Title

Moth Pollination in a Changing Climate: Illuminating Risks and Conservation Strategies in Pollination’s Darkest Hour

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Moth Pollination in a Changing Climate: Illuminating Risks and Conservation Strategies in Pollination's Darkest Hour

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by

Christopher T. Cosma

March 2024

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Dedication

This dissertation is dedicated to my parents, who instilled in me my love and wonder for the natural world; and to my beloved dog, Jasper, who taught me to treasure the time that we have with our loved ones.

ABSTRACT OF THE DISSERTATION

Moth Pollination in a Changing Climate:
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Christopher T. Cosma

Doctor of Philosophy, Graduate Program in Evolution, Ecology, and Organismal Biology
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Dr. Nicole E. Rafferty, Chairperson

Anthropogenic global climate change can disrupt plant-pollinator interactions by altering the traits, phenologies, and distributions of interacting species, exacerbating insect declines and compromising ecosystem function. However, most research has focused on diurnal pollinators, and little is known about the prevalence, importance, and vulnerability of nocturnal moth pollination. This knowledge gap limits our ability to predict and mitigate the effects of climate change and other stressors on moths and their pollination services. In this dissertation, I investigate the ecology of moth pollination interactions, how moths and their host and nectar plants will be impacted by climate change, and how to apply this knowledge in conservation strategies. I focus on native plants and moths in California, a biodiversity hotspot that is particularly impacted by climate change. I employ techniques ranging from greenhouse experiments to DNA metabarcoding to explore impacts spanning the levels of functional traits to ecological networks. In Chapter 1, I document hundreds of previously undescribed moth pollen-transport interactions along an elevational gradient spanning desert to conifer forest. I also find that moths are smaller, less diverse, and more sensitive to the simulated loss of their nectar plants in hotter and drier conditions. In Chapter 2, I reveal that experimental warming and drought alter diel patterns of floral nectar quantity and quality in a generalist plant. This may differentially affect interactions with diurnal and nocturnal pollinators, scaling up to alter the structure and stability of plant-pollinator interaction networks. In Chapter 3, I analyze and compare Lepidoptera-host and -nectar plant interaction networks across California, revealing structural differences and spatial patterns that inform management priorities. I also analyze species roles in networks to identify spatially-explicit keystone plant species to be used in butterfly and moth conservation efforts. Together, my results reveal that moth pollination interactions are diverse, complex, and vulnerable to climate change, and that data-driven conservation strategies can help protect them. Ultimately, this dissertation highlights the importance of considering the nocturnal components of plant-pollinator networks in research and management.

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Foreword

This dissertation comes during a great transition in Earth's history. It is clearer now than ever that we have entered a new epoch defined entirely by the influence of one species, *Homo sapiens*. When measured in geologic time, this new epoch, the Anthropocene, has only just begun. In 1760, at the onset of the Industrial Revolution, there were fewer than one billion humans on the planet. It took less than three centuries to reach our current eight billion. In that short amount of time—less than the average lifespan of a tree—humans have completely transformed the Earth. We have cleared 35% of the world's forests, dammed 60% of its rivers, and increased the concentration of carbon dioxide in the atmosphere by over 50%. While it is difficult to put a number on extinction, especially when so many species are still undescribed, these impacts have likely already wiped out hundreds of thousands of life forms. In fact, this current era has been given another name that is perhaps synonymous with the Anthropocene: the Sixth Mass Extinction.

A mass extinction event is characterized by a large proportion of Earth's species going extinct in a relatively short period of time. The five other mass extinctions in Earth's history were caused by global cataclysms ranging from volcanoes to asteroid impacts. This time, the cataclysm is us. In this dissertation, I address one aspect of the Sixth Mass Extinction—what has been dubbed, in turn, the Insect Apocalypse—and how anthropogenic global climate change is exacerbating it. And while I believe that the direness of the situation should not be underplayed, I also believe that the pervasive pessimistic narrative surrounding the biodiversity crisis, fueled by sensationalized news articles, shrouds a faint but much-needed silver lining.

Spending the past five years of my life immersed in the science of our dying planet has undoubtedly made it difficult at times to see any hope. However, I have also seen, and taken part in, the emerging solutions. I have seen students delight in the simple pleasure of witnessing a butterfly land on a flower they've planted. I have seen Western scientists work with Indigenous communities to repair broken relationships and to improve conservation outcomes. I have seen a new generation of youth standing up to the systems and the entities that bring both them and the planet down. And through it all, I have glimpsed on the horizon the faint glow of an essential cultural shift. It is a collective rethinking of our relationship with nature—a shift from parasitism to mutualism, and an acceptance of the responsibility that comes with it.

I put my hope in that old proverb: The darkest hour is just before the dawn. For although life on Earth may indeed be entering its darkest hour, I can see the light on the horizon. And into that new dawn, I believe that we can bring the knowledge that we need to better steward the only planet that we've got. It is my hope that this dissertation will contribute to this knowledge in some small way.

Introduction

2023 was the hottest year in recorded history. The global mean temperature was 1.4°C above the preindustrial average (GISTEMP Team 2024), and it is now inevitable that we will exceed the 1.5°C limit set by the Paris Climate Agreement (Ripple et al. 2023). 2023 also saw record heat waves across the globe, the worst fire season in Canada's history, and less ice form around Antarctica's coasts than ever recorded (Ripple et al. 2023). These are the impacts from centuries of reckless burning of fossil fuels, which has increased the concentration of carbon dioxide in the atmosphere from the preindustrial value of 280 ppm to over 420 ppm (Lan 2024). With each new broken heat record, with each new "1,000 year event", it becomes increasingly clear that anthropogenic global climate change is the greatest challenge that humanity has ever faced.

Rising death tolls and forced migrations due to heatwaves, floods, and other natural disasters put the spotlight on the direct effects of climate change on humans (Cattaneo et al. 2019; Lüthi et al. 2023). However, the indirect effects, while perhaps less conspicuous, are even more serious (Pecl et al. 2017; Díaz et al. 2019). Human health depends critically on the services provided by intact and biodiverse ecosystems (Daily 1997; Balvanera et al. 2006; Hernández-Blanco et al. 2022). These ecosystem services include water purification (Grizzetti et al. 2019), control of infectious diseases (Barbier 2021), and food production (Klein et al. 2007). Climate change, through its pervasive impacts across all levels of biological organization (Scheffers et al. 2016) and its synergies with other stressors (Brook et al. 2008), is compromising many of these services (Millennium Ecosystem Assessment 2005; Pecl et al. 2017; Bastien-Olvera et al. 2024). With a current extinction rate of up to 1,000 times the background rate (De Vos et al. 2015), at least a million species are threatened with extinction in the coming decades (IPBES 2019). The global collapse of life is eroding ecosystem integrity, and there is concern that we are approaching tipping points beyond which ecosystem functions cannot be recovered (Scheffer et al. 2012; Willcock et al. 2023; Flores et al. 2024). This anthropogenic destruction of the biosphere represents the destruction of our own life-support system—a planetary murder-suicide in the making. Thus, in the self-interest of our species, it is imperative to better understand and mitigate the effects of climate change on biodiversity. From a more pluralistic perspective, addressing these impacts is integral to solving interconnected social-environmental challenges and achieving a high quality of life for all of Earth's inhabitants—human or otherwise (Díaz et al. 2020).

Like humans, organisms across the tree of life are directly affected by climate change in many ways. Altered temperature and precipitation regimes impact organismal physiology and morphology, population demographics, species ranges and phenologies, and evolutionary processes (Walther et al. 2002; Parmesan & Yohe 2003; Williams & Jackson 2007; Pörtner & Farrell 2008; Scheffers et al. 2016; Hill et al. 2021). These direct effects have contributed to the declines and extinctions of numerous species worldwide (Foden et al. 2007; McMenamin et al. 2008; Adams et al. 2009; Trape 2009; Waller et al. 2017). However, the indirect effects of climate change on species—through disruptions to their ecological interactions—are even more formidable (Tylianakis et al. 2008; Cahill et al. 2013; Ockendon et al. 2014). The survival of every species on Earth depends on interactions with other species, ranging from antagonistic to mutualistic (Bascompte et al. 2006; Bronstein 2015; Andresen et al. 2018). There is ample evidence that climate change is disrupting ecological interactions, exacerbating species declines and extinctions (Tylianakis et al. 2008; Blois et al. 2013; Gérard et al. 2020). Indeed, interactions are often more sensitive than species themselves to environmental change, and can be lost before species go extinct (Tylianakis et al. 2008; Dunn et al. 2009; Valiente-Banuet et al. 2015). Moreover, ecological interactions influence species' vulnerabilities to climate change (Norberg et al. 2012; Bascompte et al. 2019), and interactions can modify the direct effects of climate change on species (Suttle et al. 2007; Post 2013; Forrest & Chisholm 2017; Rafferty et al. 2019). It is therefore essential to integrate species interactions into climate change research to better understand how biodiversity will be impacted. Since species interactions underpin the supply of ecosystem services (Kremen et al. 2007; Hector et al. 2009), this also provides a way to track how the impacts of climate change scale from species to communities and ultimately to the services that humans depend on (Montoya & Raffaelli 2010; Traill et al. 2010; Harvey et al. 2017).

Mutualisms—interspecific interactions in which both species receive fitness benefits (Boucher et al. 1982; Bronstein 1994)—are ubiquitous in nature (Bronstein 2015), and influence how species respond to climate change (Bascompte et al. 2019). Even if they are not themselves vulnerable to climate change, species may be threatened by the loss of their mutualists (Dunn et al. 2009; Aizen et al. 2012; Brosi et al. 2017). In this way, mutualisms may bind species to a common fate, accelerating and expanding the effects of climate change (Dunn et al. 2009; Kiers et al. 2010). Some of the best-studied mutualisms are those between plants and insects, which have played a central role in the generation and maintenance of life on Earth (Bronstein et al. 2006; Weber & Agrawal 2014; Peris & Condamine 2024). In particular, insect pollination—whereby insects transfer pollen from the male to the female sex organs of plants—has received considerable attention because it represents a globally important ecosystem service that is currently threatened by climate change and other stressors (Kremen et al. 2007; Potts et al. 2010; Vanbergen et al. 2013; Gérard et al. 2020). Approximately 88% of all flowering plant species depend in part on animal pollination to reproduce, and the majority of these services are provided by insects (Ollerton et al. 2011). These pollination services are responsible for 35% of human food crop production (Klein et al. 2007) and have an annual global value of up to US $387 billion (Porto et al. 2020). Thus, mounting evidence for pollination deficits in both wild (Biesmeijer et al. 2006; Acoca-Pidolle et al. 2023) and agricultural plants (Garibaldi et al. 2016; Reilly et al. 2020) signals a dire threat to ecosystem function and human food security (Vanbergen et al. 2013; Smith et al. 2015, 2022; Potts et al. 2016).

Global insect decline drives pollination deficits (Biesmeijer et al. 2006; Garibaldi et al. 2016; Reilly et al. 2020; Acoca-Pidolle et al. 2023). Despite significant spatial and taxonomic heterogeneity in insect population trends (Wagner et al. 2021a), recent reports, mainly from temperate countries, suggest an average global abundance decline for terrestrial insect taxa in the range of 1-2% per year (Conrad et al. 2006; Dirzo et al. 2014; Hallmann et al. 2017; Wepprich et al. 2019; van Klink et al. 2020; Forister et al. 2021; Wagner et al. 2021b). These declines are driven by the interacting effects of habitat loss, climate change, pesticide use, invasive species, and other anthropogenic stressors (Wagner et al. 2021b). Insect decline threatens the many ecologically, economically, and culturally important ecosystem services that insects provide, including pollination (Wilson 1987; Losey & Vaughan 2006; Potts et al. 2010; Basset & Lamarre 2019). However, insect decline is both a cause and a symptom of disrupted plant-pollinator interactions. The fitness of at least 352,000 arthropod species worldwide is tied to the floral resources that they gather while pollinating plants (Bowers 1986; Scaven & Rafferty 2013; Wardhaugh 2015). Climate change can disrupt plant-pollinator interactions by driving asynchronous range and/or phenological shifts in interacting partners, or by altering physiological and/or morphological traits that mediate the interaction (Scaven & Rafferty 2013; Gérard et al. 2020). These mismatches can have fitness consequences for both plants and pollinators (Ogilvie et al. 2017; Schenk et al. 2018; Kudo & Cooper 2019; de Manincor et al. 2023), emphasizing the interdependence of plant and pollinator conservation in the era of climate change.

The majority of pollination research and pollinator conservation efforts have focused on bees and other diurnal pollinators (Macgregor & Scott-Brown 2020; Cox & Gaston 2024). In contrast, moths, the dominant nocturnal pollinators, have been greatly understudied (MacGregor et al. 2015; Macgregor & Scott-Brown 2020; Buxton et al. 2022). This reflects the entrenched bias in biological research for studying diurnal species and phenomena (Gaston 2019), and is highly problematic for several reasons. First, with approximately 160,000 species worldwide, moths are one of the most diverse groups of organisms on the planet (Kristensen et al. 2007)—notably encompassing 10 times more species than butterflies, despite the inordinate focus on the latter (Young 1997; Goldstein 2017). In fact, moths are the single most diverse group of pollinators: with over 123,000 species likely visiting flowers, they are over seven times more species-rich than pollinating bees (Ollerton 2017). With mounting evidence of their global importance to both wild and agricultural plants (MacGregor et al. 2015; Hahn & Brühl 2016; Macgregor & Scott-Brown 2020; Buxton et al. 2022), studies have shown that moths provide unique functions compared to other pollinators—transporting pollen longer distances (Barthelmess et al. 2006; Skogen et al. 2019; Lewis et al. 2023), and pollinating certain plant species more efficiently (Anderson et al. 2023). Moreover, moths are critical components of terrestrial food webs: in their roles as herbivores and as prey for birds, bats and other animals, moths transfer more energy from plants to other organisms than all other herbivores combined (Janzen 1988; Wagner et al. 2021a).

In addition to their clear importance in ecosystems, moths make an excellent study system in climate change research because they possess a variety of traits with known climate sensitivities (Hill et al. 2021). For this, butterflies and moths are recognized as useful indicator taxa (Thomas 2005), and their responses to climate change are well-characterized at multiple biological levels—from their genes to their interactions with other species (reviewed in Hill et al. 2021). For example, due to their coevolved relationships, Lepidoptera are particularly affected by shifts in the availability and quality of their host plants (Altermatt 2010; Pelini et al. 2010). Since the majority of adult moths are nectarivores (Krenn 2010), it is likely that moths are also negatively impacted by climate change-driven reductions to or mismatches with their floral nectar resources. While these topics have not been investigated in moths, they are known drivers behind butterfly declines (Wallisdevries et al. 2012; Inamine et al. 2016). Nevertheless, moths are declining globally in response to multiple anthropogenic stressors, with the direct and indirect effects of climate change playing a major role (Conrad et al. 2006; Fox 2013; Fox et al. 2014; Young et al. 2017; Boyes et al. 2021; Blumgart et al. 2022). Since moth declines represent the loss of both a large portion of biodiversity, and important ecosystem functions and services, conserving them should be a top priority. However, moths are often left out of insect conservation programs, in part because they are predominantly recognized as agricultural pests (New 2004; Macgregor & Scott-Brown 2020). Expanding the understanding of their importance as pollinators is a potentially effective way to galvanize support for their inclusion into pollinator conservation programs (Hart & Sumner 2020). Information on moth pollination interactions can in turn guide conservation efforts, such as restoring key nectar sources (Williams et al. 2015; Alison et al. 2017).

Research on plant-animal mutualisms has historically focused on interactions involving only a few interacting partners (Boucher et al. 1982; Faegri & Van Der Pijl 2013). Likewise, most historical knowledge of moth pollination came from highly specialized, coevolved interactions such as the yucca-yucca moth relationship (Pellmyr & Huth 1994). While such studies helped shape our current understanding of mutualism, the lack of research on systems involving multiple interactions limited our knowledge of how species interactions operate in the community context (Waser et al. 1996). In the past few decades, studies of ecological networks—which describe interactions across entire communities—have revolutionized our understanding of the ecological and evolutionary processes determining community structure and stability (Jordano 1987; Waser et al. 1996; Memmott et al. 2004; Bascompte et al. 2006; Thompson 2006; Bascompte & Jordano 2014). These studies have revealed that plant-pollinator networks are relatively generalized (Waser et al. 1996), and display common structural features, including asymmetric specialization (Vázquez & Aizen 2004), nestedness (Bascompte et al. 2003), and modularity (Olesen et al. 2007). This "Architecture of Biodiversity" (Bascompte & Jordano 2007) determines species persistence, community stability, and ecosystem function (Memmott et al. 2004; Bascompte et al. 2006; Rohr et al. 2014; Saunders & Rader 2019; Bartomeus et al. 2021). Because of their ability to account for complex direct and indirect effects, ecological networks have also greatly improved our ability to predict and mitigate the impacts of environmental change on ecological communities and ecosystem services (Memmott et al. 2004; Tylianakis et al. 2008; Lavergne et al. 2010; Bascompte et al. 2019).

Recent network studies have started to reveal that moths, like diurnal pollinators, are relatively generalist pollinators of entire plant communities, including agricultural species (Banza et al. 2019; Macgregor et al. 2019; Walton et al. 2020; Buxton et al. 2022). However, most of our understanding of how plant-pollinator networks will be impacted by global change comes from diurnal networks, and even the basic structure of nocturnal moth pollination networks remains poorly understood (Macgregor & Scott-Brown 2020). While studies focused on diurnal taxa have revealed that nestedness and temporal interaction plasticity make plant-pollinator networks remarkably resilient to disturbances (Memmott et al. 2004; Kaiser-Bunbury et al. 2010; Thébault & Fontaine 2010; Aizen et al. 2012), there is some evidence that moth pollen-transport networks may lack these features, perhaps rendering them more sensitive (Ellis et al. 2023; Ho & Altermatt 2023). To improve predictive power, it is important to employ methods that elucidate the processes through which climate change will impact ecological networks (Woodward et al. 2010; Byers 2017; Valdovinos 2019). For example, because species functional traits determine their contributions to ecosystem function and their responses to environmental change, analyzing functional traits provides a lens through which to understand the effects of climate change at multiple biological levels (Díaz et al. 2007; Schleuning et al. 2015, 2020; Cantwell-Jones et al. 2024). Additionally, while most network studies are confined to a single place and time and focus on one interaction type, analyzing spatiotemporal variation and integrating multiple interactions (e.g., pollination, herbivory) can help untangle the processes governing species persistence and community stability (Burkle & Alarcón 2011; Byers 2017; Tylianakis & Morris 2017; Garcia-Callejas et al. 2018; Pellissier et al. 2018; Hutchinson et al. 2019).

In this dissertation, I explore the impacts of climate change on moth pollination, combining analyses of functional traits with investigation of how networks vary through space and time, and between different interaction types. My fundamental objectives are to (1) improve our understanding of the basic ecology of moth-nectar plant interactions, (2) investigate how climate change will impact them, and (3) apply this knowledge to help mitigate the impacts of anthropogenic stressors on moths and their ecosystem services. To achieve these goals, I use a variety of techniques in the field, lab, and greenhouse aimed at elucidating patterns and processes at multiple spatial, temporal, and biological scales. Additionally, I address questions from both the plant and pollinator perspective, leveraging results to inform biodiversity conservation. I focus on native plants and moths in the Southwestern US, a climate change hotspot (Diffenbaugh et al. 2008; Zhang 2023). The mean annual temperature in the region is already 0.89°C above the historical average, and it is expected to rise another 2.7-4.8°C by 2100 (Vose et al. 2017). The frequency, duration, and intensity of droughts and extreme precipitation events in the region are also increasing (Mazdiyasni & AghaKouchak 2015; Zhang et al. 2021; Zhang & Gillies 2022). The xeric ecosystems of the Western and Southwestern US are especially vulnerable to these changes (Huang et al. 2017), and both plant (Fettig et al. 2019) and insect (Forister et al. 2021) populations are declining. While the region is home to several iconic examples of specialized moth pollination systems (e.g., the yucca-yucca moth relationship: Pellmyr & Huth 1994), community-level moth pollination has not been explored in the Southwestern US. However, nocturnal pollination may be especially prevalent in this region, as more plant species flower and produce rewards at night in xeric ecosystems as an adaptive strategy to avoid water loss (Borges et al. 2016).

In Chapter 1, I use light trapping and pollen DNA metabarcoding to characterize moth pollen-transport networks along an elevational gradient. In addition to expanding our knowledge of the structure and spatiotemporal variation of moth pollen-transport networks, the natural experiment provided by the elevational gradient enables us to examine the impacts of climatic factors on moth diversity, body size, and network stability. In Chapter 2, I subject a plant that is visited by both diurnal and nocturnal pollinators to experimental warming and drought in a greenhouse to explore how climate change alters 24-hour patterns of nectar quantity and quality. This experiment sheds light on a mechanism through which climate change may disrupt plant-pollinator interactions at diel timescales, potentially reshuffling the interactions between plants and different suites of pollinators (e.g., diurnal and nocturnal). Finally, in Chapter 3, I construct a statewide database of Lepidoptera-host and -nectar plant interactions in California involving both moths and butterflies, and compare network structure through space and between the different interaction types. I also analyze species roles in networks to identify habitat-specific keystone plant species to improve Lepidoptera conservation and to increase the resilience of plant-pollinator communities to climate change and other stressors. While these chapters provide novel insights into an important, vulnerable, and understudied group of pollinators, they also have significant implications for other groups of pollinators. Indeed, my results reinforce the growing understanding that rather than being isolated, diurnal and nocturnal pollination networks, and their responses to climate change, are connected through shared plant species (Knop et al. 2018; García et al. 2024). Therefore, this dissertation contributes to a more holistic understanding of the ecology and climate change-vulnerability of pollinator communities, and advances conservation strategies to protect the ecosystem services they provide.

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Chapter 1

Variation in moth pollen-transport networks along an elevational gradient reveals potential climate change impacts

Abstract

Climate change affects insects, plants, and their interactions, threatening essential ecosystem services such as pollination. While the historical focus of moth pollination was on highly specialized interactions such as the yucca-yucca moth relationship, there is mounting evidence that moths are important nocturnal pollinators of entire plant communities. However, a strong diurnal bias in pollination ecology has resulted in a dearth of knowledge about the composition, structure, and stability of moth pollen-transport networks. This limits our ability to predict and mitigate the effects of climate change and other stressors on moths and their pollination services. Here, we sampled moth communities with light traps along an elevational gradient spanning desert to conifer forest in Southern California and built moth pollen-transport networks via pollen DNA metabarcoding. We analyzed how moth diversity, size, and pollen-transport interactions varied in relation to monthly and multi-decadal climate conditions and floral resources along the gradient. We found that moth diversity was positively correlated with greater short and long term precipitation, and that moth size was negatively correlated with hotter temperatures during the year before sampling. Sixty percent of moths from over 100 species were carrying pollen from 138 plant taxa representing 61 families including native, invasive, ornamental, and crop plants. Network structure was predominantly correlated with relatively short term (one month) precipitation patterns. Moth robustness to the simulated loss of their nectar plants was lower in drier conditions, which may have resulted from increased competition for reduced floral nectar. Our results reveal that moth pollen-transport networks are diverse,

complex, and potentially vulnerable to climate change, underscoring the risks of ignoring them in research and management.

Introduction

Global insect declines (Potts et al. 2010; Wagner et al. 2021b) have garnered considerable attention (Didham et al. 2020), largely due to the essential roles insects play in maintaining ecosystem function (Wilson 1987; Yang & Gratton 2014; Saunders & Rader 2020). The most salient example is insect pollination services, which the majority of flowering plant species require for reproduction (Ollerton et al. 2011), and which are responsible for about a third of human food crop production (Klein et al. 2007). Contrary to widely-held perceptions, the majority of these services are provided by wild pollinators and not managed honeybees (Breeze et al. 2011; Ollerton et al. 2012; Rader et al. 2016; Smith & Saunders 2016). Thus, pollination by wild insect communities represents an ecosystem service that is critical not only for maintaining the integrity of natural ecosystems, but also for securing human food supply. There is mounting evidence that both wild (Biesmeijer et al. 2006) and agricultural plants (Reilly et al. 2020; Smith et al. 2022) are encountering reduced insect pollination services. Accordingly, a monumental research effort is underway to elucidate the drivers behind insect decline and the disruption of pollination services, and to apply this knowledge in national and international pollinator conservation programs (Dicks et al. 2016; Potts et al. 2016a, 2016b; Bartomeus & Dicks 2019; Harvey et al. 2020).

A first step in this effort is to understand the ecology of plant-pollinator systems by documenting which insects visit which plants. This basic data can provide insight into why species are declining (Van der Putten et al. 2010; Wagner 2020), as well as guide pollinator conservation efforts (e.g., by identifying key plant species to use in pollinator plantings: Williams et al. 2015; Otto et al. 2017). However, there is a strong bias in pollination ecology for studying diurnal species and interactions, and we still lack basic ecological information for the majority of nocturnal insects (Hahn & Brühl 2016; Knight et al. 2018; Macgregor & Scott-Brown 2020; Cox & Gaston 2024). Considering that over 60% of insects are nocturnal (Hölker et al. 2010), including many groups of pollinators (e.g., moths, and certain bees, flies, beetles, and thrips: Macgregor & Scott-Brown 2020), this represents a fundamental gap in our knowledge of plant-pollinator systems.

Moths, the most diverse and ubiquitous nocturnal insects, have long been known to be important pollinators (Darwin 1862; Pellmyr & Huth 1994; Holland & Fleming 1999). However, most early studies were restricted to highly specialized, coevolved interactions such as the yucca-yucca moth relationship (Pellmyr & Huth 1994). Devoto et al. (2011) pioneered the study of moth pollination networks, which describe interactions between entire communities of moths and plants. Since then, a growing number of studies from around the world have revealed that moths, like bees and other diurnal insects (Waser et al. 1996), are generalist flower visitors to entire plant communities, forming interaction networks that rival their diurnal counterparts in diversity and complexity (Banza et al. 2015, 2019; Knop et al. 2018; Macgregor et al. 2019; Walton et al. 2020; Ribas-Marquès et al. 2022). For example, a study in Portugal found that 70% of moths in the community transport pollen from 83% of the flowering plant species present (Banza et al. 2019). Given the logistical challenges associated with studying plant-pollinator interactions at night, cutting-edge techniques like pollen DNA metabarcoding–which detects interactions from DNA in the pollen carried on insect bodies–have become increasingly important to this work (Pornon et al. 2016; Macgregor et al. 2019). Among the many plants recently discovered to be visited by moths are crops like apples, avocados, and berries (Macgregor et al. 2019; Walton et al. 2020; Buxton et al. 2021; Robertson et al. 2021; Buxton et al. 2022b). There is also mounting evidence for the importance of these interactions to plant reproduction (Macgregor & Scott-Brown 2020;

Alison et al. 2022; Buxton et al. 2022a, 2022b; Anderson et al. 2023). Indeed, moths may be more efficient than bees at pollinating certain plant species (Anderson et al. 2023).

Despite this shift in our understanding, moth pollination has generally been overlooked in global change research (Gaston 2019; Macgregor & Scott-Brown 2020; Gaston et al. 2023; Cox & Gaston 2024; but see Ellis et al. 2023). In turn, moths are rarely considered in pollinator conservation efforts, which rely on knowledge of insect resource requirements and environmental sensitivities. This is problematic because moths face many of the same threats as diurnal pollinators, in addition to unique ones such as light pollution (MacGregor et al. 2015; Boyes et al. 2021; Grenis et al. 2023). Consequently, moths are declining in richness and abundance globally (Conrad et al. 2006; Fox 2013; Habel et al. 2019; Salcido et al. 2020; Burner et al. 2021; Blumgart et al. 2022). In the Northeastern US, for example, 44% of hawkmoth (Sphingidae) species declined from 1900 to 2012 (Young et al. 2017). Besides their importance as pollinators, moths–as prey in both their larval and adult stages for birds, bats, and other organisms–are fundamental components of terrestrial food webs (Janzen 1988; Wagner et al. 2021a). Moreover, with approximately 160,000 species globally, moths are one of the most diverse groups of insects (Kristensen et al. 2007; Regier et al. 2009). Together they represent almost 10% of all insect species worldwide (Willmer 2011), with approximately 10 times more species than butterflies (Janzen 1988; Young 1997), and eight times more than bees (Michener 2000). Thus, neglecting moths in pollinator research and management may leave a substantial portion of pollinator biodiversity and pollination services unprotected.

Among the many anthropogenic threats implicated in insect declines, climate change is the most pervasive (Scheffers et al. 2016; Halsch et al. 2021). Organismal, phenological, population-level, and community-level responses to climate change are well-documented in Lepidoptera, which have served as a model group in climate change research (reviewed in Hill et al. 2021). For example, warming can increase egg mortality (Rocha et al. 2017), or accelerate development in immature stages leading to early emergence (Kearney et al. 2010). However, responses are highly species-specific, and while Lepidoptera are declining on average worldwide, many species are stable or even increasing (Wagner et al. 2021a). Species-specific vulnerability has been linked to life history traits including ecological generalism, voltinism, and overwintering stage (Mattila et al. 2008; Pöyry et al. 2009, 2011). In several studies, moth body size (forewing length) was the single best predictor for population declines (Mattila et al. 2009; Coulthard et al. 2019; Wagner et al. 2021a), in line with evidence across taxa that large-bodied organisms are generally more vulnerable to climate change (McCain & King 2014). Furthermore, body size reductions, in both ectotherms and endotherms, are commonly observed in response to climate warming (Ohlberger 2013; Coulthard et al. 2019). This has been documented in both butterflies (Bowden et al. 2015) and moths (Wu et al. 2019), and may occur due to increased metabolism (Sheridan & Bickford 2011), and/or accelerated development (the temperature-size rule: Atkinson 1994; Sheridan & Bickford 2011).

While these direct effects of climate change on Lepidoptera contribute to their declines (Coulthard et al. 2019), the indirect effects–through reductions to or mismatches with their plant resources–are perhaps even more serious (Scaven & Rafferty 2013; Ockendon et al. 2014; Gérard et al. 2020; Hill et al. 2021). Since many Lepidoptera species have highly specialized larval host plant relationships (Forister et al. 2015), they are particularly affected by shifts in the availability and quality of their host plants (Pelini et al. 2010). In fact, across studies, larval diet breadth is the trait most frequently correlated with moth population declines, with specialists at greater risk (Wagner et al. 2021a). As they are predominantly nectarivores (and therefore, potential pollinators) in their adult stage (Krenn 2010), moths also rely on nectar plants, and adult feeding can be as important as larval herbivory for Lepidoptera survival and reproduction (Gilbert 1972;

Murphy et al. 1983; Pivnick & McNeil 1985; Moore & Singer 1987; Hill & Pierce 1989; Mevi-Schutz & Erhardt 2005). Warming and drought can reduce the quantity and quality of floral nectar (Scaven & Rafferty 2013; Phillips et al. 2018; Descamps et al. 2021; de Manincor et al. 2023), which has been linked to declines in multiple diurnal pollinator taxa, including butterflies (Wallisdevries et al. 2012; Baude et al. 2016). While this has not been investigated in moths, climate-change driven disruptions to the interactions between moths and their nectar plants have the potential to impact plant and moth fitness, and ecosystem function.

