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CASCADING EFFECTS OF CLIMATE STRESS ON PLANT–POLLINATOR INTERACTIONS

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Abstract

Cascading effects of climate stress on plant-pollinator interactions

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Plant-pollinator communities vary over landscapes, seasons, and years. This inherently high variation can be linked at least in part to seasonal climates. Early spring temperature and rainfall are important cues for plant germination (Ackerly 2004, Carta et al. 2013, Nonogaki and Nonogaki 2016), flowering (Glover 2008, Tooke and Battey 2010) and other life-cycle events (Chuine et al. 2013). Rearing temperature also influences the growth, development, and consequently emergence time of pollinating insects (Kemp and Bosch 2005, Kingsolver and Huey 2008). While these plant-pollinator cues often align, physiological responses to climate stress in plants or pollinators can induce mismatches that could negatively impact the larger community of interacting species.

Recent climate change alters ecologically important mutualistic interactions that may have far-reaching consequences for ecosystems (Tylianakis et al. 2008, Hegland et al. 2009). For instance, increasingly warmer temperatures cue some plant species to flower at unusual times (Wolkovich et al. 2012) that their pollinators do not always track (Inouye 2008, Lambert et al. 2010, Kudo and Ida 2013, Caradonna et al. 2014). Resource-based mismatches may also develop as plants physiologically respond to environmental stress by allocating resources away from reproduction and toward survival (Memmott et al. 2007, Scaven and Rafferty 2013, De la Luz 2018a).

On a geographical scale, species' ranges may shift as pollinators expand their foraging ranges to higher elevations or latitudes over time to escape temperature stress (Kelly and Goulden 2008, Parmesan and Hanley 2015).

Resource exchange between plants and pollinators ultimately influences population-level reproductive success for both trophic levels (Wang and Smith 2002, Bascompte and Jordano 2014a). Despite the ecological importance of pollination for both biodiversity and ecosystem function (Potts et al. 2006, Bascompte and Jordano 2007, Albrecht et al. 2012, Hanley et al. 2015, IPBES 2016) there are only a few known plant-pollinator datasets that cover a long enough time frame to specifically address the potential negative impacts of recent climate change or other stressors on those communities in natural ecosystems (reviewed in Burkle and Alarcón 2011, Burkle et al. 2013).

My dissertation explores how individual physiological responses to temperature and water stress, scale up to impact plant-pollinator network structure, ecosystem function, and biodiversity. I examine how plant-pollinator networks in two habitats are structured in relation to intrinsic abiotic stressors, and how those habitats have changed over the past twenty years. In the lab and greenhouse, I test how temperature and water availability influence nectar output in three native plant species. Then I follow bumblebee foraging patterns on those plant species in controlled choice trials. In a separate set of experiments, I test how nectar diet influences a bumblebee's ability to metabolically cool their bodies while under acute heat stress.

Plant-pollinator networks in two habitats over time: Chapter one builds upon an existing dataset to examine how plant-pollinator network structure and stability vary over time in two habitats. Plant-pollinator communities are made up of pairs of mutualistic species. These communities are sometimes represented as bipartite interaction networks, where pollinator and plant species are 'nodes' and the interactions between them are 'links' (Bascompte and Jordano 2014b). Mutualistic ecological networks are often nested, a structure that develops out of an asymmetric arrangement of species interactions. Specifically, nestedness is a tendency for rare, usually specialist species to interact with generalist, usually common partners and for generalist species to most often interact with other generalists. This creates properly nested subsets of interacting species (Bascompte et al. 2003, Guimarães et al. 2006, Pires et al. 2011) that should buffer a community against 'extinction cascades', which are secondary extinctions due to the loss of an interaction partner(s) (Bascompte and Stouffer 2009). In a perfectly nested network, the inner-most species subset are 'hub' species, highly linked species that disproportionately contribute to ecosystem function (Olesen et al. 2007, Bascompte 2009, Guimarães et al. 2011). Identifying 'hub' species in a network and understanding how those species might directly respond to stress is important given the outsized role they likely play in species and network maintenance.

Mutualistic network theory predicts that a diverse and highly nested network should be more robust to climate stress than one that is less diverse or with a less defined structure (Bascompte and Jordano 2014a). To test this, I created plant-bee

networks from historical survey data (years: 1991-1993) for two California habitats with different baseline temperatures and water availabilities: coastal grasslands and endemic Santa Cruz sandhills. I evaluated nestedness as a metric of network stability and identified changes in composition and structure in each network. Next, I resurveyed (years: 2013-2015) a subset (n=9) of the original survey sites (n=14) and evaluated how plant-pollinator networks changed in the ~20 years between surveys. During that time, both habitats experienced combined temperature stress, drought, and species invasions.

