to the foraging habits of the beetle pollinators. Like many other optimally foraging pollinators, beetles were attracted to high nectar volumes, but individual beetles only consumed small amounts of nectar in experimental foraging bouts, feeding from one to two flowers before stopping for long periods of time. Neighbor effects are a consequence of the behavior of individual consumers. My work suggests that consumers with low energy needs, such as beetles, may be more likely to respond to the relative availability of nectar among individuals than to the average availability of an entire group of plants, a pattern commonly seen in larger or social pollinator species.

Chapter 4 experimentally examined how variation in an environmental factor that induced plasticity in nectar traits, thereby changing the phenotypic distribution exposed to selection, impacted selection on nectar resources. Changing the water environment plants experienced shifted both phenotypic variance and mean plant reproductive fitness, but these changes did not significantly alter selection among the two water environments. I also found a change in the form of multilevel selection compared to my results from Chapter 3: plants in the second selection study experienced a component of group selection via their fruit set fitness measure. These results indicated that selection on nectar traits may be robust to temporal changes in the environment, even while pollinator behaviors may change with dramatic shifts in community-scale resource availability. Lack of change in the direction of selection across water environments further suggested no apparent costs of nectar production in *A. muscaetoxicum* even under extreme water limitation, indicating that the benefits of pollinator interactions may far outweigh costs of nectar production in this self-incompatible species.

My empirical work with *Amianthium* in Chapters 3-4 reinforced my theoretical framework of resource evolution in consumer-resource mutualisms: nectar traits can experience strong, consistent directional selection mediated in part by the behaviors of foraging pollinators. This work is the first, to my knowledge, to demonstrate statistically significant directional selection acting on nectar volume and total sugar content in any natural plant population. Furthermore, selection on nectar traits remained

largely consistent across two years with very different background environmental conditions, even while these environmental conditions appeared to induce different patterns of pollinator behavior. The selection study in Chapter 4 occurred during a much drier flowering season compared to the conditions of the selection study in Chapter 3. Despite these differences, direct and net selection on nectar traits remained consistent and positive, even strengthening in the drier study year (e.g., direct selection β' on total sugar via a plant's total seed set = 0.17 in 2022, 0.24 in 2024). Consistent selection in the face of environmental changes across time further corroborates the lack of difference in selection detected across water environments in the 2024 experiment. In both chapters 3 and 4, the multilevel components of selection appeared to reinforce the activity of individual-level selection, preserving directional selection for higher nectar volumes and higher total sugar contents. Future work in *Amianthium* should examine the heritability of nectar traits in this population to determine how selection will translate into evolutionary change in nectar traits over time.

The present body of work further showed that pollinator foraging behavior, and the consequences of these behaviors for selection on plant traits, depended on overall nectar availability in the environment. In Chapter 3's behavior experiment, beetles only significantly changed their behavior towards a focal nectar source when nectar availability in the environment was low. In Chapter 4, extremely low nectar availability across the population appeared to cause pollinators to shift their behavior from discerning among the traits of individuals, as evidence from Chapter 3 suggested, to increasing their foraging intensity on all individuals in higher nectar-producing neighborhoods. I did not detect any evidence of group selection in the 2022 study when nectar availability at the population scale was higher (population mean in Chapter 3 = 3.2 μL nectar, population mean in Chapter 4 = 1.7 μL). My theoretical work demonstrated that changes in the size of the resource pool had important consequences for the ecological and evolutionary trajectories of plant-pollinator populations. My empirical work with *Amianthium* further emphasized this point by showing how pollinator behavior responded to variation in resource availability at the scale of small neighborhoods, larger patches, and across the entire population.

Chapter 1: The evolution of resource provisioning in pollination mutualisms

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ABSTRACT

Resource dynamics influence the contemporary ecology of consumer-resource mutualisms. Suites of resource traits such as floral nectar components also evolve in response to different selective pressures, changing the ecological dynamics of the interacting species at the evolutionary equilibrium. Here we explore the evolution of resource provisioning traits in a biotically pollinated plant that produces nectar as a resource for beneficial consumers. We develop a mathematical model describing natural selection on two quantitative nectar traits: maximum nectar production rate and maximum nectar reservoir volume. We use this model to examine how nectar production dynamics evolve under different ecological conditions that impose varying cost-benefit regimes on resource provisioning. The model results predict that natural selection favors higher nectar production when ecological factors limit the plant or pollinator's abundance (e.g., a lower productivity environment or a higher pollinator conversion efficiency). We also find that nectar traits evolve as a suite in which higher costs of producing one trait select for a compensatory increase in investment in the other trait. This empirically explicit approach to studying the evolution of consumer-resource mutualisms illustrates how natural selection acting via direct and indirect pathways of species interactions generates patterns of resource provisioning seen in natural systems.

INTRODUCTION

Many species relationships involve exchanges of energy or material resources that alter the population dynamics of the interacting species. Herbivores consume plants, thus increasing their own abundance and in many cases reducing the size of the plant population (Crawley 1989). Plant species compete with one another for nutrients, depressing each other's abundances by consuming those limiting resources (Tilman 1982; Goldberg 1990). Consumer-resource dynamics have long been a unifying theme in theoretical considerations of antagonistic species relationships such as predation, parasitism, and resource competition (Murdoch et al. 2003). Crucially, consumer-resource dynamics are influenced by species traits. For instance, plants with higher concentrations of inducible toxic alkaloids may experience reduced herbivory (e.g., Karban et al. 1997), and plants with a stronger ability to take up nitrogen will be better competitors for that limiting resource (e.g., Gutschick 1981). As these traits experience natural selection and evolve, the ecological dynamics of the consumer-resource interaction will evolve as well (e.g., Roughgarden 1972; Slatkin 1980; Taper and Chase 1985; Abrams and Chen 2002; Vasseur and Fox 2011; McPeek 2017a,b, 2019). By studying how natural selection acts on traits that affect consumer-resource dynamics, we gain insights into the ecological conditions that shape the evolution of energy flow in communities.

Many mutualisms can also be classified as consumer-resource interactions because they too are trait-mediated interactions that involve an exchange of energy or materials (Herre et al. 1999; Holland et al. 2005; Holland and DeAngelis 2009, 2010; Jones et al. 2012; Bronstein 2015). In consumer-resource mutualisms, the resource is not the individual itself, but rather a product that the individual produces in exchange for some form of benefit from its consumer. For example, larvae of some lycaenid butterflies provide nutritive secretions to ants, which directs the ants' predatory behaviors towards lycaenids' natural enemies rather than towards the lycaenids themselves (Pierce et al. 2002). Many plants produce sugar-rich fruits that are fed upon by animals that then disperse the seeds, increasing the likelihood that some will germinate (Simmons et al. 2018). To date, consumer-resource mutualisms have received little attention as a major component of consumer-resource theory (but see Holland *et al.* 2005). This is a severe oversight,

as consumer-resource mutualisms can have profound impacts on the structure of diverse ecological communities (e.g., Stachowicz 2001; Johnson 2015). Further, the benefit of product consumption for the resource provider is often an indirect result of how its own resource provisioning affects the consumer's behavior, providing a unique opportunity to study how trait-mediated indirect effects influence ecological and evolutionary dynamics in consumer-resource interactions (Abrams 1995; Werner and Peacor 2003).

In consumer-resource mutualisms, the dynamics of the resource can be treated separately from the population dynamics of the interacting species. This differs notably from consumer-resource antagonisms wherein species deplete each other's abundances, not each other's resource products. Explicitly modeling mutualistic resources has provided critical mechanistic insights into how resource dynamics may shape mutualism ecology (Soberon and Martinez Del Rio 1981; Valdovinos et al. 2013; Revilla 2015; Bachelot and Lee 2018; Valdovinos 2019). For example, a provider has a finite ability to produce resources. Its maximum rate of resource provisioning will constrain the consumer and resource-provider's population growth rates, and thus the fitness benefits that can be accrued by each from the interaction (Soberon and Martinez Del Rio 1981; Revilla 2015). The consumer species' foraging behavior further affects the resource dynamics of the interaction by determining the rate of resource depletion (Valdovinos et al. 2013; Revilla 2015). Overall, consumer-resource theories of mutualism suggest that a provider species' optimal rate of resource provisioning balances the costs and benefits of provisioning for a partner (Pyke 1981; Soberon and Martinez Del Rio 1981; Bachelot and Lee 2018). If we further conceptualize resource dynamics as trait dynamics, we can add an evolutionary perspective to this cost-benefit framework: species should evolve resource trait combinations that maximize their fitness via their provisioning for a mutualistic consumer.

Incorporating natural selection on resource traits into theories of consumer-resource mutualism can generate empirical predictions about how resource provisioning traits, as well as the species relationships they mediate, may evolve under varying cost-benefit regimes. For instance, an evolutionary perspective on consumer-resource mutualism holds potential for explaining the astonishing diversity of plant traits associated with pollination mutualisms. Many pollination interactions are consumer-resource mutualisms

in which plants produce floral nectar and pollen that feed animals, which then move pollen between flowers as they seek more food from the plants (Willmer 2011). Crucially, traits that influence a plant's nectar supply dynamics, such as a plant's rate of nutrient uptake from the environment, the quantity and chemical composition of nectar a plant produces, and the rate at which a plant can replenish its nectar as the consumer depletes its standing crop, all play a key role in mediating a plant's interaction with pollinators (Nicolson et al. 2007). While empirical studies have examined diverse aspects of nectar quality and quantity in natural plant populations, we have little understanding of the causes and strengths of natural selection on the traits that underlie its production dynamics in different ecological settings (Parachnowitsch et al. 2019). An empirically explicit theory of mutualism evolution will provide directions for future inquiry into the past, present, and future ecological forces that drive the evolution of resource provisioning in consumer-resource mutualisms, including pollination.

To this end, we develop a consumer-resource model of a mutualist plant's evolving nectar provisioning dynamics in a pairwise plant-pollinator interaction. The model describes the fitness landscapes of two plant traits that influence nectar quantity, nectar production rate and nectar reservoir volume, under a range of ecological conditions and various pollinator foraging capabilities. Nectar production rate captures a plant's physiological capacity to produce and secrete nectar and determines how fast depleted nectar can be replenished; nectar reservoir volume captures a plant's total nectar-holding capacity, reflecting traits such as floral corolla depth and the number and size of flowers on a plant individual. Together, these traits define the total resource pool available to consumers, which directly affects the local abundance of pollinators and the frequency of plant-pollinator interactions and indirectly affects the abundance of plants via interactions with pollinators. Our resource trait-centered model generates testable predictions about how consumer-resource mutualisms evolve in natural communities.

THE MODEL

Model Of Nectar Evolution In A Plant–Pollinator Interaction

401 We begin by defining the dynamics of a plant population with a population size R_1. To attract
402 pollinators, individuals produce nectar. Plants have two quantitative traits that determine their nectar
403 production dynamics. We assume that the average values of these traits are constant over an individual's
404 lifetime, and we treat a population's mean trait values as averages across all flowers on all plants. We also
405 assume that these two traits are genetically uncorrelated and can evolve independently of one another.
406 The first quantitative trait is the maximum rate at which an individual can produce and secrete nectar into
407 flowers: we represent this trait as z_{NPR}. The second trait is the maximum volume of nectar that an
408 individual plant can hold, summed across all flowers on the plant (hereafter, the reservoir volume): we
409 represent this trait as z_{RV}. The model analyzed here does not consider whether reservoir volume is
410 distributed across many small or a few large flowers (e.g., Cohen and Shmida 1993; Venable 1997). For
411 simplicity, we assume that plants always replenish nectar to its maximum holding capacity within an
412 individual flower. We also assume all other properties of nectar remain constant (e.g., sugar and amino
413 acid content and concentration). Additionally, we do not consider selection on flowering phenology, floral
414 longevity, or seasonal variation in nectar production, and we also do not consider pollen as an additional
415 resource for consumers. We are primarily concerned with the population's average trait expression across
416 the entire flowering period since these measures describe the average total resource pool available to
417 consumers at a given time.

418 Plants in this model replenish nectar dynamically as pollinators deplete their supply. We define the
419 standing volume of nectar currently available to pollinators on a single plant (hereafter, standing nectar
420 volume) as S_1. At any moment, the total volume of nectar on all plants in the population is thus $R_1 S_1$.
421 Individual plants produce nectar to fill their reservoirs according to a simple logistic resource renewal
422 function:

423 $$z_{NPR}\left(1-\frac{S_1}{z_{RV}}\right). \qquad (1)$$

424 The production rate when $S_1 = 0$ in all flowers on a plant is equal to the maximum nectar production rate
425 z_{NPR}, and this rate decreases linearly until a plant's reservoir is full (Fig. 1A). For a given constant non-
426 zero rate of nectar depletion, an increase in either z_{NPR} or z_{RV} will increase the equilibrium volume of
427 nectar S_1 on a plant. (Descriptions of all state variables and model parameters used in this model are
428 summarized in Table 1.)

429 We assume that the plant population displays logistic growth in the absence of pollinators, such that
430 its per capita growth rate is

431 $$c_1\left(z_{NPR}, z_{RV}\right) - d_1 R_1 \qquad (2)$$

432 (Verhulst 1838; Pearl and Reed 1920). Here, $c_1\left(z_{NPR}, z_{RV}\right)$ is the plant population's intrinsic rate of
433 increase, which is a function of the values of the two traits (see below), and d_1 is the strength of density
434 dependence from limiting factors that regulate its population size but are not explicitly modeled (Pianka
435 1972; Schoener 1973; Schaffer and Leigh 1976; Schaffer 1981). In the absence of pollinators, the plant
436 population will increase to an equilibrium population size of $R_1^* = c_1\left(z_{NPR}, z_{RV}\right)/d_1$ if the plant exists in
437 favorable environmental conditions and does not need the pollinator's fitness benefit to maintain a
438 population, i.e., $c_1\left(z_{NPR}, z_{RV}\right) > 0$. In this scenario, the plant is a facultative mutualist. However, if the
439 plant is an obligate mutualist (i.e., it cannot reproduce without pollinators) or is in poor environmental
440 conditions where it cannot maintain a population without the pollinator's fitness benefit, i.e.,
441 $c_1\left(z_{NPR}, z_{RV}\right) < 0$, the population will decline to extinction when pollinators are absent.

442 We assume that individual plants may pay three different costs of producing nectar depending on the
443 values of the two traits. First, producing the machinery necessary to make nectar may be costly (e.g.,
444 nectaries: Nicolson *et al.* 2007). This cost is expressed as a decrease in the plant population's intrinsic rate
445 of increase (in equation (2)) according to a quadratic function of z_{NPR}. Second, producing the structures
446 to hold nectar (e.g., increasing the number or depths of flowers) may be costly as well, also decreasing the

447 plant population's intrinsic rate of increase according to a quadratic function of z_{RV} (e.g., (Nobel 1977;
448 Ashman 1994). Based on these assumptions, the population's intrinsic rate of increase is then

449 $$c_1\left(z_{NPR}, z_{NV}\right) = c_1 - \gamma_{NPR} z_{NPR}^2 - \gamma_{RV} z_{RV}^2, \quad (3)$$

450 where c_1 is the maximum intrinsic rate of increase when $z_{NPR} = z_{RV} = 0$, and γ_{NPR} and γ_{RV} scale the
451 decline in the plant's intrinsic rate of increase as z_{NPR} and z_{RV} increase. From an evolutionary
452 perspective, these two scaling parameters also modulate the steepness of the selection gradient that acts
453 on a plant's nectar production traits via individual fitness effects on the population's intrinsic rate of
454 increase. Finally, individuals may pay an incremental cost for every unit of nectar produced:

455 $$\psi_1 z_{NPR}\left(1 - \frac{S_1}{z_{RV}}\right), \quad (4)$$

456 which is simply the realized nectar production rate (equation (1)) times a constant ψ_1 that scales the
457 fitness cost for each unit of nectar a plant produces.

458 The plant population interacts with a pollinator that has a population size N_1. Just like any consumer,
459 a pollinator uses a resource, in this case the nectar produced by the plant, to gain energy and produce
460 offspring. Pollinators consume nectar at a rate that scales with the amount of available nectar on each
461 plant according to the Michalis-Menten/Monod relationship

462 $$a_{11}\left(S_1\right) = \frac{a_{11} S_1}{\vartheta_{11} + S_1}, \quad (5)$$

463 where a_{11} is the asymptotic maximum consumption rate, and ϑ_{11} is the half-saturation constant (Fig. 1B)
464 (Michaelis and Menten 1913; Monod 1949). We also assume that the pollinator population has an
465 intrinsic density-independent death rate given by f_1. These assumptions entail that the nectar provided by
466 the plant is the main factor limiting local pollinator abundance.

467 While foraging for nectar, pollinators incidentally provide a fitness benefit to plants by transferring

pollen between flowers. This benefit is also a function of the nectar consumption rate $a_{11}(S_1)$. Consistent with Holland and DeAngelis (2009, 2010), we assume that this fitness benefit saturates with increasing pollinator population size according to

$$B(S_1, N_1) = \left(\frac{a_{11}(S_1) N_1}{1 + a_{11}(S_1) \phi_1 N_1} \right) = \frac{\frac{a_{11} S_1}{\vartheta_{11} + S_1} N_1}{1 + \frac{a_{11} S_1}{\vartheta_{11} + S_1} \phi_1 N_1} \quad (6)$$

where ϕ_1 defines the maximum fitness benefit that an individual plant can receive from pollinator foraging. This maximum fitness benefit when pollinator abundance is very large is thus $1/\phi_1$ (Fig. 1C). This fitness benefit can be measured as the increase in female plant fitness due to a greater number of ovules being fertilized by the actions of the pollinators as they forage for nectar. Specifically, $c_1(z_{NPR}, z_{RV})$ is a fitness component of the plant that defines the plant population's rate of increase independent of the pollinator's actions (i.e., how many plant ovules are fertilized by vectors other than pollinators); $B(S_1, N_1)$ defines the supplement of this fitness component given local pollinator population size; and $1/\phi_1$ is the maximum value of $B(S_1, N_1)$ if pollen is deposited in excess of the amount needed to fertilize all ovules. Note that we do not consider male fitness in this model. As stated above, if $c_1(z_{NPR}, z_{RV}) > 0$, the plant can maintain a population in the absence of pollinators under the local conditions it experiences. However, if $c_1(z_{NPR}, z_{RV}) < 0$, the plant must receive a sufficient fitness increase from the actions of pollinators (i.e., pollinators must fertilize enough additional ovules to generate a sustaining per capita fitness) in order for $c_1(z_{NPR}, z_{RV}) + B(S_1, N_1) > 0$: otherwise, the plant population will go extinct when no pollinators are present.

Given the above assumptions, the dynamics of the plant population R_1, its total nectar pool $R_1 S_1$, and the pollinator population N_1 are given by the following set of differential equations (note that the

plant and pollinator equations are expressed in their per capita forms but the nectar equation is expressed in its total growth rate form, i.e., change in the plant population's total nectar volume):

$$\frac{1}{R_1}\frac{dR_1}{dt} = \left(c_1 - \gamma_{NPR} z_{NPR}^2 - \gamma_{RV} z_{RV}^2\right) - d_1 R_1 + \frac{\frac{a_{11} S_1}{\vartheta_{11} + S_1} N_1}{1 + \frac{a_{11} S_1}{\vartheta_{11} + S_1} \phi_1 N_1} - \psi_1 z_{NPR}\left(1 - \frac{S_1}{z_{RV}}\right)$$

$$\frac{d\left(R_1 S_1\right)}{dt} = R_1 z_{NPR}\left(1 - \frac{S_1}{z_{RV}}\right) - \frac{a_{11} S_1}{\vartheta_{11} + S_1} R_1 S_1 N_1 \qquad (7)$$

$$\frac{1}{N_1}\frac{dN_1}{dt} = b_{11}\frac{a_{11} S_1}{\vartheta_{11} + S_1} R_1 S_1 - f_1$$

In the pollinator equation, b_{11} is the conversion efficiency describing the rate at which pollinators convert consumed nectar into pollinator offspring.

The per capita population growth equation for the plant species in equations (7) also expresses the average per capita fitness of the plant with mean trait values of z_{NPR} and z_{RV} (Lande 1982, 2007). In other words, this equation defines the fitness topography against which the plant population evolves. The various terms in equation (7) define how z_{NPR} and z_{RV} influence fitness components that act in combination to determine the plant's total fitness. However, the equation given in (7) only explicitly relates the relationships of these traits to the fitness costs. The relationships of these traits to the benefits of nectar production for the plant are not apparent because they are embedded in the dynamic variable for the standing nectar volume S_1.

To incorporate both the fitness benefit and cost relationships with the two quantitative plant traits into the plant equation, we assume that standing nectar volume is always at equilibrium with plant and pollinator population size (i.e., $d\left(R_1 S_1\right)/dt = 0$) and solve the nectar dynamics equation for the equilibrium standing nectar volume. This results in a quadratic function and the root associated with positive equilibrium standing nectar volume is

508 $$S_1^+ = z_{NPR}\left(\frac{z_{RV} - \vartheta_{11} + \sqrt{\left(z_{RV} + \vartheta_{11}\right)^2 + 4z_{RV}^2\vartheta_{11}a_{11}N_1/z_{NPR}}}{2\left(z_{NPR} + z_{RV}a_{11}N_1\right)}\right). \quad (8)$$

509 This derivation is then substituted into the plant population dynamics equation in (7) to express the plant
510 per capita fitness explicitly as a function of its two quantitative traits.

511 The resulting equation can then be used to model the evolution of nectar production rate and nectar
512 reservoir volume in response to the various selection pressures outlined above. We follow Lande's (1982,
513 2007) approach to trait dynamics using the continuous time breeder's equation formulation to study the
514 evolution of the mean nectar production phenotype in this population. We favor Lande's approach over
515 other possible approaches (e.g., adaptive dynamics) because it is based on the breeder's equation from
516 quantitative genetics. Thus, the evolutionary process is modeled in the same framework empiricists use to
517 study the dynamics of selection in natural systems. The dynamics of trait evolution are then given in Box
518 1.

Box 1: Dynamics of trait evolution.

The two nectar dynamics traits of the plant evolve according to

$$\frac{dz_{NPR}}{dt} = G_{NPR}\frac{\partial \dfrac{dR_1}{R_1 dt}}{\partial z_{NPR}}$$

$$= G_{NPR}\left(-2\gamma_{NPR}z_{NPR} + \frac{a_{11}\vartheta_{11}N_1 \partial S_1^+ \big/ \partial z_{NPR}}{\left(\vartheta_{11}+S_1^+\right)^2\left(1+\dfrac{a_{11}S_1^+}{\vartheta_{11}+S_1^+}\phi_1 N_1\right)^2} - \psi_1\left(1-\frac{S_1^+}{z_{RV}} - \frac{z_{NPR}\,\partial S_1^+ \big/ \partial z_{NPR}}{z_{RV}}\right)\right), \quad (9)$$

$$\frac{dz_{RV}}{dt} = G_{RV}\frac{\partial \dfrac{dR_1}{R_1 dt}}{\partial z_{RV}}$$

$$= G_{RV}\left(-2\gamma_{RV}z_{RV} + \frac{a_{11}\vartheta_{11}N_1 \partial S_1^+ \big/ \partial z_{RV}}{\left(\vartheta_{11}+S_1^+\right)^2\left(1+\dfrac{a_{11}S_1^+}{\vartheta_{11}+S_1^+}\phi_1 N_1\right)^2} - \psi_1\frac{z_{NPR}}{z_{RV}}\left(\frac{S_1^+}{z_{RV}} - \frac{\partial S_1^+}{\partial z_{NPR}}\right)\right)$$

where

$$\frac{\partial S_1^+}{\partial z_{NPR}} = \frac{2z_{RV}a_{11}N_1\left(z_{RV}-\vartheta_{11}\right)}{\left(2z_{NPR}+2z_{RV}a_{11}N_1\right)^2} + \frac{2z_{RV}a_{11}N_1\left(z_{NPR}\vartheta_{11}^2 + z_{NPR}z_{RV}^2 + 2z_{RV}^2a_{11}\vartheta_{11}N_1\right)}{\left(2z_{NPR}+2z_{RV}a_{11}N_1\right)^2\sqrt{\left(z_{NPR}z_{RV}+z_{NPR}\vartheta_{11}\right)^2 + 4z_{NPR}z_{RV}^2\vartheta_{11}a_{11}N_1}},$$

$$\frac{\partial S_1^+}{\partial z_{RV}} = \frac{2z_{NPR}\left(z_{NPR}+\vartheta_{11}a_{11}N_1\right)}{\left(2z_{NPR}+2z_{RV}a_{11}N_1\right)^2} + \frac{2z_{NPR}^2\left(z_{NPR}\vartheta_{11} + z_{NPR}z_{RV} + 3z_{RV}\vartheta_{11}a_{11}N_1 - \vartheta_{11}^2a_{11}N_1\right)}{\left(2z_{NPR}+2z_{RV}a_{11}N_1\right)^2\sqrt{\left(z_{NPR}z_{RV}+z_{NPR}\vartheta_{11}\right)^2 + 4z_{NPR}z_{RV}^2\vartheta_{11}a_{11}N_1}},$$

and G_{NPR} and G_{RV} are the additive genetic variances in nectar production in the two corresponding traits, and the terms in parentheses are the selection gradients on z_{NPR} and z_{RV} from the various fitness components of the plant.

519 A model of this complexity prohibits analytical analysis. Therefore, we use computer simulations

(numerical integration of the model using the ode45 solver of Matlab) to analyze patterns emerging from interesting and biologically reasonable areas of parameter space. Matlab code is provided as Supplemental Material.

RESULTS

Defining the Fitness Surfaces of Nectar Production Traits

We first examine how the fitness landscape of the plant's two nectar production traits, maximum nectar production rate z_{NPR} and maximum nectar reservoir volume z_{RV}, define the ecological and evolutionary trajectory of the plant population R_1. We partition the plant's total fitness into three components that depend on its trait values z_{NPR} and z_{RV} (equation (7)): the fitness contributions of the intrinsic rate of increase, of pollinators, and of nectar production.

First, consider a plant population that receives no attention from pollinators. The population's total and component fitness surfaces at ecological and evolutionary equilibrium are shown in Figure 2. Without pollinators, the costs of producing nectar for no beneficial returns push the plant population to evolve to a fitness maximum at a zero nectar production rate (Fig. 2A) and a zero nectar reservoir volume (Fig. 2B). Notice that the plant population's total fitness surfaces for nectar production rate (Fig. 2A) and reservoir volume (Fig. 2B) are identical to the component surfaces measuring the cost of making nectar-secreting and nectar-holding structures on its intrinsic rate of increase $c_1\left(z_{NPR}, z_{RV}\right)$ (Figs. 2C, D). The other component surfaces remain completely flat because plants in this case earn no fitness benefit from making nectar (Figs. 2E, F), and they pay no incremental cost ψ_1 of producing nectar because the nectar reservoir has a volume of zero (Figs. 2G, H).

Now, consider the total and component fitness surfaces of the same plant population when pollinators are present (Figure 3). The structural costs on the plant's intrinsic rate of increase of making nectar-producing and nectar-holding structures (Figs. 3C, D) are identical in magnitude to those experienced by an abiotically pollinated plant (Figs. 2C, D). However, in this population these fitness costs are offset by

543 the direct benefits pollinators provide to plants by fertilizing ovules while they forage for nectar.
544 Therefore, plants with faster nectar production rates (Fig. 3E) and larger nectar reservoir volumes (Fig.
545 3F) attain higher fitness benefits by inducing pollinators to consume nectar at faster rates $a_{11}(S_1)$ from
546 larger total nectar pools S_1. Plants with faster nectar production rates also provide more food for
547 pollinators, directly increasing pollinator population size and indirectly increasing plant fitness and plant
548 population size by elevating the number of pollinator individuals that interact with plants (Fig. 1C).
549 However, this benefit saturates with higher pollinator population size because females have a finite
550 number of ovules, and more pollinators cannot continue to confer benefits to female fitness once all plant
551 ovules have been fertilized. Lastly, a plant's nectar production is further constrained by the per capita cost
552 ψ_1 of filling a larger nectar reservoir as pollinators consume nectar at faster rates (Figs. 3G, H).

553 *Altering Selection on the Benefits of Nectar Production*

554 In both previous cases, the plant population evolves to a phenotypic optimum that balances the costs
555 and benefits of nectar production for total plant fitness. We now examine how different selective
556 environments affect the evolution of nectar production dynamics in this pairwise plant-pollinator
557 relationship.

558 Both nectar production traits generally increase in a similar manner as the maximum fitness benefit
559 from the pollinator to the plant increases (Figs. 4A, B). Figure 4 shows the equilibrium trait values,
560 population sizes, and standing nectar volume along gradients of c_1, the plant's maximum intrinsic rate of
561 increase, and $1/\phi_1$, the maximum fitness benefit a plant can earn from the actions of pollinators. The plant
562 evolves both a faster nectar production rate and a larger nectar reservoir volume when the benefit it earns
563 from pollinators $1/\phi_1$ is high (e.g., $1/\phi_1$ =4.0, Figs. 4A-B). Additionally, plants evolve larger nectar
564 reservoir volumes and faster nectar production rates in an environment affording a lower c_1 (i.e., a lower
565 intrinsic rate of increase independent of the actions of pollinators) compared to plants in environments

566 with higher c_1 (Figs. 4A-B). Below a certain level of pollinator benefit $1/\phi_1$, plants with a lower c_1
567 cannot maintain a population in that environment, causing plants to evolve lower and lower nectar
568 provisioning until both the plant and pollinator populations go extinct (Figs. 4A-B). Likewise, a higher
569 minimum intrinsic death rate for pollinators, f_1, which reflects ecological conditions that limit the growth
570 of the pollinator population independent of its relationship with the plants, also causes the plant to evolve
571 higher nectar provisioning (results not shown).
572 Plant and pollinator population sizes also increase with elevated fitness benefits from pollinators
573 (Figs. 4C and E). The pollinator population increases because the plant provides more nectar via an
574 increased nectar production rate and a larger reservoir volume (Fig. 4E). The plant population increases
575 because plants receive a greater fitness benefit via the larger number of interacting pollinators (Fig. 4C).
576 Note that while the nectar production rate and the total nectar reservoir volume both increase with higher
577 $1/\phi_1$, the standing nectar volume per plant decreases (Fig. 4D) because more pollinator individuals with
578 higher nectar consumption rates are continuously depleting the plant's standing nectar volume (Fig. 4E).
579 Varying properties of the pollinators that affect the frequency of their interactions with plants also
580 cause corresponding evolutionary responses in the plant's nectar production rate and nectar reservoir
581 volume (Figs. 5A, B). Figure 5 depicts the effects of these interaction-limiting factors, the pollinator's
582 maximum nectar consumption rate a_{11} and its maximum nectar conversion efficiency b_{11}, on plant traits,
583 population sizes, and standing nectar volume, with fitness benefits $1/\phi_1$ and fitness costs γ_{NPR}, γ_{RV}, and
584 ψ_1 held constant. When the pollinator's maximum nectar consumption rate is low (i.e., low a_{11}), the
585 plant evolves a larger nectar reservoir volume (Fig. 5B) and produces nectar to fill that reservoir at a
586 faster rate (Fig. 5A), both of which cause the plant to supply nectar to the pollinator at a faster rate.
587 Likewise, when the pollinator's maximum nectar conversion efficiency is low (i.e., low b_{11}, meaning that
588 each pollinator must consume more nectar to produce one offspring), plants that supply nectar at faster

589 rates and have larger reservoir volumes earn higher fitness benefits by increasing the number of
590 pollinators, thereby also increasing the number of interactions they receive (Figs. 5A-B).
591 Plant population size and standing nectar volume respond identically to a pollinator with a higher
592 nectar consumption rate and a higher nectar conversion efficiency, while pollinator population size
593 responds differently to these interaction-limiting factors (Fig. 5C, D). Plant population sizes are largest
594 when their pollinator's nectar conversion efficiency and nectar consumption rate are high, because plants
595 receive a greater number of visits from a larger population of pollinators while also experiencing weaker
596 selection to produce more nectar, thus paying lower production costs (Fig. 5C). Correspondingly, the
597 standing nectar volume per plant is highest when the opposite is true: when the pollinator's nectar
598 conversion efficiency and nectar consumption rate are low there are fewer pollinators consuming plant
599 nectar, and each is consuming nectar at a slower rate (Fig. 5D). Pollinator population sizes are largest
600 when their nectar conversion efficiency is high and their nectar consumption rate is low (Fig. 5E) because
601 individual pollinators deplete less of the nectar resource pool but produce more offspring.

602 *Modulating Selection on the Costs of Nectar Production*

603 In contrast to changes in the benefits from pollinators, nectar production rate and nectar reservoir
604 volume respond differently to changes in the various nectar production costs. The surfaces in Figure 6
605 display how the plant traits, population sizes, and standing nectar volume change with varying fitness
606 costs of nectar production traits (i.e., various combinations of γ_{NPR} and γ_{RV}) on the plant's intrinsic rate
607 of increase. High fitness costs on the plant's intrinsic rate of increase resulting from higher costs of
608 making larger nectar-secreting structures (i.e., larger values of γ_{NPR}) select for a slower nectar production
609 rate (Fig. 6A). Additionally, at high costs for increasing production rate but low costs for increasing
610 nectar reservoir volume (i.e., small γ_{RV}), the plant evolves a larger reservoir volume that compensates
611 for a slower production rate (Fig. 6B). In contrast, increasing the fitness cost of a larger nectar reservoir
612 volume has almost no effect on the evolution of a plant's maximum nectar production rate (Fig. 6A).