Space-for-time substitutions–which use climatic variation through space as a proxy for climate change through time (Pickett 1989)–can provide important insights into the effects of climate change on biodiversity (Blois et al. 2013; Wogan & Wang 2018; Blüthgen et al. 2022, but see Damgaard 2019; Angert 2024). Since temperature decreases and precipitation increases with elevation, elevational gradients provide excellent natural experiments for climate change while controlling for factors, such as day length, that confound similar studies along latitudinal gradients (Fukami & Wardle 2005). Insects show distinct responses to elevation at multiple biological levels, which can vary by location and between taxa depending on biological requirements, life histories, and sensitivities to abiotic and biotic factors (Hodkinson 2005; Shelomi 2012). For example, moth body size (forewing length) can either increase (Brehm et al. 2019), show no change (Brehm & Fiedler 2004), or decrease with elevation (Brehm & Fiedler 2004), depending on the taxa and location. In a global meta-analysis, Beck et al. (2017) showed that geometer moths usually show mid-elevation peaks in species richness. Insect responses to elevation also depend strongly on their host plants, which can vary elevationally in diversity and climate-dependent traits such as productivity and phenology (Hodkinson 2005). This can affect interactions with insects, contributing to variation in species interaction networks along elevational gradients.

Recent studies comparing ecological networks along elevational gradients have yielded key insights into the effects of climate change on species interactions (Tylianakis & Morris 2017; Pellissier et al. 2018). Variation in network structure across space and time reflects the shifting influences of ecological and evolutionary processes, such as environmental filtering and competition, that together shape community stability and ecosystem function (Thompson 2005, 2006; Schleuning et al. 2012; González et al. 2015). Thus, examining how networks vary across space, time, and in relation to abiotic and biotic factors along elevational gradients can contribute to a mechanistic understanding of the effects of climatic change on plant-pollinator interactions (Burkle & Alarcón 2011; Tylianakis & Morris 2017; Pellissier et al. 2018). For example, Classen et al. (2020) found that plant-pollinator networks were more generalized at cold, high elevation sites on Mt. Kilimanjaro because the increased metabolic cost of activity at low temperatures causes insects to forage more generally. As temperatures rise due to climate change, they predict that networks will become more specialized and less stable. However, responses often vary between regions and taxonomic groups. For example, Adedoja et al. (2018) found that harsher conditions and lower plant diversity at high elevations on Jonaskop Mountain, South Africa, caused a breakdown of interaction networks involving bees and beetles, but not those involving wasps and flies. Moreover, relative to bees and other diurnal pollinators, very little is known about the composition and structure of moth pollen-transport networks, let alone their spatial variation. Moth networks may be structured by different factors than diurnal ones (e.g., the distribution of larval host plants: Altermatt & Pearse 2011), and this may influence their response to climate change.

Here, we used light trapping and pollen DNA metabarcoding to investigate how moth diversity, size (forewing length), and pollen-transport networks varied in relation to climate conditions and floral resources along an elevational gradient. The study was conducted along the historic Deep Canyon Transect (Zabriskie 1979), an approximately 2,500 m elevational gradient in Riverside County, California, US. The transect lies within the Colorado Desert, part of the broader Sonoran Desert Region, which has a semiarid to arid climate and mainly cool-season precipitation. While specialized moth pollination systems are well-known in this region (e.g., the yucca-yucca moth relationship: Pellmyr & Huth 1994, and hawkmoth pollination of *Datura* and *Agave* species: Alarcón et al. 2008a; Riffell et al. 2008), there is no community-level moth pollen-transport study from the Southwestern US. We generally expected to find a high proportion of moths transporting pollen, consistent with recent findings from other regions with seasonally hot and dry climates (Banza et al. 2015, 2019; Ribas-Marquès et al. 2022). We hypothesized that along the Deep Canyon Transect, conditions for both plants and insects are generally harsher at the hotter and drier low elevations, and become especially harsh during short term heat and drought. We therefore expected that the abundance and diversity of floral resources would be lower in hotter and drier conditions and at the lower elevations, and that this, in combination with thermal limits for moth larvae and adults, would result in a lower diversity of moths. We also expected moth forewing length to be lower in hotter and drier conditions and at the lower elevations because of reduced plant resources for caterpillars and because of the various shrinking effects of heat on ectotherms (Verberk et al. 2021). In terms of interaction network structure, we had two contrasting hypotheses: (1) harsher conditions and limited resources in hotter and drier conditions and at lower elevations would cause moths to forage more generally. Alternatively, (2) higher interspecific competition for limited plant resources in hotter and drier conditions and at lower elevations would make moth species more specialized. We expected these contrasting predictions to be reflected in niche overlap and several structural network properties, such as network connectance, nestedness, robustness, and modularity.

Methods

Field work

We conducted field work from February-August 2022 at five elevational sites along the Deep Canyon Transect in Riverside County, California, US (Supplementary material Appendix 1, Figure A1.1; see deepcanyon.ucnrs.org/mayhew-line-transects/). The transect begins near Boyd Deep Canyon Desert Research Center (BDCDRC: ~200 m), and continues to Toro Peak in the Santa Rosa Mountains (2657 m). The region is part of the California Floristic Province, a biodiversity hotspot, and over 700 plant species have been recorded along the transect (see deepcanyon.ucnrs.org/species-lists/). We chose sites to encompass the range of habitat types along the gradient (creosote bush scrub at 210 m, desert succulent scrub at 828 m, pinyon-juniper woodland at 1289 m, chaparral at 1967 m, and Sierran mixed conifer forest at 2450 m). Annual mean monthly temperature along the study sites decreases from 24.23 ± 0.75°C (mean ± SD) at the lowest site to 9.08 ± 0.60°C at the highest site, while mean annual precipitation increases from 85.71 ± 46.26 mm at the lowest elevation to 644.58 ± 239.36 mm at the highest elevation (Supplementary material Appendix 1, Figure A1.2). At each site, we established a 300 m long transect to estimate flower abundance and peak flowering phenology. Weekly throughout the field season at each site, we counted the number of flower units (flowers or inflorescences) per plant species in 1x1 m quadrats adjacent to each transect. During each day, we performed one AM and one PM flower count in 30 quadrats each to include within-day variability (for a total of 60 quadrats per day). Every 10 m along the transect, we placed quadrats semi-haphazardly along a perpendicular line to the transect such that (1) AM and PM quadrats did not overlap and (2) if we saw a species that was not included in any of the AM quadrats, we placed it into a PM quadrat in order to detect rare species. We conducted these surveys during the day, and although they may miss flowers open only at night, they capture most of the floral resources available for moths.

We sampled moth communities with light traps approximately every two weeks throughout the flowering season at each site, for a total of 33 sampling nights. We avoided excessively windy nights, and sampled only between the first and third quarter lunar phases as excess moonlight can reduce moth light trap catches (Yela & Holyoak 1997). At each site, we haphazardly established three trap locations adjacent to the transect. During each sampling night, we randomly deployed three custom bucket-style light traps, one to each trap location. Each trap used two UVA LED lights (Anglian Lepidopterist Supplies LED03) with a peak wavelength of 360-365 nm, which has been shown to maximize moth attraction (Brehm et al. 2021). Trap locations were at least 100 m apart, well beyond the attraction radius of the lights (Truxa & Fielder 2013). During sampling, we placed two 0.28 L paint cans filled with ethyl acetate into each trap, which was diffused with wicks to quickly euthanize entrapped moths. Each night, we turned lights on at sunset and let them run until sunrise.

While there is negligible pollen cross-contamination between moths in comparable traps (Del Socorro & Gregg 2001), we took several steps to minimize this risk. First, we collected pollen for DNA metabarcoding only from moth proboscides, which are kept tightly coiled and sheathed by the labial palps when not feeding (Krenn 2010), and thus do not easily transfer pollen to adjacent moths. Second, we placed two metal filters within each trap–one with 0.95 cm diameter holes, and one with 0.48 cm diameter holes–to physically separate moths by size class. Finally, we placed pieces of egg carton into each trap, which gives moths surfaces to settle on and helps keep them separated.

We collected moths from traps each morning by lifting them gently by the legs or forewing margins with forceps (avoiding the head and proboscis), and retaining them individually in glassine envelopes. After collecting each moth, we cleaned the forceps with ethanol. We collected only macrolepidoptera, gauged as any moths with forewing lengths estimated to be over

1 cm. The metal filters in the traps ensured that the majority of microlepidoptera fell to the very bottom layer, which we did not sample from. When we estimated that there were less than 60 individual moths in the trap, we collected all of the moths. When there were over 60, we haphazardly sampled 30 moths from each trap layer. In the field, we immediately placed glassine envelopes containing moths into plastic bags and on dry ice before storing them in a -20°C freezer for downstream analysis. After each sampling night, we cleaned all trap components first with soapy water, followed by 0.5% sodium hypochlorite (bleach) solution, then ethanol, then several rinses in water.

Climate data

We downloaded monthly temperature and precipitation data from 1992-2022 from the BDCDRC weather stations (deepcanyon.ucnrs.org/weather-data/), which were available for the three lower elevations. Since there were no weather stations near the two higher elevations, we retrieved monthly temperature and precipitation data from 1992-2022 at 800 m resolution, interpolated to the coordinates of each site, from PRISM (prism.oregonstate.edu). To verify whether the PRISM data was suitable, we compared PRISM data to weather station data at the bottom three elevations by performing correlation tests with the 'cor.test' function in R version 4.3.2 (R Core Team 2021), using the Spearman rank correlation coefficient since the data were not normally distributed. The results confirmed strong correlations ($\rho > 0.9$, $p < 0.001$) between the data for both precipitation and temperature (Supplementary material Appendix 1, Figure A1.3).

From these weather data, we calculated various metrics describing past climatic conditions at each site. The region's climate is characterized by hot, dry summers, and cool season precipitation. Therefore, we chose to differentiate the wet and dry season in these calculations. Using climate data from the past 30 years, we determined the average driest and wettest six month period at each site to designate the dry season and the wet season for each. We then calculated the mean monthly temperatures for the dry season, wet season, and annually at each site for the following sliding time windows prior to each sampling date: one month, four months, one year, three years, 10 years, and 30 years. We chose these intervals to encompass variation in climatic conditions within and between seasons, annually, and over decades. For precipitation, we calculated the mean dry season, wet season, and annual precipitation for the three, 10, and 30 year time windows, and the total precipitation for one month, four month, and one year time windows. We also calculated the deviation in precipitation and temperature of the month prior to each sampling date from the 30 year normal for that month.

Moth identification, measuring, and proboscis excision

We analyzed moths from 21 individual sampling events, with 3-5 sampling dates per site corresponding approximately to community-level flowering onset, peak, and end (Supplementary material Appendix 1, Figure A1.4). We photographed the dorsal, forewing view of each moth in resting position. Using the photographs and the physical specimens, we identified moths to the lowest possible taxonomic level using Powell and Opler (2009) and online resources including Moth Photographers Group (mothphotographersgroup.msstate.edu), PNW Moths (pnwmoths.biol.wwu.edu), iNaturalist (inaturalist.org), and BugGuide (bugguide.net). All identifications were validated by an expert taxonomist, D. Wikle. For several cryptic taxa, we could assign only genus-level identifications, and within these, further morphospecies discriminations were not possible. During photography and identification, we minimized the amount of time each moth was out of the -20°C freezer to protect the pollen DNA. To avoid pollen cross-contamination, we handled moths only by the legs or forewing margins with sterilized forceps, and placed them on fresh slips of parchment paper. Using the photos and the computer software ImageJ (imagej.net/ij/), we measured the forewing length of each moth (the longest straight-line distance from the wing base to the wing tip; Supplementary material Appendix 1, Image A1.1A), a proxy for moth body size (Miller 1977; Miller & Miller 1997).

We examined moth proboscides under a 40x dissection scope, carefully searching the entire length and each side of the proboscis for pollen grains (Supplementary material Appendix 1, Image A1.1B). We examined a maximum of six randomly-chosen moths from each species or morphospecies or until we found three individuals with at least five pollen grains on their proboscis, our threshold for inclusion in the analysis. This is similar to the approach used for networks constructed via pollen microscopy (Devoto et al. 2011; Banza et al. 2015, 2019), and may help minimize the risk of attributing interactions to pollen cross-contamination within traps (Banza et al. 2019). We barcoded only the pollen loads of moths in the families Noctuidae, Geometridae, Erebidae, and Sphingidae since these were the most abundant macrolepidoptera and are known pollinator groups (MacGregor et al. 2015; Hahn & Brühl 2016). We excised proboscides using miniature dissection scissors and scalpels, with each moth placed on a fresh slip of parchment paper contained in a sterilized petri dish. Between each proboscis excision, we sterilized the dissection tools by first submerging and swirling them in 0.5% sodium hypochlorite (bleach) solution for 10 seconds to destroy any residual pollen DNA, then in fresh DI water for 10 seconds to remove any bleach residue, and then drying on a Kimwipe. We completed this entire process as quickly as possible, immediately retaining the proboscides individually in DNA extraction tubes and returning them to the -20°C freezer to protect pollen DNA.

DNA extraction, library prep, and sequencing

We extracted DNA using DNeasy Blood and Tissue kits in 96-well format (Qiagen, Valencia, CA). We first added 180 μL of buffer ATL, 20 μL Proteinase K and two sterile stainless steel beads to each tube containing an excised moth proboscis. We then used a Qiagen

TissueLyser II to bead beat each sample for 6 min at 30 hz. We incubated the samples ~16-18 hr at 56°C on a rocking platform, and then followed the DNeasy standard extraction protocol.

Following methods from McFrederick and Rehan (2016), we used a dual-index inline barcoding approach to prepare libraries for sequencing on the Illumina MiSeq (Illumina, San Diego, CA). We used custom barcoded primers that amplify 180–220 bases of the plant ribulose bisphosphate carboxylase large chain (RBCL) gene. The primers include either the forward or reverse Illumina sequencing primer, an eight nucleotide long barcode, and the forward or reverse genomic oligonucleotide (RBCL7 CTCCTGAMTAYGAAACCAAAGA and RBCL8 GTAGCAGCGCCCTTTGTAAC). These primers have been used successfully in pollen DNA metabarcoding studies (McFrederick & Rehan 2016).

To generate amplicons for Illumina sequencing, we performed two PCR reactions: the first using the above primers, and the second using primers containing the Illumina adapter sequence. For the first PCR reaction, we used 5.06 µL ultrapure water, 0.13 µL GoTaq (Promega, Madison, WI), 4 µL Clear GoTaq Reaction Buffer, 1.6 µL of 10mM (total) dNTPs (2.5 mM each), 1.6 µL MgCl2 (15mM), 1.6 µL 1X BSA (4mM), 1 µL each of 10 µm primer stock, and 4 µL of template DNA. For these reactions, we used a 50°C annealing temperature, 35 cycles, and negative controls. To remove unincorporated primers and dNTPs after the first PCR, we used 15 µL of PCR product in an ExoSap reaction with 0.16 µL Exonuclease 1 (20 U/µL), 0.81 µL SAP (1 U/µL), and 9.16 µL ultrapure water per sample. We incubated the ExoSap reaction at 37°C for 30 min, then 80°C for 20 min.

For the second PCR reaction, we used HPLC purified primers to complete the Illumina sequencing construct as in Kembel et al. (2014): CAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGC and AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACG. These reactions included 11.83 µL ultrapure water, 0.17 µL GoTaq, 5 µL Clear GoTaq Reaction Buffer, 2 µL of 10mM (total) dNTPs (2.5 mM each), 2 µL MgCl2 (15mM), 2 µL 1X BSA (4mM), 0.5 µL each of 10 µm primer stock, and 1 µL of the cleaned PCR product as a template. For these reactions, we used a 58°C annealing temperature, 14 cycles, and negative controls. To normalize the amount of PCR product from each reaction, we used 18 µL of these reactions and SequalPrep Normalization plates (ThermoFisher Scientific, Waltham, MA). We pooled 5 µL of each of the normalized samples, performed a left- and right-side select bead clean up (SPRIselect, Beckman Coulter, Brea, CA) to select between 200-700 bp, and then assessed the quality of our libraries using a 2100 Bioanalyzer (Agilent, Santa Clara, CA). After quality control, we sequenced the libraries using the MiSeq Reagent Kit v3 with 2 × 300 cycles.

Bioinformatics

To format the RBCL sequence and barcode data for demultiplexing, we used the extract_barcodes.py script in macqiime 1.9.1 (Caporaso et al. 2010). Then, in qiime2 2023.7, we demultiplexed and used the DADA2 pipeline to generate amplicon sequence variants (ASVs), including denoising paired-end sequences, dereplicating, filtering chimeras, and trimming low quality sequence regions. To assign taxonomy to the ASVs, we used both local BLAST searches (blastn: Altschul et al. 1990), against NCBI's nucleotide collection database (ncbi.nlm.nih.gov/nucleotide/), and the RDP Naive Bayesian Classifier (Wang et al. 2007) trained to the Eukaryote RBCL reference set from Porter (2020). To perform local blastn searches, we used the ‘metblastr’ package (Benoit & Drost 2021) in R version 4.3.2 (R Core Team 2021), searching against a local database of RBCL sequences created with the following query of the NCBI nucleotide database: “rbcl[All Fields] AND (plants[filter] AND biomol_genomic[PROP] AND is_nuccore[filter])" (accessed 14 September 2023). We retrieved taxonomy for all accession numbers with the R package ‘taxonomizr’ (Sherrill-Mix 2023), and assigned a final BLAST taxonomy to each ASV by determining which taxa had (1) the lowest E-value, then for identical hits (2) the highest percent query cover, then for identical hits (3) the highest percent max identity. To determine the final consensus taxonomy for each ASV, we compared the RDP classifier results, the BLAST results, and a local plant list from the study sites combining the comprehensive Deep Canyon plant list (deepcanyon.ucnrs.org/species-lists/) with the plant species identified from our flowering phenology surveys. We also searched iNaturalist (inaturalist.org) and CalFlora (calflora.org) for possible plant species that were not on our list, including ornamental and crop plant species. In all downstream analyses, we included only taxa that we could identify to genus level. We also removed any solely wind-pollinated plant taxa, since Lepidoptera normally do not consume pollen, and these species rarely produce floral nectar. Pollen from wind-pollinated taxa may show up on insect bodies due to its presence in the environment and because Lepidoptera are often in close proximity to plants other than their nectar plants (e.g., plants they shelter on during the day or their larval host plants).

After examining the sequence reads and taxa in the negative control samples included in each 96-well plate, we discovered contaminant plant RBCL ASVs in several of the negative controls corresponding to taxa that are found at the study sites. 50% of the contaminated reads came from just one ASV in one negative control. In light of this, we performed a rigorous decontamination protocol, using the ‘isContaminant’ function with the “prevalence” method from the R package ‘decontam’ (Davis et al. 2017), which statistically identifies and removes contaminant ASVs based on their prevalence in negative controls. We used a highly conservative probability threshold of 0.5, below which the null-hypothesis (not a contaminant) is rejected in favor of the alternate hypothesis (contaminant). We also removed any ASVs that comprised less than 2% of the total reads in each sample.

Data analyses

We performed all analyses in R version 4.3.2 (R Core Team 2021). For all network analyses, we used the package 'bipartite' (Dormann et al. 2008). We conducted analyses using binary (unweighted) interaction networks because interaction frequencies cannot be accurately estimated from pollen metabarcoding data (Pornon et al. 2016; Bell et al. 2019), and because we analyzed only up to three individuals per moth species. We only included moth and plant taxa with species-level identifications in the networks. We used the function 'frame2webs' to build moth pollen-transport networks from each collection night and site, pooling the data from the three traps used each night. We visualized networks with the 'plotweb' function. We used rarefaction and extrapolation methods to estimate the sampling completeness of moth species, pollen types, and interactions across all sampling events (Chao & Jost 2012). We performed this analysis using the function 'iNEXT' from the package 'iNEXT' (Hsieh et al. 2016), using abundance-based estimates for moths, and incidence-based estimates for pollen types and interactions.

To examine how moth pollen-transport interactions varied with abiotic and biotic factors, we calculated six binary indices describing network structure and the degree of generalization at each elevation and date. (1) Connectance (C) is the proportion of realized links (interactions) out of all possible links, ranging from 0 (no connectance) to 100 (perfectly connected). (2) Nestedness quantifies the degree to which specialized species interact with proper subsets of more generalized species' interaction partners (Bascompte et al. 2003), and has been linked to network stability (Bascompte et al. 2003; Song et al. 2017). We measured nestedness in two ways: first, we calculated the NODF metric (Nestedness measure based on Overlap and Decreasing Fill: Almeida-Neto et al. 2008), which ranges from 0 (no nestedness) to 100 (perfectly nested). This metric has been used extensively in plant-pollinator network studies, and thus serves as a useful metric for comparison. Second, to compare nestedness across different networks, we calculated the normalized NODF metric, NODFc, proposed by Song et al. (2017), which is independent of network size and therefore comparable across different networks (Song et al. 2017). (3) Modularity (Q) quantifies the degree to which subsets of interacting species are grouped into compartments (modules) where they interact more closely with one another than with species from other modules (Olesen et al. 2007). We used Newman's (2006) modularity calculation for binary networks using the 'computeModules' function. (4) Robustness (R) quantifies how sensitive a level of the network is to the simulated removal of species from the other level, and ranges from 0-1 (Memmott et al. 2004). Values close to 0 indicate low robustness, with abrupt species losses following removals from the other level, while values close to 1 indicate high robustness, with few species losses following removals from the other level. We took the mean robustness value from 9,999 bootstrapped simulations. Both modularity and robustness have been shown to be relatively insensitive to network size (Rivera-Hutinel et al. 2012). (5) Niche overlap for each level of the network quantifies the mean similarity in species' interactions, with values ranging from 0 (no similarity) to 1 (perfect niche overlap; Dorman et al. 2009). We used the Jaccard distance metric to calculate niche overlap. (6) Species degree describes the number of interaction partners for each species, and is a simple binary metric for specialization/generalization. We tested for significance in network metrics by comparing the observed values to a null distribution from 999 randomized networks produced with the 'nullmodel' function. For this, we used the 'shuffle.web' method for binary networks, which preserves the number of interactions and links from the observed network in the randomized networks. Using the full dataset, including individuals not analyzed for pollen-transport, we calculated moth species richness and Shannon diversity (the exponential of Shannon entropy) using Hill numbers via the function 'hill_taxa' from the package 'hillR' (Li 2018). Using data from the flowering phenology surveys, we also calculated the species richness of plants in bloom, the mean abundance of flowers per quadrat, and the Shannon diversity (exponential of Shannon entropy) of flowers, using the abundance of flowers per species.

We assessed whether species degree varied between moth and plant families with analyses of variance (ANOVAs) using the function ‘aov’, following by post hoc Tukey tests using the function ‘TukeyHSD’. To determine whether moth community composition and pollen composition differed between elevations, we performed permutational multivariate ANOVAs (PERMANOVAs) with the function ‘adonis2’ from the package ‘vegan’ (Oksanen 2010). We used the same approach, instead with matrices of interactions by site and date, to analyze the beta diversity of interactions between elevations. We tested for pairwise differences using the function ‘pairwise.adonis2’ from the package ‘pairwiseAdonis’ (Martinez Arbizu 2017), using the ‘false discovery rate’ p value correction method (Benjamini & Hochberg 1995). We visualized results by performing principal coordinate analyses (PCoAs) with the ‘betadisper’ function from ‘vegan’, and plotting with the ‘gg_ordiplot’ function from the package “ggordiplots’ (Quensen et al. 2023). We also analyzed moth species, plant species, and interaction turnover through space and time with the function ‘turnover’ from the package ‘codyn’ (Hallett et al. 2020), which calculates the proportion of species or interactions either gained or lost compared to the total number of species or interactions observed across time periods or sites. We built a phylogenetic tree of all the plant species detected in the pollen loads on moth proboscides with the ‘U.PhyloMaker’ package (Jin & Qian 2023), using the plant megatree based on the World Plants database (Hassler 2024) nomenclature. We visualized the tree using the ‘ggtree’ package (Yu et al. 2017).

To assess how moth diversity, size, and pollen-transport interactions varied with abiotic and biotic factors, we first performed variable selection using L1-penalized estimation via the ‘glmmLasso’ function from the ‘glmmLasso’ package (Groll & Tutz 2014). Then, we used the package ‘glmmTMB’ (Magnusson et al. 2017) to build (generalized) linear mixed effects models ((G)LMMs) with the various diversity and network metrics and moth forewing length as response variables and the various climate metrics and flowering diversity/abundance metrics as predictors. In each model, we included the site as a random effect to account for the non-independence of networks from the same locations. In models with multiple terms after variable selection, we checked for multicollinearity among model terms by calculating the variance inflation factor (VIF) with the function ‘check_collinearity’ from the package ‘performance’ (Lüdecke et al. 2021), and sequentially dropped terms with VIF values > 5. We then performed model selection with the ‘dredge’ function from the ‘MuMIn’ package (Barton 2015), selecting the best fitting model based on Second-order Akaike Information Criterion (AICc). We also investigated how the various diversity and network metrics and moth forewing length varied by elevation in (generalized) linear models ((G)LMs) with (numerical) elevation as the predictor. For all (G)LMMs and (G)LMs, we used the Gaussian family distribution in models with Shannon diversity, moth richness, forewing length, and NODFc as the response. For all models with plant richness, mean abundance of flowers, or degree as the response, we used the Poisson family distribution. For all models with connectance, modularity, niche overlap, and robustness as the response, we used the Beta family distribution. We assessed model fits by examining residual distributions using the ‘DHARMa’ package (Hartig 2017). For all (G)LMMs, we determined variable significance by performing likelihood ratio tests with the ‘anova’ function. To visualize the fixed effects in each model, we calculated predicted (marginal) effects using the ‘effect’ function from the ‘effects’ package (Fox & Weisberg 2018). We produced all plots using the ‘ggplot2’ package (Wickham et al. 2016).

Results

Moth diversity

The sampling completeness of moth species across all sampling events was 98.5% (Supplementary material Appendix 1, Table A1.1 and Figures A1.5A and A1.5B). We identified 3,132 individual moths belonging to 12 families, of which 80% could be identified to species level, comprising 143 total species. 47.7% of the individual moths were in the family Noctuidae, 33.9% in Geometridae, 7.9% in Erebidae, and 5.7% in Sphingidae. The remaining 5% were in the families Pyralidae, Pterophoridae, Crambidae, Lasiocampidae, Cossidae, Prodoxidae, Saturniidae, and Hepialidae. Based on abundance, Noctuidae was the dominant moth family at all sites except 1289 m (the pinyon-juniper woodland habitat), where Geometridae had more individuals (Supplementary material Appendix 1, Figure A1.6A). For the moths identified to species level, 58.0% of species were in the family Noctuidae, 23.8% in Geometridae, 12.6% in Erebidae, and 2.8% in Sphingidae. The remaining 3% of species were in the families Crambidae, Lasiocampidae, Saturniidae and Hepialidae. Based on species richness, Noctuidae was the dominant moth family at all sites (Supplementary material Appendix 1, Figure A1.6B).

Moth community composition varied significantly between sites ($F_{4,18} = 3.78$, $R^2 = 0.46$, $p = 0.001$; Figure 1.1A), with all pairwise differences significant except for 210 m vs. 828 m. Species richness in the dominant moth family, Noctuidae, increased with elevation, while species richness in the other families did not vary by elevation (Table 1.1; Figure 1.1B). Across all families, moth species richness was positively correlated with greater total precipitation during the month before sampling (Table 1.1; Figure 1.1C). Moth Shannon diversity was positively correlated with greater mean dry season precipitation during the three years before sampling (Table 1.1; Figure 1.1D).

Moth size

We measured the forewing lengths of 2,836 individual moths spanning 11 families and 139 species. Forewing length increased with elevation in each of the four dominant moth families (Table 1.2; Figure 1.2A). Moth forewing length was negatively correlated with warmer mean dry season temperatures during the year before sampling ($\beta = -0.017$, 95% CI [-0.024, -0.010], random effect (SD) = 0.029, $p = < 0.001$; Figure 1.2B). Finally, there was a marginally-significant, positive correlation between moth forewing length and moth degree ($\beta = 0.062$, 95% CI [-0.0025, 0.12], $p = 0.056$; Supplementary material Appendix 1, Figure A1.7).

Moth pollen transport

We examined 1,294 individual moths under a dissection scope, representing 135 species from the four dominant moth families (Noctuidae, Geometridae, Erebidae, and Sphingidae). Of these, 781 individuals (60.4%) from 111 (82.2%) species were carrying visible pollen grains on their proboscis, with 572 individuals (44.2%) from 102 (75.6%) species carrying five or more grains. 98.9% of individual moths in the family Sphingidae were carrying visible pollen, followed by 71.1% in Erebidae, 61.8% in Noctuidae, and 43.6% in Geometridae. We dissected the proboscis and barcoded the pollen loads of 569 individual moths, 113.4 ± 15.96 (mean ± SD) moths per site, and 27.0 ± 12.62 moths per sampling date. Reflecting the diversity trends, the majority of barcoded moths (56.1%) were in the family Noctuidae, with the remaining approximately evenly divided between the families Geometridae (16.6%), Sphingidae (16.6%), and Erebidae (10.8%). 46 (8.1%) of the barcoded moth pollen samples did not contain any plant RBCL sequences that we could confidently identify to at least genus level, and these were removed from subsequent analysis. For the remaining samples, we could not identify 15 (2.9%) of the moths to at least genus level, and these were removed from subsequent analysis.