Plant floral rewards and bumblebee foraging: Chapter two tests resource-based mismatches as one possible mechanism that disrupts plant-pollinator interactions. Plants often allocate resources toward survival in favor of lifetime fitness when they are stressed (e.g., life history trade-off; Stearns 1989, Ashman et al. 1994). For instance, perennial plants might postpone reproduction within a season, or across years until conditions are more favorable (Willmer 2011a); annual plants have less flexibility and may produce fewer, smaller flowers that are less attractive to bees. For plants, these strategies conserve resources to support reproduction through to seed (Pleasants and Chaplin 1983, Galen 2000, Carroll et al. 2001, Liu et al. 2012). However, small, resource-poor flowers often receive shorter and less frequent visits from bees (McCallum et al. 2013), which reduces seed production in many plant species (Wright and Schiestl 2009, Burger et al. 2010, Willmer 2011b, Essenberg et al. 2015, Milet-Pinheiro et al. 2015).

I initially grew 8 California native plant species (all visited by bumblebees in the

field) in five temperature and humidity–controlled growth chambers and under three levels of water stress. Only three of those species survived under the most stressful temperature and water conditions with enough replication to measure plant and floral traits. Then, for each plant species, I offered one plant from each of the 15 temperature/water combinations to single bumblebee workers in foraging choice trials, where I recorded each bee's behaviors over a full array of experimental plants for 20 min. To determine the downstream impact of temperature and water availability on plant fecundity through foraging, I measured seed production in choice trial plants that survived to the end of the experiment.

Bumblebee diet and heat stress: In Chapter three I test how nectar diet could limit a bumblebee's ability to cool down when heat stressed. The interface of nectar diet and temperature regulation in bumblebees, or other bees that can thermoregulate, has been studied though largely in the context of warm-up to prepare the flight muscles for foraging at cooler temperatures (Heinrich 1976). The recent large-scale declines in bumblebee diversity and abundance (National Academy of Sciences 2007, Goulson et al. 2008, Potts et al. 2010, Cameron et al. 2011, Koch 2011, Hatfield et al. 2015a, Thomson 2016) have been attributed in part to disease, a decline in floral resources, habitat fragmentation, and climate stress, but, the specific mechanisms driving species losses remain unclear.

Bumblebees are among a group of large-bodied insects capable of active thermoregulation (Heinrich 1979, May 1979). This physiological adaptation allows bumblebees to persist in cold climates as far north as Alaska (Heinrich and Vogt

1993). In temperate climates, bumblebees "shiver" to warm flight muscles so they can forage longer and at cooler temperatures than smaller bees that remain in torpor when cold (Esch et al. 1991, McCallum et al. 2013). Bumblebees use carbohydrate rich nectar to offset the energetic costs of metabolic warm-up and flight (Esch et al. 1991, Heinrich and Vogt 1993, McCallum et al. 2013). High temperatures induce increased activity and foraging rates in bumblebees; however bumblebees can also easily overheat due to their high flight metabolism (Heinrich 1977). Bumblebees could also use metabolic energy to quickly offload excess body heat (via convection) in response to temperature stress. One study addresses bumblebee thermoregulation in the context of climate stress, but at the colony level (Holland and Bourke 2015), and I have not found any papers that address the impact of nectar diet on the ability of individual bumblebees to thermoregulate in response to heat stress.

To address the question of thermoregulation and nectar diet in bumblebees, I tested three bumblebee species' abilities to offload excess heat after being fed a pre-determined nectar diet in the lab. I restrained individual bumblebees on a Styrofoam platform with insect pins around the waist (petiole) and partitioned the abdomen and thorax with an aluminum heat–shield. I used a heat-lamp to heat the head and thorax (but not to the shielded abdomen) and measured the temperature change between body segments every 30 seconds for five minutes. Understanding how pollinators tolerate high temperature stress is important, particularly as average spring temperatures exceed record highs in a trend that is likely to continue moving forward (Parmesan 2006)

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This work would not have been possible without the significant collections work by Randall Morgan (1947–2017). Randy was a passionate naturalist who explored and documented the flora and fauna of Santa Cruz county. His instinct to collect plant phenology and interactions data, even without formal scientific training, is a testament to the impact that citizen science can have on ecological research. Randy's contribution to this work, to UC Santa Cruz, and to Santa Cruz county cannot be understated and I only regret that he is not here to see this outcome of his work.