The per-capita cost of replenishing nectar ψ_1 affects the evolution of the two plant traits in a similar way, but responses to this cost vary between the two traits depending on the fitness cost of increasing nectar production rate (γ_{NPR}) on the plant's intrinsic rate of increase (Figure 7). Overall, a higher incremental cost of replenishing nectar at a given rate (i.e., larger ψ_1) selects for both a slower nectar production rate (Fig. 7A) and a smaller reservoir volume (Fig. 7B). The plant evolves the largest reservoir volume when the cost of replenishing nectar at a given rate (ψ_1) is low and the cost of a faster nectar production rate on the plant's intrinsic rate of increase (γ_{NPR}) is high (Fig. 7B), again demonstrating a compensatory evolutionary response of increasing reservoir volume when nectar production costs are high. The plant evolves the fastest nectar production rate when both costs are low (Fig. 7A). Interestingly, the cost of replenishing nectar at a given rate (ψ_1) has a greater effect on the evolution of a plant's maximum nectar reservoir volume than it does on a plant's maximum nectar production rate. This further supports the evolution of trait combinations that cause an increase in standing nectar volume in compensation for high nectar production rate costs (Fig. 7D).

Varying the costs of nectar production also has differential impacts on equilibrium population sizes. Steeper production costs on either nectar production rate (larger γ_{NPR}) or nectar reservoir volume (larger γ_{RV}) reduce plant population size (Fig. 6C) and pollinator population size (Fig. 6E) and increase the standing nectar volume available from each plant individual (Fig. 6D). However, population sizes and standing nectar volumes respond much more strongly to increasing costs of nectar production rate than they do to the cost of a larger reservoir volume. Standing nectar volume primarily increases when nectar production rate decreases because there are fewer pollinators to deplete the plant's nectar pool when plants provide fewer resources (Figs. 6D, E). Plant population size generally decreases as any of these costs increase (Figs. 6C and 7C). Pollinator population size increases when both production costs are low because the plants provide the most nectar under these conditions (Figs. 6E and 7E).

DISCUSSION

The contemporary ecology of consumer-resource mutualisms is shaped by past selection on a species' resource provisioning dynamics (Parachnowitsch et al. 2019). In this paper, we explicitly model how ecological processes generate natural selection on two plant resource provisioning traits to shape the evolutionary trajectory of the plant's interaction with a pollinator. We show that consumers with low functional or numerical responses (i.e., lower nectar foraging rates or lower nectar conversion efficiencies) select for increased nectar provisioning by plants, which has the effect of boosting their population size (Fig. 5). Additionally, high nectar production costs select for suites of nectar traits that minimize the effects of these costs on the level of resource provisioning, thus increasing the plant's population by maintaining high levels of pollination interactions (Figs. 6, 7). In particular, selection against larger values of one trait may cause a compensatory increase in the other. These model results extend existing theories of consumer-resource mutualisms into an evolutionary framework and provide testable predictions that can guide empirical research on the evolution of consumer-resource mutualisms in nature.

Providers Evolve Resource Production Dynamics That Increase Interactions With Consumers

Several researchers have shown that consumer functional and numerical responses play a key role in shaping the consumer-resource dynamics of mutualisms (e.g., Holland et al. 2005; Holland and DeAngelis 2009; Valdovinos et al. 2013; Revilla 2015; Hale et al. 2020). Our findings demonstrate how these consumer characteristics may also serve as agents of natural selection on resource provisioning traits. The model results show how pollinators' behavioral (e.g., traits that influence nectar foraging rate a_{11}) and physiological (e.g., traits that influence nectar conversion efficiency b_{11}) properties have identical effects on nectar trait evolution (Fig. 5A-B), but the effects of these two different selective agents are mediated through distinct indirect pathways.

First, our model predicts that plants evolve trait combinations that provide more resources for consumers with lower intrinsic functional responses (i.e., lower a_{11}, Fig. 5A, B) via a trait-mediated

indirect effect (Abrams 1995; Werner and Peacor 2003). Specifically, plants that provision more nectar enact a trait-mediated indirect effect on their own fitness by causing pollinators to consume nectar at faster rates. Increasing the pollinator's nectar foraging rate indirectly enhances the plant's own fitness by increasing the frequency and duration of its interactions with pollinators. Further, a plant earns a greater fitness benefit from increasing its nectar production when pollinator foraging rates are low (Fig. 5A, B). If pollinators already forage frequently from the plants and pollinate most of a plant's ovules in the process, individuals earn only a marginally greater benefit by provisioning more nectar, and in fact evolve to provision less nectar, causing a slight decrease in pollinator abundance (Fig. 5E). One empirical prediction emerging from this result is that plants with generalist pollinators that visit infrequently may evolve to produce more nectar than plants with pollinators that forage exclusively on that species. Indeed, Johnson and Nicolson (2008) found this exact pattern in bird-pollinated plants: species that interact with a large group of generalist pollinators produce up to ten times more nectar than those with highly specialized pollinators. These patterns are typically attributed to larger body sizes of generalist species, but our results suggest an alternative explanation: by increasing their resource supply, generalist-pollinated plants garner higher visitation rates and thus higher fitness benefits, whereas specialist-pollinated plants earn only a marginal fitness increase from provisioning more resources for an already active consumer. We note that while our model assumes saturating pollinator foraging on the plant's resource, the same qualitative evolutionary patterns will hold for pollinators with linear functional responses (e.g., Feldman 2006).

Second, our model predicts that plants should evolve trait combinations that provision more resources for consumers with lower intrinsic numerical responses (lower b_{11}, Fig. 5A, B) via an abundance indirect effect. High provisioning plants generate a positive indirect effect on their own abundance via their effect on the consumer's abundance (Fig. 5C, E). Specifically, plants increase the consumer's population size by increasing its food supply, thus indirectly enhancing the plant's own fitness benefit and increasing its population growth rate. As evidence for positive effects of resource provisioning on small pollinator

685 populations, Crone (2013) found that abundant floral resources increase pollinator population sizes from
686 one flowering season to the next. This effect was especially pronounced for solitary species over social
687 species, perhaps because solitary foragers must accumulate all the energy required for reproduction while
688 social foragers share energetic resources to grow their colony (Crone 2013; Maia et al. 2019).
689 Furthermore, this model result is consistent with a widespread natural pattern: plants visited by pollinators
690 that have higher energy needs, such as bats, hawkmoths, and birds, produce larger volumes of nectar
691 compared to plants with small insect pollinators that have lower energy requirements (Heinrich and
692 Raven 1972; Cruden et al. 1983). This relationship would not evolve unless plants earn some fitness
693 benefit from provisioning more nectar for more needy consumers. Here we have demonstrated a potential
694 mechanism for that benefit that merits further investigation in empirical systems: enhancing the
695 pollinator's abundance by provisioning more nectar may enhance the plant's own fitness benefit.
696 Although our model collapses many biological properties of pollinators (e.g., specificity, metabolic
697 efficiency, sociality) into a few parameters describing consumer population growth, it points to specific
698 pollinator traits that may be important selective agents on a plant's resource provisioning traits.
699 *Providers Evolve Trait Combinations That Lower Demographic Consequences Of Costly Production*
700 Costs of provisioning can substantially impact ecological consumer-resource dynamics in mutualisms
701 (Revilla 2015; Bachelot and Lee 2018; Cropp and Norbury 2018, 2019). Our model further highlights
702 how production costs can shape the form of stabilizing selection on resource traits, which will in turn
703 affect the ecological dynamics of consumer-resource interactions. In previous ecological models, a slight
704 imbalance of provisioning costs over benefits often resulted in mutualism collapse (Holland and
705 DeAngelis 2009, 2010). By allowing resource traits to evolve in response to these trade-offs, plants in our
706 model sustain nectar production over a wide range of fitness costs. In fact, our model predicts that
707 resource provisioning traits evolve in ways that minimize these trade-offs between resource production
708 and individual growth (Figs. 6, 7). This response is possible because the model treats resource
709 provisioning as a suite of traits that can each respond independently to production costs. Thus, plants can
710 still evolve higher provisioning via the less costly trait, thereby compensating for limitations affecting the

more costly trait. This theoretical result presents a potential explanation for why some plants replenish large volumes of nectar slowly and others replenish small volumes of nectar rapidly (e.g., Luo et al. 2014): contrasting values of a plant's nectar production traits may result in part from differential costs of making nectaries and nectar-holding structures. Some empirical evidence suggests that high nectar production rates can impose fecundity costs (e.g., Pyke 1991; Rutter and Rausher 2004; Whitehead et al. 2012), potentially influencing selection on nectar production in the ways our model predicts. Evaluating these predictions in nature will require more studies that examine the individual and demographic costs of various nectar production traits in a wide range of plant systems (Pyke 1991; Whitehead et al. 2012; Parachnowitsch et al. 2019).

Mechanisms that minimize resource production costs will likely be favored in environments with harsh abiotic conditions, such as those characterized by water, nitrogen, or other nutrient limitations. Our model predicts that plants evolve higher nectar provisioning when their intrinsic rate of increase (C_1) is low (Fig. 4A, B) because allocating more resources toward the pollinator boosts the plant's abundance by greatly increasing the pollinator's population size and foraging rate (Fig. 4C, E). As a caveat, abiotic resource limitations may constrain a plant's ability to produce nectar (e.g., Shuel 1955, 1957; Dudley 1996; Boose 1997). However, these populations may also experience lower growth rates when pollinators are scarce, potentially favoring a higher proportional investment in resource provisioning despite higher individual costs. Alternatively, the costs of provisioning may themselves evolve via selection on traits that are not modeled here such as water uptake capacity or nutrient use efficiency (e.g., Brodribb et al. 2009), particularly in nutrient-limited environments. This may be the case in desert honey mesquite, where Golubov et al. (2004) found no measurable fitness differences between nectar-producing individuals and nectarless individuals. Absence of evidence for resource production costs is not necessarily evidence of their absence in an evolutionary sense. Instead, we suggest that selection may act on non-resource traits that affect nutrient uptake or nutrient metabolism, thus indirectly lowering the costs of resource production.

Dynamic Resource Provisioning in an Evolving Community

Our model explores the evolutionary response of two plant nectar provisioning traits that determine the standing nectar volume available to the pollinators. Since we are principally concerned with the evolution of traits that impact average resource provisioning in a plant population, our model does not capture the full intricacy of pollinator foraging observed in nature (e.g., Zimmerman 1983; Conner and Rush 1996; Makino and Sakai 2007; Knauer and Schiestl 2015). For example, the spatial patterning of nectar distribution among flowers may particularly impact a plant's male fitness, which we do not explicitly model here, by affecting how pollinators move pollen between flowers and plants (Klinkhamer and de Jong 1993; Pyke 2016a). We expect spatial complexity to affect the quantitative but not qualitative patterns of provisioning dynamics observed in our model. Additionally, the foraging strategies of pollinators will shape patterns of natural selection on resource provisioning if individuals in the focal plant population compete with other nectar-producing species. Several empirical studies have found that high plant density contributes to increasing pollinator visitation to all species in the patch (e.g., (Moeller 2004; Mesgaran et al. 2017). Our results suggest that resource provisioning among co-occurring species will play a key role in shaping community dynamics by impacting not only pollinator behavior (e.g., (Valdovinos et al. 2013, 2016), but local pollinator abundance as well. Sharing the burden of supporting a pollinator population may lessen the selective pressure for increased provisioning by each individual species. Hence, plant species with higher production costs may have higher likelihoods of persisting in communities with multiple high nectar-producing species. Alternatively, competition for optimally-foraging pollinators may increase the fitness benefits of producing more nectar, initiating an evolutionary race for higher and higher resource production. If nectar is costly to make, intense competition for forager attention may also drive selection for traits that lower the costs of resource provisioning.

The insights of our evolutionary model stem from its explicit examination of how two plant traits that determine the nectar supply available to pollinators interact to generate a plant's resource provisioning dynamics. It would also be valuable to develop models that ask the same questions about resource quality, such as sugar or amino acid content of nectar. For instance, while the model of total nectar quantity finds

762 that populations of both species respond more strongly to provisioning rates than they do to provisioned
763 volumes, incorporating nutritional content may increase the value of holding larger volumes of nectar,
764 particularly if sugar is costly. Studies of nectar concentration often find an inverse relationship between
765 nectar volumes and sugar concentrations (Johnson and Nicolson 2008; Pyke 2016a). Costs of sugar
766 production are one hypothesis for this pattern, while others include biophysical limitations of different
767 nectar sipping morphologies (e.g., Kim et al. 2011) or evolved behavioral manipulations by plants that
768 force pollinators to visit more plants and thus transfer more pollen to meet their energy needs (Pyke
769 2016a,b). In general, we expect sugar concentrations will follow similar evolutionary patterns as nectar
770 production rates and volumes because nutrient content will also impact the population dynamics of
771 pollinators.

772 By linking population and trait dynamics with consumer-resource ecology, our evolutionary model
773 provides testable predictions about how natural selection from pollinators and other ecological sources
774 may affect the evolution of a provider species' resource provisioning. Further, our model highlights how
775 the mechanics of natural selection acting on quantitative traits, often via indirect pathways, can produce
776 observed but little understood patterns of consumer-resource trait dynamics, such as the close covariation
777 between a plant's nectar volume and its pollinators' energy requirements (Heinrich and Raven 1972;
778 Cruden et al. 1983; Johnson and Nicolson 2008) and the growing mosaic evidence of varying nectar
779 production costs in different plant species (Pyke 1991; Golubov et al. 2004; Rutter and Rausher 2004;
780 Whitehead et al. 2012). While we focus on nectar here, our modeling framework could be modified to
781 explore trait evolution and its ecological consequences in other unidirectional consumer-resource
782 mutualisms such as seed-dispersal and defense mutualisms as well as bidirectional consumer-resource
783 mutualisms such as plant-mycorrhizal and by-product mutualisms. Overall, the core insight of the
784 evolutionary model is this: ecological dynamics define the evolution of resource provisioning in
785 consumer-resource mutualisms. The rest, as with all patterns in nature, depends on ecological context.

Figures and Tables

Table 1. List of state variables and parameters used in the model presented here.

State Variable	Description
R_1	population abundance of plant
N_1	population abundance of pollinator
S_1	mean standing volume of nectar on a plant individual
z_{NPR}	mean trait value for nectar production rate
z_{RV}	mean trait value for reservoir volume

Parameter	Description
c_1	maximum intrinsic rate of increase for plant when
d_1	strength of intraspecific density dependence for plant
a_{11}	maximum consumption rate for pollinator feeding on nectar
b_{11}	conversion efficiency in fitness for pollinator feeding on nectar
f_1	intrinsic death rate for pollinator
γ_{NPR}	scaling parameter for decline in plant intrinsic rate of increase with increasing values of z_{NPR}
γ_{RV}	scaling parameter for decline in plant intrinsic rate of increase with increasing values of z_{RV}
ϕ_1	scaling parameter determining the maximum fitness benefit that an individual plant can receive from interacting with the pollinator population
ϑ_{11}	Michaelis-Menten half saturation constant for pollinator feeding on nectar
ψ_1	scaling parameter for fitness cost of producing one unit of nectar

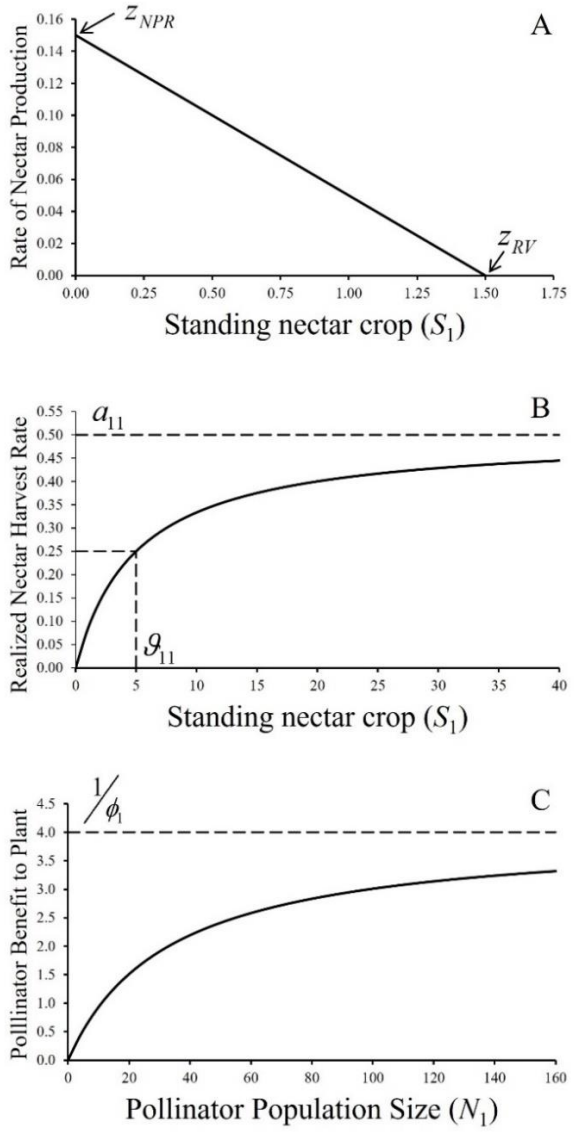

Figure 1. Illustrations of the functional relationships for various components of the plant and pollinator dynamics. (A) The rate at which nectar is produced to fill the nectar reservoir of the plant decreases linearly as nectar volume increases in the reservoir (equation (1)). The maximum rate (z_{NPR}) of filling occurs when the reservoir is empty and stops when the volume reaches z_{RV} . In this panel, z_{NPR} =0.15 and z_{RV} =1.5. (B) The realized nectar consumption rate (i.e., the attack coefficient) increases with the plant trait according to a Michaelis-Menten function (equation (5) $a_{11}(S_1) = \frac{a_{11}S_1}{\vartheta_{11} + S_1}$), in which a_{11} is the asymptotic maximum, and ϑ_{11} is the half-saturation constant (i.e., the trait value at which the nectar consumption rate is at half the asymptote). In this panel, a_{11} =0.5 and ϑ_{11} =5.0. (C) The fitness benefit received by the plant from benefits with pollinators follow Holling's saturating functional response that saturates at $1/\phi_1$ (equation (6)). In this panel, ϕ_1 =0.25.

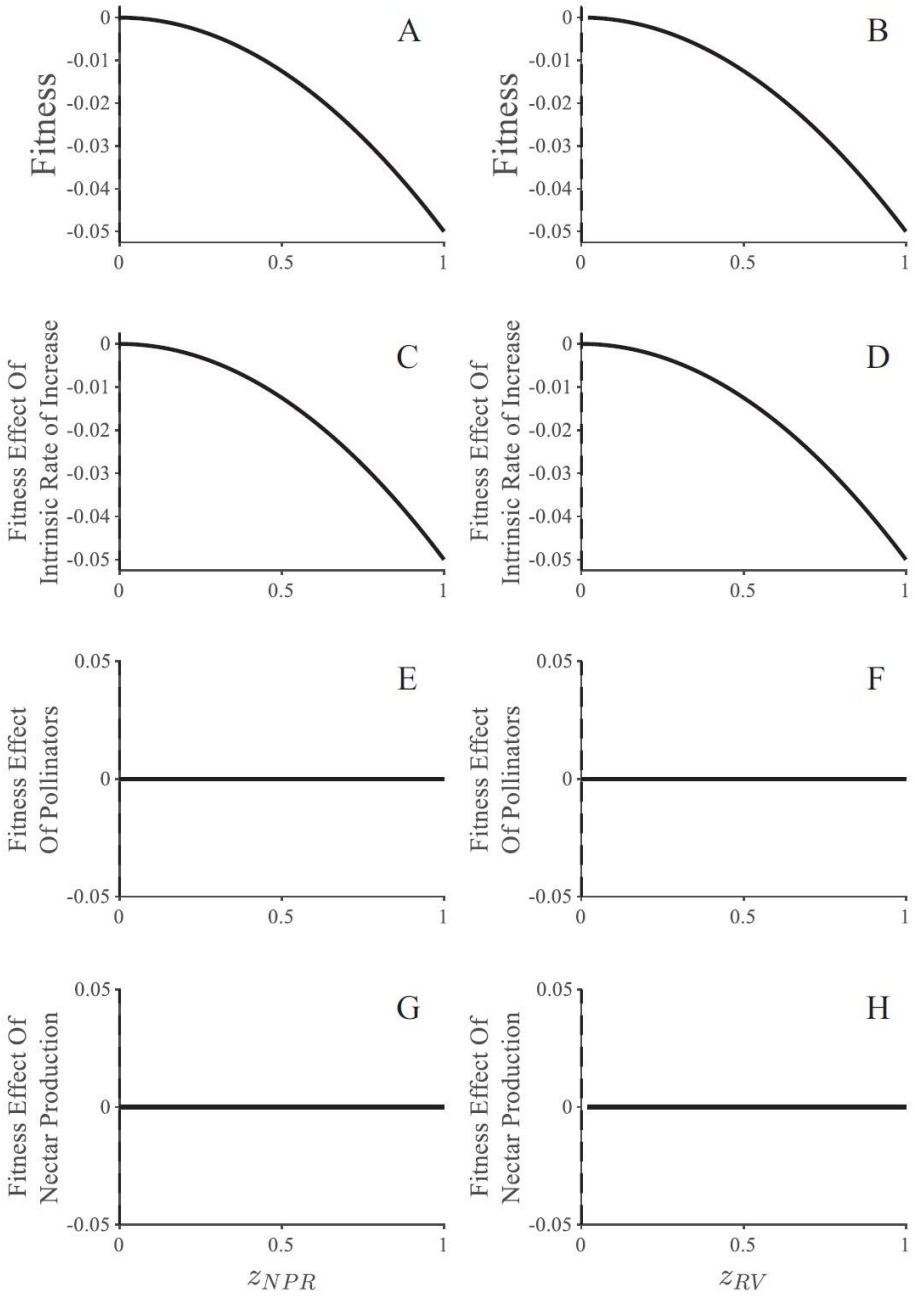

Figure 2. Determinants of plant fitness for nectar production rate and nectar reservoir volume without pollinators at evolutionary equilibrium. The top row of panels shows the topography of overall fitness for (A) nectar production rate z_{NPR} and (B) reservoir volume z_{RV}. These overall fitness relationships are composed of component topographies due to the effects that each trait has on (C and D) the plant's intrinsic rate of increase, (E and F) the benefits accrued from pollinator visits, and (G and H) the cost of nectar production. Because pollinators are absent in this example, the plants accrue neither of these benefits or production costs, and so the overall fitness topography is identical to the trait effects on the intrinsic rate of increase. Model parameters are as follows: c_1=2.0, d_1=0.02, $\gamma_{NPR} = \gamma_{RV} = \psi_1$=0.05, ϕ_1

=0.25, a_{11}=0.25, b_{11}=0.1, ϑ_{11}=0.05, f_1=0.15, $G_{NPR} = G_{RV}$ =0.2.

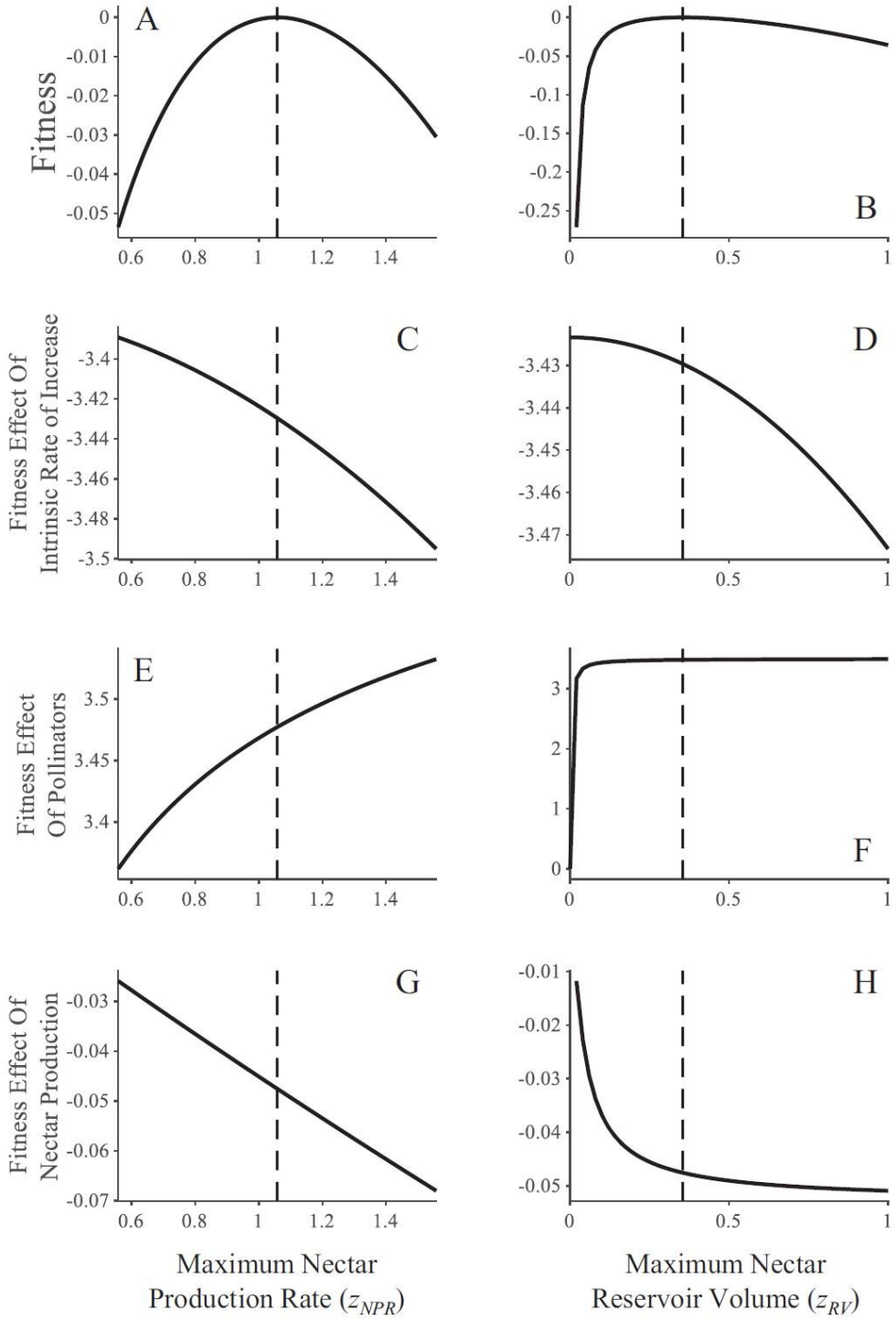

Figure 3. Determinants of plant fitness for nectar production rate and nectar reservoir size with pollinators at evolutionary equilibrium. The panels here correspond to the same fitness topographies as shown in Figure 2, but now with the pollinator population present and at its equilibrium abundance. Parameters are also as given in Figure 2.

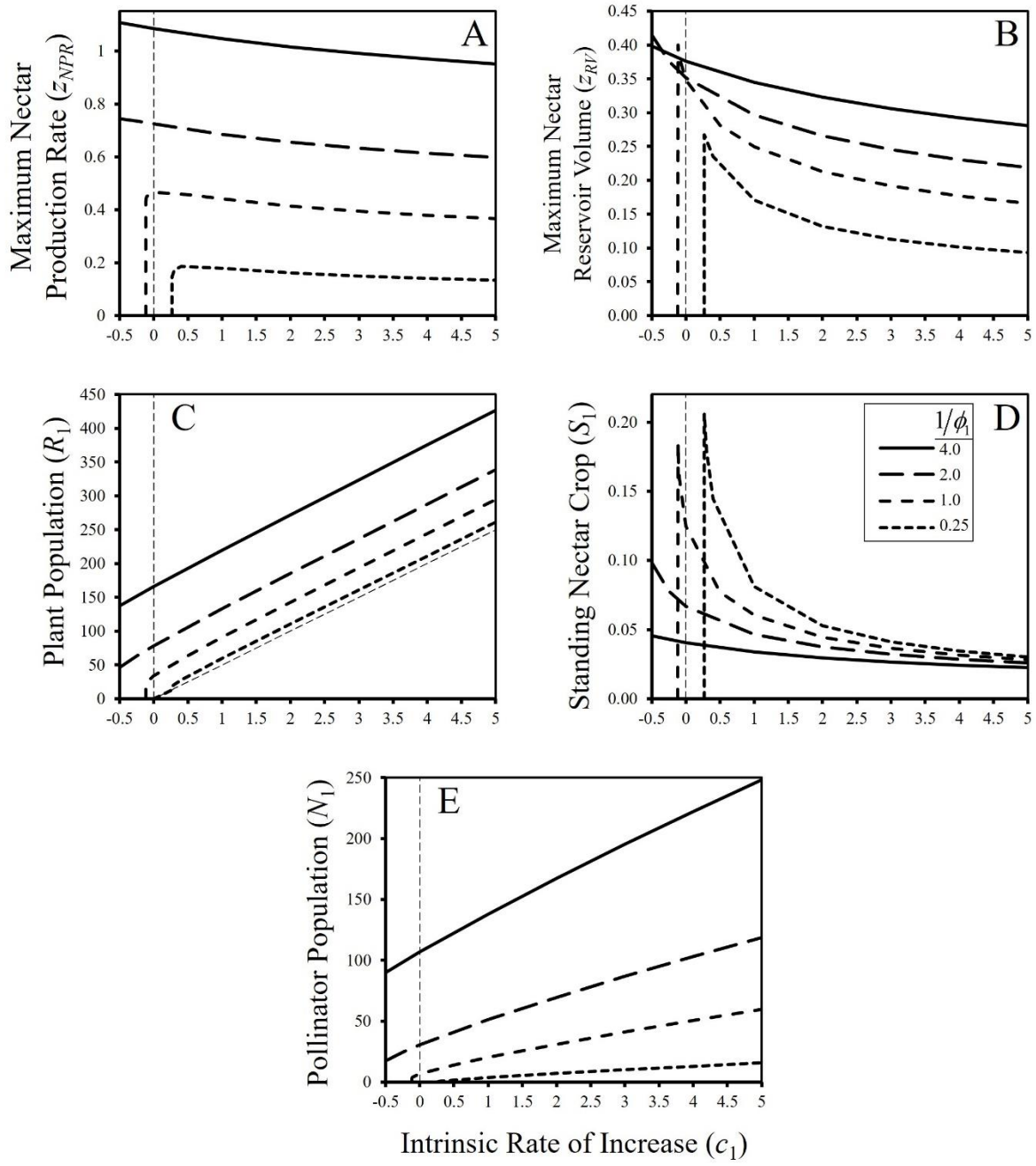

Figure 4. Plants evolve trait combinations that increase nectar provisioning when their maximum intrinsic rate of increase c_1 is low. Panels display the effects of plant maximum intrinsic rate of increase c_1 and maximum fitness benefit from pollinators ϕ_1 on the equilibrium values for (A) maximum nectar production rate, (B) maximum nectar reservoir volume, (C) plant abundance, (D) standing nectar volume per plant, and (E) pollinator abundance. All other model parameters are as given in Figure 2.