With all moths and plants identified to at least genus level, we detected 1,183 total pollen-transport interactions representing 850 unique interactions between a moth taxon and a plant taxon across all dates and elevations. Altogether, these involved 508 individual moths from 99 taxa, and 138 plant taxa. Plant taxa spanned 126 genera, 61 families and 28 orders (Figure 1.3). The top three plant families based on the number of unique taxa detected were Fabaceae (16 taxa, 11.6% of total), Asteraceae (13 taxa, 9.4% of total), and Rosaceae (8 taxa, 5.8% of total). 82 (59.4%) plant taxa were native, 21 (15.2%) were ornamental, 18 (13.0%) were crops, 15 (10.9%) were potentially invasive, introduced species, and two (1.4%) could not be determined by the genus alone (Figure 1.3). 94 (68.1%) of the plant taxa were not listed on the Deep Canyon Transect plant list (deepcanyon.ucnrs.org/species-lists/). Native plant species spanned 41 families. Introduced species spanned nine families, and included common invasives such as Tamarisk (*Tamarix* sp.) and Sahara mustard (*Brassica tournefortii*). Ornamental plants–which may also have been escaped invasives–spanned 16 families, and included popular garden flowers such as lantana (*Lantana camara*), butterfly-bush (*Buddleja davidii*), and frangipani (*Plumeria rubra*), and ornamental trees such as Southern magnolia (*Magnolia grandiflora*) and Chinaberry (*Melia azedarach*). Crop plants–which may have also been escaped invasives or ornamental plants in residential areas–spanned 13 families and included many commercially-important crop species in the area (e.g., citrus, celery, olives, melons, and mangos).

Out of the genus-level data, we could not identify 39 (8.4%) of the individual moths to species level, nor 52 (38.5%) of the plant taxa. When including only species-level identifications for both moths and plants, we detected 561 total pollen-transport interactions representing 442 unique interactions across all dates and sites. These involved 330 individual moths from 85 species, and 82 plant species. The sampling completeness of pollen types found on moth proboscides across all sampling events was 89.3% (Supplementary material Appendix 1, Table

A1.1 and Figures A1.5C and A1.5D). The sampling completeness of interactions across all sampling events was 17.0% (Supplementary material Appendix 1, Table A1.1 and Figures A1.5E and A1.5F). In individual networks, on average, each moth species transported pollen from 2.37 ± 1.09 (mean ± SD) plant species, while each plant species had its pollen transported by 1.69 ± 1.15 moth species. This varied significantly between both moth families ($F_{3,197} = 5.39$, $p = 0.001$; Supplementary material Appendix 1, Figure A1.8A) and plant families ($F_{17,165} = 1.92$, $p = 0.02$; Supplementary material Appendix 1, Figure A1.8B). Post hoc analysis revealed that moths in the family Sphingidae transported the pollen of more plant species than the other families, while no pairwise differences were significant for plants. With the data pooled between dates and sites, on average each moth species transported pollen from 5.20 ± 4.28 plant species and 4.56 ± 3.44 plant families, and each plant species had its pollen transported by 5.39 ± 6.10 moth species. This did not vary significantly between plant families, but again, Sphingidae transported significantly more pollen types than the other moth families ($F_{3,81} = 7.54$, $p < 0.001$; Supplementary material Appendix 1, Figure A1.8C). Pollen composition from moth pollen loads differed significantly between sites ($F_{4,15} = 1.58$, $R^2 = 0.30$, $p = 0.001$; Supplementary material Appendix 1, Figure A1.9). Post hoc analyses indicated that 1967 m differed from 210 m, 828 m, and 2450 m, while 1289 m differed from 210 m.

We constructed 19 individual pollen-transport networks, with at least three networks per site corresponding approximately to community-level flowering onset, peak, and end. We excluded two of the 21 possible networks from the analyses (210 m, 7 February 2022, and 1289 m, 30 March 2022) because they did not contain enough data to calculate the network indices. Six (31.6%) of the networks were significantly modular, while only one (5.3%) was significantly nested. Figure 1.4 shows an example network constructed from an individual sampling event. Pollen-transport interaction composition varied significantly between sites ($F_{4,14} = 1.08$, $R^2 =$

0.24, p = 0.002; Supplementary material Appendix 1, Figure A1.10). Post-hoc analysis indicated that interactions at 828 m differed significantly from those at 1289 m, 1967 m, and 2450 m. Interaction turnover was higher than plant or moth species turnover through both space and time (Supplementary material Appendix 1, Table A1.2).

The proportion of moths carrying pollen was negatively correlated with greater total precipitation during the month before sampling (Table 1.3; Figure 1.5A). Plant robustness to the simulated loss of moth species was negatively correlated with greater positive precipitation deviations for the month before sampling from the 30 year normal for that month (Table 1.3; Figure 1.5B). Moth robustness to the simulated loss of nectar plant species was positively correlated with greater total precipitation during the month before sampling (Table 1.3; Figure 1.5C). Plant niche overlap was negatively correlated with greater positive precipitation deviations for the month before sampling from the 30 year normal for that month (Table 1.3; Figure 1.5D). Moth niche overlap was positively correlated with higher elevation (Table 1.3; Figure 1.5E) and with greater total precipitation during the month before sampling (Table 1.3; Figure 1.5F). There were no significant relationships between elevation, climate metrics, or flower diversity/abundance and network connectance, modularity, nestedness (NODFc), or moth degree.

Plant degree was positively correlated with higher elevation (β = 1.93E-04, 95% CI [6.48E-05, 3.0E-04], p = 0.002; Figure 1.6A), and with greater total precipitation during the month before sampling (β = 0.11, 95% CI [0.048, 0.18], random effect (SD) = 0.056, p = 0.003; Figure 1.6B). There were no significant relationships between elevation, climate metrics, or flower diversity/abundance and moth degree. The species richness of plants in bloom and the Shannon diversity of flowers were positively correlated with greater precipitation deviations of the month before sampling from the 30 year normal for that month (Supplementary material Appendix 1, Table A1.3 and Figures A1.11A and A1.11B). The mean abundance of flowers per quadrat was negatively correlated with warmer mean temperatures during the four months before sampling (Supplementary material Appendix 1, Table A1.3 and Figure A1.11C).

Discussion

Our results reveal that entire communities of moths in the Southwestern US transport pollen from, and likely pollinate, entire communities of plants. Sixty percent of individual moths from over 135 species in the dominant macrolepidoptera families Noctuidae, Geometridae, Erebidae, and Sphingidae were carrying pollen on their proboscides. This percentage is much higher than what has been found in the United Kingdom (Devoto et al. 2011; Macgregor et al. 2019), but comparable to findings from the Mediterranean (Banza et al. 2015, 2019). Forty-four percent of all moths carried five or more pollen grains on their proboscis–to our knowledge, the highest percentage yet found in a moth pollen-transport study. The moth family Sphingidae had the highest percentage of individuals transporting pollen (98%), and species in this family also transported pollen from more plant species than the other moth families. However, due to their higher species richness, Noctuidae were involved in the greatest number of unique interactions, consistent with other studies (Hahn & Brühl 2016). Altogether, the pollen comprised 138 plant taxa from 61 families including native, invasive, ornamental, and crop plants. This is a more diverse flora than what has been found in previous moth pollen-transport studies (Banza et al. 2015, 2019; Macgregor et al. 2019; Walton et al. 2020). Given nocturnal flowering and nectar production may be an adaptive strategy for plants to avoid water loss in xeric environments (Borges et al. 2016), our results lend support to the idea that nocturnal pollination should be prevalent in dryland ecosystems. Although pollen-transport does not equate to pollination (King et al. 2013), our results nevertheless suggest that moths are important components of plant-pollinator networks across diverse habitats.

While some of the interactions we identified have been previously documented (e.g., hawkmoths (Sphingidae) nectaring on *Agave sp.*: Alarcón et al. 2008a), the majority of these data represent previously unknown interactions. For example, the mistletoe species *Phoradendron californicum* and *Arceuthobium campylopodum* appear to be particularly important moth nectar plants. Mistletoes, which are obligate hemiparasites, are considered keystone species worldwide (Watson 2001). Due to their ability to draw on their host's nutrients and water supply, they can flower and set fruit even in extended droughts when other plants are limited by water availability (Napier et al. 2014). While this has mainly been shown to sustain specialist birds throughout droughts (Paton & Ford 1977; Watson 2001), our data suggests that mistletoes may also be sustaining nectarivorous moths and other insects in arid conditions. Many of the plant taxa we identified fit the classic moth pollination syndrome (Grant 1983; Willmer 2011a; Borges et al. 2016) of tubular, white, and heavily-scented flowers (e.g., *Lonicera* sp.: Miyake & Yahara 1998), while others are more traditionally associated with diurnal pollinator taxa (e.g., *Justicia californica)*. This has been found in other studies (Devoto et al. 2011; MacGregor et al. 2015; Souza et al. 2022; Ellis et al. 2023), and indicates that moths are not restricted to visiting only the flowers that the pollination syndrome approach would suggest (Waser et al. 1996). It also supports the idea that many plant species have flowers that remain open day and night, which is likely a generalist pollination strategy to ensure pollination (Borges et al. 2016). Our data also adds to the growing list of crop plants known to be visited by moths, including many economically-important crops in the study region (e.g., citrus, mangos, melons, olives, and celery). In a global meta-analysis, Buxton et al. (2022b) found evidence for nocturnal pollination in crop and medicinal plants belonging to 52 families, with Cactaceae, Fabaceae and Asparagaceae mentioned most frequently in the literature. We identified pollen on moth proboscides from plants in nine of these families (including Fabaceae and Asparagaceae), and we have added another four plant families to the list: Lauraceae (*Persea americana*: avocado), Anacardiaceae (*Mangifera indica*: mango, and *Pistacia vera*: pistachio), Actinidiaceae (*Actinidia arguta*: kiwi), and Ebenaceae (*Diospyros sp.*: persimmon).

Our results agree with the emerging consensus of generalized moth pollination systems at the community level (Macgregor & Scott-Brown 2020). Specialization at the species level, however, depended on whether networks from individual sampling events were analyzed separately or pooled. When networks were analyzed separately, both moth and plant species appeared to be relatively specialized. However, pooling the networks over space and time revealed that both plant and moth species tended to be more generalized. Consistent with previous studies (Petanidou et al. 2008; Brosi 2016; Spiesman & Gratton 2016; CaraDonna et al. 2017, 2021; Schwarz et al. 2020), the relative specialization in the spatiotemporally-separated networks and generalization in the pooled networks was driven by a combination of species turnover and interaction rewiring through space and time. Interaction turnover was much higher than spatial or temporal moth and plant species turnover, indicating that interaction rewiring was largely responsible for this effect. Thus, moths appear to be nectaring on different plant species through space and time, despite high temporal and spatial overlap in floral resource composition. During individual foraging bouts, pollinator diet breadth may be constrained by a variety of factors including resource quality or quantity, dispersal ability, competition with other flower visitors, and individual behavior or preferences (Brosi 2016). This may make moths more sensitive to the loss of nectar plants due to short-term disturbances, including extreme climatic events such as floods and heatwaves. Indeed, robustness values for both plants and moths were higher when networks were pooled across time points at each site. Thus, although species may appear more generalized and in turn, more robust, when interactions are pooled across space and time, they are perhaps more specialized and sensitive at biologically-relevant time scales (Burkle

& Alarcón 2011). On the other hand, our results suggest that moths have substantial ability to switch to alternative nectar plants (interaction rewiring), which has been shown to increase robustness in plant-pollinator networks (Kaiser-Bunbury et al. 2010; Sheykhali et al. 2020).

While plant-pollinator networks are thought to be relatively robust to disturbances due to their nested, asymmetric structure (Memmott et al. 2004), most of this knowledge comes from networks involving diurnal pollinators. There is mounting evidence that moth pollen-transport networks display different structural features, which may affect their resilience to climate change and other stressors (Ellis et al. 2023). In this study, moth pollen-transport networks generally showed low levels of connectance, which is typical for plant-pollinator networks (Jordano 1987). While plant-pollinator networks also tend to be significantly nested (Bascompte et al. 2003), ours showed low levels of nestedness, with only one out of 19 networks being significantly nested. Interestingly, Ellis et al. (2023) found that when compared to bee networks in an urban setting, moth networks were significantly less nested. Since nestedness is linked to network stability (Song et al. 2017), this suggests that moth pollination interactions can be more vulnerable to climate change and other stressors. Networks showed intermediate levels of modularity, with six out of 19 of the networks being significantly modular. Compared to other moth pollen-transport network studies (Banza et al. 2015; Ribas-Marquès et al. 2022), both moths and plants showed moderate levels of robustness. Plant robustness was slightly higher, suggesting that on average, moths are more sensitive to the loss of their nectar plants than the other way around. This agrees with the results of a large-scale simulation experiment testing the effects of climate change on pollination and seed dispersal networks involving over 700 plant and animal species in central Europe (Schleuning et al. 2016).

Diurnal plant-pollinator network studies have frequently found that networks become more generalized, nested, and robust at higher elevations, often attributing this to the nichebroadening effects of harsh conditions, limited resources, and lower interspecific competition between pollinators at high elevations (Miller-Struttmann & Galen 2014; Hoiss et al. 2015; Maglianesi et al. 2015; Lara-Romero et al. 2019; Classen et al. 2020). However, few studies have investigated plant-pollinator networks along dryland elevational gradients, where conditions can be particularly harsh in the hot and dry lowlands. Additionally, to the best of our knowledge, this is the first study to examine variation in nocturnal moth pollen-transport networks along an elevational gradient (but see Mertens et al. 2021 and Ho & Altermatt 2023 for moth flower-visitation networks along elevational gradients). The only significant elevational trends we observed were for moth niche overlap and plant degree, which both increased at higher elevations, potentially due to the greater moth species richness. Variation in network structure along the gradient was predominantly related to relatively short term (one month) precipitation patterns. Greater precipitation during the month before sampling was correlated with greater plant degree, moth niche overlap, and moth robustness, and with a lower proportion of moths in the community carrying pollen. Additionally, deviations from 30 year normals that resulted in wetter than average conditions during the month before sampling were correlated with lower plant robustness. Finally, a greater abundance of flowers was correlated with lower plant niche overlap. Supporting findings from diurnal plant-pollinator network studies (Miller-Struttmann & Galen 2014; Hoiss et al. 2015; Maglianesi et al. 2015; Lara-Romero et al. 2019; Classen et al. 2020), these results reveal that the structure of moth pollen-transport networks also varies in relation to abiotic and biotic factors along elevational gradients. In particular, moth pollen-transport networks in this region appear to be particularly influenced by short term precipitation, consistent with prevailing view of biological pulse-reserve dynamics in drylands driven by rain events (Noy-Meir 1973; Collins et al. 2014).

Floral resource availability, and the resulting shifts in competition between pollinators, plays an essential role in shaping plant-pollinator interactions (Jones et al. 2012; Valdovinos & Marsland 2021; Sponsler et al. 2023). We found that the species richness of plants in bloom and the Shannon diversity of flowers were positively correlated with wetter than average conditions, while the abundance of flowers was negatively correlated with warmer temperatures during the four months before sampling. Furthermore, although we did not measure nectar production, plants generally produce less nectar in hotter and drier conditions (Scaven & Rafferty 2013; Phillips et al. 2018; Descamps et al. 2021). Together, this suggests that there is less nectar available when conditions are hotter and drier. Foraging theory predicts that consumers may respond to reduced resources either by expanding their diet breadth to compensate, or by partitioning resources (reducing niche overlap) to reduce interspecific competition (Stephens & Krebs 1986; Perry & Pianka 1997; Levine & HilleRisLambers 2009). The latter can occur by either (a) narrowing diet breadths to become more specialized, (b) shifting the range of resources used to another part of the possible array, or (c) some combination of the two (Sale 1974). Since we found that moth diet breadth (degree) did not vary in relation to elevation, floral resources, or climate conditions, we have no evidence for (a). However, we do have evidence for (b) since moth niche overlap decreased at lower elevations and in drier conditions. If moths are responding to increased competition from reduced floral resources in hotter and drier conditions by partitioning the available resources while still retaining their diet breadth, it would follow that fewer moth species would be able to coexist in these conditions. And indeed, we found lower moth species richness at the lower elevations and lower moth species richness and Shannon diversity in drier conditions. In turn, decreasing niche overlap (the number of insect species visiting the same plants) can reduce network robustness (Cusser & Goodell 2013), which we also found for moths in the drier conditions. Overall, these results suggest that drier conditions expected with climate change will increase competition among moths for floral nectar, reducing the number of moth species able to coexist, and making them more sensitive to the loss of their nectar plants. It is important to note that this effect may also have been driven by host plant availability, since total plant coverage is also lower at the lower elevations (Kelly & Goulden 2008). Interestingly, plant robustness decreased when conditions during the month before sampling were wetter than average. This may have been related to greater competition for pollinators since the species richness of plants in bloom and the Shannon diversity of flowers both increased in these conditions as well.

We found that the species richness of the dominant moth family, Noctuidae, increased with elevation. High-elevation peaks in species richness can be driven by elevational species-area relationships (Romdal & Grytnes 2007), or by more efficient sampling of seasonally compressed communities at high elevations (Whittaker 1952). However, both short and long term climate conditions, and their influence on plant productivity, are also strong predictors of ectotherm diversity along elevational gradients (McCain 2010; Jonason et al. 2014; Szewczyk & McCain 2016). Indeed, we found that moth species richness was positively correlated with greater total precipitation during the month before sampling, and that moth Shannon diversity was positively correlated with greater mean precipitation during the dry season of the previous three years. This is consistent with studies from other regions with seasonally warm and dry climates (Forister et al. 2018; Uhl et al. 2022). For example, Uhl et al. (2022) found the diversity of moths with summer-developing larvae declined when their larvae were exposed to hotter and drier Mediterranean summers. Thus, our results support the idea that lepidopterans in regions already prone to heat and drought may be pushed past their environmental tolerances as climate change makes conditions more extreme (Uhl et al. 2022; Welti et al. 2022). In particular, like their pollen-transport interactions, moth diversity in this study region appears to be predominantly affected by precipitation patterns.

Declines in moth species richness in response to drought may be due to a combination of direct effects (e.g., increased larval mortality), and indirect effects (e.g., reduced host plant biomass). There is increasing evidence that extreme drought can directly affect Lepidoptera, for instance by increasing desiccation risk for pupae (McDermott Long et al. 2017). Additionally, higher net primary productivity may lead to higher consumer richness by supporting larger population sizes, thereby reducing extinction risks (the ‘more individuals hypothesis’: Evans et al. 2005; Hurlbert & Stegen 2014). In particular, free-living herbivore species richness is often lower in drier habitats because of reduced host plant productivity (Bailey et al. 2004; Seto et al. 2004; Levanoni et al. 2011). Along the Deep Canyon Transect, total plant cover increases sharply with elevation (Kelly & Goulden 2008), likely due to water limitation in the desert lowlands. Therefore, especially considering their specialized host plant relationships, it is likely that the observed increase in Noctuidae species richness in wetter conditions and at higher elevations is driven indirectly by the effects of climate on plant productivity. This may also have been influenced by the negative effects of heat and drought on the nutritional quality of host plants and the quantity and quality of floral nectar, which can affect insect development, survival, and reproduction (Wallisdevries et al. 2012; Scaven & Rafferty, 2013; Baude et al. 2016; Phillips et al. 2018; Gely et al. 2020; Descamps et al. 2021; de Manincor et al. 2023). Supporting this conclusion, we found that the species richness of plants in bloom and the Shannon diversity of flowers declined when conditions were drier than average, and that the abundance of flowers declined in hotter conditions. Therefore, as climate change continues to decrease plant survival, productivity, and nutritional quality, moth diversity may also decline.

We found that moth forewing length increased with elevation in the four dominant moth families, and that forewing length was negatively correlated with warmer temperatures during the dry season of the year before sampling. Like species richness, this may be driven by climate impacts on plant productivity or quality (Ho et al. 2010). Higher plant productivity due to more precipitation at the higher elevations may enable larvae to grow larger, resulting in larger adult body sizes (the 'resource availability hypothesis': Blackburn et al. 1999; Watt et al. 2010). Beerli et al. (2019) showed that net primary productivity predicted geographic patterns of body size in hawkmoths (Sphingidae). Another popular hypothesis for increasing body sizes with elevation, "Bergmann's rule" (Bergmann 1847), states that species in cooler environments have larger body sizes because smaller surface area to volume ratios enable them to better conserve heat (Stone 1993; Zamora-Camacho et al. 2014). An extension of this is that smaller bodies may be advantageous in warmer conditions due to a higher capacity for radiative heat loss. However, there is scant evidence that this represents an adaptive response to climate change (Teplitsky & Millien 2014), and shifts in insect body sizes may also be driven by nonadaptive plastic responses to climate (Hodkinson 2005). For example, insect development accelerates in warmer conditions, leading to smaller adult body sizes (the temperature-size rule; Atkinson 1994; Sheridan & Bickford 2011). Brehm et al. (2019) found that temperature was a better predictor than precipitation for increasing moth body size along a tropical elevational gradient in Costa Rica, attributing the trend to the temperature-size rule.

Regardless of the mechanism, our results support recent findings that hotter conditions lead to smaller Lepidoptera body sizes (Bowden et al. 2015; Wu et al. 2019). This is worrisome because larger bodies can help protect insects from starvation or desiccation, thereby buffering them against harsh environmental conditions (Cushman et al. 1993; Chown & Gaston 2010; Harrison et al. 2012; Dias et al. 2013; Gergs & Jager 2014). Climate change may therefore make moths more susceptible to the extreme conditions that it is exacerbating. It also carries implications for their pollination services. There is substantial evidence that as pollinators, moths (especially large-bodied hawkmoths: Sphingidae) facilitate long-distance gene flow in plants and thereby improve plant fitness because they travel long distances when foraging (Herrera 1987; Young 2002; Barthelmess et al. 2006; Jones et al. 2016; Skogen et al. 2019; Lewis et al. 2023). Our data supports this, indicating pollen transport between even the farthest sites (e.g., the incidence of pollen from *Larrea tridentata,* a desert species, on moths at the highest elevation site). However, smaller-bodied insects are more limited in their dispersal ability (Jenkins et al. 2007; Sekar 2012; Jahant-Miller et al. 2022), which can affect their foraging activity (Greenleaf et al. 2007; Földesi et al. 2021). Indeed, we found a positive relationship between moth forewing length and degree. Furthermore, Sphingidae, the moth family with the largest average body size, also had the highest average degree. Together this suggests that larger moths are, on average, visiting more plant species, likely because their dispersal capacity allows them to visit more plant species over longer distances (Greenleaf et al. 2007; Hjalmarsson et al. 2015; Lara-Romero et al. 2019; Sudta et al. 2022). Since we observed a reduction in moth body size in hotter climatic conditions, these results suggest that climate change may in turn decrease the distances moths are able to travel between plants while foraging, potentially reducing their efficacy as pollinators and their ability to gather adequate resources for survival and reproduction.

There are several limitations to this study. First, while the sampling completeness of moth species and pollen types across all sampling events was high (98.5% and 89.3%, respectively), the sampling completeness of interactions was quite low (17.0%). However, this is typical in plant-pollinator network studies (Chacoff et al. 2012; García et al. 2024), especially in pollen DNA metabarcoding studies which can detect rare interactions more efficiently (Macgregor et al. 2019). Second, although some amount of contamination is expected in pollen

DNA metabarcoding studies (Bell et al. 2017; Macgregor et al. 2019), we found particularly high contamination coming from a single plant ASV. However, we performed a highly conservative decontamination protocol to remove contaminants. Third, we were restricted to analyzing binary (unweighted) networks, which provide less accurate estimates of network features that are influenced by species abundances (Blüthgen 2010; Dormann 2011). Although we focused on metrics that are relatively unbiased by species abundances and network size, this work would be improved by incorporating some measure of interaction frequency, and additionally controlling for network size (Pellisier et al. 2018). Finally, although we sampled comprehensively throughout entire flowering seasons across five elevational sites, this is still just one year of data. While network structural features remain relatively constant through time (Alarcón et al. 2008b; Dupont et al. 2009), the composition of species and interactions in plant-pollinator networks display considerable interannual variability (Alarcón et al. 2008b; Caradonna et al. 2021). Future work should analyze how moth pollen-transport networks along environmental gradients vary between years.

Conclusions

Our results reveal complex pollen-transport networks involving entire communities of moths and plants in the Southwestern US, representing one of the largest datasets of moth pollen-transport interactions to date (compare to Banza et al. 2019 and Singh et al. 2022). These data show a wider diversity of moths transporting pollen from a wider diversity of plants than has been found in most other studies, underscoring the importance of moths as pollinators in this region. In addition to native, invasive, and ornamental plants, moths carried pollen from 18 different crop plant taxa, pointing to their potential importance for agricultural production. Our results highlight the dire threat that ongoing moth declines represent to ecosystem function and human food security, and stress the need for more attention on moth pollination in research and management.

We also contribute to the body of knowledge about how insects and their ecosystem services will be impacted by climate change. While the results of space-for-time substitutions must be interpreted with caution (Damgaard 2019; Lovell et al. 2023), we provide additional evidence that moth diversity and size (forewing length) decline in hotter and drier conditions, which is likely to affect their efficacy as pollinators. In agreement with recent findings (Ellis et al. 2023), our results also suggest that the structure of moth pollen-transport networks differs from diurnal networks, perhaps rendering them less resilient to climate change and other stressors. Additionally, we found evidence that increased competition for reduced nectar resources under heat and drought make moths more sensitive to the loss of their nectar plants in these conditions. Since the Southwestern US is a climate change hotspot (Diffenbaugh et al. 2008; Zhang 2023), moths, plants, and their interactions in this region may be particularly vulnerable. While our results suggest that moths are relatively flexible in their nectar plant usage, future work should investigate whether they are able to respond plastically to disruptions in their nectar supply, including at relatively short times scales in response to the extreme climatic events such as droughts and floods that are increasing in the region (Mazdiyasni & AghaKouchak 2015; Zhang et al. 2021; Zhang & Gillies 2022).

Our results reveal that moths visit many plants that are also visited by diurnal pollinators, supporting recent findings that, far from being isolated, diurnal and nocturnal pollinators form interconnected, multilayer networks, linked by shared plant species (Macgregor & Scott-Brown 2020; Souza et al. 2022; García et al. 2024). Indeed, complementary diurnal and nocturnal pollination can substantially increase plant pollination success and buffer plants against disturbances driven by climate change and other stressors (MacGregor et al. 2015; Knop et al. 2018; Alison et al. 2022). This also suggests that perturbations may spread between diurnal and nocturnal pollinators–their fates, along with the plants that connect them, perhaps as intertwined as the networks they are embedded in. Future work should investigate how integrating diurnal and nocturnal pollination networks affects estimates of their resilience to global change stressors (e.g., García et al. 2024).

From an applied perspective, our results highlight the importance of providing diverse food resources for insects, especially in hotter and more arid ecosystems where insects appear to be more vulnerable. In Supplementary material Appendix 1, Table A1.4 we provide lists of important native nectar plant species for moths in this study region, which can be used in conservation efforts aimed at counteracting pollinator losses by providing enhanced food resources and improved habitat (e.g., Merckx et al. 2012; Alison et al. 2017). The impetus for conserving moths is currently limited by poor understanding of their importance as pollinators. This research demonstrates that moths are an integral part of healthy pollinator communities, and underscores the importance of including moths in pollinator conservation programs.

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Tables and Figures

Table 1.1. Model summaries for relationships between moth diversity metrics and elevation/climatic factors. All LMMs include the random effect of site.

Response	Predictor	Model type	Random effect (SD)	*β*	95% CI	*p*
Species richness (Noctuidae)	elevation	GLM:poisson(log)	*NA*	3.11E-04	1.5E-04, 4.7E-04	< .001
Species richness	1mo. total precip.	LMM	3.3E-04	3.89	2.06, 5.72	< .001
Shannon diversity	3y mean dry season precip.	LMM	1.9E-04	0.16	0.031, 0.29	0.018

Table 1.2. LM summaries for the relationship between moth forewing length and elevation for each of the four dominant moth families.

Moth family	F	df	R2	*β* (elevation)	95% CI	*p*
Noctuidae	222.4	1, 1346	0.14	1.29E-04	1.1E-04, 1.5E-04	< .001
Geometridae	307.8	1, 1003	0.23	2.0E-04	1.8E-04, 2.2E-04	< .001
Erebidae	44.41	1, 218	0.17	2.29E-04	1.6E-04, 3.0E-04	< .001
Sphingidae	13.23	1, 134	0.09	2.86E-04	1.3E-04, 4.4E-04	< .001

Table 1.3: Model summaries for the relationships between moth pollen-transport network metrics and elevation, climatic factors, and flower diversity/abundance. All GLMMs include the random effect of site.

Response	Predictor	Model type	Random effect (SD)	*β*	95% CI	*p*
proportion of moths carrying pollen	1mo. total precip.	GLMM: beta(logit)	0.13	-0.26	-0.46, -0.062	0.004
plant robustness	1mo. precip deviation from 30y normal	GLMM: beta(logit)	0.12	-0.019	-0.031, -0.0062	0.012
moth robustness	1mo. total precip.	GLMM: beta(logit)	0.074	0.079	0.0084, 0.15	0.027
plant niche overlap	mean abundance of flowers	GLMM: beta(logit)	8.51E-06	-0.010	-0.020, -0.0010	0.022
moth niche overlap	elevation	GLM: beta(logit)	*NA*	4.5E-04	1.5E-04, 7.5E-04	0.0032
moth niche overlap	1mo. total precip.	GLMM: beta(logit)	9.68E-06	0.24	0.097, 0.39	0.0028

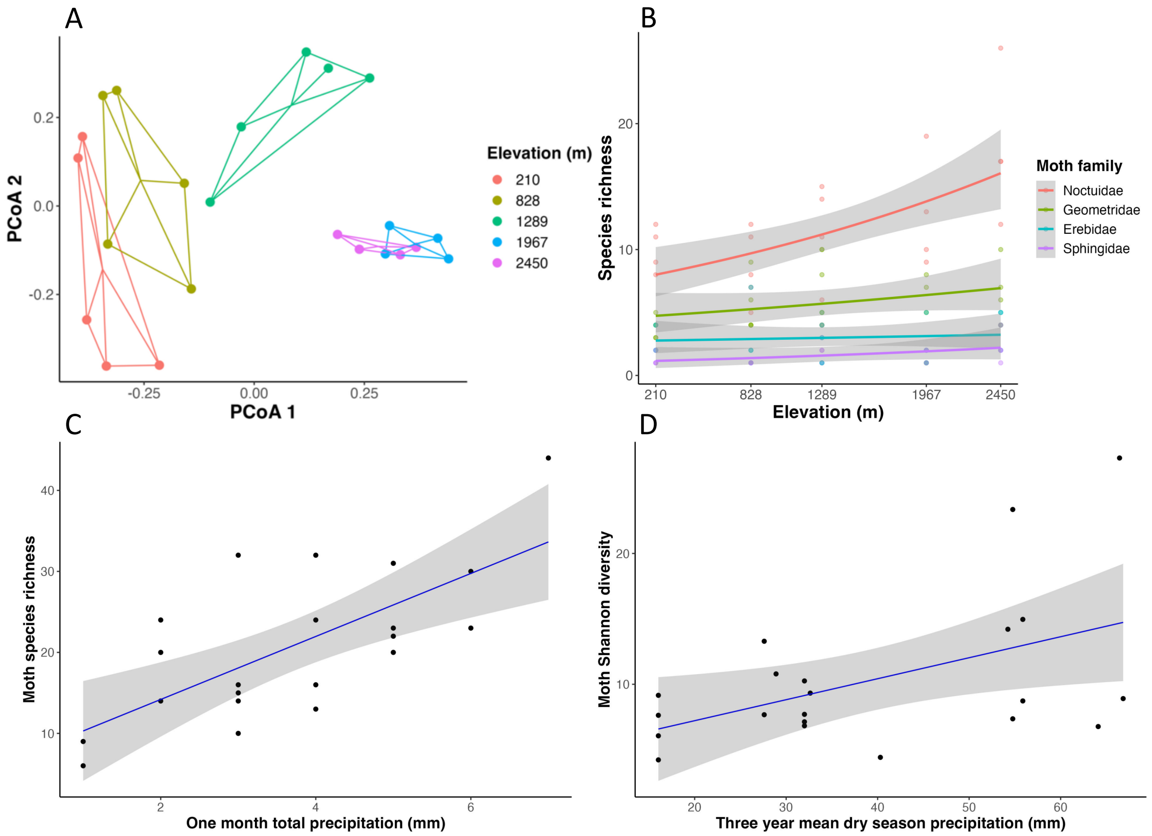

Figure 1.1. (A) PCoA plot using the Bray-Curtis distance metric displaying differences in moth community composition between sites. The relationship between (B) moth species richness and elevation, with moth families designated by color, (C) moth species richness and one month total precipitation, and (D) moth Shannon diversity and three year mean dry season precipitation. Raw data, model fits, and 95% confidence intervals are displayed.