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Chapter 1. Plant pollinator networks in two habitats are similarly complex but differentially robust to climate stress over time.

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Keywords: mutualistic network, climate change, plant-pollinator interactions, habitat filtering, environmental stress

Abstract

Environmental stressors impact important species interactions that are critical to ecosystem health and biodiversity. In abiotically stressful habitats, habitat filtering limits the overall species pool and thus influences important species interactions, such as pollination. Additional stress from climate change may decouple individual plant-pollinator interactions via species range or phenological shifts. However, it remains unclear how stress to individual species scales up to impact networks of interacting species. To address this gap, we assessed how intrinsic (habitat level) and extrinsic (climate) factors impact plant-pollinator network structure and species richness over space and time in two coastal, central California habitats: grasslands and sandhill chaparral. We predicted that plant-pollinator networks would be less complex (lower nestedness), but more resilient against species loss, in stressful (sandhill chaparral)

relative to milder (grassland) habitats. In addition, we predicted that species losses and host switching would be non-random with relation to phylogeny or origin (native vs. non-native species). We evaluated changes over time by comparing plant-pollinator networks from two periods of drought in an historical dataset (1990-1993) with resurveys (2013-2015); temperatures steadily increased during this time. Our results indicate high species turnover for both bees and plants; however, bee species richness in these habitats remained stable over time, while plant species richness declined in grassland but not in sandhill networks. Expanding on a historical dataset with resurveys at the same sites, we identify mutualistic network metrics useful to researchers and land managers for strategic targeting of ecologically important species, but suggest that nestedness may be unreliable as an *a priori* diagnostic metric of ecosystem stability.

Introduction

Environmental stressors impact important species interactions that are critical to ecosystem health and biodiversity. Changes in both plant and pollinator physiology (Scaven and Rafferty 2013, Jorgensen and Arathi 2013), behavior (Huey et al. 2012, van Loon 2016), and demography (Van der Putten et al. 2010) in response to stress modifies the dynamics of plant-pollinator interactions across a community (Benadi et al. 2013, Burkle et al. 2013). However, parsing out specific, community-level responses to chronic or acute stressors, such as temperature or species invasion, is complicated by high levels of spatial and temporal variability (e.g., seasonal, inter-

annual) that are inherent to plant-pollinator systems (Alarcon et al. 2008). Building upon existing pollination-system data with resurveys is one way to bolster those datasets to address, and partition, natural variation from that introduced by climate or other anthropogenic stressors (Burkle and Alarcón 2011). Ideally, multi-year sampling over large spatial scales will indicate how plant-pollinator networks respond to environmental stress (Pyke and Ehrlich 2010). However, very few plant-pollinator datasets have replication *both* over space (multiple sites in a single, or multiple habitats) and over time (multiple annual surveys in each site). Where those data are available, and combined with ideas from mutualistic network theory, it is possible to assess how plant-pollinator communities are structured and how they respond to environmental stress. For example, analysis of one historic long-term dataset (but with limited spatial replication) with resurveys identified large-scale declines (~50%) in bee species richness likely resulting from shifting species phenologies with climate change and increased habitat fragmentation over 120 years, (Burkle et al. 2013).

Stress from climate change affects individual plant-pollinator interactions, via several mechanisms. First, increasing temperature stress drives demographic and phenological mismatches between pairs of interacting species in multiple plant-pollinator communities (Memmott et al. 2007, Lambert et al. 2010, Caradonna et al. 2014). Second, drought and high temperatures prompt resource allocation away from flower, or per-flower nectar production in plants under stressful conditions alters foraging patterns for some pollinators (Memmott et al. 2007, De la Luz 2018). Third, exotic plant species compete with native plant species for nutrients, limiting growth

and survival in native plant species even when abiotic stress is low (Esch et al. 2018) and could undermine the ability of some native species to recover when released from high stress (Ashbacher and Cleland 2015a). Competition for pollinators with non-native plant species could similarly impact reproduction. However tests of these predictions in plant-pollinator communities are limited by the availability of long-term, community-level data on plant-pollinator interactions (but see: (Burkle et al. 2013, Stouffer et al. 2014).