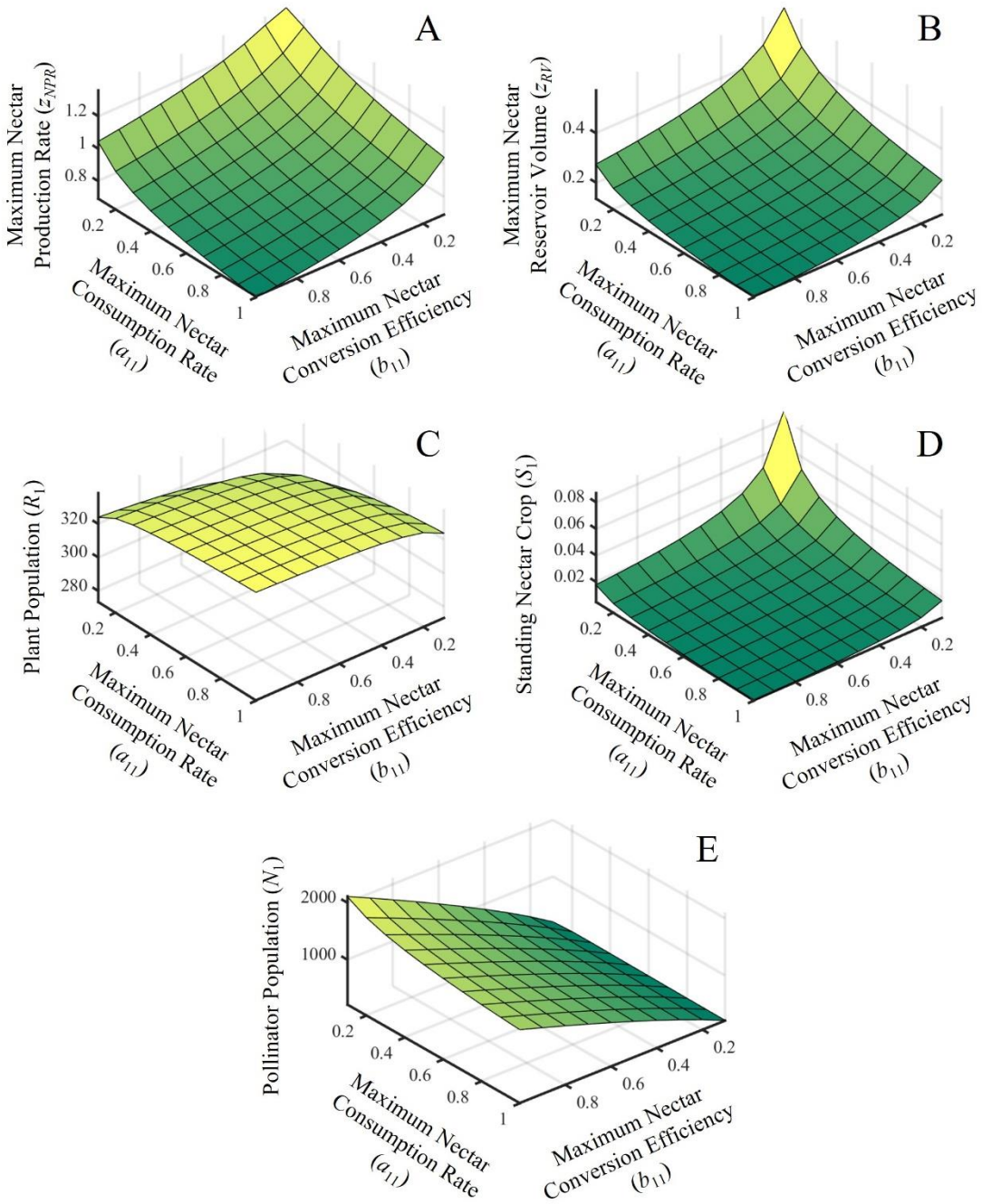

Figure 5. Plants evolve trait combinations that provision more nectar to poorer nectar foragers. Phenotypic (A, B) and population (C-E) surfaces display the effects of pollinator nectar consumption rate a_{11} and pollinator conversion efficiency b_{11} on the equilibrium values for (A) maximum nectar production rate, (B) maximum nectar reservoir volume, (C) plant abundance, (D) standing nectar volume per plant, and (E) pollinator abundance. All other model parameters are as given in Figure 2.

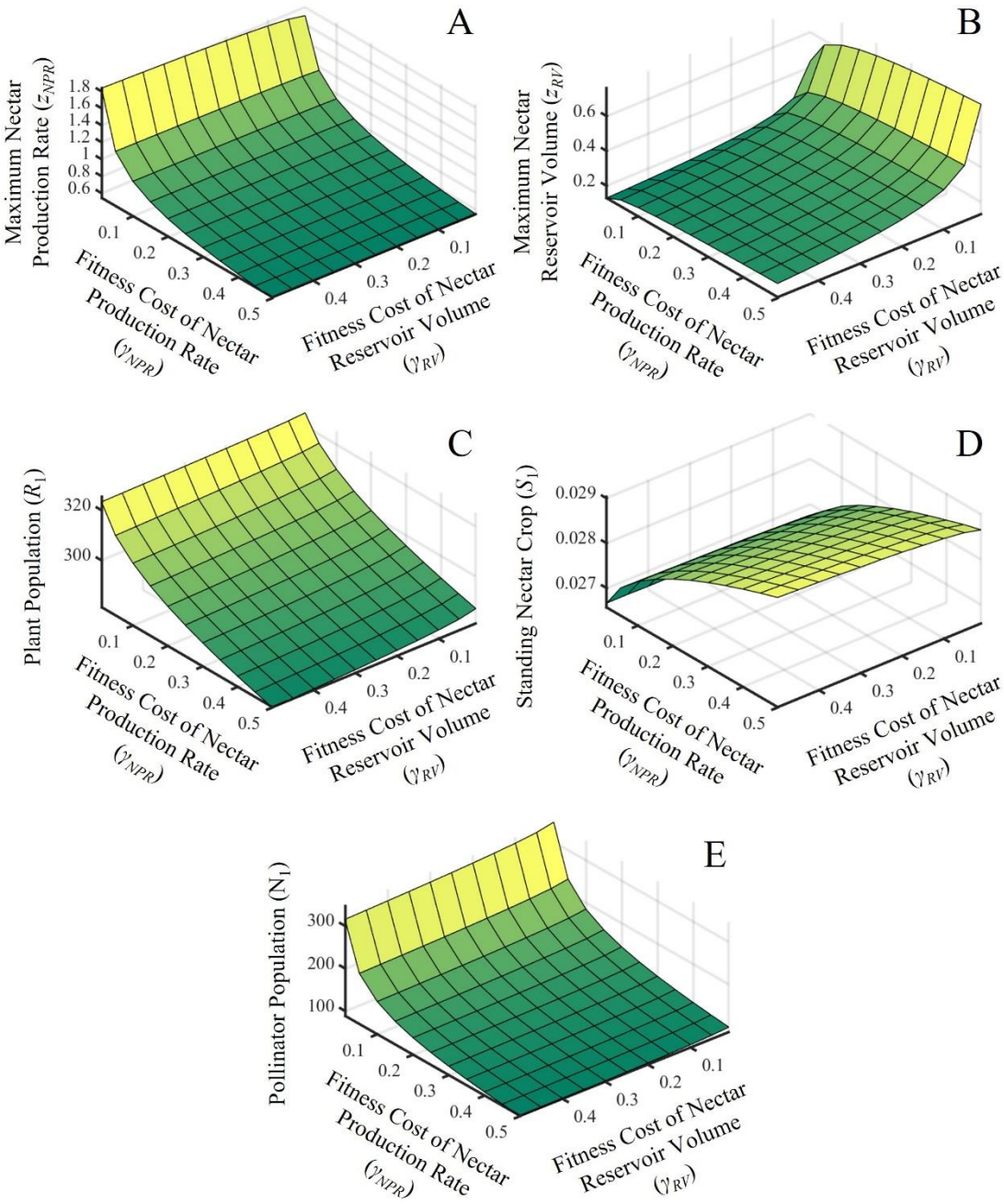

Figure 6. Plants evolve trait combinations that compensate for higher costs of one of the nectar production traits. Phenotypic (A, B) and population (C-E) surfaces display the effects of the selection strengths (γ_{NPR} and γ_{RV}) on the plant intrinsic rate of increase on the equilibrium values for (A) maximum nectar production rate, (B) maximum nectar reservoir volume, (C) plant abundance, (D) standing nectar volume per plant, and (E) pollinator abundance. All other model parameters are as given in Figure 2.

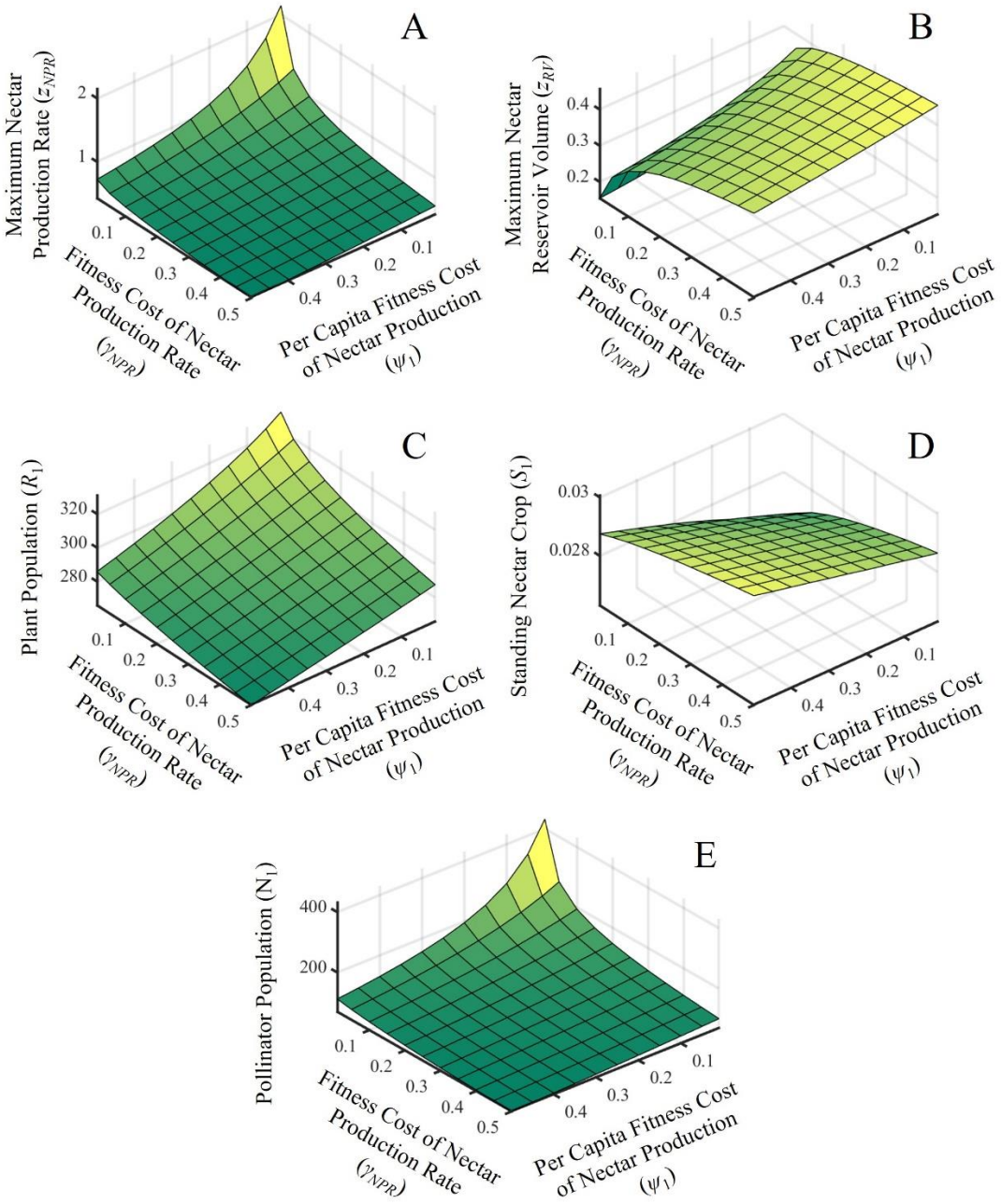

Figure 7. Plants evolve trait combinations that compensate for greater per capita costs of producing nectar. Phenotypic (A, B) and population (C-E) surfaces display the effects of the selection strength on plant intrinsic rate of increase γ_{NPR} and incremental cost of nectar production ψ_1 on the equilibrium values for (A) maximum nectar production rate, (B) maximum nectar reservoir volume, (C) plant abundance, (D) standing nectar volume per plant, and (E) pollinator abundance. All other model parameters are as given in Figure 2.

Chapter 2: Patterns of within- and among-plant variation in nectar production affect beetle foraging in *Amianthium muscaetoxicum*

ABSTRACT

Interactions with different pollinator species have shaped the evolution of a remarkable diversity of nectar production, presentation, and composition traits across the angiosperm clade. These traits allow plants to precisely manipulate and reward the behaviors of specific pollinators to enhance pollen donation and receipt. However, one group of animal-pollinated flowers notably lack characterizations of nectar trait diversity: plants that are pollinated by nectar-feeding beetles. In this study, we characterize flower-, plant-, and population-level variation in nectar traits, as well as the behavior of beetles feeding on nectar, in the beetle-pollinated perennial, *Amianthium muscaetoxicum.* We found tight correspondence between floral sexual phases and nectar production rhythms in this species. We also found significant within-plant consistency in the total nectar volume flowers produced during their lifetime. At the population scale, we observed extremely high among-plant variation in both nectar volume and nectar sugar concentration. Feeding experiments with a primary beetle pollinator further revealed that beetles changed their behavior in response to variation in nectar volume and total sugar content. These findings begin to shed light on the coevolutionary history of *Amianthium* and its beetle pollinators and on the commonalities and differences between beetles and other pollinator taxa.

INTRODUCTION

Animal-pollinated plants have evolved a remarkable diversity of nectar production, presentation, and composition traits, shaped in part by the ecology and behavior of diverse pollinator species (Cruden et al. 1983; Simpson and Neff 1983; Nicolson et al. 2007). Many of these traits can be adaptively linked to pollinator behavior. Different sugar-to-volume ratios and nectar placements attract different pollinator species (Baker 1975; Wunnachit et al. 1992; Nicolson 2002; Galetto and Bernadello 2004). Flowers with distinct sexes or dichogamous sexual phases often produce different volumes of nectar, facilitating enhanced pollinator contact with the more rewarding sex (Bawa and Beach 1981; Barrett 1998). Flowers on the same plant can also display different volumes and sugar contents, encouraging enhanced pollinator movement among flowers and between plants (Pyke 1978b; Best and Bierzychudek 1982a; Fisogni et al. 2011; Lu et al. 2015; Zhao et al. 2016). All these patterns of variation in nectar traits across species have presumably evolved to optimize pollinator-mediated reproduction in plant populations.

In addition to cross-species dimensions and within-plant of nectar trait variation, populations also display pronounced variation among plants. Individuals within a single population can vary in their nectar production rates, e.g., the hummingbird-pollinated *Epilobium canum* (Boose 1997); their total nectar volumes, e.g., the bumblebee-pollinated *Kalmia latifolia* (Real and Rathcke 1991); their sugar concentrations and compositions, e.g., the honeybee-pollinated *Leptospermum scoparium* (Noe et al. 2019); or a combination of these traits, e.g., the hawkmoth-pollinated *Mirabilis multiflora* and the generalist-pollinated *Asclepias curassavica* (Hodges 1993; Broyles 2019). Some plants, such as the hummingbird-pollinated *Aphelandra sinclairiana*, display significant among-plant variation in nectar volume but not in sugar content (McDade and Weeks 2004). Exploring variation at the population level is especially important because such among-individual trait variation is the basis for active pollinator- and non-pollinator-mediated selection (Parachnowitsch et al. 2019). Studies of plant crosses and genotypically structured nectar variation further show that among-plant variation can have a heritable component, suggesting that nectar traits may respond to pollinator-mediated selection across generations (Hodges 1993; Boose 1997; Bertazzini and Forlani 2016; Mallinger and Prasifka 2017). Hence, characterizing

among-plant nectar variation separately from cross-species nectar variation is key to examining the processes of pollinator-mediated selection on nectar traits and pollinator-mediated nectar evolution.

While species-level and population-level diversity in nectar traits has been characterized in a variety of plant species, the pollinator diversity represented by those species is comparatively narrow, focused primarily on bumblebee-pollinated, hummingbird-pollinated, and hawkmoth-pollinated species. Beetle-pollinated species are a large group of animal-pollinated plants that are notably missing from all these studies of nectar variation. This is a severe knowledge gap because beetles contribute to the pollination of many nectar-producing plants worldwide, particularly in tropical regions (Bawa 1990; Momose 2005; Muinde and Katumo 2024). Further, beetles are the earliest known consumers of floral resources and the earliest known pollinators, and may thus have played a pivotal role in the evolution of nectar production across the vastly diverse Angiosperm clade (Gottsberger 1977; Grimaldi 1999).

The dearth of attention toward nectar-feeding beetles may be driven by a historic perception of beetles as messy and 'unspecialized' pollinators that may not be attracted to nectar (Labandeira 2000; Nicolson 2007). However, nectar serves as the primary resource in many beetle-pollinated species (Woodell et al. 1997; Wäckers et al. 2007; Kirmse and Chaboo 2018, 2020). Several beetle pollinators from diverse families including *Mecometopus* Thomson *sp.* (Cerambycidae), members of the *Lycus* genus (Lycidae), and members of the Cetoniinae subfamily (Scarabaeidae) all exhibit elongated mouthparts with bristly hairs, a clear adaptation for nectar-feeding which allows them to sweep nectar droplets into their mouths (Fuchs 1974; Stamhuis 1993; Johnson and Nicolson 2001; Karolyi et al. 2016; Kirmse and Chaboo 2020). In the only known study to date that examined nectar traits in a beetle-pollinated flower, Johnson *et al.* (2007) discovered that *Satyrium microrrhynchum* (Orchidaceae) presents its nectar openly on projecting 'lollipop hairs.' We know very little about the dynamics of nectar production, nectar volumes, and sugar contents of any beetle-pollinated species, let alone scales of trait variation within those species. Beetles may respond differently to nectar traits than other pollinator species. For example, small-bodied beetle pollinators may consume smaller volumes of nectar than larger pollinator species and may thus respond differently to within- and among-plant variation in nectar traits.

Here, we describe the production dynamics, presentation, and among-plant nectar trait variation in an Appalachian population of the beetle-pollinated monocot, *Amianthium muscaetoxicum* (Liliales, Melanthiaceae). We also relate those elements of trait variation to the foraging behaviors of the predominant beetle pollinator, *Strangalepta abbreviata*. We asked the following questions: 1) What are the dynamics of nectar production in *Amianthium*, and how are these dynamics related to the floral life cycle? 2) How consistent is nectar production within an individual? 3) How variable is nectar production at the population scale? 4) How do different nectar trait components affect the feeding behaviors of beetles? This work provides the first detailed characterization of nectar traits, nectar trait variation, and nectar feeding behaviors in a beetle pollination mutualism.

METHODS AND STATISTICAL ANALYSES

Study Species: *Amianthium muscaetoxicum* (Fly poison, Melanthiaceae) is a perennial wildflower in a monotypic genus (Britton and Brown 1970; Karolyi et al. 2016). *Amianthium* is almost fully self-incompatible, requiring insect-mediated pollen transfer to produce viable seeds (Travis 1984). Flowers only produce fruits if ovules are fertilized. Flowers will set fruit with self-pollen, but seeds are largely inviable (Travis 1984). After a flower's reproductive period ends, the tepal tissue transitions from a pale cream color to a leafy yellow-green regardless of its ovule fertilization status.

Amianthium displays its flowers as a raceme: the inflorescence flowers from bottom to top over the course of two to three weeks between mid-June and late July. Inflorescence sizes vary widely from fewer than 50 flowers to over 200 flowers. Individual flowers are approximately one to one and a half centimeters in diameter. Flowers on a plant are partially dichogamous: flowers experience a two- to three-day phase of pollen availability within a six-day period of pistil receptivity (Palmer et al. 1989).

We conducted all the work reported here in the *Amianthium* population at Mountain Lake Biological Station (MLBS, Giles County, VA), where the species grows to high densities in the forest understory. A guild of beetles at MLBS, most prominently the cerambycid *Strangalepta abbreviata* (Lepturinae, Figure 1A) and the scarab *Trichiotinus affinis* (Cetoniinae, Figure 1B), accumulate pollen on their bodies as they forage for nectar and pollen from the plants (Travis 1984). Both species exhibit bristly mouthparts that

allow them to mop up nectar droplets, as is characteristic of other nectar-feeding beetle species (Fuchs 1974; Stamhuis 1993; Johnson and Nicolson 2001; Kirmse and Chaboo 2020).

Measuring nectar: *Amianthium* presents its nectar openly (Figure 1C), allowing us to non-destructively collect all of a flower's nectar using glass microcapillary tubes (Drummond Microcaps). We used two sizes of tubes for nectar collection: 5 µL tubes for flowers with visibly higher nectar volumes and 2 µL tubes for flowers with visibly lower nectar volumes. We marked the meniscus of the filled volume of the tube after collection to correct for any subsequent loss due to evaporation or dripping, thus ensuring more accurate volume measurements. For flowers that produced more than 5 µL of nectar, we used multiple microcapillary tubes and marked each with the same pen color to indicate that the nectar in these tubes was collected from the same flower. In lab, we converted the height of liquid in each tube (mm) into the volume of liquid in each tube (µL).

Nectar production dynamics and within-plant consistency: To characterize the nectar production dynamics of *Amianthium* across a flower's lifetime, we sampled nectar from flowers at different stages. On July 6, 2021, we selected 13 plant individuals and marked 20 to 25 unopened buds on the individual's inflorescence with a felt-tip pen. We covered these inflorescences with bridal veil bags to exclude foragers. After 48 hours, we uncovered plants and sampled nectar from flowers at five different stages: partially opened flowers (female phase only), opened flowers that had not dehisced (female phase only), partially dehisced flowers (male and female phase), fully dehisced flowers (male and female phase), and fully dehisced flowers with low remaining pollen content (male and female phase) (Palmer et al. 1989). After sampling nectar from a flower, we marked one of its tepals with a felt-tip pen to prevent resampling. We sampled three to five flowers per stage on each plant. We used a multiple linear regression model to examine the effects of flower stage and plant ID on flower-level nectar volume.

Next, we examined variation in nectar production over the lifetime of individual flowers by collecting nectar from the same flowers on every day of their active period. On July 12, 2021, we selected 16 plants spread across three separate regions of the woods. We marked eight to ten unopened buds at different heights on each inflorescence, giving each flower a color ID by marking the buds with different colored

felt tip pens. We covered individuals with bridal veil between sampling periods. For the next 18 days, we measured all marked flowers' flowering schedule (days since opening) and nectar production (daily nectar volume) between 9:00 am and 12:00 pm. Each day's measurement captured the total nectar that accumulated in a flower over roughly 24 hours. At the end of sampling, we summed all of a flower's measurements to obtain their total nectar volume. We used a random effects model to examine whether flowers on a plant produced similar cumulative volumes of nectar: *cumulative volume* ~ (1|*plant ID*).

Population-wide variation in nectar volume and sugar concentration: We measured the nectar volume and sugar concentration of recently dehisced flowers on 235 plant individuals spread throughout the White Pine and John's Creek regions of MLBS. We used a felt-tip pen to mark eight to twelve flowers in bud stage on each plant. We then covered the inflorescence with a waterproof fine-mesh bag (Firlar) to prevent animal foragers and other environmental factors from altering nectar accumulation. These bags did not affect in-bag humidity compared to bridal veil bags. We gave each plant approximately 500 mL water daily on the two days prior to nectar sampling to ensure similar water environments. We collected all nectar measurements between 8:00 and 15:00 hours in a two-day period with ambient temperatures ranging from 20°C - 22°C. We sampled nectar from three recently dehisced flowers per plant. To gauge the timing of an inflorescence's flowering period, we noted the approximate number of rows of buds remaining on the inflorescence. We also counted how many inflorescences each plant produced. We used a BRIX refractometer (0-30% BRIX) to sample the pooled sugar concentration from an individual's three sampled flowers (N = 202). We were unable to recover sugar concentration measures for plants that produced fewer than 0.5 µL of nectar per flower. Lastly, we destructively counted flowers on 100 haphazardly chosen inflorescences at the end of their flowering period.

We performed Pearson's product moment correlation tests to evaluate the relationships among our three trait variables: nectar volume, sugar concentration, and total flower number. We then used multiple linear regression to test which nectar and plant traits best predicted each nectar trait. For nectar volume, we used the formula: *nectar volume ~ sugar concentration + inflorescence size + inflorescence number + unopened flower rows* and for sugar concentration, we used the formula: *sugar concentration ~ nectar*

928 *volume + inflorescence size + inflorescence number + unopened flower rows.* These models tested
929 whether each nectar trait was dependent only on the other nectar trait or on other plant traits such as
930 inflorescence size and number. We also ran models excluding one nectar trait as a predictor of the other
931 trait to determine whether plant traits by themselves significantly predicted nectar traits. We used
932 ANOVA tests to identify significant contributors to variance in the two nectar traits.
933 **Beetle nectar feeding experiments:** We captured 80 *S. abbreviata* beetles from the John's Creek area of
934 MLBS and housed them in small containers (7.5 cm in diameter, 4.5 cm tall) at 22 degrees C prior to
935 testing. We assigned each beetle a unique two-letter identification code. All housed beetles received a 6-
936 µL aliquot of 8% sucrose solution for a total sugar content of 0.48 mg of sucrose every afternoon. Each
937 beetle was used in only one trial and kept for no longer than one week before being returned to the field.
938 In feeding trials, three adjacent sculpey plastine blocks (1.5 cm^2 in area by 1 cm tall), each holding
939 three *Amianthium* flowers, were positioned on one side of a small plastic arena (11.5 cm^2 in area and 3 cm
940 tall, Figure 6). Before testing, we collected inflorescences from the field, washed them with distilled
941 water, dried them with Kimwipes (Kimtech Science), and haphazardly removed dehiscing or dehisced-
942 stage flowers. We emasculated flowers prior to placement in the blocks to ensure that nectar was the only
943 accessible resource for beetles. We kept track of the plant ID of the flowers in each arena to account for
944 possible individual variation in floral traits such as scent production. Each flower block received a
945 different nectar treatment that was manually pipetted onto the flowers' tepals, mimicking the natural
946 presentation of nectar as closely as possible. We randomized the left to right order of treatment blocks in
947 an arena and placed every beetle in the same starting position at the beginning of their trial. We recorded
948 trials with an infrared camera (Basler AG acA4096 – 30 µm, Ahrensburg, Germany) with a 25 mm/F1.8
949 lens (Edmund Optics, Barrington, New Jersey), taking four pictures every second for one hour. We
950 spliced these time-lapse photos into a video played at 30 frames per second.
951 All feeding trials occurred between 9:00 and 12:00 hours from July 2 - July 8, 2024. After recording,
952 we watched each beetle's video trial and observed the number of minutes a beetle spent interacting with
953 each flower block during the one-hour trial period. We counted beetle visits to a flower block as any time

954 a beetle paused for longer than five seconds of video time while its head region was positioned over one
955 of the flower blocks. For example, if a beetle stopped with its abdomen over flower block one but its head
956 was over flower block two, we counted this as an interaction with flower block two.
957 We performed a sequence of five feeding experiments with *S. abbreviata.* First, we performed a
958 preliminary experiment to determine if artificial nectar (sucrose dissolved in distilled water) could serve
959 as an adequate proxy for natural nectar, as we could more precisely manipulate the components of
960 artificial sucrose solutions. In this experiment, one flower block received 3 µL of natural nectar per flower
961 (pooled from ten individuals in the field), one block received 3 µL of sucrose solution per flower
962 (identical sugar concentration as pooled wild sample: 4.4% BRIX), and one block received 3 µL of
963 distilled water per flower as a control for the visual presence of liquid on flowers. Beetles spent a
964 significantly longer time on flowers with nectar (either false or natural nectar) than they did on flowers
965 with water, but they did not discriminate between flowers with natural nectar and flowers with sucrose
966 solution (Table 1), allowing us to proceed with artificial nectar for all subsequent experiments.
967 We ran four experiments with artificial nectar to examine how different aspects of nectar variation
968 affected beetle feeding behaviors. In experiment A, we asked if beetles spent more time interacting with
969 flowers with higher sugar concentrations among three options (0.4 mg/µL, 0.1 mg/µL, 0 mg/µL). In
970 experiment B, we asked if beetles spent more time interacting with flowers with larger nectar volumes
971 among three options (7 µL, 2 µL, 0 µL) while holding sugar concentration constant at 10% BRIX. In
972 experiment C, we asked whether beetles spent more time on flowers with larger nectar volumes or
973 flowers with higher sugar concentrations when the total sugar content of both nectars (sugar concentration
974 multiplied by volume), was identical (0.4 mg). The three options in this third experiment were: 0.4 mg/µL
975 in 1 µL, 0.08 mg/µL in 5 µL, and 0 mg/ µL in 0 µL. Finally, in experiment D we asked whether beetles
976 showed the same behavioral pattern seen in experiment C if we doubled the total sugar content of one
977 nectar treatment over the other. The three options in this final experiment were: 0.4 mg/µL in 3 µL, 0.08
978 mg/µL in 7.5 µL, and 0 mg/µL in 0 µL.
979 We used GLMMs with a Tweedie model family to test whether beetles spent significantly different

mean times on different nectar treatments, including beetle ID, plant ID, and treatment order as random effects. We performed post hoc pairwise comparisons using the package *emmeans* (Lenth 2024) to determine which treatments significantly differed from each other in their effects on beetle behavior.

RESULTS

Nectar production varies with flower stage: *Amianthium* displays a flower stage-structured pattern of nectar production (Figure 2). Flowers increased nectar production until all anthers dehisced, then slowed and eventually ceased production as pollen levels diminished. All plants exhibited less than one µL of nectar in their opening-stage flowers. All plants also exhibited their largest nectar volumes during or following anther dehiscence, ranging from near 0 µL from some plants to over 5 µL from others. Dehiscing and dehisced-stage flowers exhibited greater variation in nectar production within and among plants than did earlier-stage flowers (Figure 2). Overall, plant ID was a more significant predictor of variance in flower-level nectar volume than was flower stage (GLM, ANOVA, flower stage: $F_{1,221}$ = 11.49, p = 0.0008, plant ID: $F_{12,221}$ = 7.91, p < 1e-12). This means that same-stage flowers on the same plant are more similar to each other than same-stage flowers on different plants.

By repeatedly sampling the same flowers every day of their active period, we found that all flowers produced nectar for a maximum of four days and stopped secreting nectar by day five (Figure 3A). Production rates were low: the maximum nectar production rate of any sampled flower was 0.2 µL per hour. Unsampled flowers that progressed through their entire active period and transitioned to fruiting stage, characterized by greening tepals, showed no evidence of nectar reabsorption (Figure 3B).

Plants produce consistent cumulative nectar volumes: Flowers on the same plant produce similar cumulative nectar volumes, but plants significantly vary in how much nectar their flowers produce (Figure 4, ANOVA, plant ID: $F_{15,127}$ = 32.88, p < 0.0001). The plant with the highest nectar production had a mean volume of 5.54 ± 1.10 µL per flower, while the plant with the lowest nectar production had a mean volume of only 0.11 ± 0.21 µL per flower. These data reveal that averaging the measurements of a few recently dehisced flowers on a plant provides a reliable estimate of that plant's mean total nectar production phenotype.

Total nectar production varies widely among plants: By sampling a much larger number of plant individuals in 2022, we found that plants vary in their nectar production by an order of magnitude (Figure 5A, N = 235 individuals). Plants produced a mean of 3.2 µL of nectar, with a total variance of 5.72 μL^2 and a range of trait values between 0 and 12.96 µL of nectar. Plants also varied in their nectar sugar concentrations. The sampled population displayed a mean of 0.1 mg sugar/µL, a variance of 0.002 $mg/\mu L^2$ and trait values ranging from 0.03 to 0.3 $mg/\mu L^2$ (Figure 5B). Plants that produced higher nectar volumes produced significantly lower sugar concentrations on average (Figure 5B, $p = 1.74e^{-08}$).

In evaluating the relationships between floral traits and nectar volume, sugar concentration was the only significant predictor of among-plant variance in nectar volume (ANOVA, $F_{4,96} = 12.01$, $p = 7.93e^{-4}$). Inflorescence traits did not significantly predict variance in among-plant nectar volume (ANOVA, total flowers on inflorescence: $F_{4,96} = 0.74$, $p = 0.39$, inflorescence number: $F_{4,96} = 0.07$, $p = 0.79$, unopened flower rows: $F_{4,96} = 0.04$, $p = 0.83$). With respect to among-plant sugar concentration, nectar volume was again the strongest predictor of variance in sugar concentration (ANOVA, $F_{4,96} = 12.01$, $p = 7.93e^{-4}$). Neither inflorescence size nor the number of unopened flower rows significantly predicted variance in sugar concentration (ANOVA, inflorescence size: $F_{4,96} = 0.9$, $p = 0.35$, unopened flower rows: $F_{4,96} = 0.14$, $p = 0.7$). When nectar volume was removed as a predictor of sugar concentration from the model, inflorescence number became a significant predictor of a plant's sugar concentration (ANOVA, $F_{3,96} = 4.29$, $p = 0.04$). Plants with fewer inflorescences produced slightly higher sugar concentrations (estimate $\pm$ *SE* = 0.007 $\pm$ 0.003). The other plant traits remained nonsignificant predictors of nectar traits when the other nectar trait was removed from the multiple regression model.

Beetles spend more time interacting with flowers with high sugar concentrations and high volumes: When presented with a choice between flowers with high sugar concentrations, flowers with low sugar concentrations, and flowers with only water, beetles visited flowers with sugar for significantly longer periods of time than they visited flowers with water (Figure 6A, Table 1). Beetles did not spend significantly different amounts of time on flowers with higher or lower sugar concentrations (Figure 6A, Table 1). When presented with choices in nectar volume, beetles visited flowers with high nectar volumes

for significantly longer periods of time than they did flowers with low nectar volumes (Figure 6B, Table 1). Beetles spent nearly four times as long on high-volume flowers (mean = 19.5 minutes) compared to low-volume flowers (mean = 4.95 min). Beetles spent the most time visiting flowers with higher nectar volumes when presented with flowers containing nectar with equal sugar contents per flower but different sugar-volume ratios (Figure 6C, Table 1). Beetles spent nearly three times as long on flowers with high volumes (mean = 31.5 min) but low sugar concentrations as they did on flowers with small volumes but high sugar concentrations (mean = 13.5 min). Beetles did not spend significantly different amounts of time visiting flowers with 1x and 2x sugar contents (Figure 6D, Table 1). In experiments B-D, beetles spent significantly more time on flowers with nectar than they did on nectar-less flowers (Figure 6, Table 1). Beetles spent very little time visiting nectar-less flowers (mean = 2.2 min) across experiments B-D. GLMM summaries including variance attributed to random effects can be found in Table S1.