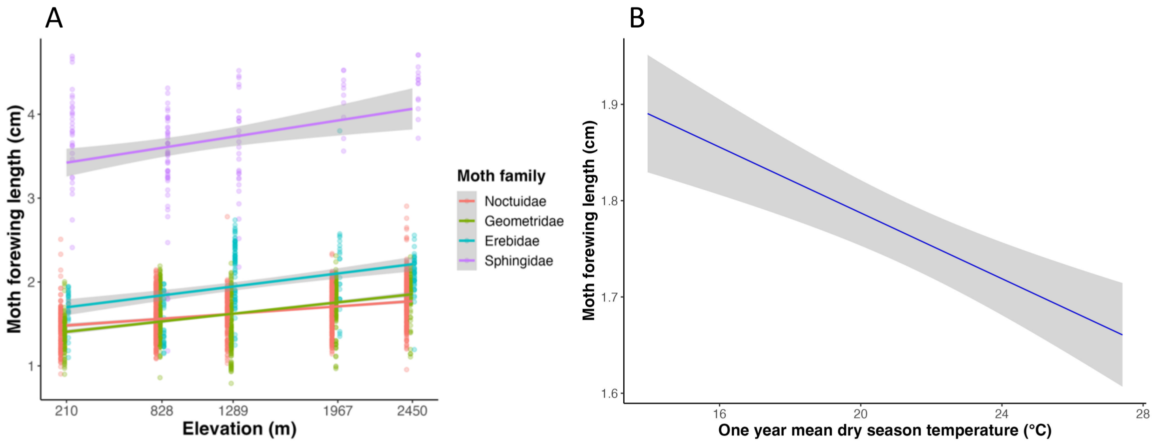

Figure 1.2. (A) The relationship between moth forewing length and elevation, with the families designated by color. Raw data, model fits, and 95% confidence intervals are displayed. (B) The relationship between moth forewing length and one year mean dry season temperature. Model fit and 95% confidence interval are displayed, with raw data removed to improve visibility.

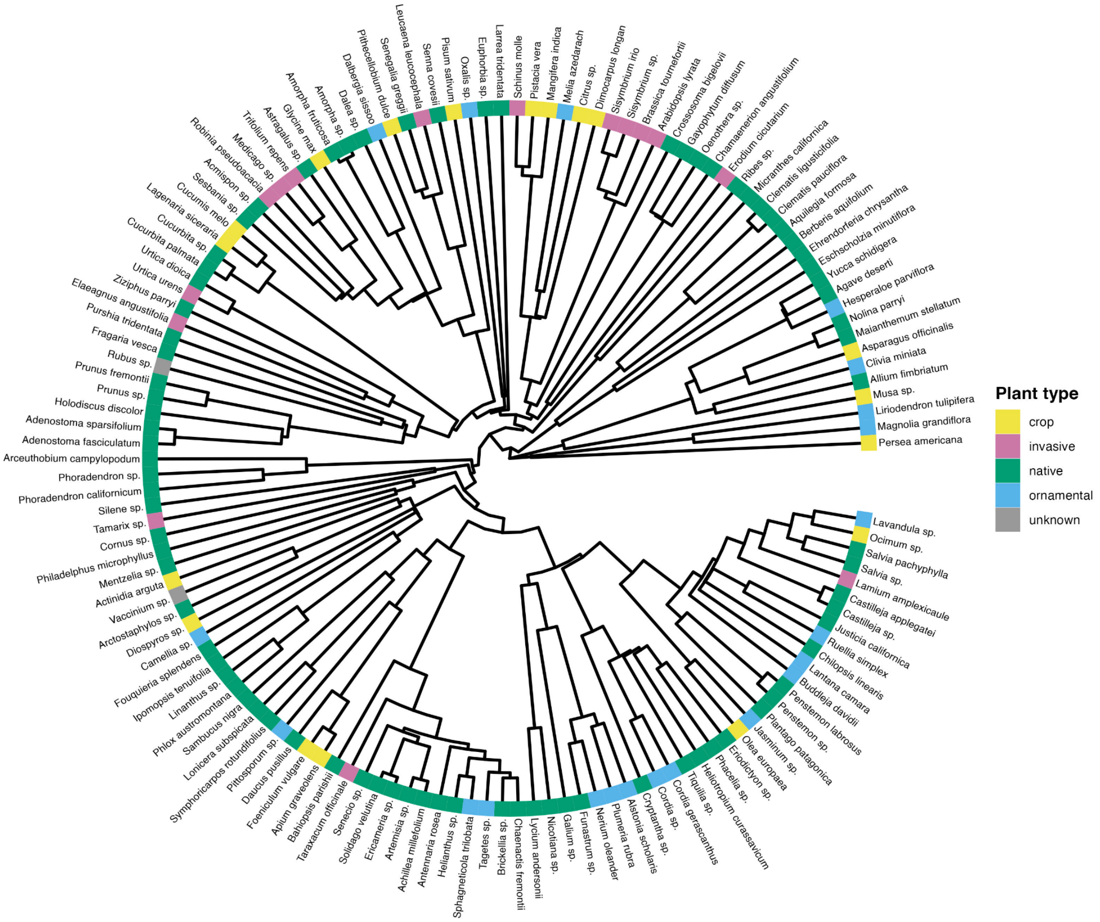

Figure 1.3. Phylogenetic tree displaying all plant taxa detected from the pollen loads carried on moth proboscides. The colors of branch tips designate the type of plant (crop, invasive, native, or ornamental).

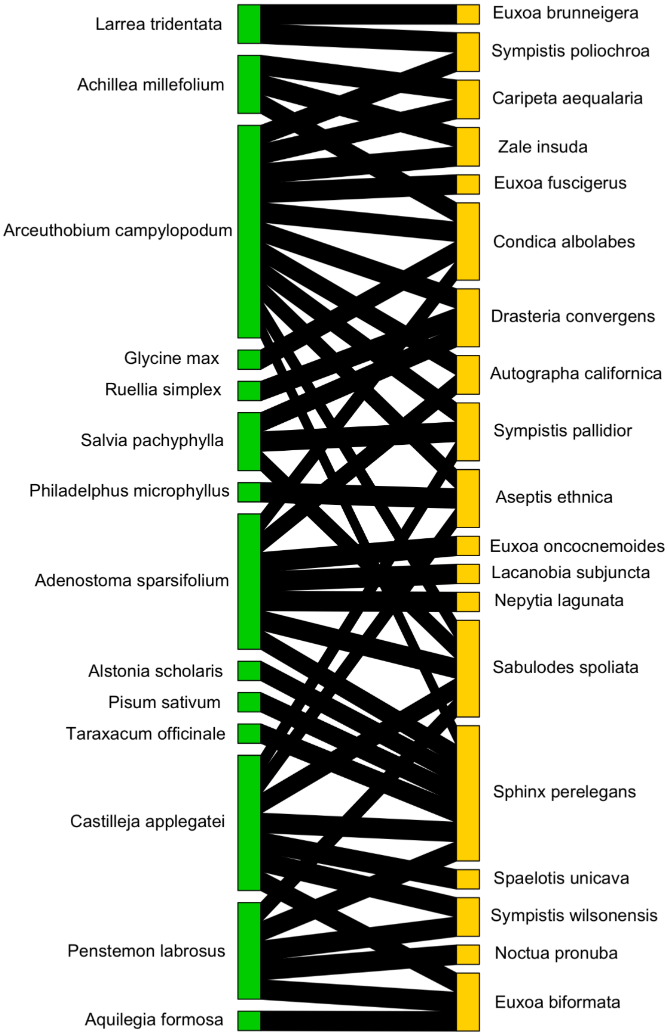

Figure 1.4. A moth pollen transport network from a single sampling event (site = 2450 m, date = 27 July 2022). Species are represented by colored boxes (plant species on the left in green, moth species on the right in yellow), and pollen-transport interactions are represented by black lines. The width of boxes corresponds to the species' degree.

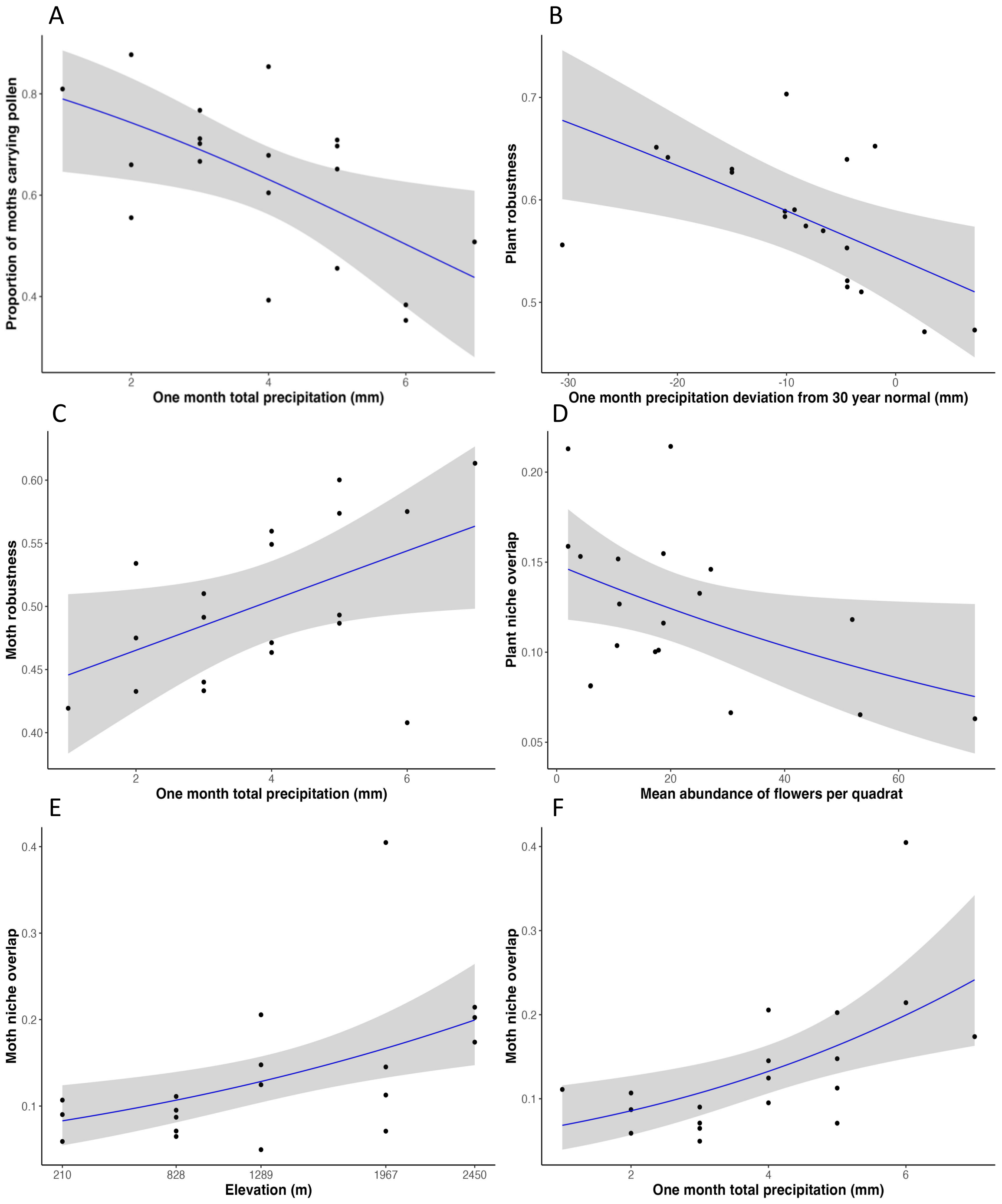

Figure 1.5. The relationship between (A) the proportion of moths carrying pollen and one month total precipitation, (B) plant robustness and one month precipitation deviation from the 30 year normal, (C) moth robustness and one month total precipitation, (D) plant niche overlap and the mean abundance of flowers per quadrat, (E) moth niche overlap and elevation, and (F) moth niche overlap and one month total precipitation. Raw data, model fits, and 95% confidence intervals are displayed.

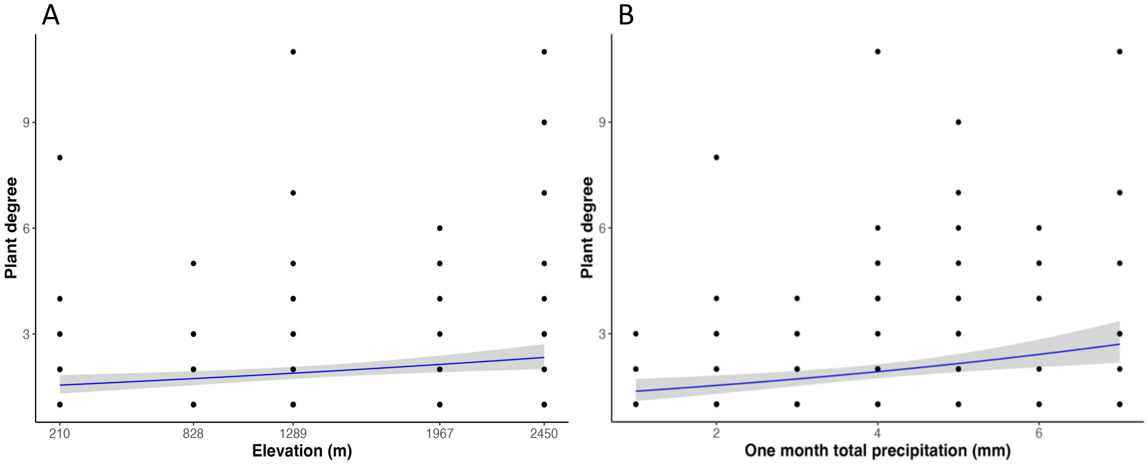

Figure 1.6. The relationship between (A) plant degree and elevation, and (B) plant degree and one month total precipitation. Raw data, model fits, and 95% confidence intervals are displayed.

Chapter 2

The combined effects of warming and drought on floral nectar vary throughout the day: Implications for pollination in a changing climate

Abstract

Climate change alters plant physiological and morphological traits, including floral traits important for pollinator attraction and reward. While warming and drought are known to affect the volume and sugar concentration of floral nectar, how these effects vary diurnally is poorly understood. Plant circadian rhythms and the daily foraging times of pollinators are often synchronized, and climate change-driven shifts to these patterns may cause diel mismatches with potential consequences for the mutualism. Using a greenhouse experiment, we investigated whether warming and drought alter nectar volume, sugar concentration, and total calories at three daily time points (morning, afternoon and night) in *Nicotiana obtusifolia*, a wild tobacco species native to the Southwestern US and visited by both nocturnal and diurnal pollinators. We also investigated whether these traits display plasticity in response to rapid environmental change. We found that alone, neither warming nor drought affected nectar volume and total calories. The combined effects of warming and drought, however, reduced nectar volume and total calories, but only during the afternoon and night. We also found that warming increased nectar sugar concentration during the morning and afternoon. Finally, we found that nectar traits displayed plasticity in response to rapid shifts in temperature and moisture, but that this response depended on the direction of the shift and the time of day. Together, our results suggest that the combined effects of increased temperatures and droughts expected with climate change in many regions may reduce floral nectar, and that the strength of this effect can vary throughout the day. These responses may differentially affect interactions with different suites of pollinators (e.g., diurnal

vs. nocturnal), ultimately altering the structure and function of plant-pollinator interaction networks.

Introduction

Global climate change is one of the most severe threats to biodiversity (Thomas et al. 2004; Brook et al. 2008; Pimm 2009; Shivanna 2022). Altered temperature and precipitation regimes affect all levels of biological organization–from genes, to species, to ecosystems (Parmesan & Yohe 2003; Pörtner & Farrell 2008; Walther 2010; Scheffers et al. 2016)–with impacts propagating through the complex networks of interactions that weave the biosphere together (Tylianakis et al. 2008; Kiers et al. 2010; Traill et al. 2010; Norberg et al. 2012). For example, since many physiological processes and corresponding traits are sensitive to temperature, global warming is likely to have large effects on species traits (Crawley 2009; Huey et al. 2012). Indeed, climate change can induce both plastic changes (Nicotra et al. 2010; Stotz et al. 2021) and rapid evolutionary changes (Jump & Peñuelas 2005) to plant traits, and these responses are often maladaptive (Franks et al. 2014). Climate change-induced changes to traits may in turn disrupt ecological interactions that are important for the provisioning of ecosystem function and services, such as pollination (Petchey et al. 2008; Scaven & Rafferty 2013; Ma et al. 2021). Thus, by quantifying how traits respond in experiments that manipulate environmental conditions, such as temperature and water availability, we can gain a more predictive understanding of the effects of climate change on community stability and ecosystem function (Woodward et al. 2010; Scaven & Rafferty 2013).

Plant floral traits, such as scent and nectar quantity, shape the attractiveness, availability and accessibility of rewards for pollinators (Scaven & Rafferty 2013). In turn, animal pollination services, mainly provided by insects, contribute to the sexual reproduction of 88% of all angiosperm species (Ollerton et al. 2011) and are responsible for 35% of human food crop production (Klein et al. 2007). Thus, plant floral traits are informative in tracking how the impacts of climate change on species traits may scale to disrupt ecological interactions, and ultimately the provisioning of ecosystem services. Indeed, one of the primary mechanisms by which climate change will disrupt plant-pollinator interactions is by altering plant and pollinator physiological and/or morphological matching traits (Scaven & Rafferty 2013; Miller-Struttmann et al. 2015; Borghi et al. 2019; Gérard et al. 2020). There is substantial evidence that warming and drought alter floral traits including flower size, longevity, color, scent, nectar, and pollen (Scaven & Rafferty 2013; Phillips et al. 2018; Gérard et al. 2020; Descamps et al. 2021c), which can disrupt interactions with pollinators (Scaven & Rafferty 2013; Descamps et al. 2021a; de Manincor et al. 2023). For example, in *Borago officinales* (Boraginaceae)*,* water and temperature stress caused reductions in nectar volume and pollen quantity, which decreased pollinator visits in a greenhouse study (Descamps et al. 2018). Different climate change impacts may interact to affect plant traits in complex and species-specific ways (Hoover et al. 2012); for example, altered precipitation can either exacerbate or alleviate the effects of higher temperatures (Bussotti et al. 2014; Cook & Wolkovich 2016). By preventing pollinators from gathering the plant resources that they rely on for nutrition (Scaven & Rafferty 2013) and reducing conspecific pollen transfer important for reproduction and maintaining genetic diversity in plants (Ellstrand 2014; Smith et al. 2022; Acoca-Pidolle et al. 2023), these effects can reduce plant and pollinator fitness (Wilson Rankin et al. 2020; de Manincor et al. 2023). In fact, the indirect effects of climate change on pollinators, through reductions to floral resources, may be even more severe than the direct effects (Ogilvie et al. 2017; Ropars et al. 2020). Given the precipitous insect declines occurring across the globe (Wagner et al. 2021), this topic requires further attention.

Most studies examining the effects of climate change on floral traits compare trait values averaged across multiple time points, or taken only at one time point (usually during daylight hours). Variation in floral traits in response to climate change at finer temporal scales has received comparatively little attention. This is problematic since the processes determining the realization of plant-pollinator interactions occur across multiple temporal scales (Kronfeld-Schor et al. 2017; CaraDonna et al. 2021). For example, diel (24 hour) variation in the quantity and quality of pollinator attractants and rewards, such as nectar and scent, can influence the timing and identity of pollinator visits (Bischoff et al. 2014; Juergens et al. 2014). This variation, in turn, has important implications for plant fitness (Baldwin et al. 1997; Yon et al. 2017). In many plant species, distinct diel rhythms in floral attraction and reward traits have coevolved with the daily foraging windows of their most important pollinators (Dudareva et al. 2000; van Doorn & van Meeteren 2003; Pacini & Nepi 2007; Chapurlat et al. 2018), and this synchronization can help maximize plant reproductive output (Herrera 1990). Depending on the species, diel floral trait rhythms can be under true circadian control (i.e., endogenous and persisting under continuous environmental conditions), or regulated by external factors related to the day/night cycle (e.g., light and temperature: Bloch et al. 2017). In either case, plant diel rhythms can be influenced by both abiotic and biotic factors (Rietveld et al. 1993; Castellanos et al. 2002; Millar 2004; Fründ et al. 2011; Kronfeld-Schor et al. 2017; Oravec & Greenham 2022). While this is better-studied for traits like stomatal opening and closing (Hotta et al. 2007; Gil & Park 2019; Xu et al. 2022; Laosuntisuk et al. 2023), diel rhythms in floral traits, including flower opening and closing, can also be influenced by temperature, humidity, and light (Fründ et al. 2011; Bloch et al. 2017). However, the potential impacts of climate change on the diel rhythms of floral attractants and rewards, and how this may affect plant-pollinator interactions, is poorly understood.

A few studies suggest that warming and/or drought may alter the diel rhythms of floral attractants and rewards (flower opening and closing: Prieto-Benitez et al. 2016; floral scent emission: Wu et al. 2023; floral nectar: Arroyo et al. 2020). For example, Prieto-Benitez et al.

(2016) found that *Silene colorata* (Caryophyllaceae) closed petals earlier in the morning and opened them later at night in response to drought, thus constricting the window of time that flowers were available to pollinators. In the same way that plants close stomata to minimize water loss in arid conditions (Hetherington & Woodward 2003; Pirasteh-Anosheh et al. 2016), plasticity in diel patterns of flower opening and closing is likely an adaptive response to avoid water loss through evapotranspiration in arid conditions (Prieto-Benitez et al. 2016; Borges et al. 2016). Indeed, flowering is a water-intensive process, and is mediated in part by water availability (Mohan Ram & Rao 1984; Galen et al. 1999; Galen 2000; De la Barrera et al. 2009). Borges et al. (2016) proposed the "arid or water-stress hypothesis for nocturnal flowering", which suggests that nocturnal flowering is an adaptive strategy for plants to avoid water loss through evapotranspiration in xeric conditions. This hypothesis is supported by the observations that globally, there are more nocturnally-flowering plant species in families with xerophytic adaptations and that nocturnal pollination is more frequent in plant families in xeric environments (Fleming et al. 2009; Borges et al. 2016). However, to what degree plants are able to plastically modify circadian floral trait rhythms, especially in response to rapid shifts in environmental conditions that involve both altered temperature and precipitation, is poorly understood (Franks et al. 2014; Harvey et al. 2020; Costaz et al. 2023). Climate change is increasing the frequency, duration and severity of extreme climatic events, including heatwaves, droughts and floods (Meehl & Tebaldi 2004; Trenberth 2011; Dai 2013; Papalexiou & Montanari 2019; Zhang et al. 2021; Robinson 2021; Capua & Rahmstorf 2023). Thus, knowledge of whether plants can mount a timely plastic response to rapid environmental shifts, and to which types of shifts, will be important for predicting and managing the effects of climate change on ecosystems (Anderson et al. 2012; Costaz et al. 2023).

However, selection of diel floral trait rhythms by the abiotic environment may also conflict with those selected by pollinators (Carroll et al. 2001; Prieto-Benitez et al. 2016). Plastic responses of diel floral rhythms to environmental change may alter the suite of pollinators with which plants interact over the course of the day. For instance, many plant species remain open both day and night, and are visited by both diurnal and nocturnal pollinators (Valdivia & Niemeyer 2006; Borges et al. 2016). This provides redundancy of pollination services, making plants more resilient to reduced pollinator visits during any one time period (Waser et al. 1996; Fleming et al. 2001; DAR et al. 2006). For these species, climate change-driven shifts to diel floral trait rhythms may decrease synchrony with all or some suites of pollinators, or alternatively increase synchrony with certain suites of pollinators depending on their foraging times (e.g., nocturnal, crepuscular, or diurnal). As different suites of pollinators vary in their contribution to plant reproduction (Wilson & Thomson 1991; Ivey et al. 2003; Scopece et al. 2018; Koski et al. 2018; Hattori et al. 2020), this may impact plant fitness. In turn, diel pollinator activity times have evolved in part in response to diel floral resource availability (Kawahara et al. 2018, 2019), and pollinators may be affected by climate change-driven shifts to floral rhythms if they are unable to modify their foraging times. Thus, in the same way that climate change-driven shifts in seasonal flowering phenology can cause asynchrony with pollinators (Burkle et al. 2013; Polce et al. 2014; Rafferty et al. 2015; Kudo & Cooper 2019), shifts in diel floral trait rhythms may push plants out of sync with the daily activity patterns of their pollinators.

Diel variation in climate change response remains virtually unexplored for many important floral traits, including nectar production (but see Arroyo et al. 2020). Nectar is the primary floral reward for pollinators and plays a key role in pollinator attraction (Pyke 1991; Raguso & Willis 2005; Pyke et al. 2020). Nectar volume and total sugar content per flower are generally reduced under both warming and drought (Carroll et al. 2001; Takkis et al. 2018;

Descamps et al. 2018, 2020; Phillips et al. 2018), and decreasing nectar supply due to climate change and other anthropogenic stressors has been linked to declines in multiple pollinator taxa (Wallisdevries et al. 2012; Baude et al. 2016). Investigating the link between floral traits and plant water economy can help reveal the mechanisms through which climate change may disrupt the supply of floral resources for pollinators. For example, since the majority of plant water loss occurs through the stomata, stomatal conductance plays a key role in regulating plant water balance (Hetherington & Woodward 2003). In order to reduce water loss, plants often close their stomata in response to drought stress (Pirasteh-Anosheh et al. 2016). However, this can also reduce photosynthetic carbon gain, leading to a lack of carbohydrates for growth and reproductive processes, such as the supply of sugars to floral nectar (Cornic 2000; Lemoine et al. 2013; Mencuccini et al. 2015). This may have significant consequences for plant-pollinator interactions, including nocturnal ones as the majority of nocturnal pollinators consume nectar as a reward (Borges et al. 2016). However, logistical challenges associated with conducting research at night has resulted in a strong diurnal bias in biological research, and how anthropogenic pressures will alter the balance between diurnal and nocturnal communities, their interactions, and ultimately their contribution to overall ecosystem functioning is a major outstanding question (Gaston 2019). This knowledge gap represents a critical barrier to our ability to predict the consequences of environmental change, since the ecological impacts of global change stressors are likely to depend on species circadian rhythms and diel activity patterns (Cox & Gaston 2024).

Here, we investigate the link between warming and drought, plant water status, and the diel variation of floral traits important for pollinator attraction and reward in *Nicotiana obtusifolia* (Solanaceae), a wild tobacco species native to the Southwestern US that is visited by both diurnal and nocturnal pollinators. The Southwestern US is a climate change hotspot (Diffenbaugh et al. 2008; Zhang 2023). Average temperatures in the region, which have already risen 0.89°C in the last century, are expected to climb another 2.7-4.8°C by 2100 (Vose et al. 2017), and droughts and extreme precipitation events are intensifying (Mazdiyasni & AghaKouchak 2015; Zhang & Gillies 2022). Using a greenhouse manipulation, we ask (1) How do warming and drought affect the diel variation in floral nectar volume, sugar concentration, and caloric content in *N. obtusifolia*? We hypothesize that flowering and the production of floral rewards are water-intensive, and that in more arid conditions, plants should produce more rewards at night to avoid water loss through evapotranspiration (Borges et al. 2016). We therefore predict overall reductions to nectar volume and caloric content, and proportionally more of the total daily nectar production to shift towards night under warming and drought. We also ask (2) Do floral traits and their diel variation display plasticity in response to rapid shifts in environment conditions? We hypothesize that because *N. obtusifolia* evolved in environments where rainfall has been historically variable, rapid plastic responses in water use efficiency and the expression of water-intensive floral traits may have been selected for (Via & Lande 1985; Heschel et al. 2002; Ghalambor et al. 2007). We therefore predict that when individual plants are moved between temperature and water treatments, the diel variation of plant traits will also quickly shift.

Methods

Study species

Nicotiana obtusifolia (Solanaceae), commonly known as desert tobacco, is a perennial, diploid herb native to the Southwestern US, from California to Utah to Texas, and Mexico (Baldwin et al. 2012). It occurs in sandy/rocky washes and slopes below 1,600 m, and grows to 20-80 cm in height (Baldwin et al. 2012). The funnel-shaped flowers are white to green-tinged, have a tubular throat between 1.5-2.6 cm long, and bloom predominantly from March-June (Baldwin et al. 2012), although they can produce flowers year round (Wells 1959). Various *Nicotiana* species have been used as model organisms for studying traits important for ecological interactions (Loughrin et al. 1991; Raguso et al. 2003; Wang & Wu 2013; Yon et al. 2017; Tiedge & Lohaus 2017). Many species in the genus display distinct diel rhythms, under circadian control, in floral traits that attract both diurnal and nocturnal pollinators (Haverkamp et al. 2018). Like many other *Nicotiana* species, *N. obtusifolia* is fully self-compatible, but is visited both diurnally by bees and butterflies and nocturnally by moths (Austin 2010; Caldwell 2020; Cosma, unpublished data). The related and also predominantly selfing species, *Nicotiana attenuata,* opportunistically employs a mixed-mating system in which hawkmoths (Sphingidae) contribute to seed set (Sime & Baldwin 2003). *Nicotiana obtusifolia* has several characteristics that made it suitable for this study: it continually produces many flowers per plant over a relatively long flowering season in the first year of growth, the flowers persist for several days, and the flowers have detectable nectar.