Interaction networks in diverse plant-pollinator communities are complex and can be highly nested, impacting how these communities respond to species loss during stressful periods. A network is nested when the community is arranged into proper subsets of interacting species (Bascompte et al. 2003, Pires et al. 2011). More specifically, nested structure occurs in a network when specialist species (i.e., diet specialists) mostly interact with generalist species, while generalist species interact with a range of species, from specialist to generalist; this pattern forms proper subsets of interacting species (Bascompte et al. 2003, Pires et al. 2011). Nestedness may buffer most species in a network from extinction cascades, (i.e., secondary species losses that result from the loss of an interaction partner; Bascompte and Stouffer 2009), but there are few tests relating nestedness to network stability in natural systems over time.

The most highly nested and interconnected species in a network are hub species, which may be particularly important to pollination network stability. Hub species are, by definition, linked to the largest proportion of partners in the overall

network (Olesen et al. 2007, Bascompte 2009, Bascompte and Jordano 2014a). Usually, only a few species in a community are hubs. Because hubs play such a key role in a plant-pollinator community, it is important to understand how those species in particular might respond to large-scale stress (Burkle and Alarcón 2011). If hub species in particular are unable to tolerate large-scale climate stress, or if non-hub species are unable to switch to novel partners, network complexity, and thus stability (Bascompte et al. 2003, Bastolla et al. 2009, Benadi et al. 2013), could be compromised.

Optimal foraging theory (OFT; (Charnov 1976, Zimmerman and Url 1981)) predicts foraging behaviors of pollinators under low and high stress: it assumes that bees show 'adaptive foraging', host switching to more profitable partners (Arstensen et al. 2016), depending on environmental stresses. OFT predicts that individual pollinators are more choosy (i.e., more specialist about where and when they forage when stress is low (Charnov 1976, Heinrich 1979, Pleasants 1981, Willmer 2011a) but are more flexible (i.e., more generalist) when stress is high. If OFT predictions apply at the community level, then there should higher nestedness, fewer links per species, and higher network specialization when more bee species in the community preferentially visit the most rewarding and accessible plants (usually hubs) when stress is low. When stress is high, then nestedness should be low because foraging is more random, links per species could be higher, though this also depends on plant species richness and abundance. Network specialization would be lower if bees compensate for unprofitable or absent partners by visiting additional plant species

within a community/network at random (Arstensen et al. 2016). Alternatively, if OFT predictions do not apply at the community level (i.e., no host switching with increasing stress) then we would not expect any differences in nestedness, links per species, or network specialization with stress.

Life-history trade-offs and OFT together help predict how plants and pollinators might jointly respond to high and low stress. For instance, some perennial plants might postpone reproduction within a season, or across years, until conditions are more favorable while annual plants must attempt reproduce each year (Willmer 2011a). Both annuals and perennial plants might also produce fewer, smaller, less rewarding flowers that are less attractive to bees foraging under stressful conditions. Further, important floral attraction cues, such as volatile scents (Essenberg et al. 2015) or nectar quality (De la Luz 2018), may not be as attractive to pollinators when plants are grown under stress (Halpern et al. 2010). While these strategies can conserve resources to support reproduction in some plants, small, resource-poor flowers often receive shorter and less frequent visits from bees, which reduces seed set in many plant species (Pleasants and Chaplin 1983, Carroll et al. 2001, Liu et al. 2012, Galen 2015). Conversely, when stress is low, plant resources are allocated to maximize growth and reproduction; plants grown under low stress produce large, showy, and rewarding flowers that are more attractive to pollinators relative to those grown under temperature and water stress (Ishii 2006, De la Luz 2018) and visitation and seed set are generally higher in these plants (Willmer 2011b). In this case, nestedness would increase because interaction partners can make foraging decisions

based on floral cues (Essenberg et al. 2015) and specialize on the most appropriate partners (higher network specialization).

In inherently stressful habitats, strong habitat filtering reduces species diversity and invasion rates because of the low likelihood that novel (i.e., exotic) species possess a suite of traits that would allow them to pass through a strong habitat filter. Based on OFT, networks in these stressful habitats should have lower nestedness, more links per species, and lower network specialization than those in milder climates where species richness is often higher. Habitat filtering, the likelihood of a species establishing in a habitat, is usually strongest and most limiting in the most extreme habitats (Cornwell and Ackerly 2009). Some species lack the appropriate traits, or trait plasticity, to tolerate more intense or more frequent abiotic stressors, such as high temperatures and drought. Once through a strong habitat filter however, trait plasticity and broad stress tolerances are common (Blum 2011, Pratt and Mooney 2013). Species in extreme environments can thus be resilient to stress if they are not already near their stress tolerance threshold (Louthan et al. 2018).