DISCUSSION

Our work reveals that nectar traits may be important mediators of the relationship between *Amianthium* and its nectar-feeding beetle pollinators. Relative to its small flowers, *Amianthium* produces large volumes of nectar that it displays openly. The plant's nectar production rhythms correspond to flowers' sexual phases, as seen in other partially dichogamous species (e.g., *Salvia hierosolymitana* and *Helleborus foetidus*, Canto et al. 2011; Leshem et al. 2011). Its raceme flowering structure also creates a stage-structured pattern of decreasing nectar volumes moving from bottom to top on the plant, mirroring a common pattern in other indeterminately flowering species (e.g., *Aconitum gymnandrum*, *Digitalis purpurea*, Best and Bierzychudek 1982a; Lu et al. 2015). Together, these production and display patterns suggest that *Amianthium* nectar traits may have evolved to manipulate beetles' foraging movements. Consistent within-plant nectar volumes and broad among-plant nectar trait variation further indicate that nectar traits could experience ongoing pollinator-mediated natural selection. Our pollinator behavior experiments provide support for these possibilities: pollinators varied the amount of time they spent on flowers with different nectar traits. Below, we examine what the natural history of nectar production and variation in *Amianthium* may reveal about its interactions with beetle pollinators.

Nectar production patterns in *Amianthium* track its reproductive receptivity as previously characterized by Palmer et al. (1989), suggesting that nectar may play a role in shaping both male and female reproductive function. Production begins at anthesis and continues through anther dehiscence. The female pistil is receptive for pollen donation during this entire period. This finding, combined with the finding that beetles spend more time visiting flowers with more nectar, suggests that nectar production could affect rates of pollinator-mediated pollen deposition across a flower's entire active period. Patterns of sexual phase-related nectar production often correlate with increased pollinator visitation to the more rewarding sex, presumably enhancing reproduction via that sex's function (Jennersten et al. 1988; Delph and Lively 1992; Shykoff 1997). Longer visits to higher-rewarding flowers can lead to higher pollen removal and deposition for those flowers (Mitchell and Waser 1992; Manetas and Petropoulou 2000), though this needs to be tested directly with *S. abbreviata* and *Amianthium.* If longer interactions with flowers lead to higher pollen deposition, then high nectar production during anther dehiscence could enhance pollinator activity during the short male phase. Cessation of nectar production also coincides with a reduction in pollen viability in four- to five-day-old flowers (Palmer et al. 1989). In addition to conserving energy, cessation of production at this stage may discourage feeding activity at late-stage flowers, focusing pollinator attention on flowers with viable pollen.

Stage-structured nectar production creates a micro-landscape wherein pollinators encounter earlier-stage flowers, and thus flowers with lower nectar volumes, as they move upwards on an inflorescence. We predict this display pattern will cause beetles to visit fewer higher up flowers, as beetles in our behavior experiments spent less time visiting flowers with lower nectar volumes. Many other raceme-bearing species exhibit a similar pattern, though flower-level variation is not always driven by floral sexual phases (Percival and Morgan 1965; Menge and Sutherland 1976; Pyke 1978a; Best and Bierzychudek 1982b; Fisogni et al. 2011; Lu et al. 2015). For example, the vertically declining structure of nectar rewards in *Aconitum gymnandrum* encourages bumblebee pollinator movement to new plants, minimizing self-pollen transfer from lower down flowers to higher up flowers on the inflorescence and thus enhancing outcrossed reproductive success (Lu et al. 2015; Zhao et al. 2016). Vertically declining

nectar volumes caused by floral sexual phases may also be advantageous for *Amianthium*, another virtually self-incompatible species (Travis 1984). Stage-structured nectar production in *Amianthium* may have evolved in part as a mechanism to decrease self-pollination by encouraging beetles to forage on flowers with viable pollen, and to depart after visiting those few highly rewarding flowers.

Despite the structured pattern of within-plant nectar variation, we also detected high within-plant consistency in the total volume flowers produced during their active period. On a physiological level, this indicates that nectar production likely shares a common basis across the plant and does not substantially vary among nectaries, unlike in some other species (Freeman and Wilken 1987; Davis et al. 1998; Herrera et al. 2006). The relatively large nectar volumes produced by some individuals are also remarkable. A pollinator visiting a plant that produces an average of five μL per flower could theoretically encounter upwards of 40 μL of nectar in eight dehisced and previously unvisited flowers. However, beetles in our feeding experiments would often drink from only a couple flowers at a time and would sit for long stretches of time between feeding bouts. Our observations in the field corroborate this pattern: *S. abbreviata* in the wild typically drink from only a few flowers on an inflorescence before departing, suggesting their energy budgets may be lower than other larger pollinator species. Thus, risks of single individuals draining large numbers of flowers at a time may be lower for *Amianthium* than they are for species that interact with more intensive foragers such as hummingbirds, hawkmoths, or bumblebees.

Further, beetles may rarely experience large volumes of nectar at single *Amianthium* flowers. While we did not directly measure standing crops in this population, visual observations suggest that flower-level nectar availability is generally low across the woods. Larger nectar droplets are mainly visible the day after rainfall, and they are quickly depleted in a matter of hours. High levels of depletion could be driven by frequent animal foraging. Additionally, open nectar presentation presents risks of depletion due to environmental sources, such as washout after heavy rainfall or rapid evaporation under intense sunlight (Corbet et al. 1979; Boose 1997; Keasar et al. 2008). *Amianthium* also experiences heavy loads of smaller beetles, particularly the tumbling flower beetle *Anaspis rufa*, that consume nectar but do not contact the anthers during feeding. In the face of these uncontrollable sources of standing crop manipulation,

producing large quantities of visible nectar in all flowers may be an adaptive form of bet-hedging. High overall production could increase the chance that some flowers will exhibit enough nectar to engage pollinators at any given point during the flowering season.

At the population scale, *Amianthium* displays extremely high among-plant variation in total nectar volumes and sugar concentrations (Figure 5). Within the same three-to-four-day period of unhindered nectar accumulation, some plants produced over ten times as much nectar as others. Ten sampled plants produced so much nectar per flower (> 8 μL) that their nectar nearly covered the whole flower (Figure 5A). At the other extreme, 22 plants produced less than 0.5 μL of nectar, barely enough to dampen a flower's tepals, and six of those plants produced no nectar at all (Figure 5A). High levels of among-plant variance in nectar volume (greater than one order of magnitude of trait variation) are known from a few species including the hawkmoth-pollinated species *Mirabilis multiflora* (Hodges 1993) and *Polemonium brandegeei* (Kulbaba and Worley 2012), several neotropical hummingbird-pollinated species (McDade and Weeks 2004), and two generalist-pollinated species, *Asclepias quadrifolia* (Pleasants and Chaplin 1983) and *Turnera ulmifolia* (Benitez-Vieyra et al. 2010). Our work adds to this pattern of high levels of standing nectar trait variation in plant populations, suggesting that natural selection from pollinators and other sources may maintain variation in nectar traits in this population through time.

A variety of intrinsic and extrinsic factors could contribute to extreme variation in nectar traits in this population. The negative relationship between nectar volume and sugar concentration could indicate energetic tradeoffs in sugar production: if sugar is expensive to produce, secreting smaller volumes of highly concentrated nectar can be one evolutionary mechanism to lower this cost and still attract pollinators (Southwick 1984; Pyke 1991; McPeek et al. 2021). Our inflorescence data casts some doubt on this possibility: neither nectar volume nor sugar concentration were related to floral display size as measured by the number of flowers on the inflorescence, a commonly hypothesized tradeoff with sugar concentration (Pleasants and Chaplin 1983; Southwick 1984). In *Asclepias quadrifolia*, plants with larger root masses produced nectar at faster rates, giving credence to the idea that well-resourced plants may acquire more of the raw materials needed to produce nectar (Pleasants and Chaplin 1983). Among-plant

variation in nectar traits could also be affected by environmental factors such as local variation in soil nutrients and moisture levels (Boose 1997; Gijbels et al. 2014), sunlight availability (Harder and Barrett 1992; Boose 1997), and relationships with beneficial soil microorganisms (Gange and Smith 2005; Toby Kiers et al. 2010). Among-plant variation may also be driven in part by genetic variation. Previous work in *Petunia, Nicotiana alata,* and *Digitalis purpurea* identified quantitative genetic components of inheritance for nectar volume, nectar production rates, and total sugar content, all of which vary widely in *Amianthium* (Galliot et al. 2006; Kaczorowski et al. 2008; Romero-Bravo and Castellanos 2024). We currently know nothing about nectar trait heritability in this system and highlight this gap as another important avenue for future research. Characterizing genetic variation will be particularly important for assessing the ability of *Amianthium* nectar traits to respond to pollinator-mediated selection (Mitchell 2004; Parachnowitsch et al. 2019; Romero-Bravo and Castellanos 2024).

Pollinator responses to both nectar volumes, sugar concentrations, and the combination of these traits have rarely been examined in an experimental context as we have done here. One series of experiments by Cnaani *et al.* (2006) found that bumblebees responded more strongly to high sugar concentrations than they did to high volumes. Our work suggests that beetles may prefer to visit flowers with high nectar volumes and high sugar contents, with a stronger overall preference for higher volume. It is important to note that increased time spent on a flower could reflect beetles needing a longer time to drink a larger nectar volume. The same could be true for sugar concentration: nectars with higher sugar contents exhibit higher viscosity which can slow down consumption, especially for species who consume nectar by licking or brushing (Köhler et al. 2010; Nardone et al. 2013; Zhou et al. 2024). That said, the estimated viscosity that slows down a pollinator's feeding can vary widely. One experiment in *Bombus impatiens* found that bees slowed their foraging when nectar concentrations exceeded 27%, while another experiment in *B. impatiens* found that bees fed at a steady pace until sugar concentrations were well above 40% (Harder 1986; Nardone et al. 2013). Currently, we cannot distinguish whether our behavioral data reflect a true preference for volume over sugar concentration or differences in feeding habits enforced by food sources with different properties. Regardless of the causes of these behavioral differences, our work clearly

shows that nectar traits play a large role in shaping beetle pollinator behavior on flowers.

All current and previous work on the natural history of floral traits and pollination in this species comes from MLBS, where *Amianthium* has grown in abundance for decades (Travis 1984; Palmer et al. 1988, 1989; Redmond et al. 1989). Work in other species including the hawkmoth-pollinated *Heracleum lanatum*, the bat-pollinated *Pseudalcantarea viridiflora*, and the bird-pollinated *Kniphofia linearifolia* shows that nectar traits can vary among populations in correspondence to variation in pollinator abundance, activity, and species composition (Cruden 1976; Brown et al. 2011; Aguilar-Rodríguez et al. 2022). Pollinator assemblages may vary among *Amianthium* populations in ways that could drastically alter the evolution of nectar production patterns across the species' range. The richness of the pollinator assemblage feeding on *Amianthium* at MLBS has notably declined since the 1980s, when Travis (1984) detected over ten species of beetles drinking nectar and carrying pollen. In the present day, we observe only two highly abundant beetle species. This dramatic shift in a matter of decades indicates that the pollinator community feeding on *Amianthium* could similarly vary across space. Future work should examine nectar production patterns and among-plant variation across its southern Appalachian range to gain a fuller picture of the natural history of nectar production and its role in pollination in this species.

FIGURES AND TABLES

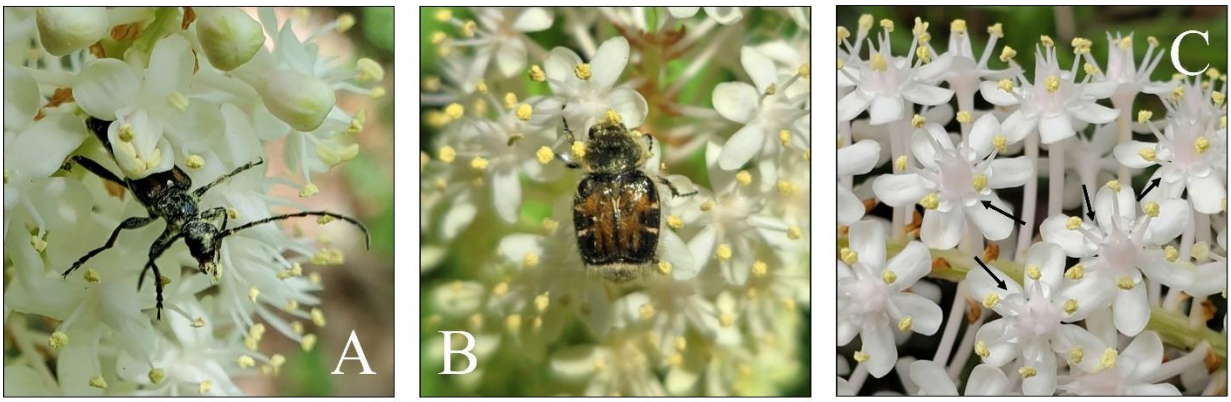

Figure 1. The two main pollinators of *Amianthium muscaetoxicum:* (A) the flower longhorn *Strangalepta abbreviata* (Lepturinae) and (B) the flower scarab *Trichiotinus affinis* (Cetoniinae). (C) Open nectar presentation in *Amianthium muscaetoxicum* (arrows highlight visible nectar). (Photos: S. J. McPeek).

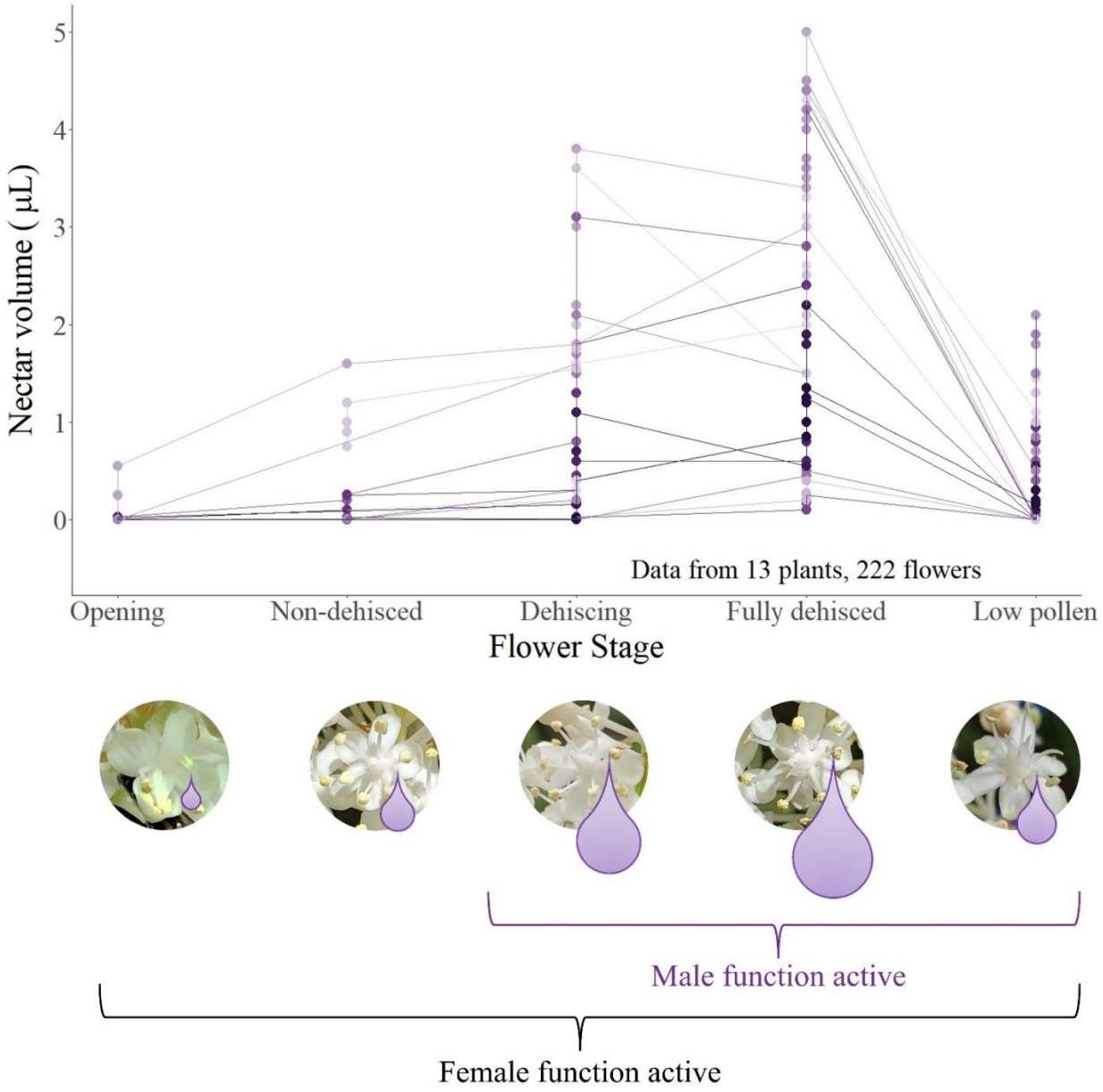

Figure 2. *Amianthium muscaetoxicum* flowers display stage-structured nectar production that coincides with floral sexual phases. Points are individual flowers sampled at each stage (N=222). Colors are plant individuals (N=13). Lines connect each plant's mean volume for each flower stage. Stages are as follows: Opening = flower displays partly open tepals, Non-dehisced = flower displays fully open tepals with anthers remaining intact, Dehiscing = flower displays partial anther-dehiscence, Fully dehisced = flower displays complete anther-dehiscence, and Low pollen = flower displays low pollen count (nearing end of viable period). (Photos: S. J. McPeek).

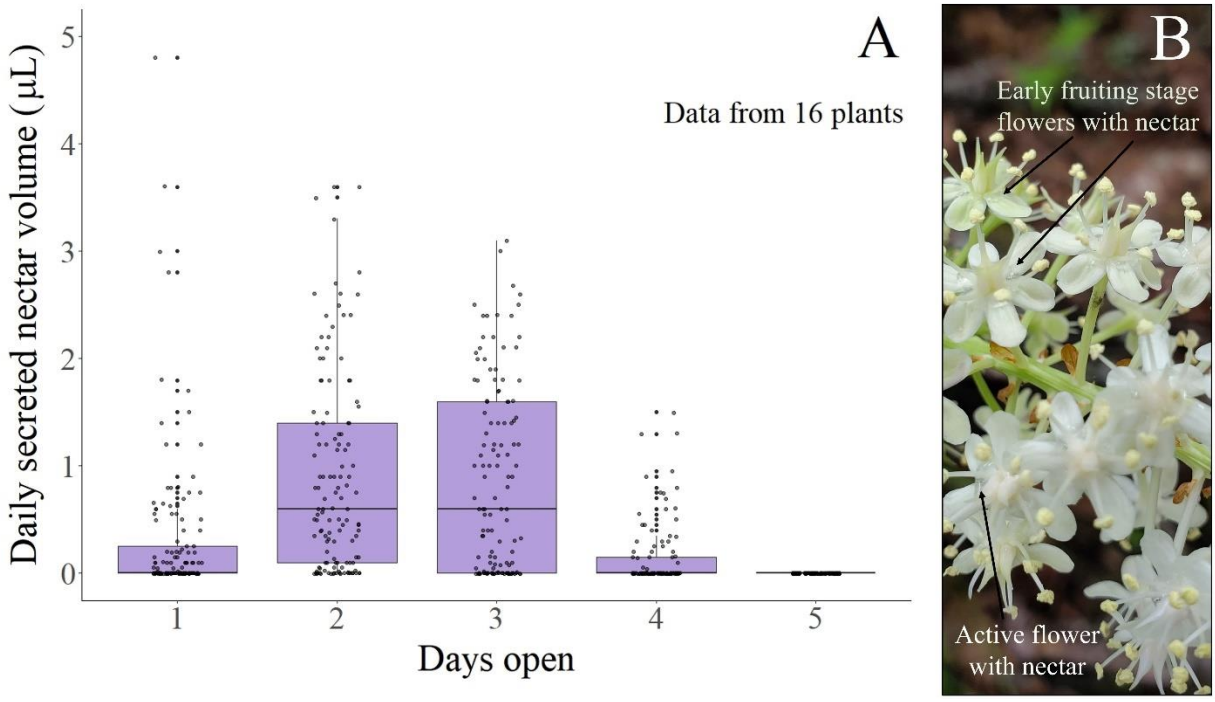

Figure 3. (A) Individual *Amianthium* flowers accumulate nectar for 1 to 4 days after opening and cease production by day 5. Boxplots capture the median (horizontal line), interquartile range (box), and total range (whiskers) for all flower measures taken each day (day 1-5 post-flower opening, N = 128 flowers from 16 plants). (B) Flowers on a plant do not reabsorb nectar, as indicated by post-receptive flowers retaining nectar on their tepals. (Photo: S. J. McPeek).

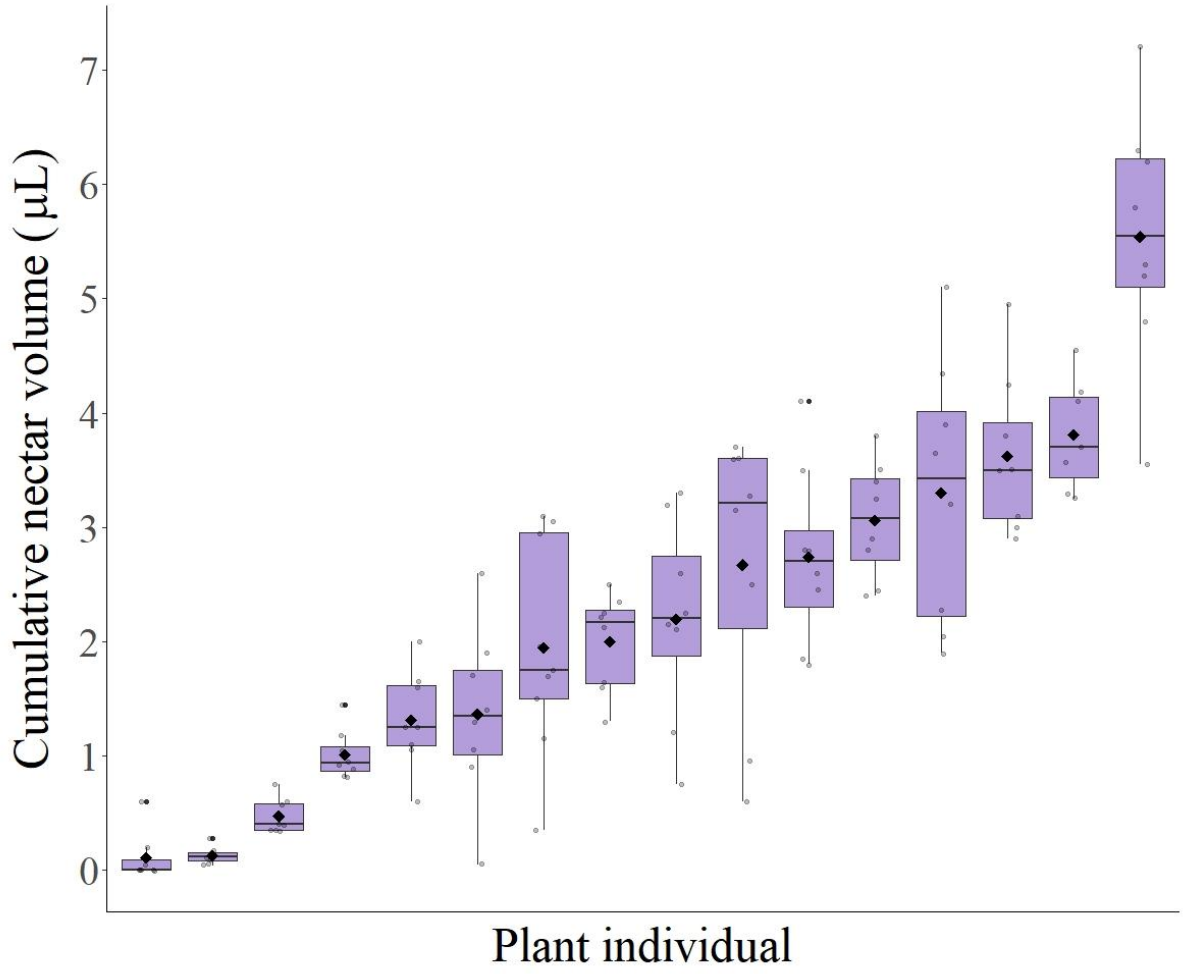

Figure 4. *Amianthium* individuals produce consistent cumulative volumes of nectar per flower. Each boxplot captures each plant's median (horizontal line), interquartile range (box), and total range (whiskers). Grey points are cumulative volumes for each flower on a plant. N = 16 plants, 8 flowers per plant. Boxplots are ordered from left to right by mean nectar volume (diamond shape).

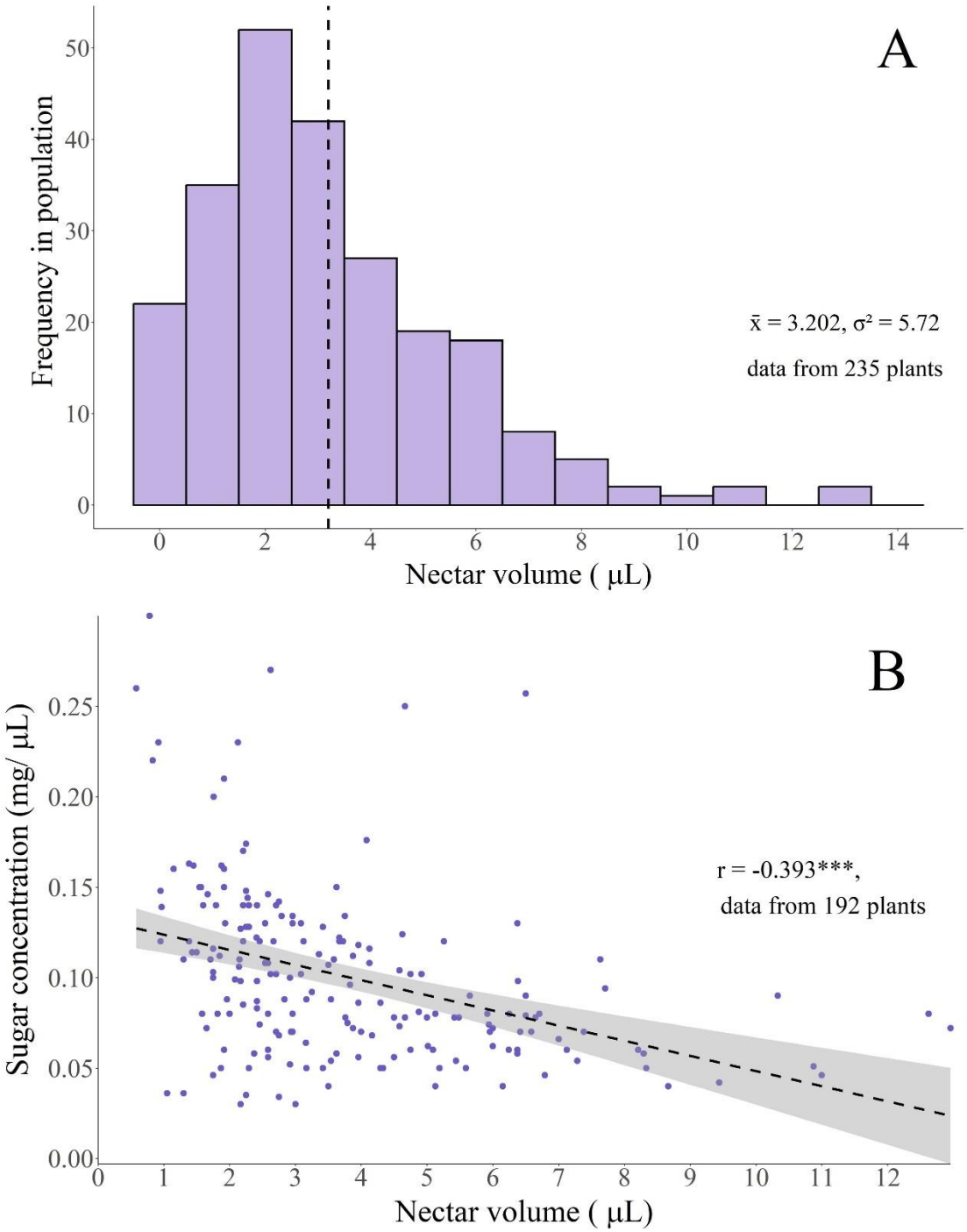

Figure 5. Individual *Amianthium* plants varies widely in (A) their mean nectar volumes and (B) their mean sugar concentrations. Nectar measurements were taken from fully dehisced flowers and averaged among three flowers on each plant. Bars in (A) display mean nectar volumes for all sampled individuals, counted in one-µL bins. Dashed vertical line is the population mean. Points in (B) are plant individuals. r is the Pearson's product moment correlation coefficient of the two plotted traits, gray shading is the standard error margins for this regression estimate. Significance level: $p < 1e^{-8}$ ***.

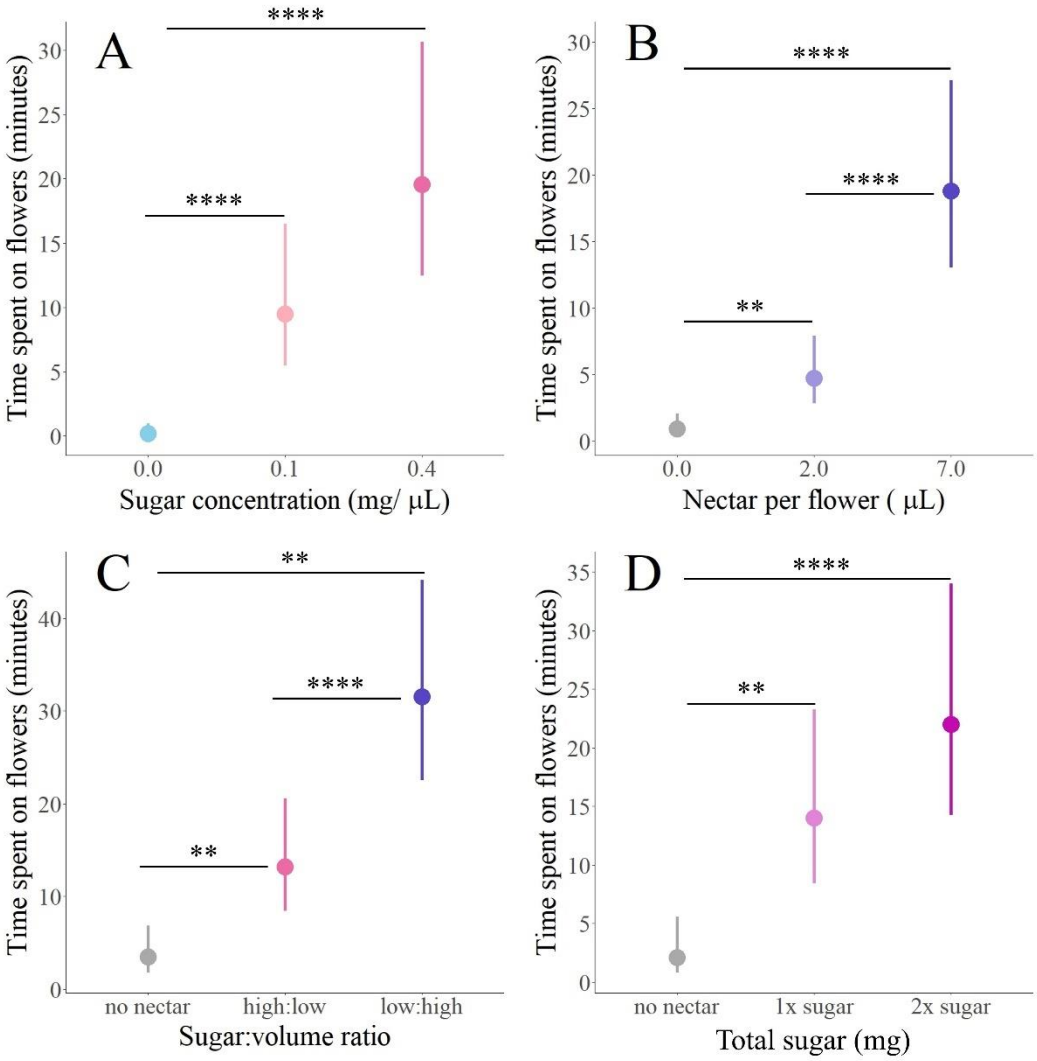

Figure 6. Beetles spent the most time visiting flowers with higher nectar volumes and higher sugar concentrations. Points are estimated marginal means, lines are upper and lower 95% confidence intervals. Samples sizes for A-C: N = 20 beetles. Sample size for D: N = 16 beetles. Significance bars show statistically significant pairwise contrasts between flower treatments as determined from pairwise contrast tests using *emmeans* (Table 1). Significance levels: $p > 0.05$ no bar, $p < 0.05$ *, $p < 0.01$ **, $p < 0.001$***, $p < 0.0001$****. P-values adjusted for multiple comparisons via the Tukey method. (Photo by S. J. McPeek.)