We obtained *N. obtusifolia* seeds from plants that were grown and allowed to self-pollinate in greenhouses at the University of California, Riverside (UCR). The parent seeds (TW143) were originally obtained from the New York Botanic Garden. We germinated seeds in January 2019 by placing them in petri dishes on filter paper dampened with DI water and incubated in a growth chamber (Conviron MTR30) at 20°C and a 12:12 light:dark cycle. We ensured that the filter paper remained damp while the seeds were germinating. Once seeds germinated (in February 2019), we planted seedlings in peat pellets and transferred them to a growth chamber at 16 hr light/8 hr dark, 25°C and light intensity of 112 mmol m^{-2} s^{-1}. We ensured the peat pellets remained damp. We kept them in these conditions until they developed the second set of true leaves. In late February 2019, we then transplanted the plants, in the peat pellets, to 2 L black plastic pots with a sandy soil mix. We transferred all the plants to a greenhouse, where they were kept in ambient temperature, humidity, and light conditions. Plants remained in ambient greenhouse light conditions for the remainder of the experiment. We ensured all plants were well-watered by giving them 100 mL of water twice a day. Once all plants were flowering (in May 2019), we subjected them to a weeklong drought-hardening period in which plants were given 30 mL of water each day, in order to avoid mortality in the drought treatment (Khan et al. 2020). To inform our drought hardening and drought treatment methods, we used additional plants not used in the main experiment to determine the volume of water that caused plants to visibly wilt but not die. To ensure growth throughout the experiment, we fertilized all plants every two weeks throughout the experiment with 25 mL of water containing .001 teaspoon/mL of 20-20-20 NPK fertilizer (Grow More All Season's), mixed in with the rest of their water for the day.

Greenhouse treatments

From June-September 2019, using a full factorial design in UCR greenhouses, we subjected plants to two temperature treatments in different temperature-controlled greenhouse rooms (cool room and warm room), and two water treatments within each room (well-watered and drought). Thus, there were four treatments (cool + well-watered, cool + drought, warm + well-watered, warm + drought), with 30 plants per treatment. We placed plants in a block design in each room, with each block containing one droughted and one well-watered plant. We randomly shuffled block positions on the greenhouse bench each week. To test for plasticity in the plant traits to rapid changes in environmental conditions, the experiment included two phases. Phase 1 lasted the first five weeks. For phase 2, we switched a random half of the plants from each treatment to the opposite temperature and water treatment, and after a week-long acclimation period, we resumed the same measurements on the switched plants for another four weeks.

Our goal was to examine the effects of heat and drought stress, rather than aim for specific temperature and moisture values expected with climate change. We verified stress in the warm and drought treatments both visually (e.g., by documenting leaf wilting: Engelbrecht et al.

2007) and with water balance measurements. To avoid plant mortality, we had to increase the amount of water in the drought treatment compared to the drought-hardening period, since the plants grew larger and the ambient temperature increased. We determined the amount of water in the well-watered treatment by testing on extra plants which amount ensured no leaf wilting throughout the day and saturated the soil but minimized water runoff during any single watering event. After optimizing the water treatments based on these criteria, plants in the drought treatment received 50 mL of water per day, while plants in the well-watered treatment received 200 mL per day. Thus, the drought treatment received 25% of the water necessary for full soil saturation, which is similar to the ratio used in other studies testing drought stress in *Nicotiana* species (e.g., Halpern et al. 2010; Hajiboland et al. 2017). Water was delivered to each plant at 8:00 am each day (after the morning measurements were taken) through individual drip irrigators placed into each pot. Temperature and relative humidity (RH) were recorded in each room every 15 minutes using data loggers (Onset HOBO). While temperature and humidity remained consistently different between the rooms, they were allowed to fluctuate based on the ambient conditions. Due to a failure of the data loggers, RH data is missing from four measurement days during phase 1 (July 17, 20, 23, and 26), while temperature data for those days were filled in from temperatures manually recorded from the data loggers during the measurement times. We measured soil moisture during each data collection time point with a soil moisture meter (Extech MO750), inserting 10 cm into the soil and allowing the measurement to equilibrate for 30 seconds.

Differences in temperature were successfully achieved between the two greenhouse rooms throughout the experiment (Supplementary material Appendix 2, Figure A2.1). The cool treatment greenhouse room had an average daily high temperature of 30.2 ± 4.5°C (mean ± SD) and an average daily low of 14.9 ± 2.7°C, while the warm treatment room had an average daily high of 36.7 ± 5.6°C, and an average daily low of 18.1 ± 3.2°C. The difference in mean daily temperature between the warm room (24.96°C) and cool room (20.34°C) was 4.6°C, which is approximately the temperature increase expected in the Southwestern US by 2100 under RCP8.5 (Vose et al. 2017). The cool room had an average daily maximum RH of 91.1 ± 6.0% (mean ± SD) and an average daily minimum RH of 51.9 ± 8.9%, while the warm room had an average daily maximum RH of 76.7 ± 7.6% and an average daily minimum RH of 34.9 ± 8.7%. The soil moisture meter we used had low sensitivity, however the results still confirm that relative differences between treatments were achieved. Soil moisture was always at 0% in the drought treatments in both rooms, while the average for the well-watered treatment in the cool room was 9.1 ± 4.8%, and the average for the well-watered treatment in the warm room was 4.4 ± 3.9%. These results confirm the efficacy of our temperature and water treatments.

Plant trait measurements

We took measurements on two non-consecutive days per week during three separate, evenly-spaced time intervals per day (morning: 6-8 am, afternoon: 2-4 pm, and night: 10 pm-12 am). We took measurements for a total of 11 days during five consecutive weeks for phase 1 and seven days during four consecutive weeks for phase 2. We measured the following traits on a different haphazardly-chosen subset of four plants per treatment each week: plant height, the number of flowers per plant, nectar volume and sugar concentration for two flowers per plant per sampling period, leaf stomatal conductance for two leaves per plant per sampling period, leaf temperature for two leaves per plant per sampling period, and soil moisture.

We measured nectar volume by inserting 4 μL microcapillary tubes (Drummond Short-Length Microcaps) into the base of the corolla, gently circling for 15 seconds or until we saw the visible nectar level in the tube stop increasing, then measuring the length of the nectar column to obtain nectar volume. We measured nectar concentration using optical handheld refractometers

(Bellingham and Stanley Eclipse Brix 30 and Eclipse Nectar 45-80). We calculated the total solution energy value in calories per flower following Dafni et al. (2005) with the following formula:

$$calories = 4 * (0.00226 + (0.00937x) + (0.0000585x^2)) * y$$

Where “x” is the nectar sugar concentration (in degrees Brix) and “y” is the nectar volume (in uL)

After opening, *N. obtusifolia* flowers take several days to dehisce. To control for the age of each flower, we tracked when each flower on each plant opened by putting small paint dots on the sepals of flowers or buds during the flower counting, using different paint colors for buds and flowers. Whenever possible, we measured only flowers that had opened within the last 48 hr period and had dehiscent anthers. We avoided repeated sampling of flowers by marking measured flowers with a third paint color. Due to the demand for a large number of flowers per plant to complete the nectar measurements each week, we had to switch which plants we took nectar measurements from at several points throughout the experiment. When this was the case, we continued taking water balance measurements on the original plants to reduce bias in these measurements.

We used several metrics to assess plant water balance and physiological stress in response to the temperature and water treatments. First, we calculated leaf-to-air vapor pressure deficit (VPD_L), measured as the difference in the water vapor pressure in the leaf minus the water vapor pressure of the ambient air (Dai et al. 1992; Day 2000; Marchin et al. 2016). VPD_L provides an accurate evaluation of leaf water balance and can be used to assess plant physiological stress (Grossiord et al. 2020). To calculate VPD_L, we first measured leaf temperature with an infrared thermometer (Raytek MiniTemp), aiming it from a distance of ~10 cm at a 90° angle to the leaf surface, and allowing the reading to equilibrate for several seconds.

We used only dry leaves that were in good condition. Using this and the RH measurements from the data loggers, we calculated VPD_L with the following formulae, assuming saturation vapor pressure within the stomatal pore:

$$\text{Air Saturation Vapor Pressure (ASVP)} = (610.78 * e\hat{}(T_A / (T_A + 237.3) * 17.2694))/1000$$

Where T_A = air temperature (in °C)

$$\text{Leaf Saturation Vapor Pressure (LSVP)} = (610.78 * e\hat{}(T_L / (T_L + 237.3) * 17.2694))/1000$$

Where T_L = leaf temperature (in °C)

$$VPD_L = LSVP - (ASVP * (RH/100))$$

Where RH = relative humidity

We also measured leaf stomatal conductance with a leaf porometer (Meter SC-1), again using only dry leaves that were in good condition. Stomatal conductance characterizes the rate of leaf water loss through stomata, normalized by the VPD_L, the force driving the evaporative flux. Plant hydraulic capacity, in both leaves and stems, is strongly related to stomatal sensitivity to VPD_L (Brodribb & Jordan 2008; Zhang et al. 2013). Before taking measurements in each greenhouse room, we allowed the porometer to rest in the ambient conditions of the room for 30 min, then calibrated the porometer in the same conditions before taking measurements. For each measurement, we allowed the porometer to rest on the leaf for 30 sec to equilibrate.

Data analyses

We performed all analyses in R version 4.3.2 (R Core Team 2021). To test whether temperature and water treatments affected plant traits at different times of day, we used the package ‘glmmTMB’ (Magnusson et al. 2017) to build generalized linear mixed-effects models (GLMMs) with the various plant trait measurements as the response. The error distribution families used for each best-fitting model are described in the tables. Predictors included temperature treatment, water treatment, time of day, and all two-way and three-way interactions among temperature, water, and time as fixed effects. In each model, we included plant identity as a random effect to account for the non-independence of repeated measurements on the same plants. To evaluate whether temperature and water treatments affected the proportion of nectar calories produced at each time point, for each treatment combination we calculated the average total nectar calories per flower per time point, and divided this by the average total calories per flower produced across all time points. We included this proportion as the response in a GLMM using the Beta family distribution, with the temperature/water treatment combination and time of day as fixed effect predictors, and plant identity as a random effect. To evaluate the relationship between VPD_L and stomatal conductance, we built GLMMs with stomatal conductance as the response variable, VPD_L, water treatment, temperature treatment, time of day, and all possible two-way, three-way, and four-way interactions among $VPD_{L,}$ temperature, water, and time as fixed effects predictors, and plant identity as a random effect. To determine whether floral and water balance traits displayed plasticity to shifting environmental conditions in phase 2 of the experiment, we built (G)LMMs with the various plant traits as the response variables, the experimental phase, the type of treatment transition, the time of day, and all two-way and three-way interactions among experimental phase, the type of treatment transition and the time of day as fixed effect predictors, and plant identity as a random effect. For all (G)LMMs, we evaluated model fit by examining residual distributions with the ‘Dharma’ package (Hartig 2017). For some of the models, fit was improved by adding zero-inflation and/or dispersion terms. We used the ‘anova’ function to perform likelihood ratio tests to assess variable significance. When there was a significant interaction term, we reported the interaction term rather than the main effects of each term. We performed pairwise post hoc comparisons by comparing estimated marginal means with the ‘emmeans’ function from the ‘emmeans’ package (Lenth et al. 2022) using the “Tukey” p value adjustment method, and converted to compact letter display with the 'cld' function from the package 'multcomp' (Hothorn et al. 2016). To visualize the main effects in each model, we calculated predicted (marginal) effects using the 'ggpredict' function from the 'ggeffects' package (Lüdecke 2018).

Since we were predominantly interested in the effects of temperature treatment, water treatment, and time of day, we did not include day of year as a predictor in the aforementioned models. However, to examine how plant height, the number of flowers per plant, and nectar traits varied throughout the experiment, we also built (generalized) linear models ((G)LMs) with the various plant traits as the response variable, and the day of year as the predictor. We repeated this analysis for each temperature and water treatment combination. We also used linear models (LMs) to examine the relationship between the various nectar traits, and the relationships between the water balance measurements and nectar traits. For the latter, due to the fact that we did not always measure water balance traits on the same plants as we measured nectar, we used averages per treatment, day, and time period. For all models with nectar sugar concentration as the response variable, we used beta regression with the 'betareg' package (Cribari-Neto & Zeileis 2010). For all (G)LMs, we verified model assumptions by examining residual plots. For all models, 95% Confidence Intervals (CIs) and p-values were computed using a Wald t-distribution (LMs) or Wald z-distribution (GLMs and GLMMs) approximation. We produced all plots using the 'ggplot2' package (Wickham et al. 2016).

Results

Plants were significantly shorter in the drought treatments than the well-watered treatments ($\beta = -0.28$, 95% CI [-0.38, -0.17], $p < 0.001$; Supplementary material Appendix 2, Figure A2.2A). Plants were also significantly shorter in the warm treatments than the cool treatments ($\beta = -0.15$, 95% CI [-0.25, -0.05], $p < 0.001$; Supplementary material Appendix 2,

Figure A2.2B). There were significantly fewer flowers per plant in the drought treatments than the well-watered treatments (β = -0.68, 95% CI [-1.05, -0.31], $p < 0.001$; Supplementary material Appendix 2, Figure A2.2C), while the number of flowers was not affected by temperature treatment. Plant height, flower abundance, and nectar volume generally increased through time in the experiment, although the significance of the trends depended on the treatment (Supplementary material Appendix 2, Table A2.1 and Figures A2.3A, A2.3B, and A2.3C). Nectar concentration did not change significantly through time in any treatment (Supplementary material Appendix 2, Table A2.1 and Figure A2.3D). Total nectar calories increased through time in all treatments (Supplementary material Appendix 2, Table A2.1 and Figure A2.3E).

Nectar traits displayed distinct diel rhythms, the general pattern of which were consistent across treatments. Nectar volume increased throughout the night, peaked in the morning, then declined again during the day in all treatments (Figure 2.1A). Total nectar calories showed the same diel rhythms as nectar volume in all treatments (Figure 2.1C), which is supported by the strong positive correlation ($R^2 = 0.96$, $F_{1, 1331} = 35128.54$, $p < 0.001$) that we found between nectar volume and total calories (Supplementary material Appendix 2, Figure A2.4A). Nectar concentration showed the opposite trend, declining during the night to the lowest concentration in the early morning, then increasing to the peak during the day in all treatments (Figure 2.1B). The contrasting trends in nectar volume and concentration are supported by the significant, although weak, negative correlation between nectar volume and sugar concentration (pseudo $R^2 = 0.06$, β = -0.17, 95% CI [-0.22, -0.12], $p < 0.001$; Supplementary material Appendix 2, Figure A2.4B). There was no significant relationship between nectar concentration and total calories (Supplementary material Appendix 2, Figure A2.4A), and this, combined with the strong relationship between nectar volume and total calories suggests that nectar caloric content is predominantly determined by nectar volume.

The effects of temperature and water treatments on plant water economy varied through the day. The interaction between temperature treatment and time of day had a significant effect on VPD_L (Table 2.1), while VPD_L was not affected by water treatment. In both warm and cool treatments, VPD_L was lowest in the morning, highest during the afternoon, and intermediate at night (Figure 2.2A). At each time point, VPD_L was significantly higher in the warm treatments than the cool treatments, especially during the afternoon (Figure 2.2A). As higher VPD_L is generally associated with higher physiological stress, these results confirm that plants were more stressed in the warm treatment and during the warmer time points in both temperature treatments. The interaction between temperature treatment, water treatment, and time of day had a significant effect on leaf stomatal conductance (Table 2.1). In all treatments, stomatal conductance was lowest at night, highest during the afternoon, and intermediate during the morning (Figure 2.2B). During the morning, stomatal conductance was significantly higher in the warm, well-watered treatment, and during the night stomatal conductance was significantly higher in the warm treatment in both water treatments (Figure 2.2B). However, during the afternoon, stomatal conductance was significantly lower in the warm, drought treatment (Figure 2.2B). While the effect was only significant in the warm treatment during the morning and afternoon, drought generally decreased stomatal conductance across most treatments and time points (Figure 2.2B). The relationship between VPD_L and stomatal conductance depended on the temperature treatment and time of day (Table 2.1). During the morning, higher VPD_L increased stomatal conductance, especially in the cool treatment (Figure 2.2C). During the other time points, higher VPD_L decreased stomatal conductance, particularly in the warm treatment during the afternoon (Figure 2.2C). Together these results suggest that the combination of excessive heat and drought caused *N. obtusifolia* plants to close their stomata to avoid water loss via transpiration, and confirms that our temperature and water treatments were causing physiological stress to the plants.

Plant water balance traits were significantly correlated with floral nectar traits. Across all treatments and time points, there was a significant negative relationship between VPD_L and nectar volume ($R^2 = 0.25$, $F_{1, 68} = 22.19$, $p < 0.001$; Supplementary material Appendix 2, Figure A2.5A), and between VPD_L and total nectar calories ($R^2 = 0.24$, $F_{1, 68} = 22.03$, $p < 0.001$; Supplementary material Appendix 2, Figure A2.5C). There was a significant positive relationship between VPD_L and nectar concentration (pseudo $R^2 = 0.18$, $\beta = 0.22$, 95% CI [0.11, 0.34], $p < 0.001$; Supplementary material Appendix 2, Figure A2.5B). The same relationships were seen between stomatal conductance and nectar volume ($R^2 = 0.1$, $F_{1, 68} = 12.01$, $p < 0.001$; Supplementary material Appendix 2, Figure A2.6A), total calories ($R^2 = 0.08$, $F_{1, 68} = 10.06$, $p = 0.002$; Supplementary material Appendix 2, Figure A2.6C), and sugar concentration (pseudo $R^2 = 0.11$, $\beta = 1.9E-3$, 95% CI [9.2E-4, 2.9E-3], $p < 0.001$; Supplementary material Appendix 2, Figure A2.6B).

While the overall diel rhythms of nectar volume, sugar concentration, and calories were consistent across treatments (Figure 2.1), the interaction between warming and drought had significant effects on these traits. Moreover, these effects also differed depending on the time of day. 49.4% of all measured flowers contained no detectable nectar, and the probability of detecting nectar varied significantly by time of day, being highest during the morning, and significantly lower both at night ($\beta = -0.85$, 95% CI [-1.41, -0.29], $p = 0.003$) and especially in the afternoon ($\beta = -2.07$, 95% CI [-2.67, -1.47], $p < 0.001$; Supplementary material Appendix 2, Figure A2.7). The three-way interaction among water treatment, temperature treatment, and time of day had a significant effect on nectar volume (Table 2.2). In all treatments, nectar volume was highest in the morning, lowest in the afternoon, and intermediate at night (Figure 2.3A). However, in the afternoon, the combination of warming and drought significantly decreased nectar volume relative to the other treatments (Figure 2.3A). Furthermore, at night, warming increased nectar volume in the well-watered treatment and decreased it in the drought treatment such that they were significantly different (Figure 2.3A). The interaction between temperature treatment and time of day had a significant effect on nectar sugar concentration (Table 2.2), while nectar sugar concentration was not significantly affected by the water treatment. In both the cool and warm temperature treatments, nectar sugar concentration was lowest in the morning, highest in the afternoon, and intermediate at night, although the difference between morning and night was not significant in the warm treatment (Figure 2.3B). At each time point, nectar sugar concentration was higher in the warm treatment, although this was only significant during the morning and afternoon (Figure 2.3B). The trends in total nectar calories were very similar to nectar volume. The interaction between water treatment, temperature treatment, and time of day had a significant effect on total nectar calories (Table 2.2). In all treatments, nectar calories were highest in the morning, lowest in the afternoon, and intermediate at night (Figure 2.3C). However, in the afternoon, the combination of warming and drought significantly decreased nectar volume relative to the warm, well-watered and cool, drought treatments (Figure 2.3C). Furthermore, at night, warming increased nectar calories in the well-watered treatment and decreased it in the drought treatment such that they were significantly different (Figure 2.3C).

We also found that the combination of warming and drought decreased the proportion of total daily nectar calories per flower produced in the afternoon in the warm, drought treatment relative to the other treatments, although this reduction was only significantly relative to the cool drought treatment ($\beta = -1.78$, 95% CI [-2.70, -0.86], $p < 0.001$; Figure 2.4). Further, although it was not significant, both the warm temperature treatments had a relatively higher proportion of total daily nectar calories per flower produced at night than the cool treatments, and the warm + drought treatment had relatively higher proportion of total daily nectar calories produced during the morning than the other treatments (Figure 2.4).

When switched to the opposite temperature and water treatments, whether plants shifted their floral traits significantly depended on the type of treatment transition and the time of day (Table 2.3). Generally, the directions of the shifts were consistent with our expectations based on the differences between treatments in phase 1 of the experiment. Plants that had previously been in the drought treatments and were switched to the well-watered treatments showed an increase in nectar volume and total calories during the morning and night in phase 2 (Figure 2.5A and 2.5C). However, during the afternoon, plants that had previously been in the cool, drought treatment and were switched to the warm, well-watered treatment showed a significant decrease in nectar volume and calories (Figure 2.5A and 2.5C). Generally, plants that had originally been in the well-watered treatments and were shifted to the drought treatments showed a decrease in nectar volume and total calories during phase 2 (Figure 2.5A and 2.5C). However, in the morning, plants that were previously in the cool, well-watered treatment increased in nectar volume and calories when moved to the warm, drought treatment, though not significantly (Figure 2.5A and 2.5C). Plants previously in the cool treatments generally increased in nectar concentration when moved to the warm treatments, and vice versa (Figure 2.5B).

When switched to the opposite temperature and water treatments, whether plants shifted their water balance traits significantly also depended on the type of treatment transition and the time of day (Table 2.4). Regardless of water treatment, for plants that were moved from the cool to the warm temperature treatment in phase 2, VPD_L increased significantly and vice versa (Figure 2.6A). The differences in VPD_L between phase 1 and phase 2 were especially pronounced during the afternoon. Stomatal conductance showed more complex trends (Figure 2.6B). During the morning, plants that started in the cool treatment and were moved to the warm treatment increased stomatal conductance significantly, while the opposite was true for plants that started in the cool treatment and were moved to the warm treatment. During the afternoon, on the other hand, all plants decreased significantly in stomatal conductance except for those that were previously in the warm, drought treatment and moved to the cool, well-watered treatment, which increased significantly. At night, the only significant shift was an increase in stomatal conductance for plants that started in the cool, well-watered treatment and were moved to the warm, drought treatment.

Discussion

Our results reveal that warming and drought negatively affect traits important for pollinator attraction and reward in *N. obtusifolia*, consistent with studies on other plant species (reviewed in Scaven & Rafferty 2013; Borghi et al. 2019; Descamps et al. 2021b). Separately, both warming and drought made plants shorter, and drought reduced the abundance of flowers per plant, as observed in other studies (Liu et al. 2012; Takkis et al. 2018). However, this study provides some of the first evidence that the effects of warming and drought on floral nectar vary throughout the day. There were significantly more nectarless flowers in the afternoon, which, in other species, has been associated with temperature stress (Petanidou & Smets 1996; Takkis et al. 2018). Alone, warming increased nectar sugar concentration, but only during the morning and afternoon. In contrast, only the interaction between warming and drought affected nectar volume and total calories, and these effects also differed depending on the time of day. Alone, drought did not significantly decrease nectar volume or total calories, which has been documented in other drought-tolerant species (Phillips et al. 2018; Suni et al. 2020). Similarly, warming alone did not reduce nectar production; in fact, warming slightly increased nectar volume and total calories at night for well-watered plants. This response is consistent with the idea that for any given plant species, there is usually an optimal amount of warming that can enhance photosynthesis by increasing stomatal conductance (Zandalinas et al. 2018), resulting in increased nectar production with moderate warming (Pacini & Nepi 2007; Nocentini et al. 2013). Indeed, we found that warming, when not combined with drought, increased stomatal conductance, likely leading to higher nectar volume and total calories at night. However, when higher temperatures and water stress are combined, photosynthetic activity can decline (Mittler 2006; Awasthi et al. 2014; Devasirvatham et al. 2016), reducing the resources available for flowers (Petanidou & Smets 1996; Scaven & Rafferty 2013; Takkis et al. 2018; Descamps et al. 2020). We observed this effect in our study: the interaction between warming and drought negated the potential benefit of warming on nectar production at night, and also significantly decreased nectar volume and total calories in the afternoon. Importantly, we also found that the interaction between warming and drought reduced the proportion of total daily nectar sugar produced in the afternoon relative to the other treatments, shifting more of the production to early morning or nighttime hours instead. Together, these results support our first hypothesis, and suggest that as both warming and droughts increase under climate change, floral nectar production will be negatively impacted, and plants may shift more of their nectar production to night or twilight hours to minimize stress during the hottest parts of the day. Over generations, natural selection may favor nocturnal flowering in additional species, as proposed by Borges et al. (2016).

Flowering is a water-intensive process (Mohan Ram & Rao 1984; Galen et al. 1999; Galen 2000; De la Barrera et al. 2009), and the effects of climate change on floral traits are likely to be mediated in part by plant water status (Carroll et al. 2001; Scaven & Rafferty 2013; Parmesan & Hanley 2015; Glenny et al. 2018). Plants can lose substantial amounts of water through floral organs (Whiley et al. 1988; Barrera & Nobel 2004), and some species respond to water limitation by producing smaller flowers (Galen et al. 1999; Lambrecht & Dawson 2007; Lambrecht 2013) or producing less nectar (Descamps et al. 2021c). However, in some species, the hydraulics of floral tissues and in turn, floral trait expression, may be independent from the water status of the rest of the plant (Lambrecht et al. 2011). Furthermore, while the xylem is the main plant hydraulic tissue, water delivery to floral structures is controlled by the phloem in some species (Elias et al. 1975; Razem & Davis 1999; Barrera & Nobel 2004; Lambrecht et al. 2011). Thus, it remains unclear to what degree plant water balance affects floral traits across species. For both VPD_L and stomatal conductance, we found a significant negative relationship with nectar volume/total calories, and a significant positive relationship with nectar sugar concentration. We observed the highest VPD_L and the lowest stomatal conductance with the combination of warming and drought during the afternoon, when nectar volume and total calories were also the lowest and nectar concentration was the highest. During the afternoon and night, we also found that stomatal conductance decreased with increasing VPD_L. Together, this suggests that nectar production in *N. obtusifolia* is in part mediated by plant water balance: when plants are more stressed due to heat and drought, they close their stomata and produce less nectar, and the remaining nectar becomes more concentrated. Although we did not measure plant reproductive output, this finding suggests plasticity in the diel rhythms of plant floral traits in response to warming and drought is an adaptive strategy to prevent water loss. Previous studies have also shown that plants that modify floral and water balance traits in response to abiotic stress have higher seed set, suggesting that plants plastically optimize fitness by reducing flower maintenance costs (Dudley et al. 2018). It is interesting to note, however, that in the morning, stomatal conductance increased in response to higher VPD_L, and there was no decrease in nectar volume and total calories in response to warming despite higher VPD_L. This may indicate that selection has shaped *N. obtusifolia* to produce floral nectar in the morning, even if conditions are unfavorable, and perhaps at the cost of increased water loss. Alternatively, plants may not have been stressed enough, even in the warming and drought treatments, to reduce nectar production in the morning, which was the coolest time point.

While plasticity is critical for plant species to persist in the face of rapid environmental change (Kozlowski & Pallardy 2002), plastic responses are not always adaptive (Franks et al. 2014). Our results from phase 2 of the experiment show that plant traits can have relatively rapid (~1 week) plastic responses to instantaneous shifts in temperature and water availability. However, plastic responses depended on both the type of treatment transition and the time of day, and it appeared that the shifts were not always beneficial in terms of balancing water regulation with the maintenance of floral rewards. We found that plants may take advantage of new favorable abiotic conditions by producing more nectar (e.g., for plants that were moved from warm + drought, to cool + well-watered conditions). However, during certain times of day, new unfavorable abiotic conditions may force plants to save water at the cost of reduced floral resources. For example, plants that were moved to warmer and/or drier conditions had lower stomatal conductance in the afternoon in phase 2. While this may help save water, it was also accompanied by a reduction in nectar volume and total calories in these treatments. At other times of day, floral trait responses were independent of water balance responses. For example, in the morning, for plants that were moved from 'warm, drought' to 'cool, well-watered' conditions, there was an increase in nectar volume and total calories despite reduced stomatal conductance. This suggests that rapid environmental change may disrupt the balance between water regulation and floral resource production, and that plants may not be able to respond in time via trait plasticity to certain types of rapid environmental shifts that are increasing with climate change. It is important to note, however, that our experimental design did not allow us to statistically separate the effects of water and temperature treatment transitions, as each temperature transition was also accompanied by a water transition.

Physiological stress expressed in the form of altered traits will also likely alter interactions with pollinators, which could affect both plant and pollinator fitness (Scaven &

Rafferty 2013). We observed heat- and drought-induced reductions to plant height and the abundance of flowers per plant–two components of floral display, which is important for attracting pollinators (Schiestl & Johnson 2013; Barragán-Fonseca et al. 2020). Additionally, reductions to the number of flowers available at a landscape scale due to climate change can drive pollinator declines, as has been observed for long-tongued bumble bees in the Rocky Mountains of Colorado in the US (Miller-Struttmann et al. 2015). Numerous studies have also documented changes to pollinator foraging behavior in response to heat- and drought-induced changes in floral nectar (Descamps et al. 2018, 2021a; de Manincor et al. 2023), and several have linked this to both reduced plant and pollinator fitness (Wilson Rankin et al. 2020; de Manincor et al. 2023). However, such studies rarely investigate diel variation. Our results reveal that the effects of climate change on floral nectar differ throughout the day, which could differentially affect suites of pollinators according to their daily foraging windows. This may also alter competition between these different suites of pollinators for floral resources, which can exacerbate insect declines (Thomson 2016; Thomson & Page 2020). Specifically, we found that warming and drought negatively impacted nectar volume and total calories most during the afternoon and night, and less during the morning in *N. obtusifolia*. This may benefit pollinators that are active during the early morning hours.