Experiment	*Mean ratio ± SE*	*LCL*	*UCL*	*z-ratio*	*p*
Sugar water versus nectar versus water					
0.044 mg/µL sucrose soln.: 0.044 mg/µL nectar	0.87 ± 0.32	0.37	2.06	-0.38	0.92
0.044 mg/µL sucrose soln.: 0.0 mg/µL water	14.93 ± 9.80	3.21	69.52	4.12	0.0001
0.044 mg/µL nectar: 0.0 mg/µL water	13.00 ± 8.58	2.76	61.10	3.88	0.0003
A: Sugar concentration					
0.4 mg: 0.1 mg	2.06 ± 0.75	0.88	4.82	1.99	0.12
0.4 mg: 0.0 mg	101.4 ± 87.83	13.32	772.04	5.33	< 0.0001
0.1 mg: 0.0 mg	49.31 ± 43.45	6.25	388.86	4.42	< 0.0001
B: Volume					
7 µL: 2 µL	3.96 ± 1.17	1.99	7.91	4.67	< 0.0001
7 µL: 0 µL	0.05 ± 0.09	0.07	0.59	-7.05	< 0.0001
2 µL: 0 µL	0.20 ± 0.09	0.069	0.59	-3.48	0.001
C: Equal total sugar content, different volumes and sugar concentrations					
0.4 mg/µL, 1 µL: 0.08 mg/µL, 5 µL	0.42 ± 0.12	0.22	0.81	-3.07	0.006
0.4 mg/µL, 1 µL: 0.0 mg/µL, 0 µL	3.77 ± 1.55	1.44	9.91	3.23	0.004
0.08 mg/µL, 5 µL: 0.0 mg/µL, 0 µL	9.02 ± 3.47	3.66	22.22	5.72	< 0.0001
D: Different total sugar content, volumes, and sugar concentrations					
0.4 mg/µL, 3 µL: 0.08 mg/µL, 7.5 µL	0.64 ± 0.22	0.29	1.41	-1.33	0.38
0.08 mg/µL, 7.5 µL: 0.0 mg/µL, 0 µL	0.15 ± 0.08	0.04	0.56	-3.40	0.002
0.4 mg/µL, 3 µL: 0.0 mg/µL, 0 µL	0.10 ± 0.05	0.03	0.34	-4.34	< 0.0001

Table 1. Pairwise comparisons of the mean times beetles spent on each treatment in nectar-feeding experiments. Estimated marginal means contrasts extracted from GLMMs. LCL and UCL are upper and lower 95% confidence intervals for each pairwise contrast mean ratio. P-values adjusted for multiple comparisons via Tukey method. Statistically significant contrasts are bolded ($p < 0.05$).

Nectar versus sugar water versus water: *Sucrose solution* *Natural nectar* *water*	*Fixed effects*	χ^2	*p*	$\sigma^2 \pm SE$
	Nectar treatment	17.53	2e-4	-
	Random effects			
	Beetle ID	-	-	2e-9 ± 5e-5
	Plant ID	-	-	0.29 ± 0.54
A: Sugar concentration *0.4 mg sucrose* *0.1 mg sucrose* *0 mg sucrose*	*Fixed effects*			
	Nectar treatment	29.66	4e-7	-
	Random effects			
	Beetle ID	-	-	3e-10 ± 2e-5
	Plant ID	-	-	4e-9 ± 6e-5
	Flower block order	-	-	2e-10 ± 1e-5
B: Volume *7 µL nectar* *2 µL nectar* *0 µL nectar*	*Fixed effects*			
	Nectar treatment	60.11	9e-14	-
	Random effects			
	Beetle ID			0.08 ± 0.28
	Plant ID			4e-9 ± 6e-5
	Flower block order			4e-10 ± 2e-5
C: Equal total sugar contents *0.4 mg/µL, 1 µL* *0.08 mg/µL, 5 µL* *0 mg/µL, 0 µL*	*Fixed effects*			
	Nectar treatment	35.24	2e-8	-
	Random effects			
	Beetle ID	-	-	2e-10 ± 2e-5
	Plant ID	-	-	3e-10 ± 2e-5
	Flower block order	-	-	3e-10 ± 2e-5
D: Different total sugar contents *0.4 mg/µL, 3 µL* *0.08 mg/µL, 7.5 µL* *0 mg/µL, 0 µL*	*Fixed effects*			
	Nectar treatment	18.84	8e-5	-
	Random effects			
	Beetle ID	-	-	10e-10 ± 3e-5
	Plant ID	-	-	7e-13 ± 9e-7
	Flower block order	-	-	3e-11± 6e-6

Table S1. Summary of GLMMs testing the effect of nectar treatment (flower blocks with three different nectars) on the total time beetles spent on a flower block. Statistically significant fixed effects are bolded ($p < 0.05$).

Chapter 3: Nectar traits of neighbors affect pollinator interactions in *Amianthium muscaetoxicum*

ABSTRACT

Neighbors can affect each other's performance and fitness in many kinds of species interactions. Many neighbor effects are mediated by the behavior of forager species, such as an herbivore or a pollinator, responding to spatial variation in the traits of their resource species, such as spatial variation in plant defenses or the quantity and quality of nectar rewards. Hence, forager behavior may be an agent of multilevel selection on traits that mediate species interactions. However, ecological neighbor effects can be divided into two categories: 1) associational effects in which forager behavior responds only to group-level variation in their resources, and 2) neighbor contrast effects in which forager behavior responds to individual traits within the context of the local group. These two non-exclusive forms of neighbor effects may have unique consequences for phenotypic evolution in resource species. In the present study, we examined how these two kinds of neighbor effects may play a role in shaping pollinator behavior and thus plant reproductive outcomes in a wild population of the beetle-pollinated wildflower, *Amianthium muscaetoxicum*. Through this combination of observational and experimental study, we find evidence for both forms of neighbor effects on pollinator behavior and supporting evidence for the neighbor contrast effect as a cause of multilevel selection in *Amianthium*. Our work reveals that neighbor effects play a role in shaping beetle interactions with flowers, but multilevel effects of neighbors do not appear to strongly impact the overall magnitude or direction of selection on individual-level nectar traits.

INTRODUCTION

Neighbors can affect each other's performance in antagonistic and mutualistic interactions with other species. Patches of plants can experience associational susceptibility or "shared doom" where a palatable, undefended species increases herbivore attacks on the whole group despite group members' own defense levels (e.g., Letourneau 1995; Wahl and Hay 1995; Enderlein et al. 2003; Emerson et al. 2012). Likewise, an attractive co-flowering species can facilitate pollinator visits to an entire patch (e.g., Thomson 1978; Feldman et al. 2004) or enhance interspecific competition for pollinator attention within a patch (e.g., Hanoteaux et al. 2013; Bruckman and Campbell 2014; Seifan et al. 2014). Many of these neighbor effects are mediated by forager species, such as herbivores or pollinators, responding to local variation in the traits of their resource species (reviewed by Underwood et al. 2014, 2020). Most work on neighbor effects has focused on effects among co-occurring species, using local species diversity as a proxy for local trait diversity. Recently, work examining conspecific variation in defense traits suggests that herbivores change their foraging behavior based on the trait composition of a resource patch (Sato and Kudoh 2016; Tamura et al. 2020). For example, groups with high levels of chemical defense experience fewer herbivore attacks than groups with low levels of chemical defense (Bustos-Segura et al. 2017; Champagne et al. 2020; Ziaja and Müller 2023). These findings further suggest that neighbors could play an underappreciated role in shaping forager-mediated selection in species interactions.

Multilevel selection presents a possible mechanism by which neighbors could affect selection on traits in species interactions. Multilevel selection occurs when group level, or contextual, traits affect individual fitness (Heisler and Damuth 1987; Goodnight et al. 1992; Okasha 2006). Field studies exploring multilevel selection in a variety of natural systems show that an individual's group context can affect their fitness in a variety of interactions such as contests over mates (e.g., Formica et al. 2011; Costello et al. 2023) and competition for ecological resources (e.g., Stevens et al. 1995; Donohue 2003, 2004; Fisher et al. 2017). Multilevel selection may also be common when individuals either facilitate each other's interactions with a partner species or compete with one another for partner attention (or deterrence in the case of herbivore interactions). To our knowledge, only one study has related multilevel selection

on plant traits to intraspecific pollinator facilitation and herbivore intensity (Aspi et al. 2003). Given the plethora of evidence that local intraspecific trait variation can affect consumer behavior, and the fact that these neighbor effects can cause variation in survival and/or reproductive fitness, multilevel selection may be an important force shaping the evolution of attraction and defense traits in wild populations. Furthermore, multilevel selection can alter the strength and directionality of individual-level selection among groups with different trait compositions (Goodnight et al. 1992; Aspi et al. 2003; Weinig et al. 2007; Formica et al. 2011; Cameron et al. 2021; Costello et al. 2023), potentially changing net selection on individual phenotypes and the evolutionary response to selection at the population scale (Moore et al. 1997; Wolf et al. 1999; McGlothlin et al. 2010). Therefore, addressing the potential for multilevel selection on attraction and defense traits may change predictions of how these traits evolve in natural, spatially variable plant populations.

In plant-animal interactions, trait-mediated neighbor effects exhibit remarkable variation, not all of which fit neatly into the group-versus-individual mold of classical contextual analysis. Ecological neighbor effects can be divided into two categories according to how forager species respond to variation within and among patches of their resource species. If an animal forages selectively among patches but consumes everyone in a patch, then resources may experience associational susceptibility/attractant effects or associational refuge/decoy effects (Tahvanainen and Root 1972; Atsatt and O'Dowd 1976; Brown and Ewel 1987; Barbosa et al. 2009). Associational effects do not depend on the phenotype of the focal individual relative to its group context and are thus a possible cause of group selection. If an animal forages selectively within a patch, consuming individuals based on their attractiveness or palatability within their group context, resources may experience neighbor contrast susceptibility/defense (Bergvall et al. 2006, 2008; Courant and Fortin 2010; Erfanian et al. 2021). Neighbor contrast effects occur when foragers make a comparison between the traits of individuals and their neighbors. Hence, this form of neighbor effects depends on the phenotype of the focal individual in relation to the phenotypes of its neighbors, causing a possibly distinct form of multilevel selection theorized by Wolf et al. (1999) that may have distinct consequences for trait evolution at the group and population scales.

These two forms of neighbor effects are not mutually exclusive in a population. For example, we have evidence for both kinds of neighbor effects in plant-pollinator interactions, though they are rarely explicitly linked to nectar traits. Neighbors can affect the frequency of pollinator visitation to entire patches (Thomson 1978; Feldman et al. 2004), which conforms to the multilevel selection/associational effects paradigm. For example, work in *Echium vulgare* shows that individuals that produce lower quantities of nectar receive more pollinator attention when growing near higher producing conspecifics (Ott et al. 1985; Klinkhamer et al. 2001; Leiss and Klinkhamer 2005). We refer to this as the associational effect. Neighbors can also exacerbate visitation disparities among individuals in patches (e.g., Bruckman and Campbell 2014; Hegland 2014), hinting at the existence of neighbor contrast effects mediated by pollinator responses to variation in nectar quantity and quality. Therefore, nectar variation in plant-pollinator interactions is a potentially instructive case for exploring both kinds of behavior-mediated neighbor effects as causes of multilevel selection on nectar phenotypes.

In this study, we explore the evidence for these two forms of neighbor effects: associational effects and neighbor contrast effects, and their roles as possible drivers of multilevel selection in the beetle-pollinated wildflower, *Amianthium muscaetoxicum*. We conducted an observational field study of multilevel selection on nectar traits that allowed us to examine the evidence for two major categories of trait-mediated neighbor effects: associational effects and neighbor contrast effects, in *Amianthium* (Figure 1). For this, we selected 12 patches of plants displaying substantial within- and among-patch variation in nectar quantity and quality. We also defined a smaller scale of neighborhood: the immediate neighbors of each focal plant individual in a patch. We compared evidence for the two forms of neighbor effects on pollinator visitation and plant reproductive success at both spatial scales (Figure 1). We combined this work with an experimental test of neighbor effects on the feeding behavior of an abundant *Amianthium* pollinator, the longhorn beetle *Strangalepta abbreviata* (Figure 2). Through this combination of observational and experimental work, we gain mechanistic insight into nectar trait-mediated neighbor effects on pollinator behavior and their potential role in shaping reproductive outcomes of plants.

STUDY SYSTEM

Amianthium muscaetoxicum is an Appalachian perennial that grows in a large patchy forest understory population at Mountain Lake Biological Station (MLBS, Giles County, VA, USA). *Amianthium muscaetoxicum* is an almost fully self-incompatible species, requiring insect-mediated outcrossed pollen transfer to produce viable seeds each year (Travis 1984). Flowers only develop into fruits if ovules are fertilized. A maximally pollinated flower produces two to three seeds in each of three locules, totaling a maximum of six to nine seeds per flower. The *Amianthium* population at MLBS displays high variability in fruit set and is pollen-limited (Travis 1984).

Amianthium flowers from mid-June through mid to late July at MLBS (Travis 1984; Palmer et al. 1988). Inflorescences flower indeterminately from bottom to top over the course of two to three weeks. Individual flowers live for six days and are partially dichogamous: male anthers dehisce two to three days after the flower opens (Palmer et al. 1989). The female pistil remains receptive over the entire six-day period (Palmer et al. 1989). Once a flower opens, nectar begins to accumulate on the floral tepals in light-catching droplets. Production ceases when a flower reaches complete anther dehiscence. Flowers on a plant produce similar volumes of nectar (McPeek et al. Chapter 2). Hence, sampling nectar from a few recently dehisced flowers and taking the average of those measurements provides an estimate of the total nectar volume and sugar content each of an individual's flowers will produce. Plants vary considerably in their mean nectar production volumes, ranging from zero to 13 µL per flower (McPeek et al. Chapter 2).

At MLBS, *Amianthium* is pollinated by a guild of small-bodied nectar-feeding beetles, with the two most abundant taxa being *Strangalepta abbreviata* (Cerambycidae) and *Trichiotinus affinis* (Scarabaeidae). These species use hairy maxillae to lap up nectar droplets, brushing pollen onto the female stigma in the process of foraging. Most individuals captured in the field retain pollen granules around their legs, abdomen, and head. In experiments where beetles were given choices between flowers presenting different nectar traits, *Strangalepta* interacted most with flowers with higher nectar volumes and flowers with higher total sugar contents (McPeek et al. Chapter 2). This work demonstrated that nectar traits may be an important driver of beetle-*Amianthium* interactions. Further, these experiments suggested that beetles may discern among the traits of neighboring flowers, or among the traits of

individuals within neighborhoods, during foraging.

METHODS

Field methods: In summer 2022, we selected 12 similarly sized patches of plants (15-21 plant individuals per group). All patches were approximately three meters in diameter and separated from each other by greater than 10 meters. We based our choice of patch size on previous observations of common cluster sizes across the forest and observations of movement patterns of beetle pollinators. To distinguish patches more firmly from the rest of the forest, we removed any *Amianthium* inflorescences within a two-meter radius of each patch. We also defined the smaller neighborhood scale as any individuals growing within half a meter radius of each focal individual (Figure 1A).

We bagged all inflorescences prior to nectar sampling to prevent consumers from affecting standing nectar crops. We also controlled the water environment plants received during the period of nectar accumulation by giving each plant 200 mL of water in the two days prior to sampling its nectar. We performed nectar sampling in all 12 patches within a two-day span with similar temperature conditions (20-22º C) between 8:00 and 13:00 hours. Hence, we are confident that our nectar measures are representative of the traits individuals across the population would express under similar environmental conditions (similar water and temperature environments, no nectar removal via consumption).

We used 5 µL and 2 µL microcapillary tubes to sample nectar from three previously marked and presently dehisced flowers on each plant. We measured the volume of nectar in each tube using a millimeter ruler. We used the average of a plant's three sampled flowers as their total nectar volume produced per flower (hereafter nectar volume). We also measured pooled sugar concentration from all three flowers' samples using a sugar refractometer (BRIX 0-30%). We pooled samples across flowers to obtain this measurement because low volumes from some flowers prevented us from taking a separate reading for each flower. After completing all nectar measurements, we removed plants' covers to allow natural insect-mediated pollination.

During this open-pollination window, we performed field observations of pollinator visitation in six patches that varied in their nectar availability, measured as the mean nectar volume of the entire patch.

We selected two patches that had lower mean nectar volumes (1.50 ± 1.18 μL and 2.04 ± 1.88 μL, mean ± standard deviation), two that had intermediate mean nectar volumes (2.87 ± 1.86 μL and 3.25 ± 1.68 μL) and two that had high mean nectar volumes (3.81 ± 1.56 μL and 5.23 ± 1.93 μL). All six patches displayed considerable among-individual variation in nectar traits, allowing us to capture beetle visitation to plants with similar traits found in different patch settings. We flagged each plant in these patches with a unique color code so we could identify it in the field without knowing its identity in the broader selection study. We conducted hour-long surveys of pollinator visitation in each patch on four separate days, visiting each patch twice in the morning (between 9 am and 12 pm) and twice in the afternoon (between 1 pm and 4 pm). During the hour-long observation period, we recorded which plants received pollinator visits, defined as any time a beetle landed on a plant and stayed for longer than five seconds.

By the time we removed plants' covers to allow natural insect-mediated pollination, plants had reached different stages of flowering. To account for variation in accessible flowers, we marked the bottom-most row of unopened buds and drew a line at that height on the stem with a felt tip pen. We only measured fruit set and seed set for the portion of the inflorescence above this mark, limiting our fitness data to flowers that experienced natural pollination.

Once all plants in our study population transitioned to fruit stage, we collected all inflorescences from the field. We retained 110 plants in our final reproductive data set, losing many to various natural causes such as trampling by wildlife, wind blowdown, intense granivory, and rust diseases. We destructively counted the number of flowers on each inflorescence, including those below the stem markings, to determine the total number of flowers on an individual (hereafter inflorescence size)

We collected three reproductive fitness components: fruit set (%), mean seeds per fruit, and a composite measurement of total seed set. For fruit set, we counted the number of open-pollinated flowers that produced visibly swollen fruits. For mean seed set per fruit, we haphazardly opened a maximum of 20 fruits per individual, counted the number of seeds, and took the mean of those counts. Initial sampling revealed that this subset is a close approximation of the mean seed set per fruit across the whole plant (accurate within 0.4 seeds). We also defined a measurement of total female reproductive success called

total seed set (mean seeds per fruit * inflorescence size * fruit set %). This measurement estimates a plant's total seed set if its entire inflorescence had experienced open pollination, capturing the expected reproductive success of an individual across the entire flowering season.

Statistical analyses: We performed all statistical analyses and constructed all figures in R version 4.3.3. Preliminary examination of the nectar data revealed that a plant's nectar volume (µL) and nectar sugar concentration (mg/ µL) were negatively correlated (r = -0.4, p =1.88e^{-5}). To account for this strong relationship, we created a composite trait measurement, total sugar, that measured the amount of sugar a pollinator would consume if it drank all the nectar a flower produced. For this, we multiplied a plant's sugar concentration by its mean nectar volume.

We used generalized linear mixed models (GLMMs) to analyze individual trait effects on pollinator visitation and reproductive success. For analyses of pooled pollinator visit counts, we used a negative binomial model family with a log link. For reproductive fitness measures, we used a Gaussian model family for analyses of fruit set and seed set fitness components and a Tweedie model family with a log link for analyses of total seed set. We used the *DHARMa* package to test whether each measure of pollinator visitation and reproductive fitness fit the assumptions of normally distributed data (Hartig 2022). All generalized linear mixed models (GLMMs) were built using the *glmmTMB* package (Brooks et al. 2017). We tested the severity of collinearity between trait variables in our models by calculating variance inflation factors (VIFs) for the terms in each model (Fox and Weisberg 2018). We excluded any models with VIFs exceeding 3 from subsequent tests (Johnston et al. 2018).

Individual-level traits: We first examined how individual-level nectar traits shaped patterns of beetle visitation. We fit a GLMM with nectar volume, total sugar, and the remaining number of unopened flower rows at the beginning of the open pollination period as fixed effects. The unopened flower rows covariate accounted for the fact that plants in our patches had reached slightly different stages of their flowering period during the open pollination window, and so pollinators could access different numbers of currently active flowers during the open pollination window. We did not include Patch ID as a random effect because preliminary examination revealed that patch ID explained a large proportion of the

variance in pollinator visitation (21%), effectively eliminating statistical power to detect trait effects on pollinator visitation. Subsequent exploration revealed that this effect was largely driven by strong effects of patch-level variation in nectar traits and not other unmeasured attributes of patches.

We estimated selection on individual-level nectar traits (nectar volume, sugar concentration, and total sugar) and inflorescence size (the total number of flowers on an inflorescence) with respect to each female reproductive fitness component. We mean-standardized fitness to obtain each individual's relative fitness (mean = 1) and variance-standardized all traits (mean = 0, units in *SD*). We chose to estimate selection stemming from both fruit set and seed set fitness components since each can provide different information about pollinator behaviors that mediate pollination. Fruit set is a common proxy for the frequency of pollinator visits to a plant (e.g., Kunin 1993), whereas seed set per flower is a signal for the visit quality of individual pollinators or the frequency of visits to an individual flower (e.g., Herrera 1987; Irwin and Brody 1998). Total seed set is the outcome of these two components. Early exploration revealed that models of reproductive fitness that included both nectar volume and total sugar violated our pre-assigned VIF threshold. Therefore, we evaluated selection on total sugar separately from the other two nectar traits. Our two selection gradient models were Model 1) *fitness ~ nectar volume + sugar concentration + inflorescence size,* and Model 2) *fitness ~ total sugar + inflorescence size*. We also estimated selection differentials for each trait to examine how estimates of direct selection (β') compared to estimates of net selection (*S'*, Lande and Arnold 1983). In all these models, we included patch ID and unopened flower rows as random effects to account for spatial and temporal variation in pollination across the flowering season. In the text and tables, we report selection gradients on inflorescence size estimated from Model 1 only, as all these β' estimates were within 0.02 standardized units of those estimated from Model 2.

One individual in our reproductive data set produced a total sugar content that was greater than 3 standard deviations above the population mean. To determine the effect of this extreme individual on our selection measurements, we re-estimated direct and net selection on total sugar with this individual excluded. This extreme individual made it challenging to visualize selection patterns in the raw data.

Thus, we excluded it from the presented visualization of selection differentials in Figure 3.

Multilevel neighbor traits: We evaluated evidence for associational and neighbor contrast effects as causes of multilevel selection in the *Amianthium* population. We created two forms of contextual traits for each focal individual in our visitation and pollination data sets (Figure 1B). For associational effects, we calculated the mean total sugar and mean nectar volume for each patch ($\bar{z}_p$) and neighborhood ($\bar{z}_n$) in our population. For neighbor contrast effects, we created neighborhood-adjusted focal traits at both spatial scales. To do this, we computed patch and neighborhood mean total sugar and nectar volume trait values for each focal individual, excluding the focal's trait from that mean (Okasha 2004). We then subtracted this focal-exclusive group mean from the focal individual's trait to obtain their nectar trait value relative to their group ($z_{i\text{-}\bar{n}}$). We also subtracted each focal's nectar volume from the global population mean ($z_{i\text{-}\overline{pop}}$) so that tests of neighbor contrast effects would evaluate whether local-scale or population-scale contrast best explained variance in pollinator visitation and reproductive fitness.

Having calculated these contextual traits, we then built GLMMs to examine the support for each neighbor effects hypothesis in the *Amianthium* population. For associational effects, we used the equation: $w_i \sim \beta_1 z_{indiv.} + \beta_2 \bar{z}_{group} + \beta_3 z_{group\ size} + e_{indiv,group}$, at both spatial scales. For neighbor contrast effects, we used the equation: $w_i \sim \beta_1 z_{indiv\text{-}\overline{pop}} + \beta_3 z_{indiv\text{-}\overline{group}} + \beta_4 z_{group\ size} + e_{indiv,group}$. We applied the same model families and random effects structures used for the previously described individual-level models, with one exception. We excluded patch ID as a random effect from models of patch-scale neighbor effects because patch ID and patch level traits had the same number of observation levels (12 patches, 12 patch level traits). We also included group size as a covariate in all models because local plant density could affect pollinator behavior, and by extension, individual fitness. Initial exploration of our data confirmed that a plant's neighbor contrast trait at the patch level was extremely highly correlated with its individual trait ($r = 0.96$, $p = 2.2e^{-16}$). Therefore, we did not evaluate neighbor contrast effects at this level because almost all the variance in that contextual trait could be captured by the individual scale.

After building the full neighbor effects models, we used Akaike's Information Criterion (*AIC*) to determine whether removing any trait terms moderately improved the model fit ($\Delta AICc \geq 2$) (Akaike 1973). Any such trait terms were subsequently dropped, and the revised model was re-run. If a neighbor effects hypothesis on a response variable was supported, we used *AIC* to compare the fit of that final model to both the full model for the other form of neighbor effect and the model at the individual-level. We used the same threshold of $\Delta AICc \geq 2$ to determine which model provided the best fit for the data. Where the best-fit model at a spatial scale differed from the individual-level model of a fitness measurement, we calculated standardized selection differentials on that contextual trait.

Due to the observed lack of an effect of individual total sugar on pollinator visitation, we only examined neighbor effects of nectar volume on pollinator visitation. We tested neighbor effects of both total sugar and nectar volume on reproductive fitness. We focused results presentation on the composite measure total sugar after finding that total sugar exhibited the strongest net selection via all three fitness measures (Table 1). In all cases, the best-fit neighbor effects total sugar model for each reproductive fitness component also emerged as the best-fit model for nectar volume.

Testing a mechanism of pollinator behavior-driven neighbor effects: We performed controlled feeding experiments with wild-caught *Strangalepta abbreviata* (Figure 2). Beetle housing followed the same protocol as in McPeek et al. (Chapter 2). We gave all housed beetles a 6-µL aliquot of 8% sucrose solution for a total sugar content of 0.48 mg of sucrose on the afternoon prior to their trial. We tested 92 beetles in total over the course of five sets of trials. Due to challenges with collecting large numbers of beetles from the field, some beetles were used in only one trial (N = 32), some were used in two trials (N = 41) and some were used in three trials (N = 16). Individual beetles experienced a different treatment in each of their trials.

We used a 2 x 2 factorial design in which we presented two focal nectars, a high-nectar focal (5 µL per flower, 0.5 mg total sugar) and low-nectar focal (2.5 µL per flower, 0.25 mg total sugar) in neighborhood contexts where the focal was either more rewarding than its neighbors (2 µL higher volume and 0.2 mg higher total sugar than neighbors) or less rewarding than its neighbors (2 µL lower volume

and 0.2 lower total sugar than neighbors) (Figure 2A). We selected focal nectar traits values that were approximately one standard deviation above or below the population mean nectar volume and total sugar in our 2022 wild data (McPeek et al. Chapter 2, 235 plants). All nectar for these trials was pulled from a single 10% sucrose solution so that all experimental flowers expressed the same sugar concentration per microliter of sucrose but varied in their total sugar due to differences in volume.

Foraging arena setup followed methods described in McPeek et al. (Chapter 2). Each arena contained three sculpey plastine blocks that each held three wild-collected, washed, and emasculated *Amianthium* flowers (Figure 2B). We kept track of which plant individual we used for each trial to statistically control for any effects of unknown floral trait variation on pollinator behavior. We randomized the left to right order of the three 'individuals' in an arena so that the focal individual could be located to the left, to the right, or between its two neighbors (Figure 2B). We included replicates of all four nectar treatments with all three block orientations in each trial set. In preliminary tests, pollinators often failed to explore multiple food sources when flower blocks were separated from each other, enforcing the adjacent placement of blocks in the arena. This behavior was most likely an artefact of the artificial environment as beetles readily fly between plants in the wild. Therefore, we used variation among adjacent clusters of flowers as an experimental proxy for variation among neighboring plants.

At the start of a feeding trial, we placed each beetle inside the circle marked in the bottom area of the arena so they entered at a standardized distance from their food source (Figure 2B). We recorded beetle foraging for one hour using a Basler ace L acA4096-30um camera shooting at 4 frames per second (Baesler AG, Ahrensburg, Germany). We recorded 164 behavioral trials: 40 low focal-lower than neighbors, 38 low focal-higher than neighbors, 36 high focal-lower than neighbors, 37 high focal-higher than neighbors. Each trial set contained between 29 and 36 arenas placed in a rectangular grid under the camera's frame of view.

We watched and scored the behaviors of beetles using InqScribe software (Inquirium, Chicago, IL). For each trial, we recorded the duration of time a beetle spent interacting with a particular flower block (methods described in McPeek et al. Chapter 2). Briefly, we counted interactions as any time a beetle's

head and thorax region were positioned over one flower block for longer than five seconds of the video. Interactions ended when these body regions were no longer over that block. It is important to note that beetles were not necessarily feeding for the entire interaction with a flower block. Beetles often fed for short bursts and then sat for long periods of time before feeding again. We counted this as part of the interaction time because beetles feeding in this manner in the wild could transfer pollen to the female stigma at any point. Indeed, we often find beetles sitting on inflorescences for long periods of time in the wild, suggesting the behavior pattern we saw in our experiment matches their natural behavior.

We examined how two beetle behaviors: the proportion of time a beetle spent interacting with the focal and the number of times it visited the focal during the hour-long trial period, changed across the two neighborhood contexts. We constructed generalized linear mixed models that tested the effect of the focal's nectar trait (categorical low-nectar focal or high-nectar focal), its neighbor context (categorical neighbors lower than focal or neighbors higher than focal), and the interaction between focal nectar and neighbor context on each behavior. We also included five random effects: the ID of the beetle being tested, the trial number for that beetle (to account for repeat uses), the order of flower blocks (focal on the left, middle, or right), the plant ID of the flowers used in a trial, and the ID of the observer who scored each trial video. Any random effects that explained less than 1e-10 proportion of the total variance in the data were removed from the final reported models. We used a Tweedie model family with a log link for the proportion behavior due to its right-skewed distribution, and a negative binomial model family with a log link for the visit count behavior. We conducted post hoc tests using the R package *emmeans* that compared beetle behaviors on the focal nectars among neighborhood treatments (Lenth 2024).

RESULTS

Individual-level nectar traits affect pollinator visitation and plant reproduction: Plants that produced higher volumes of nectar received more pollinator visits on average than plants that produced lower volumes of nectar (ANOVA, $\chi^2_{3,97} = 4.12$, $p = 0.04$, Figure 2). Plants with higher total sugar received a similar number of pollinator visits as those with lower total sugar ($\chi^2_{3,97} = 2.52$, $p = 0.11$). Plants with more rows of potentially active flowers during the open pollination window did not receive significantly

more visits than plants with fewer rows ($\chi^2_{3,97} = 3.1$, $p = 0.08$).

Total sugar experienced significant positive directional selection (both direct and net) via all reproductive fitness components (Table 1, Figure 3). Plants with higher total sugar set a greater percentage of fruits (Figure 3A), a greater mean number of seeds per fruit (Figure 3B), and a greater seed set overall (Figure 3C) than did plants with lower total sugar. Removing one outlier individual (standardized total sugar = 6.52) strengthened selection via all three fitness measures relative to the estimates reported in Table 1, but did not qualitatively change the direction of selection or the statistical significance of estimates (Figure 3).

Neither nectar volume nor nectar sugar concentration experienced net selection (*S'*) via any fitness measure, but both traits did experience net selection (*β'*, Table 1). Direct selection via any fitness component favored higher nectar volumes: plants with higher nectar volumes achieved higher reproductive success than plants with lower nectar volumes. Direct selection via two fitness measures also favored higher sugar concentrations: plants with higher sugar concentrations set greater percentages of fruits and a greater total seed set than plants with lower sugar concentrations. Selection on inflorescence size was only moderately strong ($\beta' > 0.1$, Kingsolver et al. 2001) and statistically significant via a plant's total seed set: plants with more flowers had a higher potential to produce more seeds (Table 1).

Neighbor effects on pollinator visitation differ from neighbor effects on plant reproduction: Plants experienced patch-scale effects on pollinator visitation, but not on reproductive fitness (Table 2). Plants in patches with higher mean nectar volumes received higher mean numbers of pollinator visits, supporting the associational effect (Figure 5). Associational effects were a moderately better fit model of pollinator visitation than was individual attraction ($\Delta AICc = 2$). Individual-level nectar volume was a non-significant predictor of pollinator visit counts when patch mean nectar volume was included (Table 4). Patch-scale contextual traits were not significant predictors of any fitness measure (Table 2), indicating that only individual traits predicted individual fitness at this spatial scale (Table 4). Patch density did not affect any measures of reproductive fitness (Table 2).

Plants experienced no neighborhood-scale effects on pollinator visitation, but did show evidence of a

neighbor contrast effect via the seed set fitness components (Table 3). The neighbor contrast effect and associational effect models provided nearly equivalent fits for pollinator visitation data ($\Delta AICc = 0.98$), but no terms in either neighbor effects model were statistically significant (Table 3). Only individual traits significantly predicted individual fruit set (Table 3). Among the two forms of neighbor effects, the neighbor contrast effects model provided a moderately better fit to the seeds per fruit fitness measure than did the associational effects model ($\Delta AICc = 4.62$), or the individual-level model ($\Delta AICc = 2.33$). Plants with higher total sugar than their neighbors' mean total sugar set a significantly higher mean number of seeds per fruit (Table 4, Figure 6A). Removing individual-level nectar traits moderately improved the fit of the neighbor contrast effects model for the seeds per fruit fitness measure (Table 3), indicating that the contextual trait better predicted seed set than did the individual trait. Plants with higher total sugar than their neighbors' mean also set a higher total seed set (Figure 6B), although the neighbor contrast effects model only performed substantially better over the associational effects model ($\Delta AICc = 2.83$), but did not substantially improve the model fit for total seed set over the individual traits model ($\Delta AICc = 0.5$). We found no support for associational effects at the neighborhood scale (Tables 3, 4). Neighborhood density did not affect either pollinator visitation or reproductive fitness (Tables 3, 4).

Beetles exhibit neighbor contrast effects toward the low-nectar focal flowers: Neighbor context (focal nectar higher or lower than neighbors) significantly predicted the proportion of trial time pollinators spent interacting with the focal flowers (Table 5). Beetles spent a significantly longer mean proportion of time feeding on the low-nectar focal when that focal had more nectar than its neighbors (*estimated marginal mean* ± *standard error* = 0.45 ± 0.09) than they did when the low-nectar focal had less nectar than its neighbors (*EMM* ± *SE* = 0.21 ± 0.05, Figure 7A, Table 6.). Beetles also stayed significantly longer on the low-nectar focal when the focal had more nectar than its neighbors than they did on the high-nectar focal when it had more nectar than its neighbors (Figure 7A, Table 6).

Focal nectar volume, neighbor context, and the interaction between focal nectar and neighbor context all significantly predicted how many times a beetle visited the focal during the trial period (Table 5). Beetles visited the low-nectar focal significantly more times when the focal had more nectar than its

neighbors (*EMM* ± *SE* = 3.79 ± 0.74) than they did when the focal had less nectar than its neighbors (*EMM* ± *SE* = 1.66 ± 0.4, Figure 7B, Table 6). Beetles did not visit the high-nectar focal a significantly different number of times between the two neighborhood contexts (Figure 7B, Table 6). In the context where the focal had more nectar than its neighbors, beetles visited the low-nectar focal significantly more times than they visited the high-nectar focal (Figure 7B, Table 6).

DISCUSSION

In the present study, we examined patterns of selection on nectar traits in a wild population of the self-incompatible *Amianthium muscaetoxicum* and explored how neighbor effects may play a role in shaping pollinator behavior and thus plant reproductive outcomes. Nectar traits experienced moderately strong directional selection via both fruit set and seed set fitness measures, although net selection (S') only acted strongly on total sugar across all fitness measures. We detected a complex pattern of neighbor effects acting on pollinator visitation and female reproductive fitness in this population. Beetle pollinators landed most frequently on plants in patches with higher mean nectar volumes, supporting the associational effect. However, this apparent attraction to highly rewarding groups did not translate into higher reproduction for all individuals in those groups. Our pollination data provided moderate support for the neighbor contrast effect acting at the smaller neighborhood scale: plants with higher total sugar than their nearest neighbors set a greater number of seeds per fruit and a greater seed set overall. The experimental test of pollinator foraging behavior provided a potential mechanism for this pattern in the wild data: pollinators exhibited neighbor contrast effects toward the low-nectar focal, but not the high-nectar focal. These findings reveal that neighbor effects play a role in shaping beetle interactions with flowers, but these multilevel effects on plant reproduction do not appear to strongly impact the overall magnitude or direction of selection on individual-level nectar traits.

Whether neighbor effects are competitive (neighbor contrast effect) or facilitative (associational effect) depends principally on the behavior of the forager species. Patterns of wild pollinator visitation supported an associational effect at the patch scale, suggesting that beetles perceive patch-level variation in nectar volume and may visit nectar-rich patches most frequently. However, we found no evidence of

associational effects on female reproductive outcomes. In the feeding experiment, beetles tended to stay and feed intermittently for long periods of time on flower blocks with higher nectar volumes and moved the most between flowers in the environment with the lowest nectar availability (low-nectar focal with 2.5 µL, lower nectar neighbors with 0.5 µL). If beetles follow this pattern of behavior in the wild, lower interplant movement in higher-rewarding patches may prevent lower-rewarding plants in those patches from reaping any benefits of their neighbors' attractiveness. In contrast, other pollinators such as bumblebees visit more flowers on high-rewarding plants (e.g., Waddington and Heinrich 1979; Zimmerman 1983; Zhao et al. 2016) and in high-rewarding areas (e.g., Waddington 1980; Pleasants 1981; Dreisig 1995), and may be more likely to enact associational attraction effects on plant reproductive outcomes. Future work should examine the translation of behavioral neighbor effects on pollinator behavior into reproductive outcomes in other plant-pollinator systems. Bumblebee-pollinated species such as *Echium vulgare* are excellent future targets for exploration given past support for associational effects of nectar traits on bumblebee behavior (Klinkhamer et al. 2001; Leiss and Klinkhamer 2005).

Small-bodied, solitary pollinators such as the beetles observed in the present study likely have lower energy needs than more commonly studied species such as bumblebees and hawkmoths and may be more likely to forage in shorter, spatially restricted bouts (Heinrich and Raven 1972; Heinrich 1975). As a result, they may pay more attention to individual level variation within groups than to the overall composition of groups. Our work in *Amianthium* reveals that beetle pollinators distinguish between the nectar traits of individual flowers, with possible effects on flower-level pollination among plants. We found the strongest support for neighbor contrast effects on a plant's flower-level seed set (mean seeds per fruit). Our feeding experiment provided supporting evidence for the wild data: beetles spent significantly more time interacting with the low-nectar focal when it had more nectar than its two neighbors and revisited the focal significantly more times during the trial period. Increased flower visit times could enhance flower-level seed set, as seen in other systems (e.g., Zimmerman 1983; Brandenburg et al. 2012), though this needs to be tested in *Amianthium*. Pollinators such as beetles, flies, and other solitary insects may be more likely to exacerbate trait-based competition within groups via neighbor

contrast effects, while larger-bodied and social pollinator species may be more likely to cause facilitation via associational effects. This prediction may extend to other kinds of interactions such as seed dispersal mutualisms: bird and mammalian seed dispersers may be more likely to be attracted to dense clusters of fruits and seeds (e.g., Sargent 1990; Carlo and Morales 2008; Morales et al. 2012), while smaller bodied, shorter-distance dispersers such as ants may pay more attention to variation within resource clusters.

Neighbor contrast effects on reproductive fitness were stronger for plants nearer to the mean of the population's phenotypic distribution, and weaker for plants near the extremes of the distribution (see color shading of points in Figure 5A-B). Individuals with phenotype values near the population mean appeared to experience the strongest neighbor contrast effects, suggesting that a pollinator's attraction towards plants with intermediate sugar contents may depend on the plant's neighborhood context. On the other hand, plants with nectar trait values that were farther from the population mean also tended to have substantially higher or lower total sugar contents relative to their neighborhood. In the feeding experiment, beetles only significantly changed the amount of time they spent on the low-nectar focal across its two neighborhood contexts, suggesting that beetles may only discern among nectar sources when the overall nectar level of the neighborhood is low. Previous work on neighbor contrast effects in plant-herbivore systems compared discrete trait variation among defended and undefended resources (e.g., Bergvall et al. 2006), or between discrete species (e.g., Courant and Fortin 2010; Erfanian et al. 2021). Examining neighbor contrast effects on quantitative variation in other systems, including in herbivore-plant interactions, may reveal similar threshold behavioral responses to trait variation.

In this population, the vectors of individual-scale selection on total sugar and neighborhood-scale neighbor contrast total sugar on reproductive fitness were both positive. This accordance of individual and neighborhood level selection suggests that the neighbor contrast effect reinforces individual selection by exposing trait disparities among individuals rather than concealing them. On the other hand, multilevel selection is expected to weaken the response to individual selection if the sign of group selection opposes that of individual selection (e.g., Stevens et al. 1995; Aspi et al. 2003; Weinig et al. 2007), which may be possible in cases of associational efects. With associational effects, low-rewarding plants growing near

higher-rewarding neighbors could receive spillover pollinator visits without suffering the possible physiological costs of nectar production (e.g., Southwick 1984; Pyke 1991). Neighbor contrast effects are a fundamentally competitive form of neighbor effect and may thus have different consequences for the evolution of traits that mediate species interactions. Future theoretical and empirical work should examine how neighbor contrast effects may change predicted responses to multilevel selection compared to associational effects (group selection).

We lacked sufficient statistical power to perform contextual analysis with neighbor contrast traits in this study. Therefore, we cannot definitively reject the possibility that the observed neighbor contrast effects are individual effects transformed in a way that slightly enhances their power as predictors of reproductive outcomes. This explanation may be the case for selection on total seed set: selection differentials on individual total sugar ($S' = 0.14$) and neighbor contrast total sugar were nearly identical ($S' = 0.15$). However, we doubt this explanation with respect to a plant's per flower seed set fitness component for two reasons. First, the selection gradient on neighbor contrast total sugar ($S' = 0.099$), was nearly 0.3 units stronger than that on individual total sugar ($S' = 0.07$), although the standard errors for these differentials overlapped one another. Such an increase in selection strength, combined with the fact that *AIC* comparisons favored the neighbor contrast model over the individual model, makes us suspect that the neighbor effect on reproductive fitness was more than a transformed individual effect. Second, the behavioral data from our experiment strongly supports neighbor contrast effects on pollinator behavior. While this flower cluster experiment is a not a direct match to plant-level nectar variation in the natural environment, our finding that beetles change their behavior on flowers based on the context of those flowers' nectar environments suggests that beetles may engage in similar behaviors as they forage among plants in the wild. Future work in *Amianthium* should re-examine neighborhood-level neighbor effects in a larger population sample to fully confirm the present study's findings.

Neighbor effects may also impact male reproductive fitness by changing how pollinators move among plants, a possibility not explored here. Beetles in the feeding experiment increased their movements among flowers in the low focal-low neighbor context, suggesting that pollen transfer among

plants in neighborhoods may be enhanced in low-nectar neighborhoods. Previous work by Ott et al. (1995) also found that bumblebees engaged in more near-neighbor movements when they encountered low nectar variance among flowers and the overall nectar availability in the neighborhood was low. Such pollinator movement patterns could create spatial genetic structure in low-nectar patches if near neighbors also tend to be moderately related to one another, a pattern seen in *Echium vulgare* (Leiss et al. 2009). Previous work in *Amianthium* found moderate relatedness coefficients in neighboring plants via allozyme data (Joseph Travis personal communications). In the self-incompatible *Amianthium*, pollen transfer among relatives could increase the production of inviable seeds, reducing the fitness of both the male pollen donor and the female pollen recipient (Travis 1984). On the other hand, beetles moved very little among flower clusters across the other three neighbor contexts, indicating that pollen transfer among plants in neighborhoods may be lower when the overall nectar availability in the neighborhood is high. Future work should examine the effects of neighbor effects on male and female fitness in *Amianthium* and other plant-pollinator systems. Beetle behavioral data suggests that male fitness in *Amianthium* may experience associational effects: plants in low-nectar neighborhoods may suffer reduced fitness via higher levels of pollen transfer among genetic relatives.

Quantitative neighbor contrast effects may be important factors in the evolution of many traits where competitive ability in a local context shapes fitness in conspecific interactions or species interactions. In addition to exploring neighbor contrast effects on fitness in a wider variety of plant-pollinator, plant-seed disperser, and plant-herbivore mutualisms, researchers could expand the neighbor contrast framework of multilevel selection to other traits affecting competitive ability such as plant nutrient uptake efficiency (e.g., Stevens et al. 1995; Donohue 2003). Further, research on social and species interactions should carefully consider the behaviors of partners that may shape group-dependent fitness when making hypotheses about the operation of multilevel selection. Our work reveals that foragers can exhibit diverse, scale-dependent and context-dependent responses to spatial trait variation. Leading with natural history-driven hypotheses about the mechanisms of neighbor effects in diverse natural systems, we may discover an even wider array of neighbor effects operating on phenotypic selection in nature.

FIGURES AND TABLES

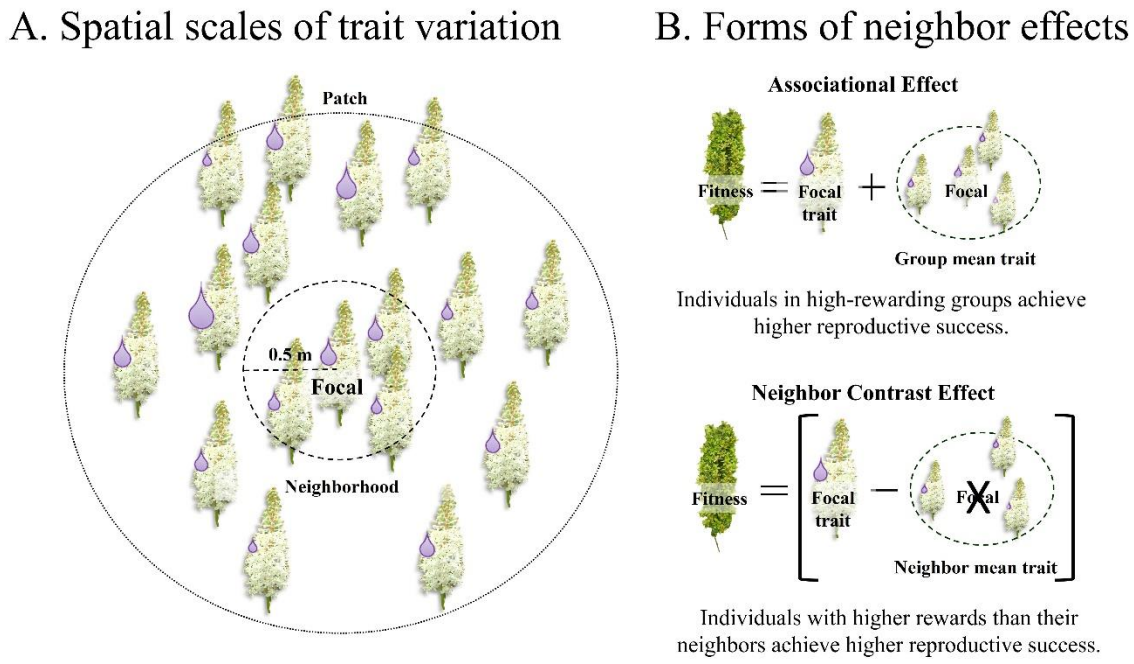

Figure 1. Summary schematic of the two forms of neighbor effects. Panel (A) shows the two spatial scales evaluated in the present study. Panel (B) summarizes the approach to calculating the associational (top) and neighbor contrast (bottom) forms of trait-mediated neighbor effects.

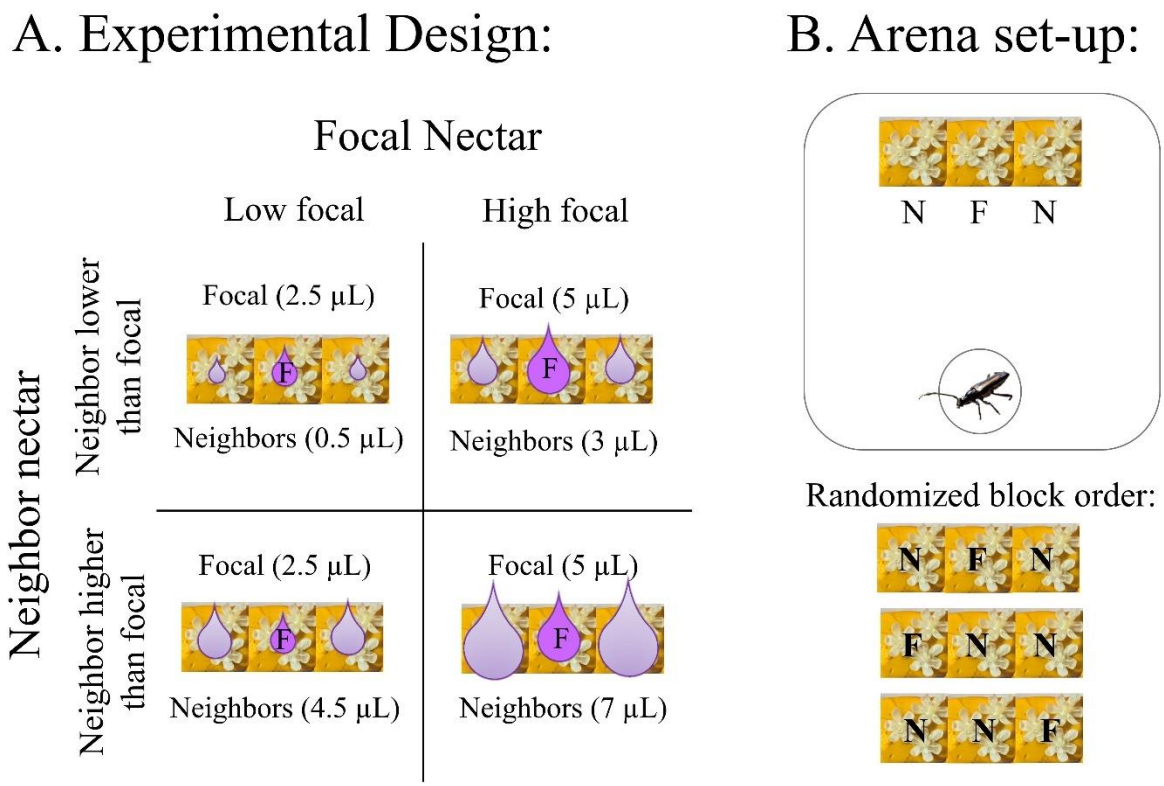

Figure 2. Design for beetle neighbor contrast effects experiment. Panel (A) summarizes the factorial design used in the feeding experiment. Beetles were presented with one of these four nectar trait combinations. All nectars used in this experiment had identical sugar concentrations (0.1 mg). Panel (B) shows the arena setup for beetle feeding trials including the beetle starting position in the bottom-middle of the arena (N = neighbor block, F = focal block). Block order was randomized from left to right across trials (focal individual can be to the left, to the right, or in between neighbors.

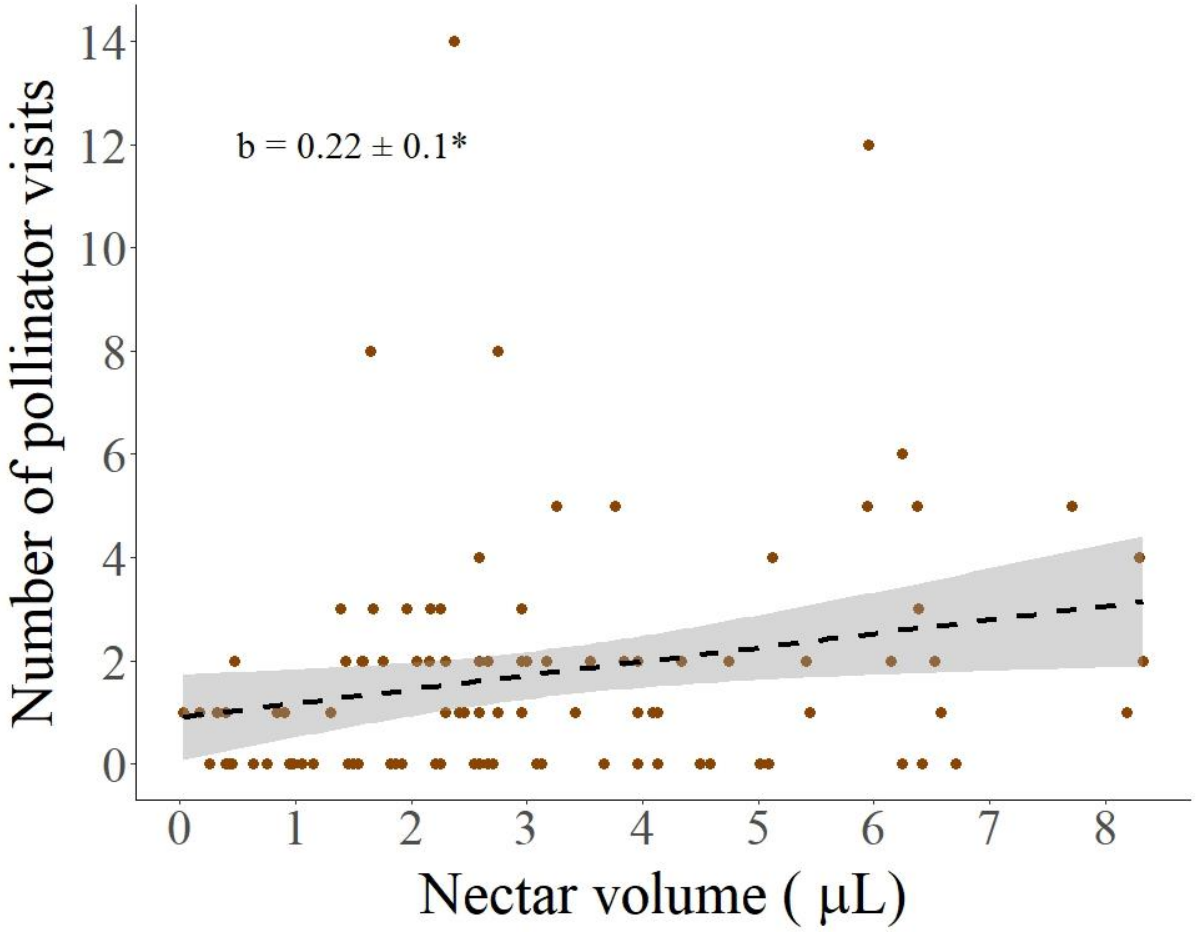

Figure 3. Beetle visitation responded to nectar volume variation. (A) Beetles visited plants with higher nectar volumes more frequently than they visited plants with lower nectar volumes. Partial regression slope drawn from the negative binomial family regression model: *number of pollinator visits ~ nectar volume + total sugar + number of active rows of flowers on inflorescence*. Significance level $p < 0.05$.

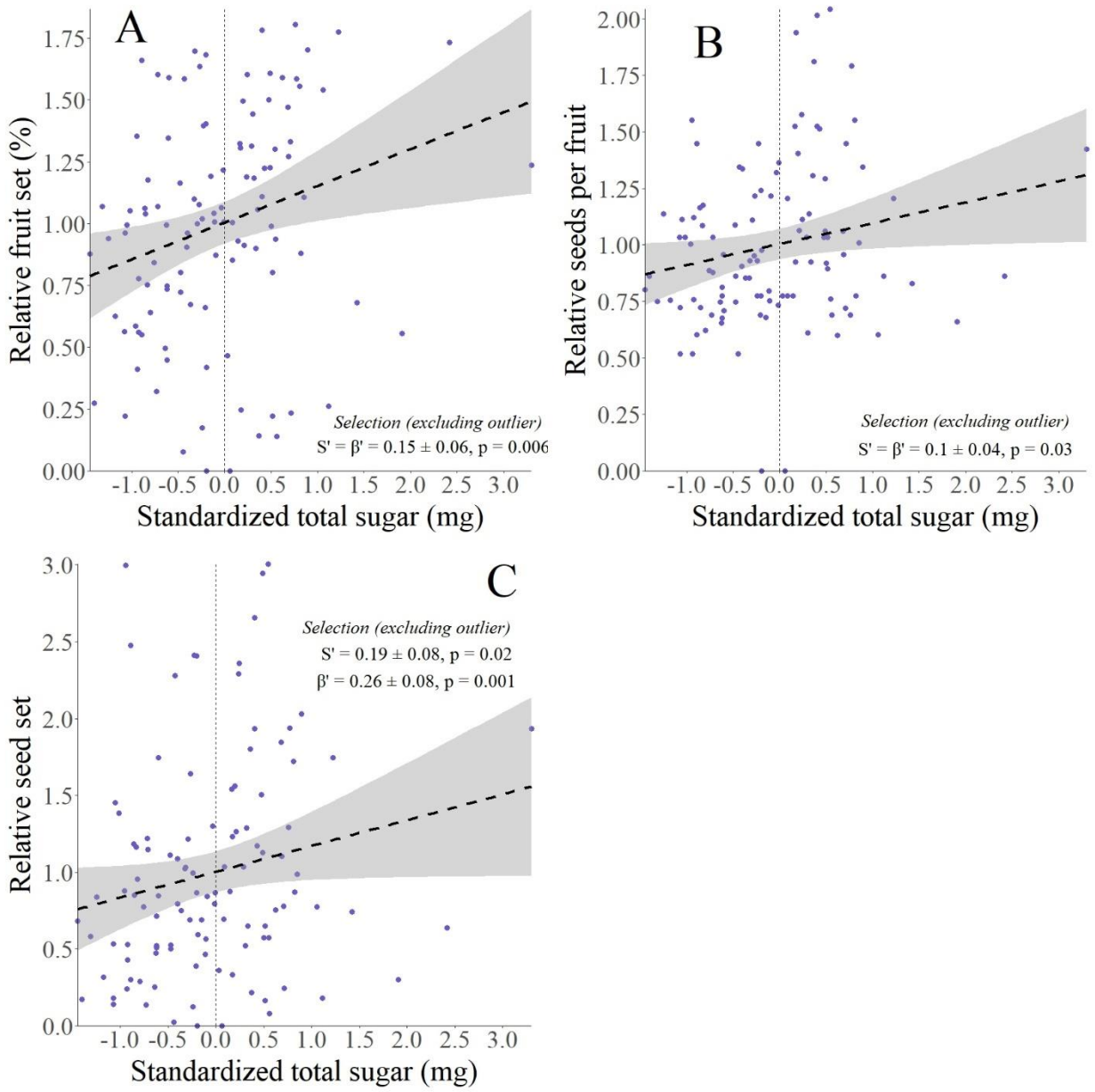

Figure 4. Individual total sugar experienced positive directional selection in *Amianthium muscaetoxicum* via all three measurements of reproductive fitness. Points represent individual plants. Regression lines are linear selection differentials with 95% confidence interval shading in gray. β' is estimated from the multiple regression model: fitness ~ total sugar + inflorescence size + (1|patch ID) + (1|remaining flower rows). Significance levels: $p < 0.05$ *, $p < 0.01$ **. Data visualization removes one outlier individual 3 standard deviations above the population mean total sugar value.

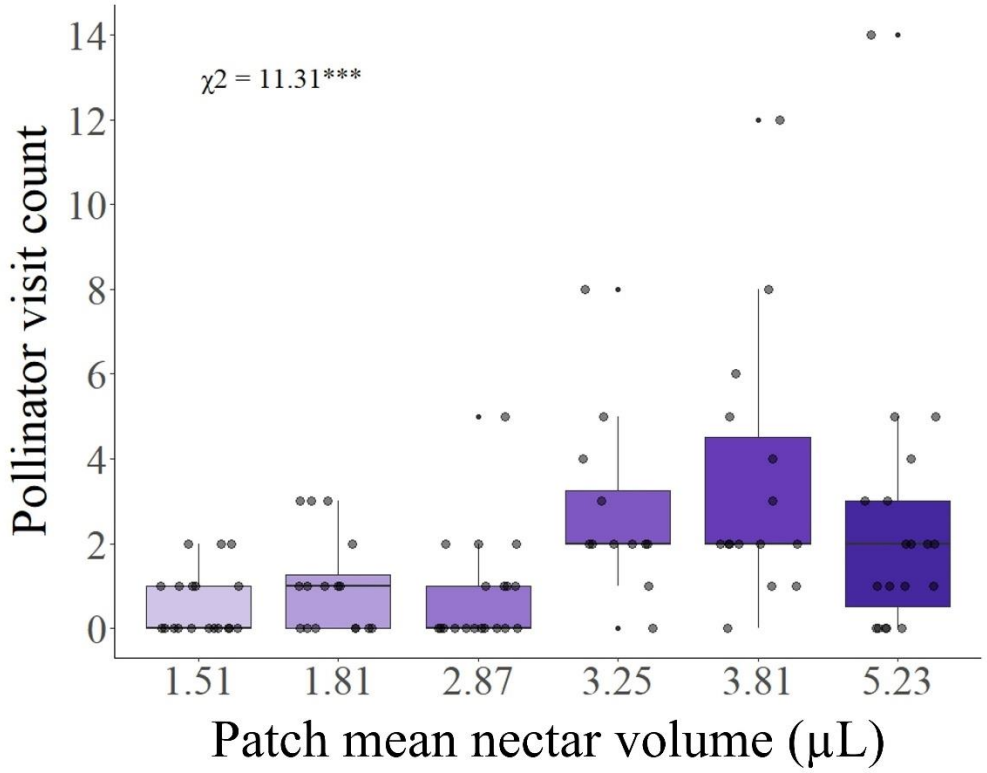

Figure 5. Patches with higher mean nectar volumes received a higher number of pollinator visits. Boxes encompass 1st quartiles, medians, and 3rd quartiles for each patch, and whiskers are minimum and maximum nectar trait values in each patch. Dots are raw visit counts for each plant in a patch. Darkening purple color = higher mean nectar volume. Chi-squared statistic from ANOVA test of model: *visit count* ~ *nectar volume* + *patch mean nectar volume* + (1| *flower rows left*).

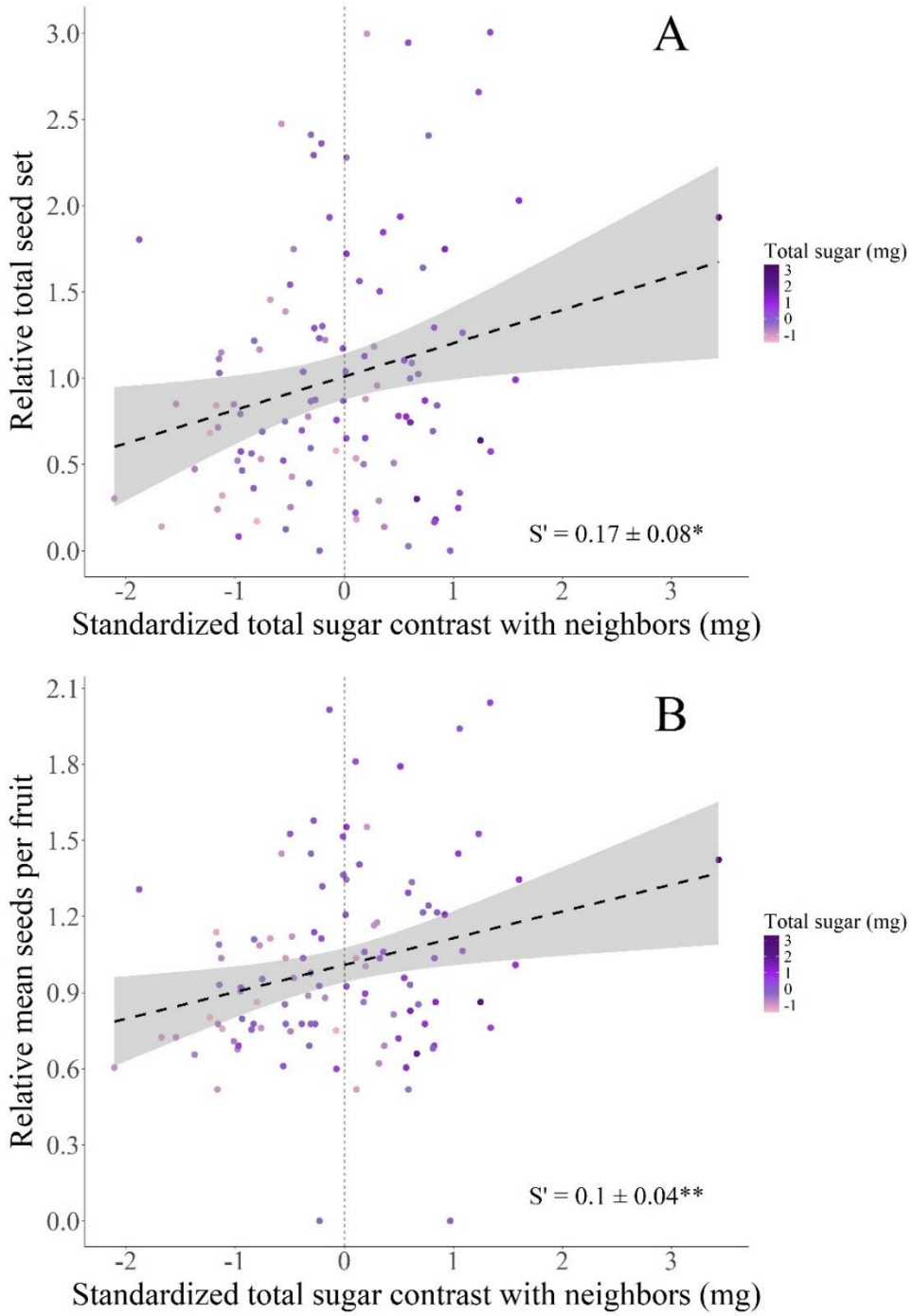

Figure 6. Neighbor contrast effects at the neighborhood scale shaped total plant seed set (A) and mean seed set per fruit (B). Plant individuals (points) are color shaded by their total sugar trait values (mg/μL nectar) relativized to the global population mean (z_i-$\bar{z}_{pop}$, darker color = higher total sugar relative to population mean). Panels A-B display least squares regression from GLMM models: *mean seed set per fruit ~ neighbor contrast total sugar + neighbor count* (A) and *predicted total seed set ~ neighbor contrast total sugar + neighbor count* (B). Dotted vertical lines denote standardized trait mean of 0. Estimates are standardized regression coefficients.