However, both the quantity and quality of floral nectar can influence pollinator foraging choices. Pollinators often modify foraging choices based on trade-offs between nectar volume and sugar concentration determined by their specific nutritional requirements and feeding mechanisms (Fowler et al. 2016; Shackleton et al. 2016). Lepidoptera generally prefer high volume, low concentration nectar, while bees prefer low volume, high concentration nectar (Waller 1972; Heyneman 1983; Krenn 2019; Wei et al. 2020). In fact, with their long, thin proboscides, butterflies and moths may be unable to drink highly concentrated, viscous nectar

(Pivnick & McNeil 1985), while bees can handle a wider range of nectar concentrations (Wei et al. 2020). Whereas previous studies suggest that nectar sugar concentration is less variable and less affected by temperature than nectar volume or total sugar content per flower (Villarreal & Freeman 1990; Nocentini et al. 2013; Takkis et al. 2018), we found that warming significantly increased nectar sugar concentration during the morning and afternoon, well above the average preferred concentration for both hawkmoths (19%) and butterflies (25%: Kim et al. 2011). By decreasing their ability to feed on the nectar that is available during these time periods, this may be detrimental for diurnal Lepidoptera (most butterflies, and some moth species), as well as crepuscular moths including many important pollinating hawkmoths (Sphingidae). Conversely, bees may benefit from the higher concentration of nectar during the day. However, the total calories per flower were lower during the afternoon, which can also influence bee foraging choices (Fowler et al. 2016) and increase marginal foraging costs by increasing the required number of flower visits to obtain the same level of nutrients (Latty & Trueblood 2020; Descamps et al. 2021b).

How plant and pollinator fitness will be affected by climate change-driven shifts to the diel patterns of floral resources will depend on how closely species rely on diel synchrony with interaction partners, which is highly species-specific (Waser et al. 1996; Kronfeld-Schor et al. 2017). Nevertheless, our results suggest that climate change may desynchronize coevolved diel rhythms in floral attraction/reward traits and the activity of the most important pollinators, which may have fitness consequences for both plants and pollinators. However, fitness costs may also be buffered by plasticity in daily pollinator foraging times (Abram et al. 2017; Kronfeld-Schor et al. 2017). While a strong circadian component to diel activity limits plastic shifts in foraging times in some insect species (Abram et al. 2017; Speights et al. 2017), others are able to adjust diel foraging times to align with peak nectar rhythms (Moore et al. 1989; Fründ et al. 2011) or to escape heat during the day (Huey & Pascual 2009; Abram et al. 2017; Speights et al. 2017; Ma et al. 2021). Indeed, Zoller et al. (2020) found that the peak diel activity of entire pollinator communities shifted towards nighttime hours during a hotter summer in the arctic. Our results suggest that plant trait rhythms may also be shifting towards night or early morning hours, indicating the possibility that plant and pollinator diel rhythms may remain synchronized under climate change. However, plant and pollinator diel rhythms can evolve independently based on their specific responses to the physical environment (Herrera 1990), and their plastic responses to environmental change may also vary (Kronfeld-Schor et al. 2017). Additionally, pollinators often possess a suite of specific adaptations that enable them to efficiently locate and forage from flowers during their temporal niche (Waser & Ollerton 2006; Patiny 2011; Fleming & Kress 2013; Borges et al. 2016; Borges 2018), and they may therefore be unable to effectively forage during other time periods. Flowers, in turn, have evolved attraction and reward traits suited to their preferred pollinators, with scent being more important for attracting nocturnal pollinators, and color more important for diurnal pollinators (Moreira et al. 2019). Thus, to what extent diel shifts will cause mismatches between plants and pollinators likely depends on each partner's current adaptations, and their plastic responses to both the abiotic and biotic environment.

Conclusions

Altogether, our results suggest that climate change, by shifting diel rhythms in floral traits, may alter the frequency and identity of plant-pollinator interactions throughout the day. This may ultimately impact community structure and stability, and ecosystem function. For example, Fründ et al. (2011) showed that alterations to the diel rhythms of flower opening and closing due to pollinator visits can scale up to affect community-level patterns in plant-pollinator interaction networks over the course of a day. However, the effects of anthropogenic pressures such as climate change in this context have not been explored. Diel variation in plant-pollinator interaction networks has only recently gained attention as an important component of community structure and stability, including resilience to climate change (CaraDonna et al. 2021; Bascompte & Scheffer 2023). Our results encourage further investigation into plant-pollinator interactions across the diel cycle, particularly through the inclusion of neglected nocturnal interactions and the factors that may affect them under global change. For instance, large scale field manipulations (e.g., Moss & Evans 2022), and natural experiments (e.g., Petanidou et al. 2018; Classen et al. 2020) aimed at elucidating the impacts of climate change on plant-pollinator networks should also quantify diel variation in floral resource availability as an important predictor of interactions. Combined with insights from controlled laboratory or greenhouse manipulations, such as the present study, this will improve our ability to predict and mitigate the effects of climate change and other stressors. Because the impacts of climate change are borne out not just over seasons and years, but also over the course of single days, biological responses at these finer temporal scales deserve more attention.

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Tables and Figures

Table 2.1: GLMM summaries for water balance measurements. Each model includes the random effect of plant identity. The intercept for temperature is cool, for water is well-watered, and for time is morning.

Response	Distribution	ZI	Dispersion	Random effect (SD)	Significant predictors	*β*	95% CI	*p*
VPD$_L$	Gamma (log)		~temp: time	1.89E-05	temp(w):time(a)	-0.51	-0.72, -0.30	< .001
stomatal cond.	Gamma (log)	~1	~water: temp: time	0.22	water(dr):temp (w):time(a)	-0.36	-0.64, -0.08	0.012
stomatal cond.	Gamma (log)	~1	~time	0.26	VPD$_L$:temp(w): time(a)	2.23	0.77, 3.69	0.003
					VPD$_L$:temp(w): time(n)	3.95	0.51, 7.39	0.025

Table 2.2: GLMM summaries for nectar measurements. Each model includes the random effect of plant identity. The intercept for temperature is cool, for water is well-watered, and for time is morning.

Response	Distribution	Dispersion	Random effect (SD)	Significant predictors	*β*	95% CI	*p*
volume	tweedie (log)	~time	0.35	water(dr):temp(w): time(a)	-1.34	-2.28, -0.39	0.005
				water(dr):temp(w): time(n)	-0.75	-1.39, -0.12	0.02
concentration	beta (logit)		0.087	temp(w):time(n)	-0.31	-0.44, -0.18	< .001
calories	tweedie (log)	~time	0.38	water(dr):temp(w): time(a)	-1.45	-2.5, -0.41	0.006
				water(dr):temp(w): time(n)	-0.81	-1.45, -0.17	0.014

Table 2.3: GLMM summaries for the phase 2 transition for nectar traits. Each model includes the random effect of plant identity The intercept for phase is 1, for transition is ‘cool, drought → warm, well-watered’, and for time is morning.

Response	Distri-bution	Disper-sion	Random effect (SD)	Significant predictors	*β*	95% CI	*p*
volume	tweedie (log)		0.17	phase(2): trans(wdr→cww):time(a)	3.64	2.09, 5.19	< .001
concentration	beta (logit)	~time	0.057	phase(2): trans(wdr→cww):time(n)	0.39	0.08, 0.7	0.014
				phase(2): trans(www→cdr):time(n)	0.51	0.19, 0.82	0.002
calories	tweedie (log)		0.2	phase(2): trans(wdr→cww):time(a)	3.96	2.36, 5.55	< .001

Table 2.4. (G)LMM summaries for the phase 2 transition for water balance traits. Each model includes the random effect of plant identity. The intercept for phase is 1, for transition is 'cool, drought → warm, well-watered', and for time is morning.

Response	Distribution	ZI	Dispersion	Rand. effect (SD)	Significant predictors	*β*	95% CI	*p*
VPD_L	gamma (log)		temp: time	0.06	phase(2):trans(wdr →cww):time(a)	0.62	0.3, 0.94	< .001
					phase(2):trans(www →cdr):time(a)	0.77	0.44, 1.09	< .001
log(stomatal cond.)	gaussian	~1	water + time	0.19	phase(2):trans(wdr →cww):time(a)	1.69	1.23, 2.14	< .001
					phase(2):trans(www →cdr):time(a)	1.36	0.90, 1.81	< .001
					phase(2):trans(wdr →cww):time(n)	0.63	0.06, 1.20	0.03

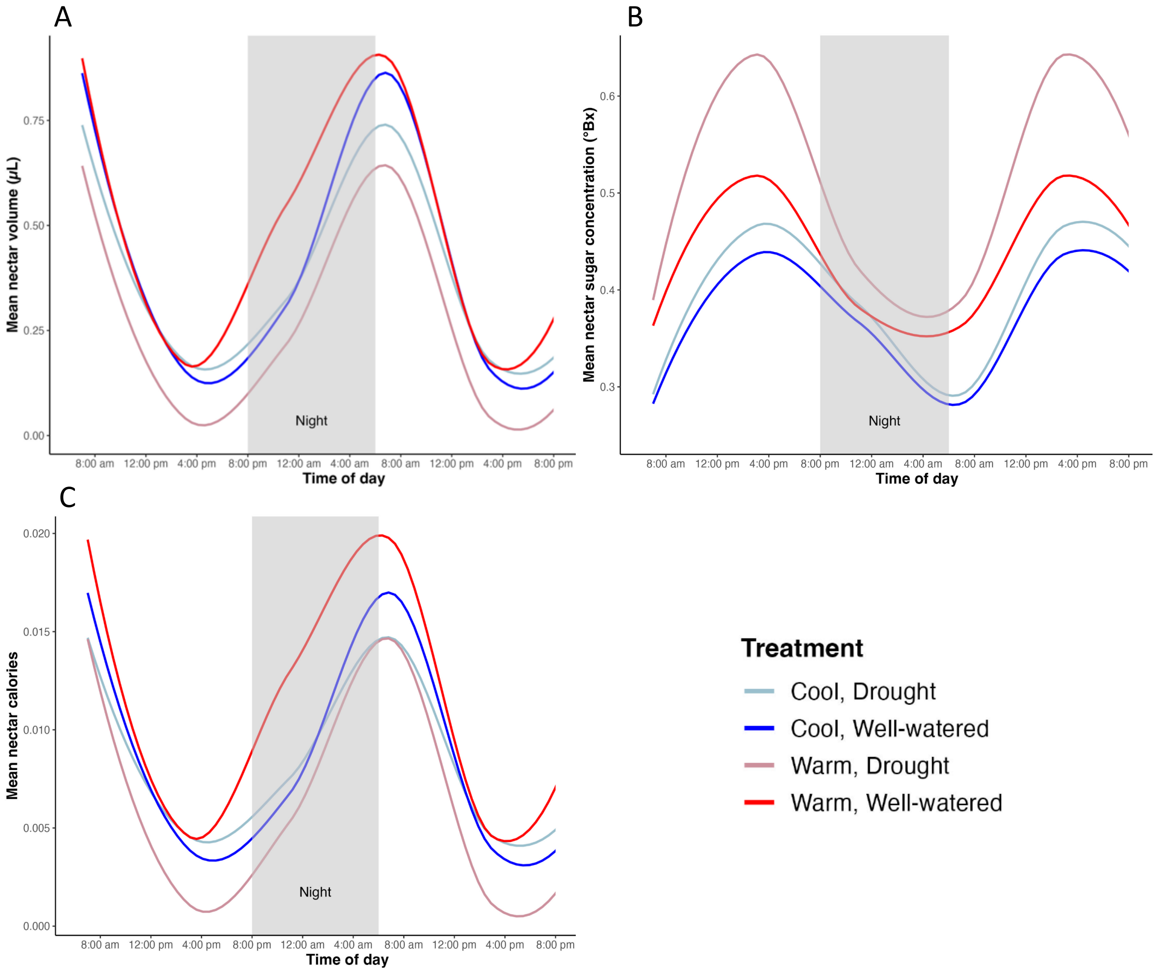

Figure 2.1. Approximated circadian rhythms in (A) nectar volume, (B) nectar sugar concentration, and (C) total nectar calories across treatments (colors), constructed by fitting a loess curve to data from the three daily collection periods (6-8 am, 2-4 pm, 10 pm-12 am), averaged within each treatment.

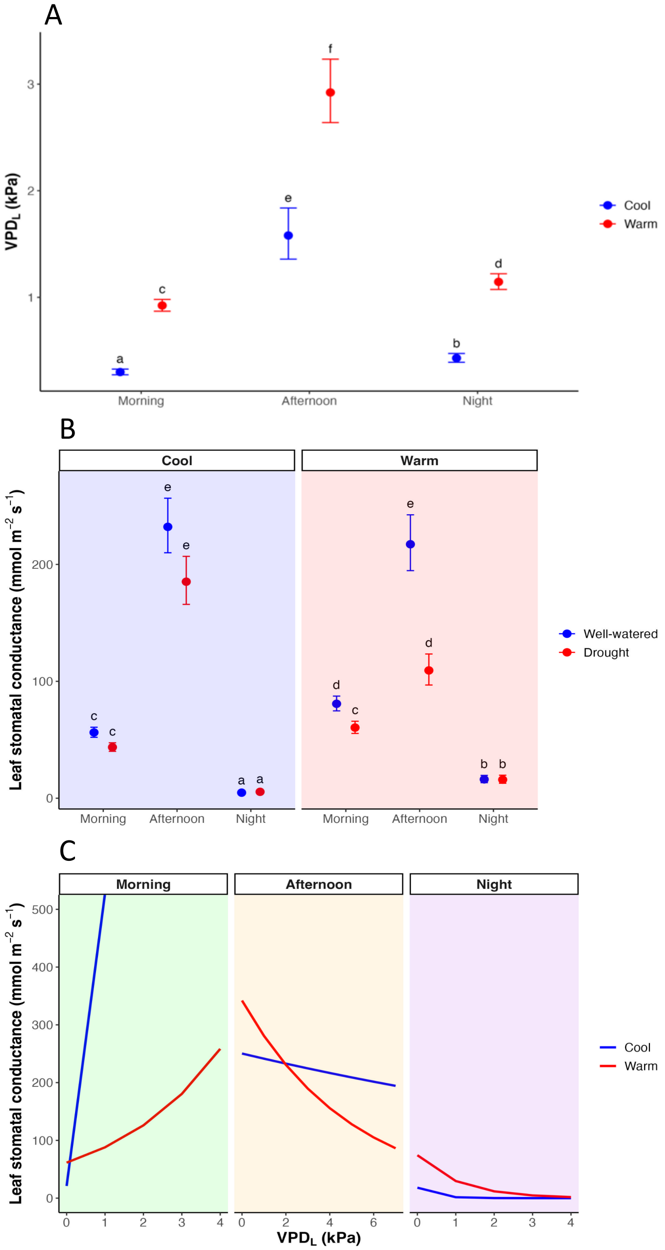

Figure 2.2. Predicted (marginal) effects of (A) the interaction between time of day and temperature treatment (cool = blue, warm = red) on VPD_L, and (B) the interaction between time of day, temperature treatment (cool = left panel, warm = right panel), and water treatment (well-watered = blue, drought = red) on leaf stomatal conductance. Mean and 95% confidence intervals are displayed. Letters represent the significance of pairwise differences. (C) Predicted (marginal) effects of the interaction between VPD_L, time of day (panels) and temperature treatment (cool = blue, warm = red) on leaf stomatal conductance. Model fits are displayed, with confidence intervals omitted to improve visibility.

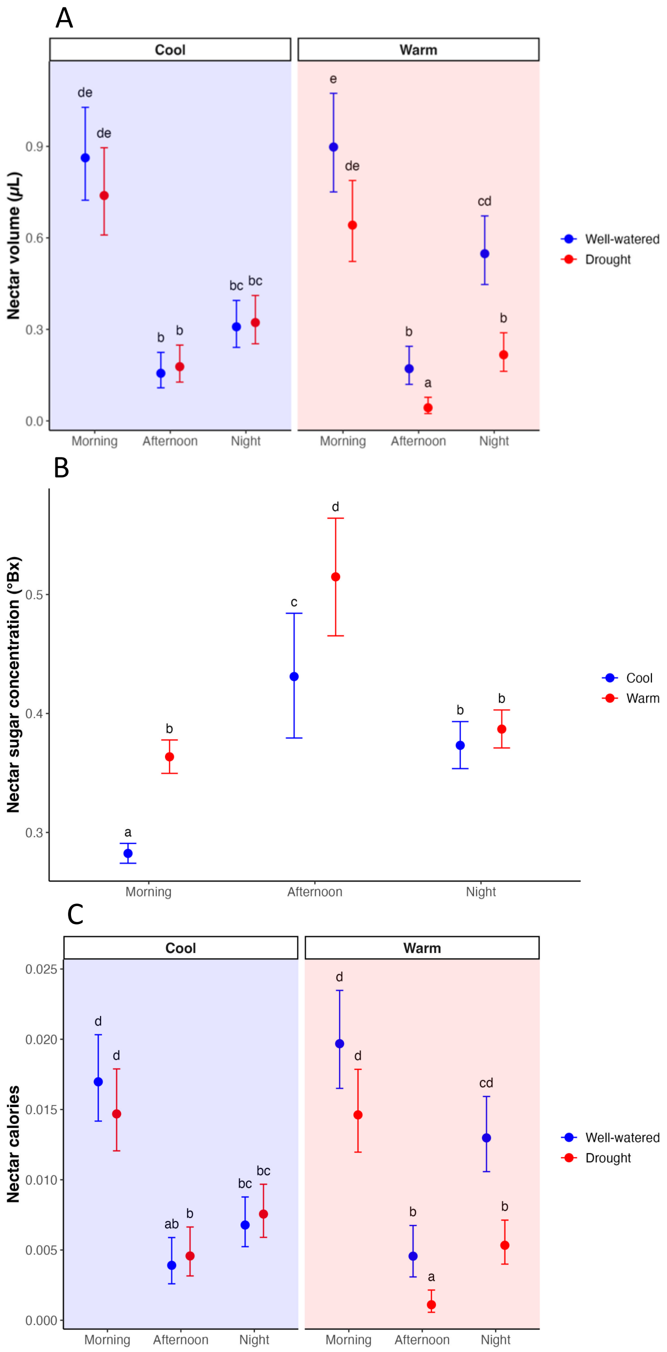

Figure 2.3. Predicted (marginal) effects of: (A) The interaction between time of day, temperature treatment (cool = left panel, warm = right panel), and water treatment (well-watered = blue, drought = red) on nectar volume. (B) The interaction between time of day and temperature treatment (cool = blue, warm = red) on nectar sugar concentration. (C) The interaction between time of day, temperature treatment (cool = left panel, warm = right panel), and water treatment (well-watered = blue, drought = red) on nectar calories. Mean and 95% confidence intervals are displayed. Letters represent the significance of pairwise differences.

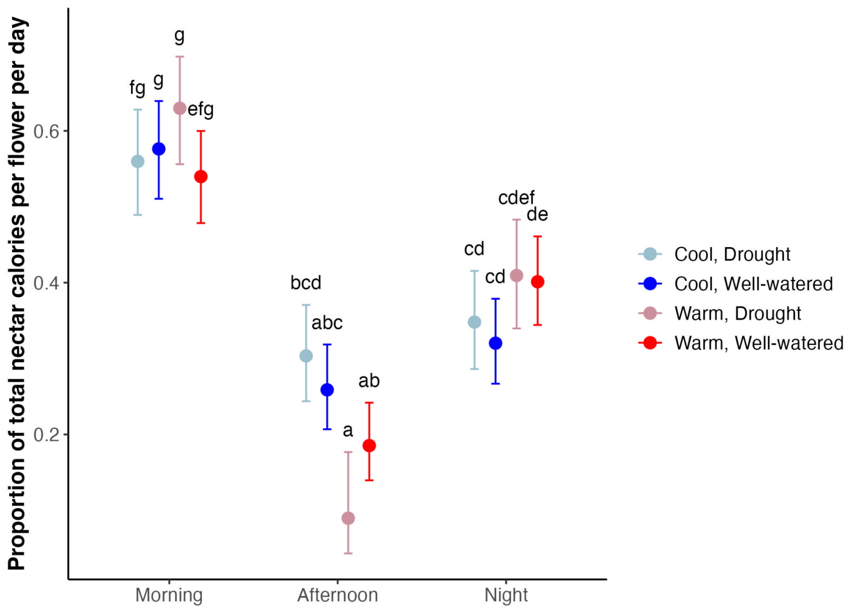

Figure 2.4. Predicted (marginal) effects of the interaction between time of day and temperature + water treatment combination (colors) on the proportion of total daily nectar calories per flower per day. Mean and 95% confidence intervals are displayed. Letters represent the significance of pairwise differences.

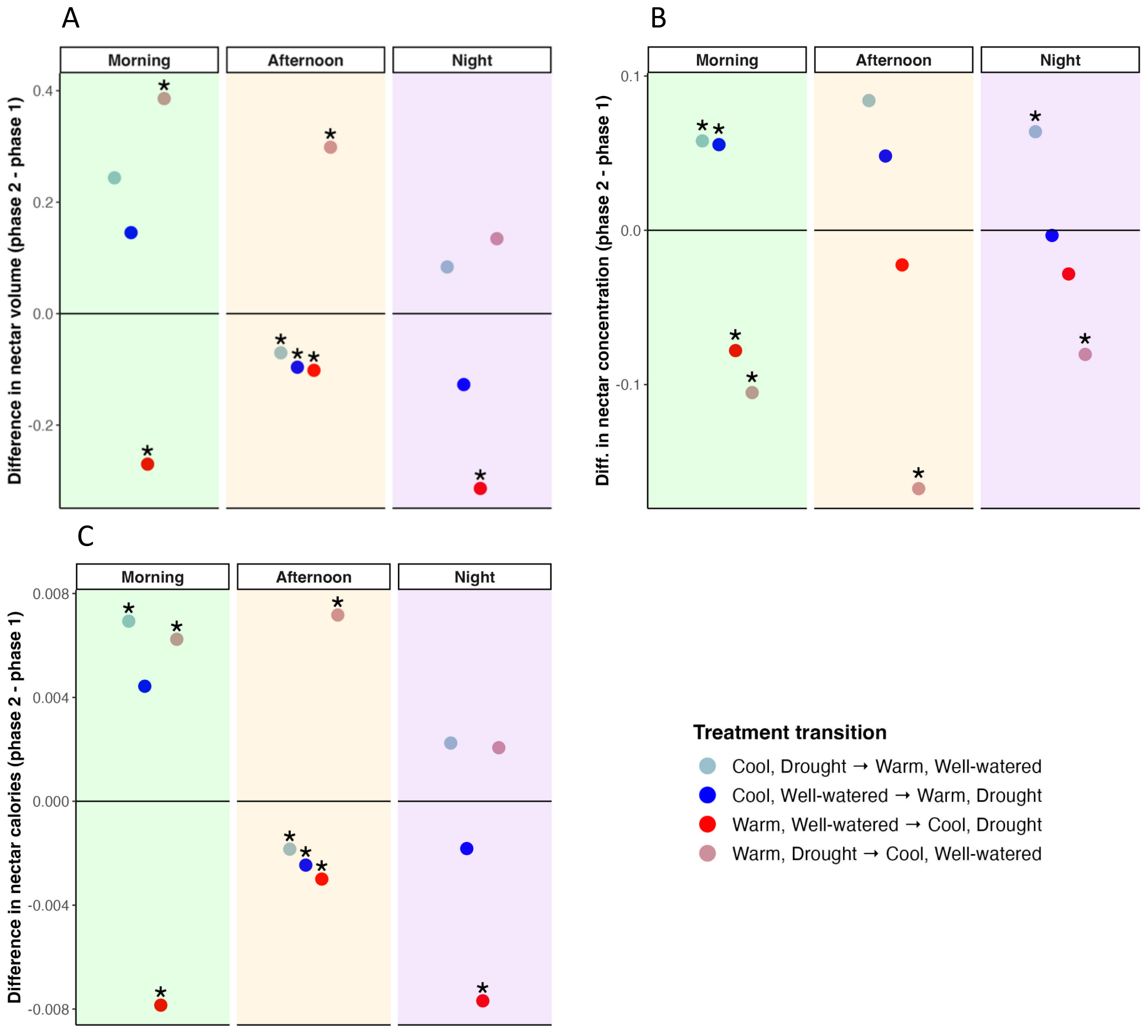

Figure 2.5. Predicted (marginal) differences in (A) nectar volume, (B) nectar sugar concentration and (C) total nectar calories between phases of the experiment (phase 2 - phase 1). For each of the treatment transitions (colors), differences between the mean values are displayed, with asterisks designating significance. Values above the y = 0 line indicate an increase in the value in phase 2, while values below the line indicate a decrease in the value in phase 2.

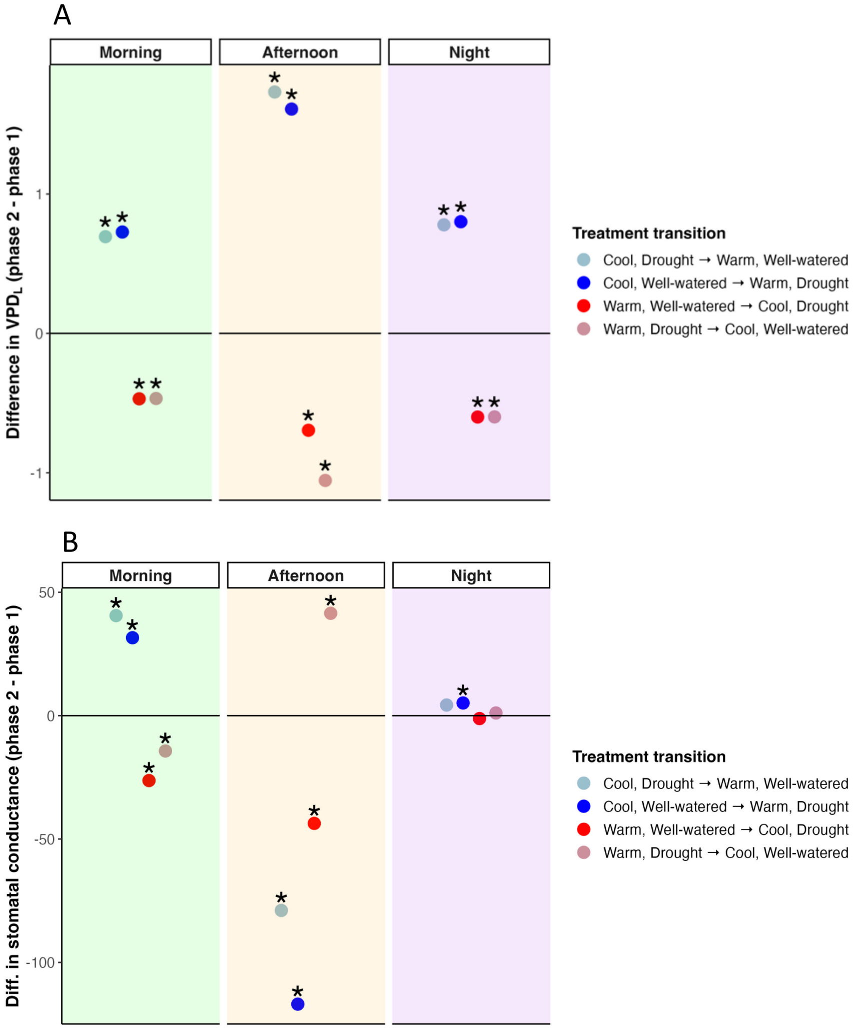

Figure 2.6. Predicted (marginal) differences in (A) VPD$_L$, and (B) leaf stomatal conductance between phases of the experiment (phase 2 - phase 1). For each of the treatment transitions (colors), differences between the mean values are displayed, with asterisks designating significance. Values above the y = 0 line indicate an increase in the value in phase 2, while values below the line indicate a decrease in the value in phase 2.

Chapter 3

Landscape-scale interaction networks reveal keystone native host and nectar plants for Lepidoptera conservation

Abstract

Like other groups of insects, Lepidoptera (butterflies and moths) are declining rapidly. The loss of native plant habitat contributes to these declines by disrupting specialized feeding relationships, which can be mitigated with native plant restoration. However, the majority of Lepidoptera conservation in the US has focused on providing milkweeds for monarchs, a single-species approach that is insufficient to conserve diverse, complex, and vulnerable Lepidopteran communities. Leveraging community science data, we constructed landscape-scale, multilayer Lepidoptera -host and -nectar plant interaction networks in California comprising over 280,000 spatially-explicit interactions and involving nearly 1,500 Lepidoptera species. Integrating network theory with biodiversity conservation, we analyzed network structure, composition, and species roles across the state to reveal clear management priorities. We found that, across ecoregions, the composition of host and nectar plant communities differs, and a minority of plant species supports the majority of Lepidoptera species. Furthermore, the identity of the most important host and nectar plant species varies significantly among ecoregions. We produce spatially-explicit lists of keystone native host and nectar plant species that should be prioritized in restoration and native plant landscaping to support more diverse and resilient Lepidoptera communities.

Introduction

Insects are declining globally in response to habitat loss, climate change, pesticide use, invasive species, and other anthropogenic stressors (Wagner et al. 2021b). These declines threaten life on Earth (Cardoso et al. 2020). Insects supply numerous ecosystem functions and services that are not only critical for maintaining the integrity of natural ecosystems but also central to human health, culture, and economies (Wilson 1987; Losey & Vaughan 2006; Yang & Gratton 2014; Potts et al. 2016; Basset & Lamarre 2019). For example, insect pollination services, provided to the majority of flowering plant species (Ollerton et al. 2011), are responsible for about a third of global food crop production (Klein et al. 2007), and worth an annual $16 billion in the US alone (Calderone 2012; Rader et al. 2016). Given the severity of reported insect declines (Conrad et al. 2006; Dirzo et al. 2014; Hallmann et al. 2017; van Klink et al. 2020; Forister et al. 2021) and the serious consequences for human (Smith et al. 2022) and environmental health (Bennett et al. 2020), scientists have made urgent calls to prioritize insect conservation (Forister et al. 2019; Harvey et al. 2020, 2023; Cardoso et al. 2020).

Lepidoptera (butterflies and moths) are one of the most diverse insect orders (Scoble 1992; Wahlberg et al. 2013; Goldstein 2017), and are suffering some of the steepest declines among insect groups in part due to specialized food and habitat requirements and temperature-sensitive life stages (Wilson & Maclean 2011; Fox 2013; Fox et al. 2014; Maurer et al. 2018; Wepprich et al. 2019; Wagner et al. 2021a). In the US, for example, Western butterfly abundance has declined by an average of 1.6% per year over the last four decades (Forister et al. 2021). Though they represent 95% of Lepidoptera species diversity (Powell & Opler 2009), much less is known about moth population trends in the US. There is some evidence from the eastern US that large moth species are declining, including over half of hawk moth species (Sphingidae: Young et al. 2017). Moreover, long-term studies from other temperate countries indicate steep declines in moth fauna (Fox 2013; Habel et al. 2019; Burner et al. 2021; Blumgart et al. 2022).

Declines in Lepidoptera are particularly concerning given their critical roles in terrestrial food webs as herbivores and as prey for birds and other organisms (Tallamy & Shropshire 2009; Narango et al. 2017; Wagner et al. 2021a; Tallamy & Shriver 2021). In these two roles, caterpillars transfer more energy from plants to other organisms than all other herbivores combined (Janzen 1988). Thus, the loss of Lepidoptera richness and abundance cascades through food webs (Wagner et al. 2021a), and has been linked to the precipitous decline of native bird populations in the US (Tallamy & Shriver 2021). Further, while butterflies are generally thought to be inefficient pollinators compared to bees (Willmer 2011), a growing body of evidence has revealed that moths supply vital pollination services to entire plant communities (Devoto et al. 2011; MacGregor et al. 2015; Macgregor et al. 2019; Banza et al. 2019; Walton et al. 2020; Ellis et al. 2023), including food crops such as apples, avocadoes, and berries (Buxton et al. 2021; Robertson et al. 2021; Buxton et al. 2022; Anderson et al. 2023a).

Perhaps the single greatest threat to insects is the reduction of habitat quantity and quality (Wagner et al. 2021b), including the loss of key native plant resources (Carvell et al. 2006; Wallisdevries et al. 2012; Scheper et al. 2014; Goulson et al. 2015; Baude et al. 2016) and, often, replacement with invasives (Dutta 2018; Salgado et al. 2019; Casoli et al. 2021). Half of all insect species are herbivorous (Strong et al. 1984; Mitter et al. 1988; Grimaldi et al. 2005), and 90% of these rely on just one or a few native plant lineages (Forister et al. 2015). These specialized relationships are the result of millions of years of evolution by insects to tolerate their host plants' chemical defenses (Dethier 1954; Ehrlich & Raven 1964; Holloway & Hebert 1979). Thus, most herbivorous insects are not able to subsist on non-native plant species (Tallamy & Shropshire 2009). This is particularly true of mandibulate (chewing) insects like Lepidoptera, as their feeding mechanism directly exposes them to toxic or deterrent phytochemicals, and often necessitates specific physiological adaptations (Dyer et al. 2007). When insects consume non-native plants, these plant species may act as ecological traps, increasing larval mortality, extending development time, and reducing mass compared to native hosts (White et al. 2008; Castells & Berenbaum 2008; Keeler & Chew 2008; Brown & Zuefle 2009; Ding & Blossey 2009; Harvey et al. 2010; Fortuna et al. 2012; Knerl & Bowers 2013; Tallamy et al. 2021). Unsurprisingly, the loss of native host and nectar plants due to habitat destruction, climate change, and pesticide use is a leading driver behind butterfly declines in the US (Crossley et al. 2021; Forister et al. 2021), including the monarch (Inamine et al. 2016).

In light of their specialized interactions, conserving Lepidoptera requires protecting the native plant species to which they are evolutionarily tied. Indeed, restoring individual, threatened Lepidoptera species' host plants has proven an effective conservation strategy—one that even small habitat patches in private yards can contribute to (Ramírez-Restrepo et al. 2017; Thogmartin et al. 2017a; Geest et al. 2019). For example, in Florida, the rare Atala butterfly (*Eumaeus atala*) rebounded after local extinction following increased use of its native host plant in urban landscaping (Ramírez-Restrepo et al. 2017). Additionally, while the factors driving population trends in the monarch butterfly are still poorly understood (Inamine et al. 2016; Thogmartin et al. 2017b), the concerted national effort to plant its obligate host plants, milkweeds, is believed to be integral to the monarch's recovery (Thogmartin et al. 2017a; Pleasants 2017). It is important to recognize, however, that the majority of Lepidoptera feed on plants at two distinct life stages: caterpillars, which feed on host plant tissue, and adults, which feed on floral nectar and thereby pollinate plants (Scoble 1992; Krenn 2010). There is some evidence that the loss of nectar plants plays a larger role in monarch decline than the loss of milkweed (Inamine et al. 2016). Indeed, many Lepidoptera species also have specialized nectar plant requirements (Hardy et al. 2007), and diet breadth in adult and larval stages can independently influence extinction risk (Koh et al. 2004; Hardy et al. 2007; Eskildsen et al. 2015). This highlights the importance of considering risks and dependencies at multiple insect life stages for insect conservation.

However, if native plant restoration is to meet its full potential for insect conservation in the Anthropocene, it must recognize that the traditional approach to conservation—which focuses on individual rare, threatened and/or charismatic species—is not suitable for insects given their immense diversity and heterogeneous responses to environmental change (Longcore & Osborne 2015; Blüthgen et al. 2023). For example, while the bulk of Lepidoptera conservation in the US has focused on a few individual species like the monarch butterfly, hundreds of other butterfly species are declining (Crossley et al. 2021; Forister et al. 2021), including widespread species and some that are at greater risk of extinction than the monarch (Forister et al. 2023). This crisis calls for new approaches to conserving insects to be applied quickly, even as we continue the basic research needed to untangle the knot of interacting factors driving their declines (Forister et al. 2019; Saunders et al. 2020; Weisser et al. 2023).

In response to this call, scientists (Elle et al. 2012; Valiente-Banuet et al. 2015; Harvey et al. 2017; Heinen et al. 2020; Bascompte & Scheffer 2023) have encouraged shifting the focus of insect conservation away from individual species and onto interaction networks that underpin the maintenance of biodiversity (Bascompte et al. 2006; Bascompte & Jordano 2007; Andresen et al. 2018) and the supply of ecosystem functions (Gómez et al. 2011; Schleuning et al. 2015; Luna et al. 2020). In particular, ecological network analysis provides a robust, quantitative approach to prioritizing plant species for restoration based on their functional importance to insect communities (Kaiser-Bunbury & Blüthgen 2015; Harvey et al. 2017). This can help maximize the conservation impact of wildlife gardening and habitat restoration, where usually only a subset of the total plant species in a given ecosystem are used (Zobel et al. 1998; Barr et al. 2017; Ladouceur et al. 2018). A body of research has revealed that native plants differ widely in the number of insects they support (Tallamy & Shropshire 2009), and that most interactions–including both mutualistic (Jordano 1987; Menz et al. 2011) and antagonistic (Narango et al.

2020)–involve a small percentage of the total plant species in any given community. For example, Narango et al. (2020) found that on average just 14% of plant genera across US counties host over 90% of Lepidoptera species. Importantly, a random selection of plant species from the regional pool cannot be assumed to support high insect diversity, nor ecosystem functions and services (Ladouceur et al. 2022). By accounting for complex direct and indirect interactions in ecological communities, network techniques–including analyses of modularity (Olesen et al. 2007), centrality (Martín González et al. 2010), and species removal or addition simulations (Messeder et al. 2020)—can identify "keystone" species or interactions that, based on their position in the network, contribute most to biodiversity maintenance, community stability, and ecosystem function (Harvey et al. 2017; Saunders & Rader 2019).

This data-driven approach can improve conservation outcomes compared to traditional methods to selecting plant mixes for insect habitat restoration. These have mainly been based on expert opinion or assumed ecological relationships (Garbuzov & Ratnieks 2014; Lundin et al. 2019; Ladouceur et al. 2022), which are not always accurate (Ollerton et al. 2009; Garbuzov & Ratnieks 2014), and can even lead to failures in conservation programs (Fleming & Kress 2013). In contrast, network approaches provide quantitative comparisons to aid in management decisions, can help to avoid potential errors and biases, and can be extended across systems (Holden & Ellner 2016; Ladouceur et al. 2022). Ultimately, network-based plant species selections can improve the ability of even small habitat patches to support high insect diversity, and in turn provide services such as pollination and natural pest control. For example, several recent studies used network analyses to identify keystone non-crop plants for agricultural hedgerows and flower strips that best support native pollinators, and in turn increase pollination services for adjacent crop plants (Peters et al. 2016; Windsor et al. 2021; Assunção et al. 2022). Analyzing multilayer networks integrating multiple interaction types (Pilosof et al. 2017; Garcia-

Callejas et al. 2018; Hutchinson et al. 2019) can also identify keystone plants that support insects at multiple life stages (e.g., host plants for immature stages and nectar plants for adults: Wang et al. 2023).

Despite their potential, network-based prioritization methods have yet to be broadly integrated into insect conservation efforts. One of the greatest hurdles is the lack of adequate species interaction data, especially of high spatial resolution across large geographic areas (Morales-Castilla et al. 2015). Many existing native plant selection resources use data that are specific to locations where studies have been conducted, often extrapolating to broader areas. This is problematic because species composition and their interactions vary across space, and therefore so do their functional importance in communities (Olesen & Jordano 2002; Thompson 2005; Poisot et al. 2015). Thus, the identity and variation of keystone plant species at landscape scales remains largely unknown (but see Narango et al. 2020). This is critical information to inform both public and professional planting decisions to improve the value of habitat restoration for insect conservation in human-dominated landscapes (Harvey et al. 2017).

The growing availability of large biodiversity databases (Poelen et al. 2014; Runting et al. 2020; Heberling et al. 2021; Musvuugwa et al. 2021; Binley et al. 2023) and community science data (Chandler et al. 2017; Binley et al. 2021; Lin et al. 2022; Fraisl et al. 2022; Johnston et al. 2023) affords an unprecedented opportunity for data-driven conservation approaches. Here, we combine species distribution data with a detailed plant-Lepidoptera interaction dataset, both compiled from multiple open source and community science data repositories, to construct and analyze spatially-explicit Lepidoptera-host and -nectar plant interaction networks across California. We investigate (1) whether the structure and composition of host and nectar plant networks differ, (2) which host and nectar plant species are the most ecologically important for Lepidoptera communities, and (3) whether the identity of these keystone plant species varies geographically. From these analyses, we produce ecoregion- and habitat-specific lists of keystone native host and nectar plant species that can maximize the ecological benefit of restoration efforts on private and public lands in California. To advance inclusive participation in insect conservation across multiple stakeholders, we developed a free, user-friendly R Shiny web application (Chang et al. 2017) to disseminate this information.

Methods

Interaction data

We conducted all analyses in R version 4.3.2 (R Core Team 2021). We manually entered into tables each pairwise host and nectar plant interaction between a Lepidoptera species and a California native plant from Caldwell (2020). This gardening and restoration guide details native host and nectar plant usage by butterfly and moth species across California. The author meticulously combed through numerous sources to compile the data, including scientific publications, natural history books, ecological interaction databases (e.g., HOSTS: data.nhm.ac.uk/dataset/hosts), entomology websites, community science databases (e.g., iNaturalist: inaturalist.org), and personal communication with experts. A full list of references can be found in the guide. We included only species-level records in the analyses.

Species distribution data

We downloaded all georeferenced native and wild (i.e., not intentionally-planted) plant species occurrence records in California from Calflora (calflora.org), and all georeferenced Lepidoptera species occurrence records in California from the Symbiota Collections of Arthropods Network (SCAN: scan-bugs.org), the Global Biodiversity Information Facility (GBIF: gbif.org), and Moth Photographers Group (mothphotographersgroup.msstate.edu). Using functions from the R packages ‘bdc’ (Ribeiro et al. 2022), ‘BeeBDC’ (Dorey et al. 2023), and ‘CoordinateCleaner’ (Zizka et al. 2019), we merged the datasets and removed records with missing or problematic coordinates (e.g., out of range, imprecise, close to biodiversity institutions).

For both the interaction data and the species distribution data, we cleaned scientific names using the ‘bdc_clean_names’ function from ‘bdc’. We then standardized scientific names to the GBIF backbone (GBIF Secretariat 2021), and retrieved higher taxonomy using the R package ‘taxize’ (Chamberlain & Szöcs 2013). We chose to distinguish subspecies in both datasets, as different subspecies are often involved in distinct interactions (Ehrlich & Hanski 2004; Forbes et al. 2017; Haan et al. 2021).

Building the multilayer metanetwork

Because the raw interaction data was not inherently spatially-explicit, we combined it with the species distribution data to create a spatial multilayer metanetwork describing binary Lepidoptera-host and -nectar plant interactions across ecoregions and habitats in California. “Multilayer” refers to the fact that multiple interaction types (host and nectar plant) are included, and “metanetwork” refers to the fact that it is composed of spatially-explicit subnetworks. Due to the general lack of moth nectar plant data, and because butterflies and moths fill ecologically similar roles, we did not separate butterflies and moths in the analyses.

To build the multilayer metanetwork, we first downloaded the level III and level IV ecoregion shapefiles from the Environmental Protection Agency (EPA: epa.gov/eco-research/ecoregions) and the California Wildlife Habitat Relationships (CWHR) shapefile from the California Department of Fish and Wildlife (wildlife.ca.gov/Data/CWHR). We converted these into raster layers using the function ‘rast’ from the R package ‘terra’ (Hijmans et al. 2022). Then we extracted the ecoregion and habitat raster values for each plant and Lepidoptera occurrence coordinate using the function ‘extract’ from ‘terra’, producing lists of all native plant and Lepidoptera species that occur in each unique EPA level III ecoregion-level IV ecoregion-

CWHR habitat combination. Hereafter, we will refer to these as “subregions”. We made these lists binary rather than considering the number of occurrences for each species, as georeferenced occurrence databases do not accurately estimate relative species abundances (Meyer et al. 2016; Daru et al. 2018). Further, while weighted interaction data may provide additional information, binary data are well-suited for analyzing species roles in networks (Corso et al. 2015; Miranda et al. 2019). We additionally excluded all anthropogenic habitat types from the analyses (e.g., agricultural, urban), as our focus was to characterize natural communities. We then built spatially-explicit subnetworks for each subregion by filtering the full interaction dataset to include only the plant and Lepidoptera species that occur within each subregion. The end result was a statewide multilayer metanetwork comprised of spatially-explicit host and nectar plant subnetworks, each in turn comprised only of the native plant and Lepidoptera species that co-occur in that subregion, and have been recorded to interact in California. Hereafter, we will refer to these as “subnetworks”. We constructed bipartite interaction networks with these data using the function ‘frame2webs’ from the R package ‘bipartite’ (Dormann et al. 2008).

Assessing survey completeness

Due to the opportunistic nature of species occurrence data, especially when drawing from community science sources like iNaturalist (contained in the Calflora and GBIF data), survey completeness can be spatially heterogeneous (Shirey et al. 2021; Geurts et al. 2023). In this study, incomplete data could result in poorly-resolved subnetworks in certain subregions. To assess the survey completeness of each subregion, we used the R package ‘KnowBR’ (Lobo et al. 2018) to calculate species accumulation curves for both plants and Lepidoptera in each EPA level IV ecoregion. ‘KnowBR’ uses the number of records contained in the georeferenced species occurrence database as a surrogate for sampling effort (Lobo et al. 2018). Using the function ‘KnowBPolygon’, we calculated three metrics—completeness, the final slope of the accumulation curve, and the ratio between the number of records and the observed species—which, in combination, can be used to select the most probable well-surveyed spatial units (Lobo et al. 2018). We calculated extrapolated richness and completeness values using the “rational” adjustment function. We estimated the accumulation curve according to the exact estimator, as well as by performing the default 200 permutations of the observed data (random estimator). We dropped subregions from the analyses unless the EPA level IV ecoregion to which they belonged met the following criteria for both plants and Lepidoptera, suggested by Lobo et al. (2018), to qualify them as well-surveyed: completeness ≥ 50%, slope ≤ 0.3, and ratio ≥ 3. To further ensure that under-surveyed networks were not included, we excluded all subnetworks that did not contain at least 10 plant species and 10 insect species.

Network analysis and keystone species

We calculated “supported Lepidoptera accumulation curves” for both host and nectar plant species by calculating, for each subnetwork within the multilayer metanetwork, the minimum number of plant species required to support any given percentage of Lepidoptera species, with support being defined as when interactions were recorded between the plants and Lepidoptera species. For this calculation, we began with the plant species in each subnetwork that interacted with the most Lepidoptera species (i.e., had the highest degree), then sequentially added plants that interacted with the most additional unique Lepidoptera species, until all Lepidoptera species were accounted for. To compare the structural properties of host and nectar plant networks, we calculated the following indices using the functions ‘networklevel’, and ‘specieslevel’ from ‘bipartite’: connectance, NODF (a measure of nestedness), the number of compartments, plant and Lepidoptera robustness, and plant and Lepidoptera degree. See Supplementary material Appendix 3 for details on each index. We included these indices as response variables in (generalized) linear mixed-effects models ((G)LMMs) with the type of network (host or nectar plant) as a fixed effect and the EPA level III ecoregion as a random effect to account for the non-independence of subnetworks from the same ecoregions. We used the Beta family distribution in models with connectance, NODF (divided by 100), and robustness as the response, the Poisson family distribution in the model with the number of compartments as the response, and the Gaussian family distribution in models with Lepidoptera and plant degree as the response. We built all (G)LMMs using the R package "glmmTMB' (Magnusson et al. 2017), and obtained p values using the 'anova' function to compare nested models with likelihood ratio tests. We calculated 95% confidence intervals with the 'confint' function, using the "wald" method. To assess model fit, we examined residual distributions using the 'DHARMa' package (Hartig 2017).

To identify keystone plant species in each subnetwork, we built upon an approach developed in recent studies of combining several different metrics (Harvey et al. 2017; Narango et al. 2020; Ulrich & Peters 2023), which can help balance the advantages and disadvantages of each method (Ulrich & Peters 2023). For each plant species in each subnetwork, we calculated, min-max normalized, then averaged six metrics into one final "importance" score, ultimately producing lists of plant species in each subnetwork ranked based on their relative importance as host and nectar plants for Lepidoptera communities in that subregion. Hereafter, we will refer to this final score as their "importance". The six metrics we chose are: (1) and (2) metanetwork C and Z scores describing each species' importance as landscape module connectors and hubs, respectively, following methods from Hackett et al. (2019); (3) and (4) subnetwork C and Z scores, describing each species importance as local module connectors and hubs, respectively (Guimerà & Nunes Amaral 2005). For this, we used the 'cluster leading eigenvector' algorithm (Newman 2006) to calculate modularity and module composition; (5) extinction sensitivity, describing the total number of specialized Lepidoptera species that rely exclusively on a plant and would be extirpated if that plant were removed (Narango et al. 2020); and (6) species degree, describing the total number of Lepidoptera species hosted by each plant species. Because we were interested in the relative importance of species within networks, we ranked each species rather than using absolute thresholds to assign module hub or connector status (e.g., Olesen et al. 2007). The contributions of each of these metrics can help identify species that are most important for maintaining diversity, stability, and function in networks (Olesen et al. 2007; Mello et al. 2015; Peters et al. 2016; Harvey et al. 2017; Saunders & Rader 2019; Narango et al. 2020; Ulrich & Peters 2023). See Supplementary material Appendix 3 for additional information on the choice of each metric.

We tested the correlations between all of the species metrics and between the final importance score with the ‘cor.test’ function in R, using the Spearman rank correlation coefficient as the data were not normally distributed. For plant species that occur in both host and nectar plant networks, we also used Spearman correlation tests to assess whether species importance in one network type was correlated with importance in the other. To investigate whether plant species importance varied significantly between ecoregions, we built generalized linear models (GLMs) using the Beta family distribution, and including species importance score as the response and EPA level III ecoregion the predictor. We performed this analysis on each species separately, and to ensure adequate sample sizes in the models, we only included plant species that occurred in at least four subregions in at least four EPA level III ecoregions.

Beta diversity analysis

To test for differences in community composition between host and nectar plant communities and between ecoregions, we first built site-by-species matrices for each subnetwork, and calculated the Jaccard distance using the function ‘vegdist’ from the R package ‘vegan’ (Oksanen 2010). Then, with either plant type or EPA level III ecoregion as the grouping factor depending on the model, we performed permutational multivariate ANOVA (PERMANOVA) tests with the function ‘adonis2’ from ‘vegan’. Because we found significant differences in host and nectar plant composition, we tested for differences in community composition between ecoregions for host and nectar plants separately. We tested for pairwise differences using the function ‘pairwise.adonis2’ from the R package ‘pairwiseAdonis’ (Martinez Arbizu 2020), using the ‘false discovery rate’ p value correction method (Benjamini & Hochberg 1995). Finally, we tested the homogeneity of group dispersions using the function ‘permutest’ from ‘vegan’. For all permutational tests, we set the number of permutations to 999. To visualize results, we performed principal coordinate analyses (PCoAs) using the function ‘betadisper’ from ‘vegan’, defining either interaction type or ecoregion as the grouping factor. We plotted the PCoAs using the ‘gg_ordiplot’ function from the R package ‘ggordiplots’ (Quensen 2018). We performed this analysis for communities containing all plant species, and for communities containing just the top 10% of plant species from each subnetwork based on their importance scores.

Results

Full interaction dataset

After cleaning and standardization, the full interaction dataset includes 1,906 native plant species and subspecies and 1,927 Lepidoptera species and subspecies: 332 (17%) butterflies and 1,534 (78%) moths. There are 13,675 unique interactions in the dataset, 6,913 (51%) of which are nectar plant interactions and 6,762 (49%) are host plant interactions. Based on the full interaction dataset, Lepidoptera species have an average of 3.74 ± 5.54 (mean ± SD) larval host plants, and an average of 13.42 ± 23.07 nectar plants (Supplementary material Appendix 3, Table A3.1). 42% of Lepidoptera species have just one documented host plant, and 72% of Lepidoptera species have three or fewer documented host plants (Supplementary material Appendix 3, Figure A3.1A). 16% of butterfly species have one reported nectar plant, and 26% have three or fewer reported nectar plants (Supplementary material Appendix 3, Figure A3.1B). When analyzing the number of nectar plants per species, we excluded moths because the lack of moth nectar plant data suggests nearly all moths are specialist nectar feeders, which is almost certainly not the case (Hahn & Brühl 2016; Macgregor & Scott-Brown 2020). Connectance and NODF (nestedness) are higher in the nectar plant networks, while number of compartments is higher in host plant networks (Supplementary Material Appendix 3, Table A3.1).

Multilayer metanetwork

We found an average EPA level IV ecoregion survey completeness of 49.34 ± 22.36% (mean ± SD) for plant species and 46.49 ± 19.05% for Lepidoptera species. We excluded 106 (59%) of 180 possible level IV ecoregions that did not make the threshold to qualify as well-surveyed (Figure 3.1). After combining the interaction, species occurrence, and ecoregion and habitat raster data to produce the multilayer metanetwork, we excluded 552 (38%) of the 1,461 total subnetworks because they had less than 10 plant and/or 10 insect species. We were left with 909 unique subnetworks from 477 subregions distributed across 12 of the 13 level III ecoregions, with only the Northern Basin and Range completely excluded (Supplementary material Appendix 3, Figure A3.2). 470 (52%) of the subnetworks are nectar plant networks and 439 (48%) are host plant networks.

There are a total of 1,631 plant species and 1,456 Lepidoptera species in the multilayer metanetwork, 277 (19%) of which are butterflies and 1,179 (81%) are moths. Across all subnetworks, there are a total of 284,300 spatially-explicit interactions comprising 11,015 unique interactions, 5,948 (54%) of which are nectar plant interactions and 5,067 (46%) are host plant interactions. 5,504 (93%) of the unique nectar plant interactions are with butterflies, while 444 (7%) are with moths. In contrast, 1,537 (30%) of the unique host plant interactions are with butterflies, while 3,530 (70%) are with moths. 522 (32%) of the plant species occurred in both network types, indicating that they serve as both host and nectar plants. 337 (23%) of Lepidoptera species occurred in both network types. 374 (3.4%) of the unique interactions were shared between network types, indicating instances of Lepidoptera species using the same host plant as caterpillars that they visit for nectar as adults. Additional details about the species distribution dataset, the full interaction dataset, and the multilayer metanetwork are provided in Supplementary material Appendix 3.

Differences between host and nectar plant networks

Lepidoptera degree, plant degree, connectance, NODF, Lepidoptera robustness, and plant robustness were all significantly higher in the nectar plant networks (Table 3.1; Figure 3.2; Supplementary material Appendix 3, Table A3.2). In contrast, the number of compartments was significantly higher in the host plant networks (Table 3.1; Figure 3.2; Supplementary material Appendix 3, Table A3.2). Across all subnetworks, the composition of Lepidoptera host plants differed significantly from the composition of nectar plants ($F_{1,907} = 39.54$, $R^2 = 0.042$, $p = 0.001$; Figure 3.3A). The group dispersions were not significantly different ($F_{1,907} = 0.18$, $p = 0.659$). These differences were stronger when looking at just the top 10% most important plant species in each subnetwork ($F_{1,907} = 64.12$, $R^2 = 0.066$, $p = 0.001$; Figure 3.2B), although the group dispersions were significantly different ($F_{1,907} = 39.54$, $p = 0.001$). These results did not change when analyzing only interactions involving the 23% of Lepidoptera species that occur in both network types (data not shown). The composition of host plants also differed significantly from nectar plants in all EPA level III ecoregions when analyzed individually (data not shown).

Keystone species and geographic variation

Across all subnetworks, on average 30 ± 12% (mean ± SD) of host plant species and 8.4 ± 7.1% of nectar plant species are required to support 90% of Lepidoptera species (Figure 3.4). Species degree was most strongly correlated with the species final importance score for both nectar plants ($\rho = 0.91$, $p < 0.001$) and host plants ($\rho = 0.77$, $p < 0.001$; Supplementary material

Appendix 3, Figure A3.3). For species that serve as both host and nectar plants (N = 522, 32% of total plant species), their importance in one network type was significantly correlated with their importance in the other network type ($\rho = 0.61$, $p < 0.001$; Figure 3.5).

When considering all plant species, nectar plant community composition differed significantly between EPA level III ecoregions ($F_{11,458} = 10.0$, $R^2 = 0.19$, $p = 0.001$; Figure 3.6A). Group dispersions were significantly different ($F_{11,458} = 4.41$, $p = 0.001$). All pairwise differences in nectar plant community composition between EPA level III ecoregions were significant. Host plant community composition also differed significantly between EPA level III ecoregions ($F_{11,427} = 9.0$, $R^2 = 0.19$, $p = 0.001$; Figure 3.6B). Group dispersions were significantly different ($F_{11,427} = 2.75$, $p = 0.002$). All pairwise differences in host plant community composition between level III ecoregions were significant. When looking at just at the top 10% of species based on the final importance scores, the differences in plant community composition were still significant for both nectar plants ($F_{11,458} = 11.88$, $R^2 = 0.22$, $p = 0.001$; Figure 3.6C) and host plants ($F_{11,427} = 9.5$, $R^2 = 0.20$, $p = 0.001$; Figure 3.6D). Group dispersions were significantly different for both the top 10% of nectar plants ($F_{11,458} = 3.19$, $p = 0.001$) and the top 10% of host plants ($F_{11,427} = 5.40$, $p = 0.001$). For the top 10% of nectar plants, all pairwise differences were significant except for the Cascades vs. the Eastern Cascades Slopes and Foothills ecoregions ($F_{1,7} = 1.78$, $R^2 = 0.20$, $p = 0.063$). For the top 10% of host plants, all pairwise differences between EPA level III ecoregions were significant.

For all plants that occurred in at least four EPA level IV ecoregion-CWHR habitat combinations in at least four EPA level III ecoregions, we found that 78.8% of nectar plant species (N = 226) varied significantly in importance between EPA level III ecoregions, and 70.4% (N = 500) of host plant species varied significantly in importance between EPA level III ecoregions. Visually examining how species importance changes across the landscape, we see (1) variation in species' importance across their range of ecoregions, and (2) differences in the relative importance between species, which can also shift between ecoregions (Figure 3.7).

Discussion

Our findings advance the understanding of how ecological interaction networks and species roles vary across space and between different interaction types, which is essential for predicting and mitigating the effects of climate change and other stressors on biodiversity and ecosystem services (Poisot et al. 2015; Hutchinson et al. 2019; Burkle & Alarcón 2011). As in previous studies (Thébault & Fontaine 2008, 2010; Fontaine et al. 2011; Astegiano et al. 2017), we found structural differences between host and nectar plant networks. Across California, Lepidoptera host plant networks were more modular and had lower connectance and nestedness than nectar plant networks, which is suggested to promote the evolution of specialization and generalism in antagonistic and mutualistic networks, respectively (Fontaine et al. 2009). Indeed, consistent with other studies (Fontaine et al. 2009; Altermatt & Pearse 2011; Fontaine & Thébault 2015), we found that caterpillars were significantly more specialized than adults. It is well known that diet specialization puts species at greater risk of extinction under environmental change (Dennis et al. 2004; Mattila et al. 2006; Biesmeijer et al. 2006; Boyles & Storm 2007; Colles et al. 2009; Fonseca 2009; Balisi et al. 2018; Palash et al. 2022). Supporting this, we found that Lepidoptera communities were significantly more sensitive to the simulated loss of host plants than nectar plants, underscoring the importance of supplying native host plants in Lepidoptera conservation efforts (Curtis et al. 2015; Thogmartin et al. 2017a).

However, we also found specialization to be common in nectar plant interactions, with 26% of butterfly species visiting three or fewer nectar plants. Our result supports previous findings that butterflies often have specialized nectar plant relationships due to specific chemical requirements or the coevolution of specialized pollination systems (Nilsson 1988; Lipani 1990;

Corbet 2000; Fenster et al. 2004; Hardy et al. 2007; Krenn 2010; Jain et al. 2016; Levin et al. 2017). Importantly, we found that the composition of host and nectar plants differed significantly in all ecoregions, a pattern that was stronger when only the top 10% most important plants were considered. This finding is supported by the fact that only 3.4% of unique interactions occurred in both network types, and together suggests that Lepidoptera species rarely nectar on the same plants as adults that they feed on as caterpillars. This contradicts the findings of Altermatt and Pearse (2011), who found that Lepidoptera use the same plants as caterpillars and adults more often than would be expected. However, our results are consistent with the recent findings of Wang et al. (2023), who suggest that selecting different plants for oviposition and nectar feeding may reflect selection in Lepidoptera to ensure adequate resources for their two life stages by preventing over-consumption of the same plant. This pattern may also be driven by selection on plant traits (Irwin 2010). For example, Kessler et al. (2010) reported that *Nicotiana attenuata*, a plant both herbivorized and pollinated by nocturnal hawkmoths (Sphingidae), can shift chemical cues to attract diurnal pollinators to avoid herbivory by hawkmoth caterpillars. There is an intriguing possibility that such tradeoffs in pollinator attraction and plant defense may be commonplace across entire plant-insect interaction networks.