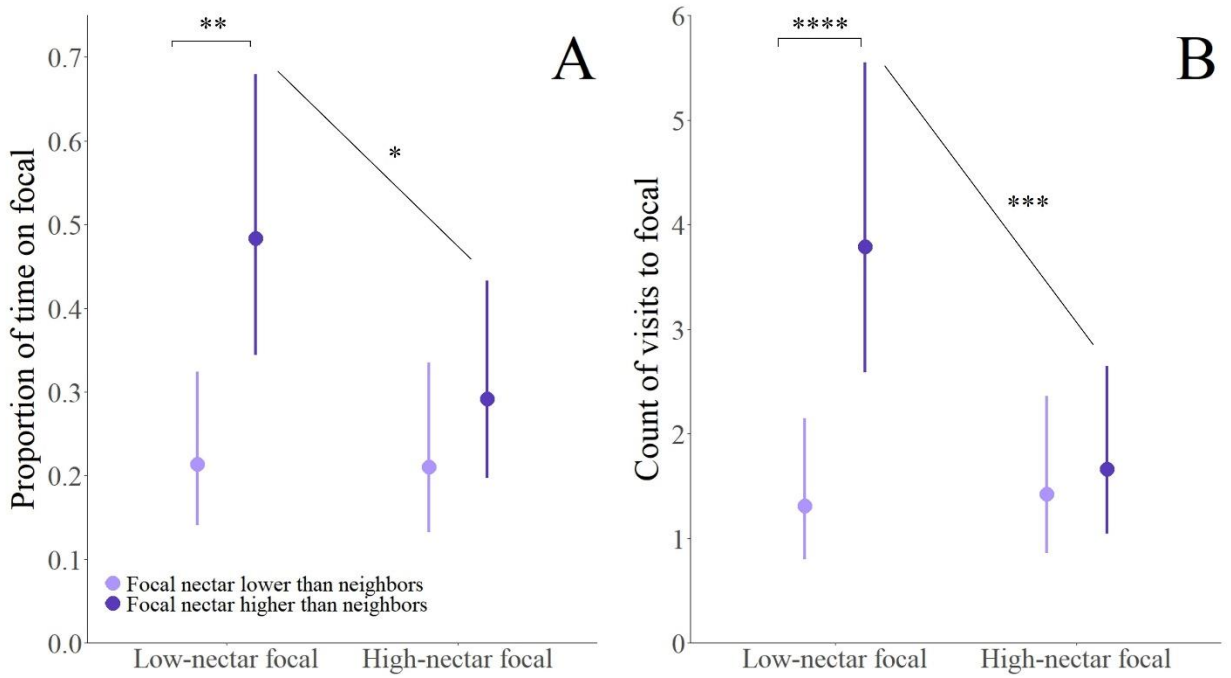

Figure 7. Beetles spent more time interacting with the low focal (A) and engaged in more repeat visits to the low focal (B) when that focal had higher nectar than its neighbors. Significance symbols depict post hoc contrasts of behavioral responses to each focal between its two neighborhood contexts (bracket), and behavioral responses to the two focals' neighborhood contexts (diagonal). Significance levels: $p < 0.05$ *, $p < 0.01$ **, $p < 0.001$ ***, $p < 0.0001$ ***. Full statistical outputs in Table 6.

Fitness component	*Trait*	$S' \pm SE$	χ^2	p	$\beta' \pm SE$	χ^2	p
Fruit set	Nectar volume	0.07 ± 0.04	2.65	0.1	**0.11 ± 0.05**	5.45	0.02
	Sugar concentration	0.06 ± 0.05	1.62	0.2	**0.1 ± 0.05**	4.11	0.04
	Total sugar	**0.12 ± 0.04**	8.23	0.004	**0.12 ± 0.04**	8.41	0.004
	Inflorescence size	0.02 ± 0.04	0.12	0.73	0.02 ± 0.04	0.21	0.65
Seeds per fruit	Nectar volume	0.06 ± 0.04	2.89	0.09	**0.07 ± 0.04**	3.94	0.05
	Sugar concentration	0.01 ± 0.03	0.08	0.78	0.03 ± 0.04	0.82	0.37
	Total sugar	**0.07 ± 0.03**	3.91	0.05	**0.07 ± 0.03**	4.25	0.04
	Inflorescence size	0.04 ± 0.03	1.51	0.22	0.05 ± 0.03	1.96	0.16
Total seed set	Nectar volume	0.01 ± 0.06	2.43	0.12	**0.21 ± 0.07**	10.13	0.001
	Sugar concentration	0.09 ± 0.06	2.17	0.14	**0.15 ± 0.07**	4.78	0.03
	Total sugar	**0.14 ± 0.06**	6.02	0.01	**0.17 ± 0.06**	9.8	0.002
	Inflorescence size	**0.27 ± 0.06**	18.72	1.5e-4	**0.29 ± 0.06**	22.03	2.7e-6

Table 1. Individual-level selection gradients (β') and differentials (S'). Selection gradients estimated from GLMMs and tested via ANOVA: Model 1) *fitness ~ nectar volume + sugar concentration + inflorescence size* and Model 2) *fitness ~ total sugar + inflorescence size*. Inflorescence size estimates taken from Model 2. Bolded estimates attain a significance level of $p < 0.05$.

PATCH-LEVEL VISITATION AND FITNESS

Fitness component	*Neighbor hypothesis*	*Trait*	*AICc*	χ^2	*p*
Pollinator visit count	Associational effect	Nectar volume + patch mean nectar volume	0.00	-	-
		nectar volume	1.51	0.49	0.48
		patch mean nectar volume	- 6.82	8.82	0.003
Fruit set	Associational effect	Total sugar + patch mean total sugar + patch density	0.35	-	-
		total sugar	-5.20	7.2	0.003
		patch mean total sugar	1.72	0.28	0.6
		patch density	1.45	0.55	0.46
Seeds per fruit	Associational effect	Total sugar + neighborhood mean total sugar + neighborhood density	0.00	-	-
		total sugar	-1.43	3.43	0.06
		neighborhood mean total sugar*	2.00	0.003	0.96
		neighborhood density	1.98	0.02	0.88
Total seed set	Associational effect	Total sugar + neighborhood mean total sugar + neighborhood density	0.00	-	-
		total sugar	-3.60	5.68	0.02
		neighborhood mean total sugar	0.80	1.19	0.28
		neighborhood density	0.10	1.94	0.16

Table 2. AIC single-term deletion comparisons of models evaluating the effect of individual and contextual traits on reproductive fitness components at the patch scale. Chi-squared tests and *p*- values taken from the full model summary. Terms that moderately improve model fit when dropped ($\Delta AICc \geq +2$) are starred. Terms that significantly predict a given fitness component via the full model ($p < 0.05$) are bolded. Neighbor contrast effects were not analyzed at the patch-scale due to extremely high correlations between patch-scale neighbor contrast traits and individual-scale traits ($r = 0.95$, $t = 33.31$, $p < 2.2e\text{-}16$).

NEIGHBORHOOD-LEVEL VISITATION AND FITNESS

Fitness component	*Neighbor hypothesis*	*Trait*	*AICc*	χ^2	*p*
Pollinator visit count	Associational effect	Nectar volume + neighborhood mean volume + neighborhood density	0.00	-	-
		nectar volume	1.91	0.90	0.71
		neighborhood mean volume	1.89	0.10	0.73
		neighborhood density	0.92	1.07	0.30
	Neighbor contrast effect	Nectar volume + neighbor contrast volume + neighborhood density	0.00	-	-
		nectar volume	1.90	0.09	0.76
		neighbor contrast nectar volume	1.51	0.49	0.49
		neighborhood density	1.52	0.47	0.49
Fruit set	Associational effect	Total sugar + neighborhood mean total sugar + neighborhood density	0.00	-	-
		total sugar	- 3.43	5.43	0.02
		neighborhood mean total sugar	1.82	0.18	0.67
		neighborhood density	1.68	0.32	0.57
	Neighbor contrast effect	Total sugar + neighbor contrast total sugar + neighborhood density	0.00	-	-
		total sugar	- 0.69	2.69	0.10
		neighbor contrast total sugar*	2.41	1e-4	0.99
		neighborhood density	1.77	0.23	0.63
Seeds per fruit	Associational effect	Total sugar + neighborhood mean total sugar + neighborhood density	0.00	-	-
		total sugar	-0.06	2.05	0.15
		neighborhood mean total sugar*	2.00	3e-4	0.99
		neighborhood density	1.79	0.20	0.65
	Neighbor contrast effect	Total sugar + neighbor contrast total sugar + neighborhood density	0.00	-	-
		total sugar*	2.00	8e-4	0.98
		neighbor contrast total sugar	-0.35	2.34	0.13
		neighborhood density	1.95	0.05	0.83
Total seed set	Associational effect	Total sugar + neighborhood mean total sugar + neighborhood density	0.00	-	-
		total sugar	-1.00	2.96	0.09
		neighborhood mean total sugar*	2.00	7e-4	0.98
		neighborhood density*	2.00	0.002	0.97
	Neighbor contrast effect	Total sugar + neighbor contrast total sugar + neighborhood density	0.00	-	-
		total sugar	1.70	0.31	0.58
		neighbor contrast total sugar	1.20	0.82	0.37
		neighborhood density	1.80	0.17	0.68

Table 3. AIC single-term deletion comparisons of models evaluating the effect of individual and contextual traits on reproductive fitness components at the neighborhood scale. Chi-squared tests and *p*-values taken from the full model summary. Terms that moderately improve model fit when dropped ($\Delta AICc \geq +2$) are starred. Terms that significantly predict a given fitness component via the full model ($p < 0.05$) are bolded.

Fitness component	*Spatial scale*	*Best-fit model terms*	*b ± SE*	χ^2	*p*	$\sigma^2 \pm SD$
Pollinator visit count	Patch		Associational Effect			
		Fixed effects				
		Nectar volume	0.05 ± 0.07	0.49	0.48	-
		Patch mean volume	0.35 ± 0.11	9.62	**0.002**	-
		Random effects				
		Unopened flower rows	-	-	-	3e-9 ± 5e-5
	Neighborhood		Individual model is best fit			
Fruit set	Patch		Individual model is best fit			
	Neighborhood		Individual model is best fit			
Seeds per fruit	Patch		Individual model is best fit			
	Neighborhood		Neighbor Contrast Effect			
		Fixed effects	*β′ ± SE*	χ^2	*p*	$\sigma^2 \pm SE$
		Neighbor contrast total sugar	0.09 ± 0.06	6.3	**0.01**	-
		Neighborhood density	0.01 ± 0.03	0.06	0.81	-
		Random effects				
		Patch ID	-	-	-	< 1e-10
		Unopened flower rows	-	-	-	< 1e-10
Total seed set	Patch		Individual model is best fit			
	Neighborhood		Neighbor Contrast Effect			
		Fixed effects	*β′ ± SE*	χ^2	*p*	$\sigma^2 \pm SE$
		Neighbor contrast total sugar	0.15 ± 0.06	6.3	**0.01**	-
		Neighborhood density	0.04 ± 0.06	0.43	0.51	-
		Random effects				
		Patch ID	-	-	-	0.003 ± 0.06
		Unopened flower rows	-	-	-	0.06 ± 0.25

Table 4. Summary of best-fit models according to *AICc* (Tables 2-4) for each performance measure or fitness component. 'Individual model is best fit' indicates that neither neighbor effects hypothesis significantly predicted variance in a given performance measure. Statistically significant fixed effects are bolded.

Beetle behavior	*Fixed effects*	*Estimate ± SE*	χ^2	*p*	$\sigma^2 \pm SD$
Proportion of time spent feeding on focal	Intercept	-1.27 ± 0.14	-	-	-
	Focal nectar	-0.18 ± 0.15	1.59	0.21	-
	Neighbor context	-0.29 ± 0.1	8.62	**0.003**	-
	Focal nectar*neighbor context	0.17 ± 0.14	1.52	0.22	-
	Random effects				
	Flower block order	-	-	-	0.003 ± 0.05
	Observer ID	-	-	-	0.007 ± 0.09
	Fixed effects	*Estimate ± SE*	χ^2	*p*	$\sigma^2 \pm SE$
Number of visits to focal	Intercept	0.62 ± 0.17	-	-	-
	Focal nectar	-0.26 ± 0.13	3.92	**0.05**	-
	Neighbor context	-0.32 ± 0.09	10.94	**0.0009**	-
	Focal nectar*neighbor context	0.32 ± 0.13	6.01	**0.01**	-
	Random effects				
	Beetle ID	-	-	-	9e-9 ± 10e-5
	Observer ID	-	-	-	0.06 ± 0.24

Table 5. Summary of GLMM models testing the effects of focal nectar, neighbor context, and the interaction between focal nectar and neighbor context, on beetle behaviors. Degrees of freedom for both models are 2 in the numerator and 153 in the denominator. Statistically significant fixed effects are bolded. Additional random effects (plant ID, repeat trial number) explained less than 1e-10 of the variance in behaviors in each model and were subsequently removed. Beetle ID explained less than 1e-10 of the variance in the proportion of time beetles spent feeding on focal (top), and flower block order explained less than 1e-10 of the variance in number of visits to focal (bottom).

Beetle behavior	*Nectar trait*	*Pairwise contrast for treatment*	*Odds ratio ± SE*	*t-ratio*	*p*
Time interacting with focal flowers	**Low-focal nectar**	**Focal > neighbor/ focal < neighbor**	0.44 ± 0.12	-3.13	0.002
	High-focal nectar	Focal > neighbor/ focal < neighbor	0.72 ± 0.21	-1.11	0.27
	Focal nectar higher than neighbor nectar	**Low focal/ high focal**	1.66 ± 0.42	2.0	0.05
	Focal nectar lower than neighbor nectar	Low focal/ high focal	1.01 ± 0.32	0.04	0.97
Count of visits to focal flowers	**Low-focal nectar**	**Focal > neighbor/ focal < neighbor**	0.35 ± 0.08	-4.45	<0.0001
	High-focal nectar	Focal > neighbor/ focal < neighbor	0.86 ± 0.24	-0.55	0.58
	Focal nectar higher than neighbor nectar	**Low focal/ high focal**	2.28 ± 0.52	3.62	0.0003
	Focal nectar lower than neighbor nectar	Low focal/ high focal	0.92 ± 0.27	-0.28	0.78

Table 6. Post hoc contrasts of focal and neighbor nectar combinations on beetle behaviors. Results are contrasts between estimated marginal means from GLMM models. Estimated using R package *emmeans*. Statistically significant contrasts are bolded.

Chapter 4: Selection on nectar traits is robust to environmental variation in the pollinator-dependent *Amianthium muscaetoxicum*

ABSTRACT

Environmental variation through time can cause temporal variation in the dynamics of phenotypic selection. Environmental change can alter phenotypic selection in three non-exclusive ways. Environmental factors that affect resource availability can change the variance in fitness, they can change the phenotypic distribution underlying selection via trait plasticity, and they can affect the relationship between traits and fitness, thus changing the magnitude and or/direction of selection on phenotypes. Environmental factors such as precipitation have the potential to alter all three dimensions of phenotypic selection on traits that respond plastically to environmental variation, such as floral nectar. In this study, we experimentally manipulated precipitation, an environmental factor we hypothesized could cause all three mechanisms of change in phenotypic selection on nectar traits, in replicate patches of the self-incompatible perennial *Amianthium muscaetoxicum*. We found that the direction of selection on nectar traits in *Amianthium*, including a multilevel component of selection, was largely robust to variation in the water environment despite water-induced trait plasticity of nectar traits and differences in the opportunity for selection among water environments. However, the magnitudes of direct and net selection on nectar volume and nectar total sugar content, as well as group selection on neighborhood mean total sugar content, were consistently stronger in the low-water environment, suggesting that the strength of selection may vary over time even if the direction of selection remains consistent in the face of environmental change.

INTRODUCTION

Environmental agents such as climatic factors, resource availability, and species interactions create phenotypic selection in natural populations. As components of the environment change, the targets and modes of selection may correspondingly vary. Indeed, studies that measure phenotypic selection across years often correlate changing selection gradients with environmental variation (Schemske and Horvitz 1989; Reimchen and Nosil 2002; Caruso et al. 2003; Campbell and Powers 2015). For example, temporal changes in the availability of a resource (Grant and Grant 1989; Siepielski and Benkman 2007), the abundance of a predator (Reimchen and Nosil 2002), or the community composition of a mutualist guild (Schemske and Horvitz 1989) correlate with changes in selection on phenotypes that mediate species interactions. While compelling, these studies cannot directly identify ecological causes of variation in selection, which is crucial for predicting the consistency or variability of selection across space and through time (Siepielski et al. 2009; Morrissey and Hadfield 2012). Determining the environmental causes of variation in phenotypic selection requires experimental manipulation of the hypothesized source (Wade and Kalisz 1990; Caruso et al. 2017).

There are three distinct, nonexclusive ways in which the environment could alter phenotypic selection. First, the environment can change both the mean and variance in fitness, thereby affecting the opportunity for selection. Second, the environment can directly alter the distribution of phenotypes exposed to selection via trait plasticity (Bradshaw 1965). Plasticity can alter the strength and form of selection acting on phenotypes through time purely by shifting the phenotypic distribution underlying the adaptive landscape (e.g., Steele et al. 2011). These first and second mechanisms can interact: environmental factors can inflate estimated covariances between phenotypes and fitness if the environment independently alters phenotypic expression (plasticity) and fitness outcomes (Scheiner et al. 2002; Stinchcombe et al. 2002). Third, the environment can alter the relationship between phenotypes and relative fitness by changing the activity of a selective agent. Shifts in the distribution of phenotypes via trait plasticity in different environments can also interact with the action of a selective agent, such as a partner species. For example, herbivore-induced plasticity in floral display size and plant height in

Brassica rapa caused bumblebee pollinators to visit disproportionately higher numbers of taller, many-flowered plants. (Dorey and Schiestl 2022). Hence, the environment could shape variation in phenotypic selection through multiple, potentially interacting, ecological mechanisms.

The interaction between plastic shifts in trait variation and the dynamics of phenotypic selection could be especially consequential for plastic traits that affect interactions with another species, such as floral nectar in the context of plant-pollinator interactions. Consumer species respond to spatial variation in their resources, producing local context-dependent patterns of species interactions (reviewed by Underwood et al. 2014). Many pollinators change their behavior in response to spatial variation in nectar traits at different scales (e.g., Pleasants 1981; Klinkhamer et al. 2001; Leiss and Klinkhamer 2005; Bruckman and Campbell 2014; Hegland 2014). Recent work by McPeek et al. (Chapter 3) in *Amianthium muscaetoxicum* suggests that such behavioral responses by pollinators can create multilevel selection on nectar traits. Specifically, plant fitness via female function was partially shaped by how their nectar traits contrasted with that of their local neighborhood, a form of neighbor effect termed the neighbor contrast effect after similar patterns in herbivory interactions (Bergvall et al. 2006). If environmental conditions reduce nectar production for all plants, this also shifts the absolute differences in nectar offered by flowers in groups, which could affect how pollinators forage on those groups. Changes in multilevel selection due to temporal environmental variation could be an underappreciated contributor to temporal variation in overall selection on nectar and other phenotypes that mediate species interactions.

Recent experiments have shown that one environmental variable, precipitation, is capable of all three sources of environmentally induced shifts in phenotypic selection on floral nectar traits. Many plant species set fewer seeds under extreme water limitation, lowering mean fitness (e.g., de Jong and Klinkhamer 1989; Klinkhamer et al. 1994; Galen 2000; Recart and Campbell 2021). Many plants also reduce nectar volume in response to water limitation, lowering overall resource availability for pollinators (e.g., Gallagher and Campbell 2017; Phillips et al. 2018; Rering et al. 2020; Suni et al. 2020; Kuppler and Kotowska 2021; García et al. 2023; Powers et al. 2024). Selection on floral and nectar traits can change under different water environments, though results vary widely among species. In *Ipomopsis aggregata*,

selection on nectar traits changed in response to the timing of snow melting but not to changes in summer precipitation (Powers *et al.* 2024). In the self-compatible *Ipomoea purpurea*, water limitation changed the variance in nectar volume and the sign of selection on volume from positive to negative, indicating a possible physiological cost of investing in nectar production when water resources are scarce (García et al. 2023). More studies are needed in self-incompatible systems that rely heavily on pollinators for reproduction, which may experience weaker or absent benefits of reducing nectar production under low-water conditions. Further, no studies have examined how water-induced changes in nectar production may affect aspects of multilevel selection on nectar traits (McPeek et al. Chapter 3).

In this study, we directly manipulated precipitation, an environmental factor we hypothesized could affect selection on nectar traits via all three mechanisms, in spatial replicates within a single flowering season in *Amianthium muscaetoxicum* (Melanthiaceae). *Amianthium* is a long-lived, partially dichogamous, self-incompatible perennial that grows in abundance in the forest understory at Mountain Lake Biological Station (MLBS) (Travis 1984; Palmer et al. 1989). *Amianthium* presents nectar for pollinators in large droplets and displays substantial among plant variation in both nectar quantity and sugar concentration (McPeek et al. Chapter 2). Previous work in this population found that a single precipitation event produced a three-fold decrease in variance in nectar volume and a nearly five-fold decrease in variance in sugar concentration. Previous work also detected positive directional selection on nectar volume, sugar concentration, and the composite measure of total sugar on female plant fitness (McPeek et al. Chapter 3). Data on seed production and pollinator behavior revealed focal trait-dependent multilevel selection on nectar traits (neighbor contrast effect). Plants with higher total sugar than their nearest neighbors produced more seeds, and beetle pollinators spent more time interacting with low-volume flowers that had more nectar than their neighbors than when those same low-volume flowers had less nectar than their neighbors (McPeek et al. Chapter 3).

We manipulated the water environment plants experienced by exposing patches of plants to either high- or low-water environments. We asked how this environmental change affected individual and multilevel selection on nectar traits in this population by 1) directly shifting the distribution of

phenotypes, 2) changing the opportunity for selection among water environments, and 3) changing the relationship between phenotypes and relative fitness. We predicted that selection on nectar traits would be weaker in the low water environment because lower trait variance would reduce pollinators' abilities to perceive differences in the quantity and quality of individuals' resources. We further predicted that this would result in a weaker or absent signal of multilevel selection in the low water environment.

METHODS

Field experiment methods: In summer 2024, we prepared seventeen patches of 12 to 36 plants for water manipulation: 9 high-water patches and 8 low-water patches, in a quarter-hectare plot within the White Pine/Moonshine Dell region of the woods. We randomly assigned these patches to either high- or low-water treatments so that both would include natural variation in soil types, moisture levels, vegetation density, and other environmental factors. Once plants reached bud stage, we constructed rain-exclusion shelters over our patches using iron t-posts staked into the ground as supports (Figure 1). We covered these frames with transparent plastic sheeting (Farm Plastics Supply, 4-Year UV Resistant Clear Sheeting) suspended between PEX piping. Any plants within 30 cm of a tarp edge were cut to eliminate the possibility that experimental plants could experience water dripping off the tarp sides.

Once a patch was covered, we begin watering every two days for the duration of the experiment using a Chapin 4-gallon backpack sprayer. Each plant in the high-water treatment received a concentrated spray of 200 mL water aimed directly at the shoot, and each plant in the low-water treatment received a 40 mL water spray to the shoot. In total, plants in our high-water treatment received 600- 800 mL of water per week, totaling 2.4-3.2 L received over the course of the 4 weeks of watering. Plants in the low-water treatment received one-fifth this amount, totaling 550-650 mL water over the course of the 4-week watering period. We determined these water levels by examining past weather station data from MLBS. The high-water environment is comparable to the level of precipitation MLBS received in July 2017 (20 cm) and June 2020 (19 cm); the two wettest summer months recorded in the past seven years. The low-water environment affords slightly more water than a plant would have experienced during the extreme drought conditions of summer 2023 (mean monthly rainfall = 3 cm), but less than plants would have

received in any prior summer since 2017 (mean monthly rainfall 2017-2022: 11.5 cm).

When plants neared flowering, we covered each plant with a mesh bag supported by a garden stake and labeled each individual with a unique ID flag. While plants were covered, we measured the distances between all plants in our patches to determine each plant's 50-centimeter neighbors, as in prior experiments (McPeek et al. Chapter 3). We sampled nectar from plants between June 18 and June 26. We only sampled plants the day after they had been watered. All plants received one full week of watering (four water applications) prior to nectar sampling. Nectar sampling and trait measurements followed procedures used by McPeek et al. (Chapters 2,3). We measured three nectar traits for each individual: nectar volume (μL) which is the total volume of nectar produced by each flower on a plant, as averaged across three flowers, sugar concentration (mg/μL), which is the concentration of sugar in that nectar converted from % BRIX to mg sugar per μL nectar, and total sugar (mg), which is the total sugar content of a flower (volume * sugar concentration). In previous work, each nectar trait component affected pollinator behavior. After sampling, we marked each plant's stem at the point where open flowers ended and new buds were opening (McPeek et al. Chapter 3).

We removed tarps after all patches had received a full month of watering and all plants in the patches had reached fruiting stage. Stopping watering at this point equalized the water levels patches received during the later stages of seed development, allowing us to isolate the effects of water on plants during the active flowering period. During the two-week period between tarp removal (July 15) and seed collection (July 26), MLBS received several rainstorms that contributed an additional 2.1 cm of rain (data from MLBS weather station). We collected all remaining inflorescences from the woods and placed inflorescences individually in water to maintain turgor pressure prior to seed collection.

We destructively counted the number of flowers on an inflorescence (hereafter inflorescence size), dividing counts between the portion of the inflorescence below the stem mark and the portion above the stem mark. We opened all fruiting flowers above these marks and counted the number of seeds in each. We collected three fitness measures: proportion of flowers above stem marks that set fruit (percent fruit set), mean number of seeds per fruit (mean seed set per fruit), and the estimated total seed set (percent

fruit set*mean seeds per fruit*inflorescence size) following methods established by McPeek et al. (Chapter 3).

Statistical analyses: We conducted all analyses and produced all figures in R version 4.3.3 (R Core Team 2024). We tested the fit of our models by examining the residuals using the *DHARMa* package (Hartig 2022). We built all mixed effects models in the *glmmTMB* package (Brooks et al. 2017).

We first tested whether our water manipulation significantly altered nectar trait distributions between the high and low-water treatments. We first used non-parametric Kruskal-Wallis tests to determine the effect of our watering treatment on nectar trait means and Levene's test for homogeneity of variance to determine whether watering treatments displayed significant differences in their trait variances. These initial tests ignored patch structure. To examine whether these effects changed when spatial structure was incorporated in the analyses of treatment-level differences, we constructed generalized mixed models (GLMMs) with the formula *nectar trait ~ treatment* + (1|*patch ID*) and fit models with a Tweedie model family with a log link function. We performed the same series of analyses on the three fitness measures to determine whether means and variances in plant fitness measures differed among water treatments.

We calculated standardized selection gradients on our nectar traits using linear mixed models (GLMMs). All these models included patch ID and the date on which nectar was sampled as random effects. Models of total seed set were fit with the Tweedie model family with a log-link due to the extreme right skew of the raw data. Fruit set and seeds per fruit fitness components were fit with a Gaussian model family. We variance-standardized floral traits (mean of 0 units in *SD*) and relativized fitness components (mean = 1) across the entire experiment, not by treatment (Lande and Arnold 1983). Relativizing fitness at the population-scale allows us to assess any potential differences in selection among treatments while also incorporating any potential differences in phenotypic distributions or fitness distributions that could be induced by the watering treatment.

We built models including nectar volume, sugar concentration, and inflorescence size as floral traits and a main effect of treatment to test whether the watering treatments independently affected fitness outcomes. To test whether selection on floral traits differed among water treatments, we included

interaction terms between each trait and the categorical watering treatment. We also estimated selection on total sugar for all fitness measures, replacing nectar volume and sugar concentration in models with the composite total sugar measure. We used ANCOVA to evaluate the effect of traits, treatments, and the interaction between trait value and treatment on each fitness measure. We reran models removing non-significant interaction terms and found no changes in the qualitative model outputs. We chose to report findings from the models including interaction terms in our results because changes in selection across treatments was an a priori hypothesis of the present study.

We also estimated selection differentials on each trait for each fitness component using models including only the standardized trait and random effects. After calculating individual selection at the population scale, we also returned and re-standardized traits and re-relativized absolute fitness within watering treatments. We used these treatment-standardized traits and treatment-relativized fitness components to estimate separate selection gradients and differentials for each water treatment using the same general model structure as the population-scale model.

We evaluated the potential for neighbor effects to create multilevel selection on fruit and seed production across water environments by repeating the contextual analysis approach used in our prior study (McPeek et al. Chapter 3). We calculated two forms of neighborhood nectar traits for each individual in our experiment: associational attraction (mean nectar trait of neighborhood including focal individual) and neighbor contrast attraction (focal trait – mean trait value of its neighbors). Forty-five individuals had no close neighbors and thus their neighbor contrast nectar traits and their global contrast nectar traits were perfectly correlated with one another. We excluded those individuals from neighbor effects analyses to deal with the statistical challenge of multicollinearity. Our model testing approach followed the procedure used by McPeek et al. (Chapter 3) for both total sugar and nectar volume. Briefly, we created full models including the neighborhood-level trait, the individual-level trait, and the neighborhood density for each of the two forms of neighbor effects. We included treatment as a fixed effect and patch ID and date of nectar sampling as random effects as we did in individual level selection models. We used Akaike's Information Criteria (*AIC*) to determine which of these two full models better

explained individual fitness measures, using the threshold of $\Delta AIC \geq 2$ as indicative of moderate support for one model over the other (Akaike 1973). We then used ANOVA tests on the best-fit model for each fitness component to determine whether contextual traits contributed to patterns of fitness, and whether these patterns differed among the two water environments. Where we detected a statistically significant effect of neighbor-level traits, we estimated standardized multivariate selection gradients and univariate selection differentials across the entire population and separately for each treatment, using the standardization protocols applied at the individual level.

RESULTS

Water significantly affected variance in nectar volume and sugar concentration: Experimental water manipulation significantly altered variation in nectar volume and sugar concentration among the two treatments (Figure 2A-B). The two water environments displayed significant differences in mean nectar volume (Kruskal-Wallis test, $\chi^2_{1,274} = 10.6$, $p = 0.001$) and variance in nectar volume (Levene test, $F_{1,274} = 4.86$, $p = 0.03$, Figure 2A). Water manipulation did not significantly alter mean sugar concentration among the two water environments (Kruskal-Wallis test, $\chi^2_{1,255} = 0.62$, $p = 0.43$), but the two water environments did display significant differences in variance in sugar concentration (Levene test, $F_{1,255} = 4.06$, $p = 0.05$, Figure 2B). These individual trait changes resulted in a marginally non-significant difference in mean total sugar (Kruskal-Wallis test, $\chi^2_{1,255} = 4.06$, $p = 0.07$), and no significant difference in the variance in total sugar between water environments (Levene test, $F_{1,255} = 0.1$, $p = 0.75$).

Excluding one extremely high nectar-producing individual (mean nectar volume = 11.88 μL) did not change qualitative results or significance levels. However, all significant differences in nectar trait distributions between water environments disappeared when patch ID was included as a random effect in the model structure (nectar volume: $\chi^2_{1,274} = 2.54$, $p = 0.11$, sugar concentration: $\chi^2_{1,255} = 0.25$, $p = 0.62$, total sugar: $\chi^2_{1,255} = 0.93$, $p = 0.33$), and patch ID explained moderate to large proportions of the variance in each trait ($\sigma^2 \pm SD$, nectar volume: 0.23 ± 0.48, sugar concentration: 0.11 ± 0.34, total sugar: 0.05 ± 0.21).

Water affected mean fitness, changing opportunity for selection: Water manipulation also

significantly altered variation in plant seed set, but not fruit set (Figure 2C-D). Plants in the high-water environment produced significantly more seeds per fruit, on average, than plants in the low-water environment (Kruskal-Wallis test, $\chi^2_{1,238} = 19.24$, $p = 1.15e\text{-}5$), but they did not experience significantly different levels of variance in seed set than plants in the low-water environment (Levene test, $F_{1,238} = 1.42$, $p = 0.23$, Figure 2C). Plants in the high-water environment also set a significantly higher percentage of fruits, on average, than plants in the low-water environment (Kruskal-Wallis test, $\chi^2_{1,238} = 9.12$, $p = 0.003$), but they experienced similar levels of variance in fruit set as plants in the low-water environment (Levene test, $F_{1,238} = 1.42$, $p = 0.23$, Figure 2D). These differences in mean seed set and fruit set among treatments resulted in significant differences in mean total seed set among water environments (Kruskal-Wallis test, $\chi^2_{1,238} = 15.43$, $p = 8.56e\text{-}5$), and no significant differences in the variance in total seed set among water environments (Levene test, $F_{1,238} = 2.92$, $p = 0.09$).

The disparity in mean fitness created a higher opportunity for selection in the low-water environment than in the high-water environment for all fitness measures: fruit set (low-water $I = 0.79$, high-water = 0.40), seeds per fruit (low-water $I = 0.28$, high-water = 0.24), and total seed set (low-water $I = 0.27$, high-water = 0.17). The effect of water environment on seed set measures held when spatial variation (patch ID) was accounted for (mean seeds: $\chi^2_{1,238} = 10.57$, $p = 0.001$, total seed set: $\chi^2_{1,255} = 4.52$, $p = 0.03$), but the effect of water environment on fruit set became marginally non-significant ($\chi^2_{1,238} = 3.21$, $p = 0.07$). Patch ID explained 0.07 ± 0.27, 0.18 ± 0.42, and 0.004 ± 0.2 proportion of the variance in mean seeds per fruit, total seed set, and fruit set, respectively.

Water did not change selection on nectar traits: Plants in different water environments experienced similar patterns of phenotypic selection (Table 1). Trait*water environment interactions were not significant contributors to any fitness measures. There was a main effect of water environment on all three fitness measures: plants in the low-water environment produced lower mean reproductive outputs via all three fitness measures than did plants in the high-water environment (Table 1). The same patterns held for selection gradients on total sugar separate from nectar volume and sugar concentration (Table 2).

All measured plant traits exhibited statistically significant directional selection gradients with respect

to at least two of the three fitness measures (Table 1, 2). Nectar volume, total sugar, and inflorescence size also experienced net selection via all three fitness measures, but sugar concentration experienced no net selection via any measure. While the direction of selection remained the same across environments, indicating no significant differences in selection between water environments, the strengths of direct and net selection differed between the two water environments (Table 3, Figure 3). Net selection on nectar volume was consistently strongest in the low-water environment (Figure 3A, D, G). Net selection on total sugar was stronger via a plant's fruit set in the low-water environment, but similar across environments with respect to seed set fitness measures (Figure 3B, E, H) Net selection on inflorescence size was consistent across water environments (Figure 3C, F, I).

Plants experience an associational effect via their fruit set fitness measure: Plants in neighborhood with higher mean total sugar set a significantly higher percentage of their fruits across the entire experiment (Table 4, Figure 4A). This pattern of associational effects held across both water environments, although the estimate of the effect was significantly stronger in the low-water environment than in the high-water environment (Table 5, Figure 4B). Otherwise, we detected no additional evidence of neighbor effects on any measure of reproductive fitness (Table 6).

DISCUSSION

Our experimental water manipulation changed the variance in both nectar volume and sugar concentration between the two water environments, highlighting the plasticity of nectar traits in response to environmental input. Changes in the water environment also created higher opportunity for selection in the low-water environment compared to the high-water environment, creating a main effect of water environment on seed set fitness measures. However, plastic shifts in nectar trait variance and shifts in the mean fitness across water environments did not result in substantial changes to the direction of selection on nectar traits across water environments. A pattern of multilevel selection via a plant's fruit set fitness measure also remained consistent across water environments. These results suggest that selection on nectar traits in *Amianthium* may be fairly robust to environmental changes, despite changes in both trait variance and reproductive fitness variance across environmental contexts.

Selection on *Amianthium* nectar traits remained significant and positive across water environments in the present study. In contrast, recent experimental manipulations in other flowering species reveal that water availability, soil nutrient availability, and herbivory can change selection on floral volatiles, flower size and number, and nectar production (Dorey and Schiestl 2022; García et al. 2023; Powers et al. 2024). In *Ipomoea,* water limitation changed the direction of selection on nectar volume, favoring lower nectar volumes under water stress (García et al. 2023). This change in the sign of selection likely results from *Ipomoea* being a self-compatible species and thereby experiencing a different balance of costs and benefits of nectar production than *Amianthium*. When physiological costs of nectar production outweigh the benefits from pollinators, we expect selection to favor reduced investment in nectar production (Pyke 1991; McPeek et al. 2021). We would not expect this response in the self-incompatible *Amianthium*, which requires pollinators to reproduce (Travis 1984). Selection for increased nectar volume was in fact consistently stronger in the low-water treatment, which may result from the high number of individuals with no seed production in the low-water treatment (relative fitness = 0). Underlying physiological responses to water availability could jointly affect nectar production and seed production, inflating their covariance due to a shared environmental response (Scheiner et al. 2002; Stinchcombe et al. 2002). Drought conditions can also limit a plant's ability to produce pollen, which could further lower pollen donation (e.g., Waser and Price 2016). The strong main effect of water environment on seed set fitness measures lends credence to this explanation. Performing a full-factorial manipulation of both water environment and pollen limitation would clarify the exact contribution of this environmental covariance to overall direct selection in this population (Caruso et al. 2019).

An alternative explanation for the lack of variation in selection is that our experimental manipulation did not create drastic enough variance shifts to see substantial changes in selection among our treatments. We conducted this experiment during an unusually hot, dry summer: MLBS received only 3.78 cm of rainfall during the entire four-week run of the experiment. These conditions could lead to more rapid loss of soil moisture and enhanced nectar evaporation (e.g., Villarreal and Freeman 1990; Plos et al. 2023), further dissipating levels of trait variation among plants. Natural differences in soil type across the woods

may have also contributed to uneven absorption of the watering treatment among patches. For example, some plants in one low-water patch produced nectar volumes upwards of six microliters, and some plants in two of our high-water patches produced almost no nectar (< 0.5 µL). These are inevitable consequences of working with existing natural variation in the wild and cannot be accounted for with the present design. Repeating this study in a year with different background climate conditions could clarify how much these unmanipulable elements of environmental variation affected the present study's findings.

While the dynamics of phenotypic selection were consistent across environments, certain patterns of selection in the present study differed from prior results in this *A. muscaetoxicum* population (McPeek et al. Chapter 3). We detected a much stronger, statistically significant signal of directional selection on inflorescence size in 2024 than we did in 2022, an overall wetter flowering season. Second, we detected no evidence that pollinators responded differently to focal individuals' nectar traits based on contrasts with traits of their close neighbors. Instead, we detected group selection that was independent of a focal individual's phenotype. Both patterns suggest that pollinators may have adjusted their foraging movements during the extremely dry season. If there was lower nectar availability across the entire population, pollinators may have concentrated their efforts on large inflorescences, where they would be more likely to encounter nectar in at least a few flowers. Work on seasonal variation in landscape-scale nectar resources corroborates this idea: foraging bumblebees displayed a strong preference for denser patches of flowers during times of year with lower nectar availability (Pope and Jha 2018). In years where nectar availability is higher, as was the case in 2022, inflorescence size may factor less into beetle foraging decisions, resulting in reduced or absent selection on floral display (McPeek et al. Chapter 3).

The presence of group-level selection on neighborhood mean total sugar further indicates that pollinators may have stayed and foraged for longer periods in areas where they encountered substantial resources, resulting in higher reproductive success for all individuals in those groups. In our previous work, we detected evidence of fine scale among-plant selectivity by pollinators for plants with higher total sugar (neighbor contrast effect) that was absent in the present study (McPeek et al. Chapter 3). Theory predicts that optimally foraging species should reduce their resource selectivity when those

resources are scarce (Stamps et al. 2005). In high-resource years, pollinators may be choosier about their resource options than they are in low-resource years, leading to enhanced interactions with the higher-rewarding individuals in small local areas. While work has documented changes in the form of neighbor effects across spatial scales (e.g., Moore et al. 2010; Emerson et al. 2012), the present study is, to our knowledge the first demonstration that the form of neighbor effects acting on a population can change over time. We suggest that such changes may be driven by shifts in the availability of resources in a habitat: neighbor contrast effects may dominate when population-level resources are abundant, and group attraction effects may appear when population-level resources are scarce. Hence, the lowest rewarding individuals may fare best in dry years when overall nectar availability is low, benefiting from spillover attention from higher producing neighbors (Klinkhamer et al. 2001; Leiss and Klinkhamer 2005).

A growing body of work demonstrates selection acting on nectar traits including volume, production rate, and sugar concentration in many plant species (e.g., Kulbaba and Worley 2012; Zhao et al. 2016; Mackin et al. 2021; García et al. 2023). However, the dynamic plasticity of nectar traits in response to environmental variation casts doubt on whether nectar traits would experience consistent selection that could translate into a substantial evolutionary response across multiple seasons (Boose 1997). Our experiment suggests that the dynamics of selection on nectar traits in *Amianthium* may be fairly robust to plastic changes in trait variance. Further, comparing selection gradients between the present study and a previous selection study that took place during a wetter year suggests that the overall dynamics of selection may remain consistent across seasons with different environmental backgrounds. That said, changes in selection on inflorescence size and group mean total sugar between these two studies suggest that seasonal variation in the environment may change pollinator behavior. As a long-lived perennial, *Amianthium* experiences substantial variation in precipitation over the course of many years of flowering. If selection on nectar traits were consistent across this time, as the present study suggests, then selection on nectar traits may translate into consistent cross-generational responses to selection. Future work in *Amianthium* should examine the genetic basis of nectar traits, and the genetic basis of nectar trait plasticity in response to water variation, to determine the magnitude of this evolutionary response.

FIGURES AND TABLES

Figure 1. Rain exclusion shelter setup in the woods at Mountain Lake Biological Station (MLBS).

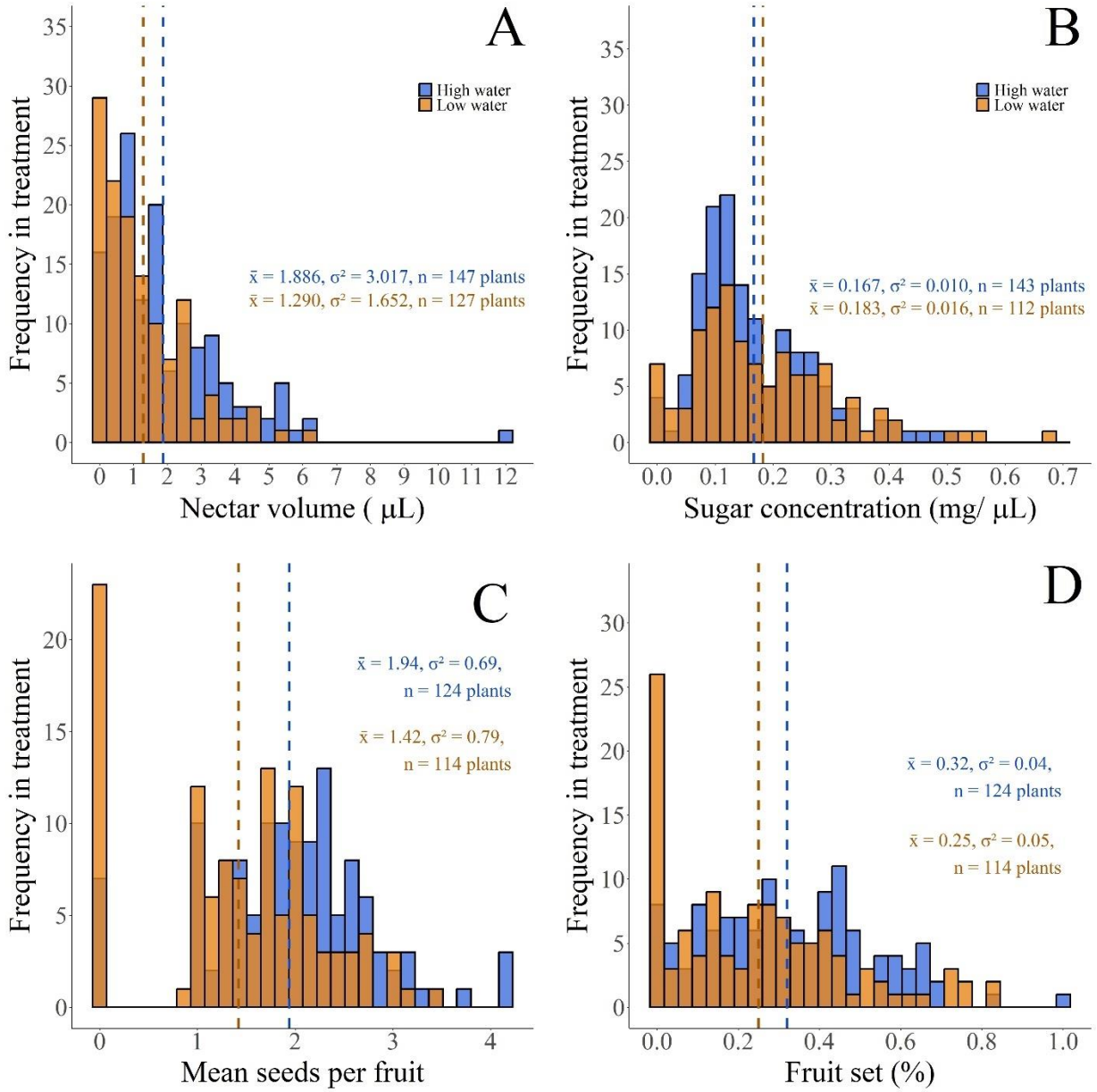

Figure 2. Water manipulation alters population-scale nectar trait variation (panels A-B) and female reproductive fitness components (panels C-D). Water treatments significantly differed in their mean nectar volume (A, $\chi^2_{1,274} = 10.6$, $p = 0.001$) but not in their mean sugar content (B, $\chi^2_{1,255} = 0.62$, $p = 0.43$). Water treatments significantly differed in both their mean seeds per fruit (C, $\chi^2_{1,238} = 19.24$, $p = 1.15e\text{-}5$) and their fruit set % (D, $\chi^2_{1,238} = 9.12$, $p = 0.003$) Vertical dashed lines mark the arithmetic mean trait of the population sample. Bars for the low-water environment are semi-transparent and overlap those of the high-water environment.

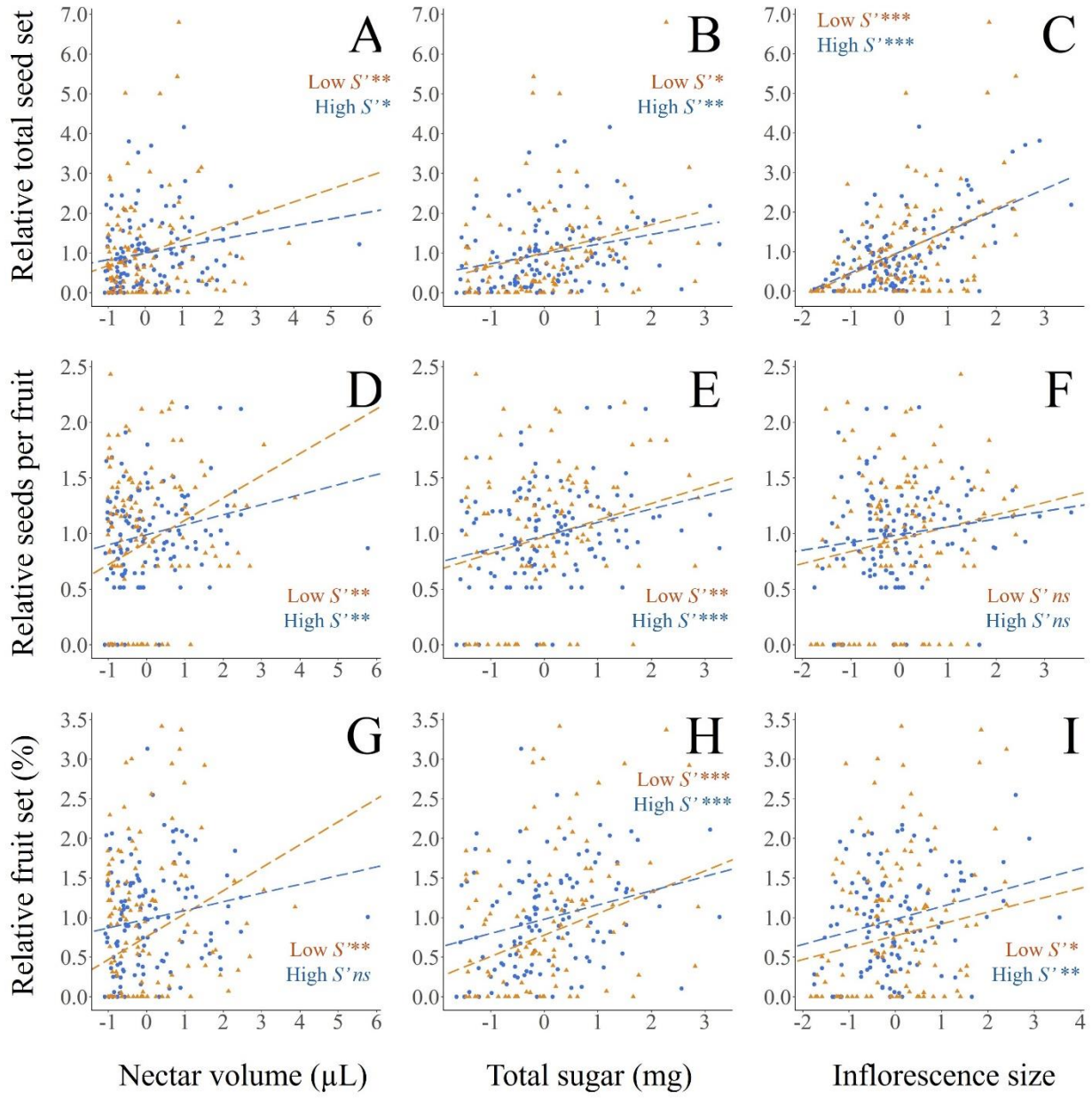

Figure 3. Standardized selection differentials for nectar volume (A, D, G) sugar concentration (B, E, H), and inflorescence size (C, F, I) do not significantly differ between high-water (blue) and low-water (orange) environments for any fitness measure. Points are plant individuals. Selection differentials are taken from regression models *treatment-relativized fitness measure ~ treatment-standardized trait* + (1| *patch ID*) + (1|*date nectar sampled*), estimated separately by water treatment. Significance symbols indicate the statistical significance level for each standardized selection differential (estimates and statistics in Table 3). Significance levels are $p > 0.05$ = no symbol, $p < 0.05$ = *, $p < 0.01$ = **, $p < 0.001$ = ***.

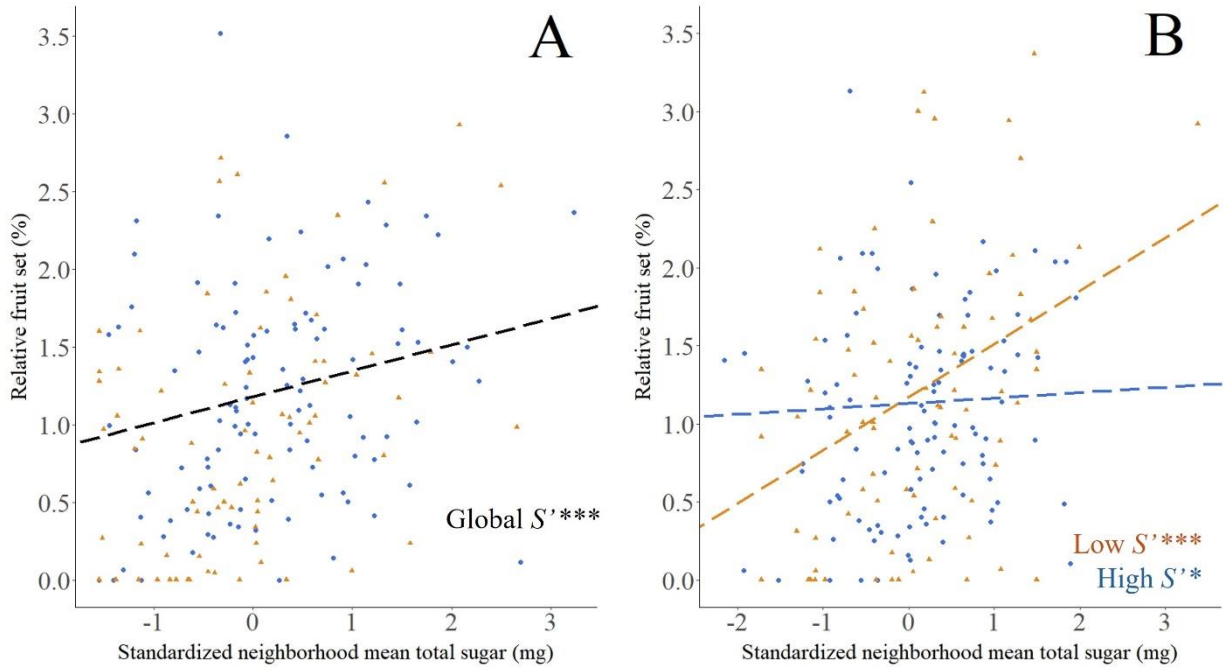

Figure 4. Plants in neighborhoods with higher mean total sugar set a higher percentage of their fruits. Panel (A) depicts the selection differentials for neighborhood mean total sugar across the entire population. Panel (B) shows the selection differentials for each water environment (traits and fitness standardized separately within each water treatment). Black solid line is the regression estimate across whole experiment, blue and orange dashed lines are the estimates for high and low-water environments, respectively. Points are plant individuals. N = 197 plants (75 low-water, 107 high-water). Significance symbols indicate the statistical significance level for each standardized selection differential (estimates and statistics in Table 5). Significance levels are $p < 0.05$ = *, $p < 0.001$ = ***.

Fitness measure	*Trait/Environmental variable*	*β' ± SE*	χ^2	*p*	*Variance ± SD*
Fruit set	Intercept	-	165.19	2e-16	-
	Nectar volume	**0.16 ± 0.04**	18.71	1e-5	-
	Sugar concentration	**0.08 ± 0.03**	6.49	0.01	-
	Inflorescence size	**0.15 ± 0.03**	31.08	2e-8	-
	Water treatment	-	4.19	0.04	-
	Nectar volume*treatment	-	1.32	0.25	-
	Sugar concentration*treatment	-	0.34	0.56	-
	Inflorescence size*treatment	-	0.10	0.75	-
	Random effects				
	Date sampled				0.01 ± 0.12
	Patch ID				0.03 ± 0.17
Mean seeds per fruit	Intercept	-	230.16	2e-16	
	Nectar volume	**0.15 ± 0.04**	13.17	0.0005	
	Sugar concentration	**0.10 ± 0.04**	6.81	0.009	
	Inflorescence size	**0.07 ± 0.03**	4.24	0.04	
	Water treatment	-	6.44	0.01	
	Nectar volume*treatment	-	0.26	0.61	
	Sugar concentration*treatment	-	1.18	0.28	
	Inflorescence size*treatment	-	0.007	0.94	
	Random effects				
	Date sampled				0.004 ± 0.06
	Patch ID				0.03 ± 0.17
Total seed set	Intercept	-	5.41	0.02	
	Nectar volume	**0.27 ± 0.08**	11.16	0.0008	
	Sugar concentration	0.10 ± 0.08	1.58	0.21	
	Inflorescence size	**0.50 ± 0.06**	73.92	> 2e-16	
	Water treatment	-	3.83	0.05	
	Nectar volume*treatment	-	1.94	0.16	
	Sugar concentration*treatment	-	0.01	0.92	
	Inflorescence size*treatment	-	0.52	0.47	
	Random effects				
	Date sampled				0.07 ± 0.27
	Patch ID				0.13 ± 0.36

Table 1. Individual-level selection gradients on nectar volume, sugar concentration, and inflorescence size do not differ among water treatments. Traits and fitness measures are standardized at the population-scale. Bolding indicates a statistically significant model term. N = 238 plants.

Fitness measure	*Trait/Environmental variable*	$\beta' \pm SE$	χ^2	*p*	$\sigma^2 \pm SD$
Fruit set	Intercept	-	188.30	2e-16	-
	Total sugar	**0.13 ± 0.03**	21.07	4e-6	-
	Inflorescence size	**0.15 ± 0.03**	32.66	1e-8	-
	Water treatment	-	5.11	0.02	-
	Total sugar*treatment	-	0.11	0.74	-
	Inflorescence size*treatment	-	0.18	0.68	-
	Random effects				
	Date sampled				0.01 ± 0.11
	Patch ID				0.02 ± 0.14
Mean seeds per fruit	Intercept	-	256.23	2e-16	-
	Total sugar	**0.12 ± 0.03**	14.43	0.0001	-
	Inflorescence size	**0.07 ± 0.03**	4.71	0.03	-
	Water treatment	-	7.48	0.006	-
	Total sugar*treatment	-	0.02	0.89	-
	Inflorescence size*treatment	-	0.02	0.89	-
	Random effects				
	Date sampled				0.003 ± 0.05
	Patch ID				0.20 ± 0.45
Total seed set	Intercept	-	5.96	0.01	
	Total sugar	**0.24 ± 0.06**	16.50	5e-5	-
	Inflorescence size	**0.50 ± 0.06**	74.63	< 2e-16	-
	Water treatment	-	4.64	0.03	-
	Total sugar*treatment	-	0.63	0.43	-
	Inflorescence size*treatment	-	0.39	0.53	-
	Random effects				
	Date sampled				0.06 ± 0.24
	Patch ID				0.11 ± 0.33

Table 2. Individual level selection gradients on total sugar and inflorescence size do not differ among water treatments. Traits and fitness measures are standardized at the population-scale. Bolding indicates a statistically significant model term. N = 238 plants.

Fitness measure	*Water treatment*	*Trait*	$S' \pm SE$	χ^2	*p*	$\beta' \pm SE$	χ^2	*p*
Fruit set	*Low*	Nectar volume	**0.29 ± 0.09**	10.40	0.001	**0.30 ± 0.10**	9.44	0.002
		Sugar concentration	0.08 ± 0.10	0.64	0.42	**0.10 ± 0.09**	1.28	0.26
		Total sugar	**0.27 ± 0.08**	12.10	5e-4	**0.24 ± 0.08**	9.99	0.002
		Inflorescence size	**0.15 ± 0.07**	4.15	0.04	0.11 ± 0.07	2.31	0.13
	High	Nectar volume	0.11 ± 0.06	3.21	0.07	**0.13 ± 0.06**	4.43	0.04
		Sugar conc.	0.02 ± 0.06	0.09	0.77	0.10 ± 0.06	2.37	0.12
		Total sugar	**0.18 ± 0.05**	11.08	9e-4	**0.17 ± 0.05**	10.57	0.001
		Inflor. size	**0.16 ± 0.05**	9.56	0.002	0.15 ± 0.05	7.73	0.005
Seeds per fruit	*Low*	Nectar volume	**0.20 ± 0.07**	8.31	0.004	**0.18 ± 0.07**	7.20	0.007
		Sugar conc.	0.03 ± 0.07	0.18	0.67	0.05 ± 0.07	0.58	0.45
		Total sugar	**0.15 ± 0.06**	7.45	0.006	**0.14 ± 0.06**	6.12	0.01
		Inflor. size	0.11 ± 0.06	3.54	0.06	0.07 ± 0.06	1.47	0.23
	High	Nectar volume	**0.09 ± 0.04**	7.05	0.008	**0.14 ± 0.04**	11.53	7e-4
		Sugar conc.	0.04 ± 0.05	0.72	0.40	**0.10 ± 0.04**	5.17	0.02
		Total sugar	**0.12 ± 0.04**	10.71	0.001	**0.11 ± 0.04**	9.70	0.002
		Inflor. size	0.07 ± 0.04	3.66	0.06	0.05 ± 0.04	1.82	0.18
Total seed set	*Low*	Nectar volume	**0.32 ± 0.12**	6.82	0.009	**0.35 ± 0.13**	7.76	0.005
		Sugar conc.	0.05 ± 0.13	0.13	0.72	0.11 ± 0.12	0.80	0.37
		Total sugar	**0.30 ± 0.11**	7.65	0.006	**0.25 ± 0.09**	7.17	0.007
		Inflor. size	**0.52 ± 0.09**	33.12	9e-9	**0.52 ± 0.09**	32.13	1e-8
	High	Nectar volume	**0.17 ± 0.08**	4.94	0.03	0.14 ± 0.08	3.44	0.06
		Sugar conc.	-0.03 ± 0.09	0.08	0.78	0.09 ± 0.09	0.97	0.32
		Total sugar	**0.25 ± 0.08**	10.18	0.001	**0.18 ± 0.07**	7.31	0.007
		Inflor. size	**0.45 ± 0.06**	51.37	8e-13	**0.45 ± 0.06**	50.88	1e-14

Table 3. Individual-level selection gradients (β') and differentials (S') estimated separately for each water environment. Selection gradients and differentials estimated from GLMMs and tested via ANOVA: Model 1) *fitness ~ nectar volume + sugar concentration + inflorescence size* + (1|*patch ID*) + (1|*date of nectar sampling*), and Model 2) *fitness ~ total sugar + inflorescence size* + (1|*patch ID*) + (1|*date of nectar sampling*). Inflorescence size β' taken from Model 1. Traits standardized within each water environment and fitness measures relativized within each water environment. Bolding indicates statistically significant estimate ($p < 0.05$). N low-water environment = 112 plants, N high-water environment = 124 plants.

Fitness measure	*Trait/Environmental variable*	*β' ± SE*	χ^2	*p*	*Variance ± SD*
Fruit set	Intercept	-	133.92	-	-
	Total sugar	**0.16 ± 0.07**	5.50	0.02	-
	Neighborhood mean total sugar	**0.17 ± 0.09**	3.88	0.05	-
	Neighborhood density	-0.09 ± 0.05	2.95	0.09	-
	Water treatment	0.05 ± 0.07	0.43	0.51	-
	Total sugar*treatment	0.03 ± 0.07	0.17	0.68	-
	Neighborhood mean total sugar*treatment	-0.13 ± 0.08	2.42	0.12	-
	Neighborhood density*treatment	-0.01 ± 0.05	0.01	0.93	-
	Random effects				-
	Date sampled	-	-	-	0.01 ± 0.11
	Patch ID	-	-	-	0.04 ± 0.21

Table 4. Plants experience an associational effect of neighborhood mean total sugar on individual fruit set. N = 197 plants. Traits and fruit set standardized at the population level. Bolding indicates a significant trait effect ($p < 0.05$).

Fitness measure	*Water treatment*	*Trait*	$S' \pm SE$	χ^2	*p*	$\beta' \pm SE$	χ^2	*p*
Fruit set	*Both*	Neighborhood mean total sugar	**0.29 ± 0.07**	18.44	2e-5	**0.17 ± 0.09**	3.88	0.05
		Total sugar	**0.24 ± 0.05**	20.74	5e-6	**0.16 ± 0.07**	5.50	0.02
	Low	Neighborhood mean total sugar	**0.52 ± 0.13**	15.53	8e-5	**0.34 ± 0.16**	4.72	0.03
		Total sugar	**0.34 ± 0.09**	14.45	1e-4	0.17 ± 0.12	1.92	0.17
	High	Neighborhood mean total sugar	**0.15 ± 0.07**	4.85	0.03	0.03 ± 0.09	0.16	0.69
		Total sugar	**0.18 ± 0.06**	9.43	0.002	**0.17 0.08**	4.86	0.03

Table 5. Group selection accounting for individual selection. Traits and fruit set are standardized globally (both) or within each treatment (low, high). Bolding of a neighbor hypothesis indicates a significant neighbor effect ($p < 0.05$). N = 75 low-water plants, 107 high-water plants.

Nectar trait	*Fitness measure*	*Neighbor hypothesis*	*Model structure*	*ΔAIC*	*Significant terms in model ($p < 0.05$)*
Total sugar	*Total seed set*	Associational effect	Total sugar + neighborhood mean total sugar + neighborhood density + treatment + total sugar*treatment + neighborhood mean total sugar*treatment + neighborhood density*treatment	38.46	None
		Neighbor contrast effect	Total sugar + neighbor contrast total sugar + neighborhood density + treatment + total sugar*treatment + neighbor contrast total sugar*treatment + neighborhood density*treatment	0.00	Total sugar
	Mean seeds per fruit	Associational	…	6.15	Total sugar
		Neighbor contrast	- - -	0.00	Total sugar
	Fruit set	**Associational**	…	0.00	**Total sugar, neighborhood mean total sugar**
		Neighbor contrast	- - -	5.72	Total sugar
Nectar volume	*Total seed set*	Associational effect	Nectar volume + neighborhood mean volume + neighborhood density + treatment + volume*treatment + neighborhood mean volume*treatment + neighborhood density*treatment	8.72	None
		Neighbor contrast	Nectar volume + neighbor contrast volume + neighborhood density + treatment + volume*treatment + neighbor contrast volume*treatment + neighborhood density*treatment	0.00	Nectar volume
	Mean seeds per fruit	Associational	…	1.93	Nectar volume
		Neighbor contrast	- - -	0.00	Nectar volume
	Fruit set	Associational	…	0.00	Nectar volume
		Neighbor contrast	- - -	1.11	Nectar volume

Table 6. AIC comparisons of models evaluating the effect of individual and contextual total sugar traits on reproductive fitness components at the neighborhood scale. … = model has same structure as the first listed associational effects model, - - - = model has the same structure as the first listed neighbor contrast effects model. Bolding of a neighbor hypothesis indicates a significant neighbor effect ($p < 0.05$).

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