The main management implication of these findings is that providing the most important host plants may do little to support Lepidoptera in their adult stage, and likewise, providing the most important nectar plants may do little to support Lepidoptera in their larval stage. As previous research has indicated (Dennis et al. 2004; Tudor et al. 2004; Croxton et al. 2005; Freese et al. 2006; Wallisdevries et al. 2012; Inamine et al. 2016; Kolkman et al. 2022), it is important to provide both specific host plants and specific nectar plants in conservation efforts to support the entire Lepidoptera life cycle. Indeed, adult nectar feeding can be as important as larval herbivory for Lepidoptera development and reproduction (Gilbert 1972; Murphy et al. 1983; Pivnick &

McNeil 1985; Moore & Singer 1987; Hill & Pierce 1989; Mevi-Schutz & Erhardt 2005). However, we also found that for the 32% of plant species in this study that served as both host and nectar plants, their importance in one network is positively correlated with their importance in the other, consistent with other studies (Wang et al. 2023; Timoteo et al. 2023). This indicates that there are some plant species that can serve as both excellent host and nectar plants, which may be of particular interest in conservation efforts. Theoretical work suggests that such plants may be especially important for maintaining community stability (Sauve et al. 2016).

Supporting the results of Narango et al. (2020), who focused only on Lepidoptera host plants at the genus level, we found that on average just 30% of host plant species and 8% of nectar plant species are required to support 90% of Lepidoptera species across the California ecoregions analyzed in this study. We also found that the composition of keystone host and nectar plant species varies significantly between California ecoregions, and that for the majority of species occurring in multiple ecoregions, their importance as host and nectar plants for Lepidoptera communities varies significantly between ecoregions. These findings support the understanding, advanced by the Geographic Mosaic Theory of Coevolution (Thompson 2005), that the ecological and evolutionary forces structuring communities of interacting species vary geographically, leading to different functional outcomes. Indeed, numerous studies have found that ecological network topology varies through space, which can affect species ecological roles (Olesen & Jordano 2002; Tylianakis & Morris 2017; Hackett et al. 2019). Our results reveal why considering this geographic variation is important for conservation and management decisions: plants that are considered keystone in one ecoregion may not be in the other ecoregions they occur in, depending on spatial variation in the structure and composition of the interaction networks to which they belong. This complements the growing understanding of the importance of selecting locally-native (i.e., indigenous) plant species in restoration by considering species' precise natural ranges (Nichols et al. 2019; Berthon et al. 2021).

Ultimately, our results provide ecoregion and habitat-specific lists of keystone native host and nectar plant species that can be used to maximize the benefit of native plant landscaping and restoration for Lepidoptera conservation in California. We disseminate this information with a free R Shiny web application (ctcosma.shinyapps.io/the_butterfly_net/). While we encourage the use of keystone species specific to each habitat and ecoregion in California, following Narango et al. (2020) we also identified host and nectar plant genera that are disproportionately important across multiple ecoregions, providing a more manageable list of plants to choose from (Supplementary material Appendix 3, Figure A3.4). The use of these keystone plant species will be particularly important in urban, suburban, and exurban areas. Urban greenspaces, including private yards and gardens, have the potential to support a high diversity of insects (Hinners et al. 2012; Gardiner et al. 2013; Baldock et al. 2015, 2019; Aronson et al. 2017; Hall et al. 2017; Kremen & Merenlender 2018; Levé et al. 2019; Prendergast et al. 2022), and are important steppingstones for broader conservation goals (Dearborn & Kark 2010; Goddard et al. 2010). However, especially for Lepidoptera, their conservation value depends on the use of appropriate plant species (Koh & Sodhi 2004; Threlfall et al. 2017; Ramírez-Restrepo & MacGregor-Fors 2017; Aguilera et al. 2019). Residential urban gardeners have traditionally selected plants based mainly on aesthetics (Goodness 2018), and as a result, urban areas are currently dominated by introduced and often invasive plant species (McKinney 2004; Qian & Ricklefs 2006) that provide few resources for native insects (Tallamy & Shropshire 2009; Tallamy et al. 2021). Even many native planting guides suggest plants based on aesthetics or expert choice (but see The National Wildlife Federation's Native Plant Finder: nativeplantfinder.nwf.org). In contrast, our network approach provides evidence-based planting recommendations, which have been shown to improve the ability of even small habitat patches to support insects (Maia et al. 2019; Saunders & Rader 2019; Windsor et al. 2021; Assunção et al. 2022; Zaninotto et al. 2023). This can also increase human wellbeing in urban areas, as the benefits of urban greenspaces for human psychological health depend on how biodiverse those greenspaces are (Wood et al. 2018).

There are several limitations to our approach. First, although we analyzed sampling completeness to exclude poorly-sampled subregions, there are still likely to be biases and gaps in the opportunistic species distribution data, as well as the final interaction networks. However, relative differences in species roles, including degree, tend to show up quickly even in under-surveyed networks (Fründ et al. 2016), and the network techniques employed can identify keystone species with incomplete data (Harvey et al. 2017). Additionally, to build the spatially-explicit subnetworks, we assumed that species that interact somewhere in California will interact wherever they co-occur. However, species co-occurrence does not always correspond with species interactions (Blanchet et al. 2020), and insects may specialize at local scales despite being more generalized at macro scales (Fox & Morrow 1981; Kuussaari et al. 2000). Likewise, the networks analyzed in this study contain temporally-aggregated interaction data. While this may capture plant species' total importance across seasons, species roles in interaction networks can be highly dynamic through time (Olesen et al. 2008; Chacoff et al. 2018; CaraDonna et al. 2021; Crespo et al. 2022). To improve this work, there should be more effort to describe interactions with higher spatiotemporal resolution, perhaps by integrating plant and insect phenology data. Lastly, species importance metrics influenced by the total number of interaction partners (degree) may prioritize the most common and widespread species, possibly overlooking the unique requirements and/or conservation value of rare or specialized species. However, the data used in this study were collated from dozens of sources, including observations from entomologists, and rare species often receive disproportionate attention (Altermatt & Pearse 2011; Astegiano et al.

2017). Further, rare or specialized species usually represent “peripheral” species in interaction networks, which in turn usually rely on the species with keystone positions (Gibson et al. 2006; Elle et al. 2012). Since these rare or specialized species are often the focus of traditional conservation efforts, a network approach can help to reconcile single-species and ecosystem-services approaches to conservation (Lindenmayer et al. 2007). Indeed, in our study, *Asclepias*, the monarch host plant genus, was identified as a keystone nectar plant across all subregions. Nevertheless, special attention should be paid to plant species selection in scenarios where the main goal is to support specialized and/or threatened insects. One promising approach is to integrate network-based prioritization with complementary data on insect risk (e.g., Forister et al. 2023). We also recommend field experiments to assess the efficacy of keystone plant species in supporting conservation goals (e.g., Maia et al. 2019).

Conclusions

Our results highlight several important considerations to improve the value of native plant restoration for insect conservation. First, it is important to provide both host and nectar plants, as the plant species used in larval and adult stages often differ. Second, selecting plant species with keystone roles in interaction networks can maximize the number of insect species supported. And third, the identity of keystone plant species varies geographically, making it important to consider spatial variation in the structure and composition of interaction networks when prioritizing plant species. Selecting plants based on these considerations can help increase habitat connectivity and the flow of resources and services across the landscape (Blitzer et al. 2012; Hackett et al. 2019), which is important for climate change resilience (Timpane-Padgham et al. 2017; Morelli et al. 2017; Anderson et al. 2023b).

This research provides an example of how to leverage the increasing amount of biogeographic data, including from community science, for conservation. With just two general requirements, data on species distributions and their interactions, this framework can be extended to any taxonomic group and geographic region. However, our results also highlight that the interactions of many important groups of insects, such as nocturnally-pollinating moths, are poorly described (Macgregor & Scott-Brown 2020). Indeed, moth nectar plant interactions comprised just 7% of the total nectar plant interactions in this study, despite the fact that there are over 10 times more moth species than butterfly species in California (Powell & Opler 2009), which visit many plant species for nectar (Cosma, unpublished data). Next-generation sequencing technologies will be important for filling in these knowledge gaps (e.g., Macgregor et al. 2019), in combination with modeling techniques capable of predicting ecological interactions and the structure of ecological networks with incomplete data (Valdovinos 2019; Strydom et al. 2021). We also point to the need to extend community science efforts to document not only where species occur, but which species they interact with.

It is important to note that the keystone plant recommendations we provide apply only to Lepidoptera species, which are just one component of complex ecosystems. To expand the utility of network theory to biodiversity conservation, more interaction types and taxonomic groups should be integrated to provide plant species recommendations that deliver broader benefits. Furthermore, integrating other sources of data, such as plant and insect traits, can tailor plant species recommendations to specific management scenarios. For example, incorporating data on flowering phenology can identify keystone plant species that sustain pollinators in agricultural landscapes during the crop dormant season (Russo et al. 2013). Incorporating data on the social and economic factors that influence planting decisions is also needed (Helfand et al. 2006; Larson et al. 2009; Peterson et al. 2012; Avolio et al. 2015; Delphia et al. 2019; Gillis & Swim 2020). For example, native planting choices are often limited by seed and nursery availability (White et al. 2018; León-Lobos et al. 2020; Pedrini et al. 2023), and data on what is locally available could make keystone plant recommendations more realistic and sustainable. This integration of the social, economic, and ecological sciences would help to stimulate cooperation among various stakeholders, which is critical for effective insect conservation in the Anthropocene.

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Tables and Figures

Table 3.1. (G)LMM summaries for models comparing network metrics between host and nectar plant networks, using all subnetworks in each category. All models include the random effect of EPA level III ecoregion. In all models, the host plant network is the intercept.

Response	Model type	Random effect (SD)	*β*	95% CI	*p*
Lepidoptera degree	LMM	0.48	5.65	5.28, 6.02	< .001
plant degree	LMM	0.32	2.38	2.24, 2.52	< .001
connectance	GLMM: beta(logit)	0.19	0.89	0.83, 0.96	< .001
NODF	GLMM: beta(logit)	0.12	1.64	1.58, 1.70	< .001
Lepidoptera robustness	GLMM: beta(logit)	0.084	0.41	0.38, 0.44	< .001
plant robustness	GLMM: beta(logit)	0.13	0.78	0.74, 0.83	< .001
number of compartments	GLMM: poisson(log)	0.19	-2.13	-2.21, -2.05	< .001

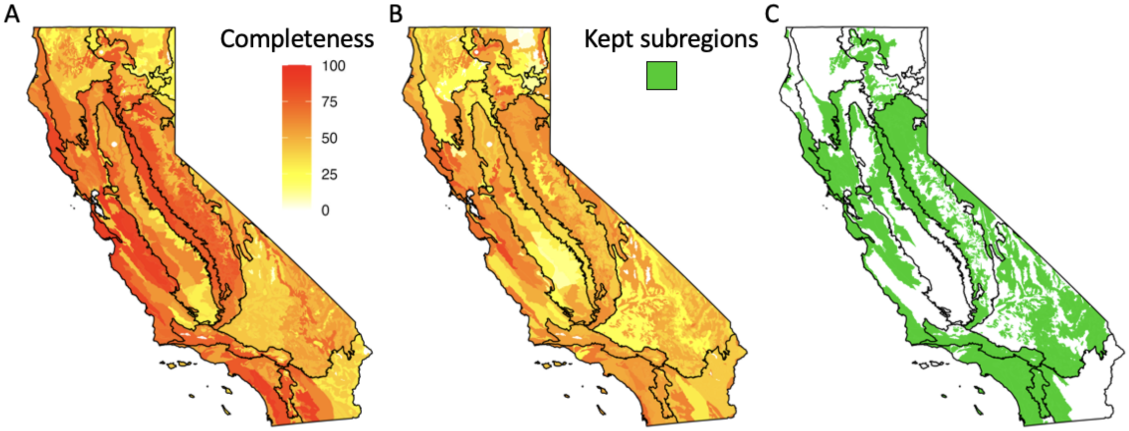

Figure 3.1. Survey completeness by EPA level IV ecoregion based on georeferenced species occurrences for (A) plant species and (B) Lepidoptera species, and showing (C) the subregions that qualified as well-surveyed for both plants and Lepidoptera (completeness ≥ 50%, slope ≤ 0.3, and ratio ≥ 3). EPA level III ecoregion outlines are displayed in black.

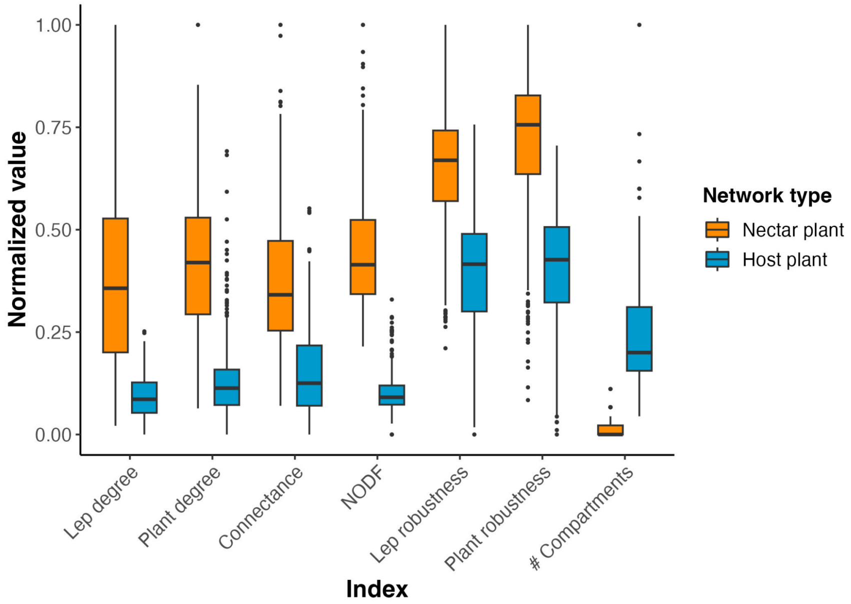

Figure 3.2. Boxplot comparing selected network indices between nectar plant (orange) and host plant (blue) subnetworks, min-max normalized for visualization. The boxes show the median (center line), first and third quartile (upper and lower hinges) and 1.5 * interquartile range (whiskers).

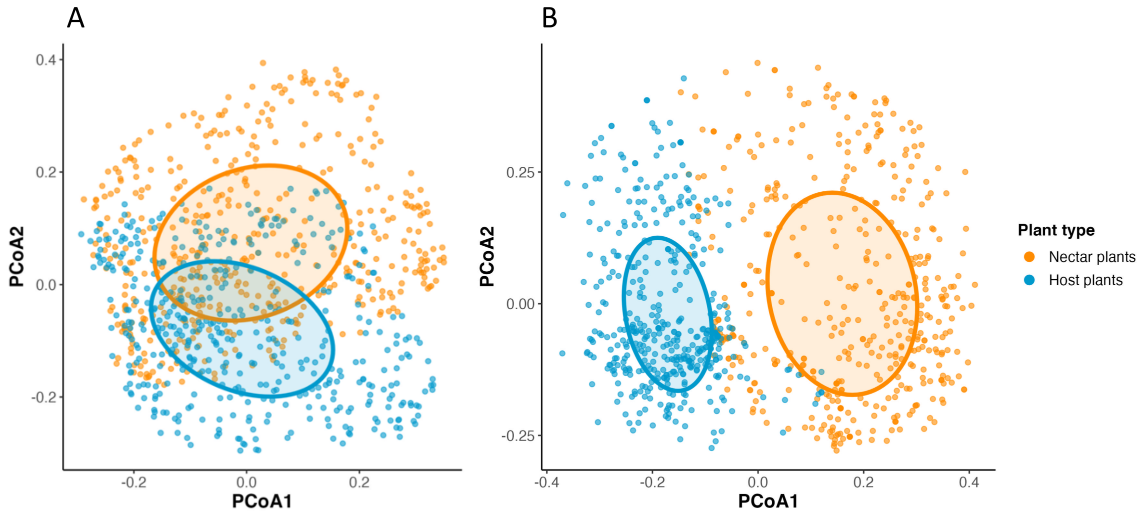

Figure 3.3. PCoA plots using the Jaccard distance metric displaying differences in community composition between nectar plant communities (orange) and host plant communities (blue) for all subnetworks. Panel A includes all plant species, while panel B includes just the top 10% of plant species from each subnetwork based on their importance scores. Ellipses are centered at the mean and encompass the standard deviation.

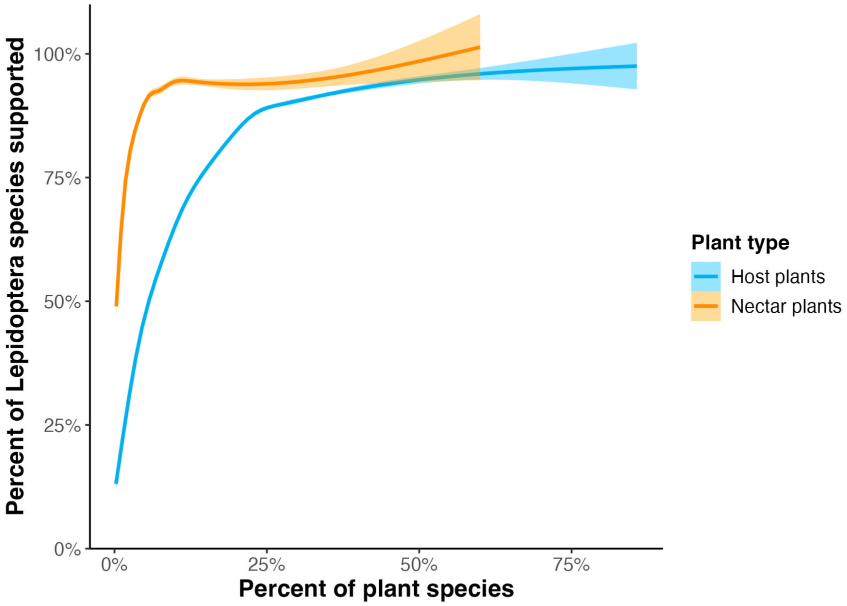

Figure 3.4. Supported Lepidoptera accumulation curves displaying the minimum percentage of host plants (blue) and nectar plants (orange) required to support any given percentage of Lepidoptera species across all subnetworks, with loess curves and 95% confidence intervals displayed.

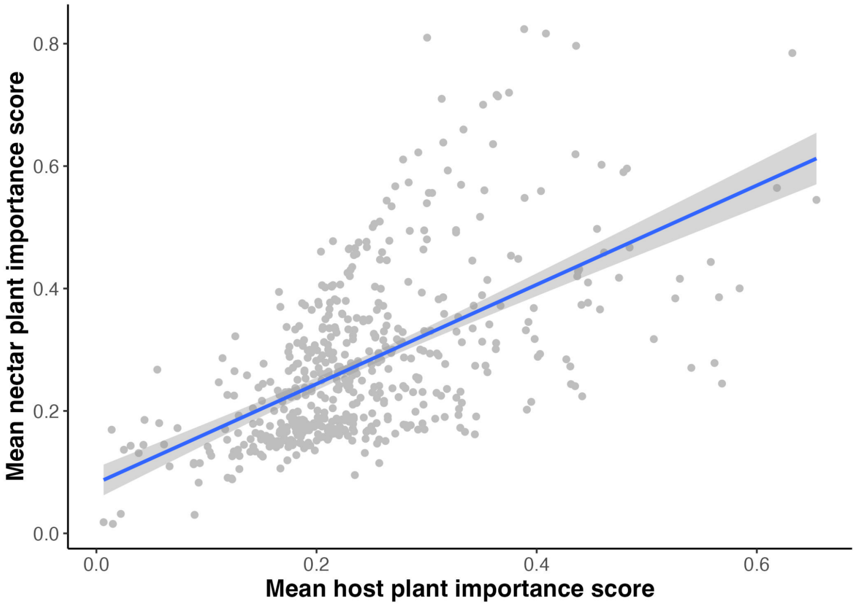

Figure 3.5. For all plant species that serve as both host and nectar plants for Lepidoptera, the mean importance score across all host plant subnetworks versus the mean importance score across all nectar plant subnetworks. Raw data, linear fit, and 95% confidence interval are displayed.

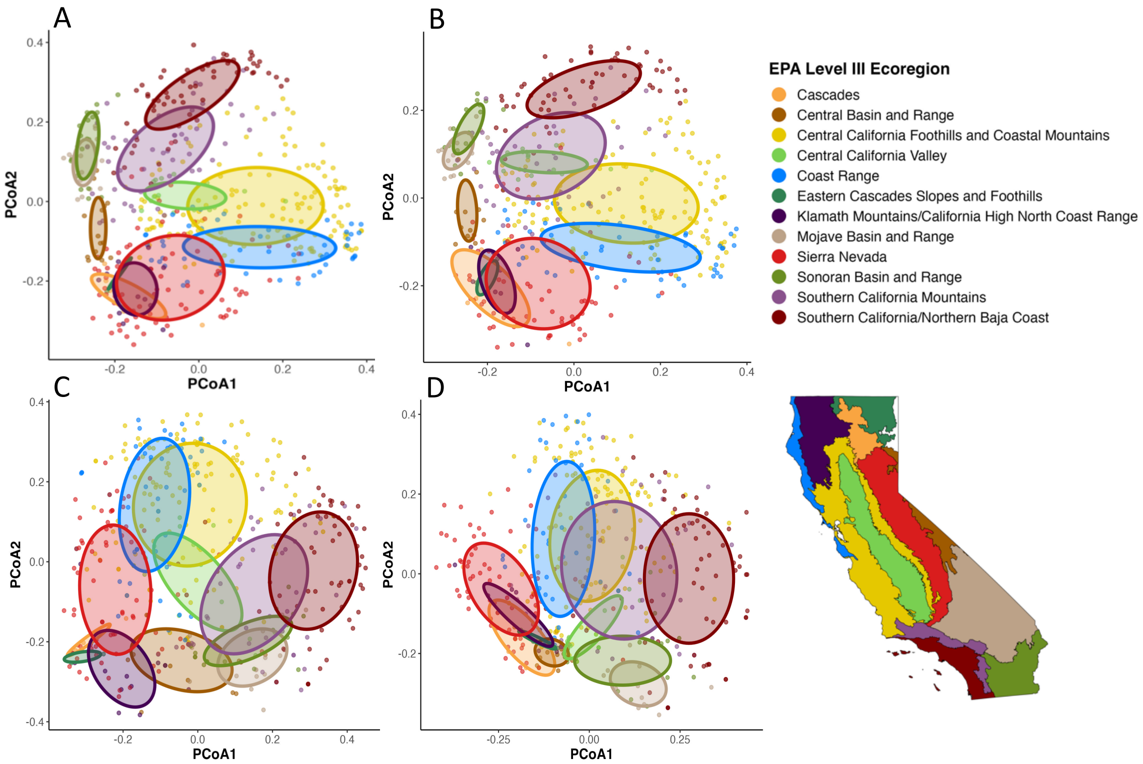

Figure 3.6. PCoA plots using the Jaccard distance metric displaying differences in plant community composition between EPA level III ecoregions for all subnetworks including (A) all nectar plants, (B) all host plants, (C) the top 10% most important nectar plants, and (D) the top 10% most important host plants. Ellipses are centered at the mean and encompass the standard deviation. The map of California shows the locations of the color coded EPA level III ecoregions.

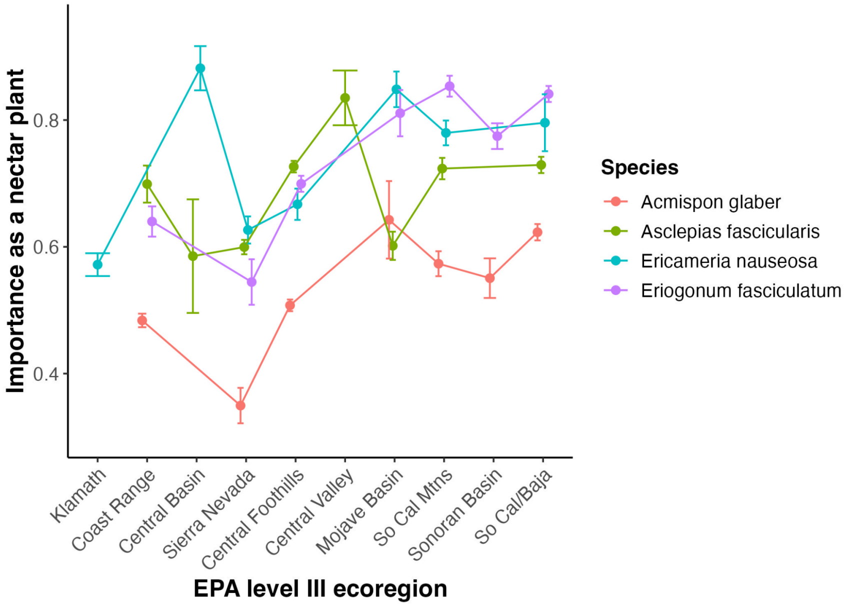

Figure 3.7. An example with four plant species showing variation in species importance as Lepidoptera nectar plants between EPA level III ecoregions, arranged by approximate latitude. Mean and standard error bars are displayed.

Synthesis

Amid the current biodiversity crisis, precipitous global moth declines are eliminating pollination services that we are only just beginning to understand (Macgregor & Scott-Brown 2020). In this dissertation, I have shed light on the diversity, structure, and stability of nocturnal moth pollen-transport networks, revealed the climate-sensitivity of plant and pollinator functional traits that mediate the interaction, and applied this knowledge to advance data-driven conservation efforts aimed at protecting moths and their ecosystem services. Importantly, our results help elucidate the mechanisms through which climate change may impact plant-pollinator interactions. In Chapter 1, we found that moths were more sensitive to the loss of their nectar plants in warmer and drier conditions, which was likely driven in part by increased competition for reduced floral resources. In Chapter 2, using a plant species that we detected in moth pollen loads from Chapter 1, we confirmed that experimental drought reduced the abundance of flowers, and that the combination of warming and drought reduced nectar volume and caloric content at night. Taken together, these results illuminate how the impacts of climate change on species functional traits (e.g., floral nectar) may propagate through their altered interactions to ultimately impact network structure and stability.

From an applied perspective, the results of Chapters 1 and 2 underscore the need to provide diverse floral resources for moths in pollinator conservation efforts. In particular, as the combined effects of heat and drought under climate change decrease the quality and availability of floral resources in native plant communities, supplemental plantings—such as those that can be established in urban greenspaces—will become increasingly important for pollinator resilience. In Chapter 3, we used a spatially-explicit network approach to identify keystone native host and nectar plant species that best support butterfly and moth communities. By presenting these results in the form of a user-friendly R Shiny tool, we aim to make this information accessible to audiences, such as urban gardeners, that have the potential to make a significant impact in insect conservation. When widely implemented, even small-scale use of native plant species can improve landscape-scale conservation efforts in heavily human-modified landscapes (Goddard et al. 2010, Riva & Fahrig 2022). For example, the use of native plants in urban greenspaces can increase habitat connectivity, which is essential for species to persist under climate change (Vos et al. 2008; Beller et al. 2019; Synes et al. 2020).

The results of Chapter 3 also highlight how the general lack of available moth pollination data limits conservation efforts. While we uncovered hundreds of previously unknown moth-pollen transport interactions in Chapter 1, this data is from one region. It will be important for researchers around the world to leverage cutting-edge techniques, including pollen DNA metabarcoding (Macgregor et al. 2019) and camera surveillance (Alison et al. 2022), to document these nocturnal interactions that are likely of global importance to ecosystem health and human food security (Macgregor & Scott-Brown 2020; Buxton et al. 2022). Indeed, our results from Chapter 1 revealed moths transporting pollen from over a dozen crop species. While there is relatively little evidence for the importance of moths to crop production (Macgregor & Scott-Brown 2020), recent experimental studies have shown that flower visits by moths contribute to production in apples (Robertson et al. 2021), gourds (Lu et al. 2021), and the forage crop red clover (*Trifolium pratense*: Alison et al. 2022). Additional experimental work, such as pollinator-exclusion experiments, are needed to further elucidate the importance of moth pollination to both wild and agricultural plants.

In Chapter 1, we found that, in contrast to most diurnal networks, moth pollen-transport networks had low levels of nestedness, which may make them more sensitive to environmental change (Song et al. 2017; Ellis et al. 2023). This has implications not just for moths, but also for the diurnal pollinators that they are connected to. Indeed, consistent with recent findings (Knop et al. 2018; Ellis et al. 2023; García et al. 2024), our results reveal that diurnal and nocturnal plant-pollinator networks are linked by shared plant species, indicating that their responses to climate change and other stressors may also be linked. García et al. (2024) showed that when diurnal and nocturnal plant-pollinator networks are analyzed together, they may be more sensitive to perturbations than their isolated analysis would suggest. Our results from Chapter 2 also indicate that by modifying the quantity and quality of floral resources throughout the day, climate change may alter competitive dynamics between diurnal and nocturnal pollinators. This is worrisome, as altered competition between pollinators via the effects of climate change on plants has been implicated in insect declines (Thomson 2016; Thomson & Page 2020).

How moths and their ecosystem services will be impacted by climate change will ultimately depend on multiple species-specific factors including distribution, life history traits, and environmental requirements (Hill et al. 2021; Wagner et al. 2021). Lepidoptera also possess a variety of adaptations, including adaptive phenotypic plasticity, that may buffer them against environmental change (Hill et al. 2021). For example, the expression of the heat tolerance-related gene, Hsp70, increases in moths exposed to higher temperatures (Bahar et al. 2013; Wang et al. 2015). Our results from Chapter 1 also suggest that moths are able to switch between alternative nectar plants, which can buffer pollinators against the effects of environmental change (Kaiser-Bunbury et al. 2010; Sheykhali et al. 2020). Plants may also be buffered by plasticity, as we observed for traits related to plant water economy in Chapter 2. However, adaptive responses may be limited when species occur in already harsh environments like the Southwestern US (Bussotti et al. 2014; Vale & Brito 2015). Additionally, selection by the biotic and abiotic environment may conflict (Carroll et al. 2001; Prieto-Benítez et al. 2016). For instance, under climate change conditions, selective pressures on plants to maintain pollinator attraction (e.g., by producing nectar) may be constrained by a trade off with plant water economy.

Thus, the intertwined fates of moths, plants, and humankind are uncertain. However, solutions at all scales are becoming clearer, and we must institute them now. We desperately need transformative change to come from policymakers and from corporations. We also need to cultivate deeper relationships with biodiversity in our day-to-day lives. This begins by breaking down the barriers that underserved communities, especially, face. Integrating biodiversity into the design of our urban and agricultural areas, giving children the opportunity to interact with plants and insects, blurring the line between what is human and what is nature—these are the steps that we must take to solve interconnected social-environmental challenges, and to usher in a new era of Earth stewardship. I have seen the difference that even a small native wildflower patch in the middle of an urban jungle can make for biodiversity and for people. As I hope this dissertation has shown, it would benefit us all if that wildflower patch also contained some good moth nectar plants.

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