In this paper, we use both historical data and recent resurveys to address three specific questions about community-level plant-pollinator network complexity in two central, coastal California habitats with different baseline levels of temperature, water stress, and species invasion. First, we compared how plant-pollinator networks differ in terms of species richness, nestedness, links per species, and network specialization between two habitats with contrasting stress regimes, and thus (*a priori*) level of habitat filtering. Coastal California grassland communities often occur on rich soils

and experience a mild climate relative to inland communities, while sandhill chaparral occurs on harsh, sandy soils that hold very little moisture, and have high temperatures relative to grasslands.

Second, we assessed how nestedness, links per species, and network specialization changed over time as temperature stress in the region has increased. We compare historic (1990-1993) and resurvey data (2013-2015) from some of the same survey sites. Both historical and contemporary surveys took place during strong droughts allowing a broad-scale temporal comparison (historic vs. resurvey) of pollination network structure during drought, but with variable temperatures in two habitats.

Third, we determined the likelihood that species were lost from each network (i.e., present in historical but not resurvey networks) and whether these changes were predictable based on phylogeny (family and genus level), and origin (native or exotic). Our specific predictions for each of these study questions are:

(a) Plant-pollinator network structure is less complex (i.e., less nested, more generalized), and species richness is lower in communities with a strong habitat filter (*a priori*: sandhills, stressful climate) than in a habitat with a milder climate (*a priori*: grasslands: more nested, less generalized).

(b) Plant-pollinator network structure in already stressful environments (*a priori*: sandhills) is resilient (i.e., change in species richness; bees or plants) to increased stress *over time* compared to communities that experience less extreme stressors (*a priori*: grasslands).

(c) Species losses *over time* are predictable based on family, genus, or origin.

Methods

Study System. We surveyed two habitat types in Santa Cruz County, near the central California coast (Supplemental figure 1) over three years (2013-2015): grasslands (5 sites) and sandhill (4 sites). We compared these new surveys (hereafter 'resurveys') to historical data from these sites that were part of a larger dataset (1989-1998) from 39 sites across five habitat types.

California grasslands have historically had among the highest levels of plant biodiversity of grasslands across North America (Stromberg et al. 2001). Most grassland plants are herbaceous annual or perennial forbs and grasses. Santa Cruz grassland soils hold moisture and plants there typically lack specific adaptations to tolerate long-term drought or temperature stress (Wright et al. 2004, Leishman et al. 2007, Funk and Cornwell 2013). Our five grassland study sites range from ca. 3–23 km from the coast.

Sandhill chaparral communities occur on remnant coastal sand deposits, ca. 7 km inland from the current coastline, at its shortest distance (McGraw et al. 2004). Sandhill plants are mainly woody perennial shrubs, but open spaces between shrubs are occupied by numerous herbaceous species. Sandhills are warmer, with more annual rainfall than grasslands (Supplemental figure 2, Supplemental table 1). However, sandy soils hold very little of this moisture (Bowman and Estrada 1980, Brady and Weil 2008). Sandhill plants have specific adaptations to tolerate

temperature and water stress; e.g., tough waxy leaves help retain water and downy white leaf hairs capture condensation from the air and reflect heat (Mahall and Schlesinger 1982, Ackerly 2004, Keeley and Davis 2007). The four sandhill sites in our resurvey occur as isolated patches in the Santa Cruz mountains ca. 7–20 km from the coast.

Historical data collection. Randall Morgan, a local naturalist, surveyed and collected pollinators and recorded host-plant species data with each observation. He took detailed notes on plant flowering phenology and floral visitors, and noted species interactions when he was unable to collect a voucher specimen. Morgan surveyed a subset of sites (ca. 6 per year; 3–4 hours/site/visit; 1989–1998) every 3-4 weeks in each calendar year, and then surveyed a new set of sites each subsequent year. Morgan walked a set transect route at each survey site (Appendix A) and used targeted net capture to collect insects in contact with reproductive structures of flowers.

Since Morgan's grassland and sandhill habitats surveys were most comprehensive earlier in his work (1990-1993), we only used those years for the historical data in our study. We worked with taxonomic specialists and species keys for species-level bee identifications and organized the bee collection using standard curation methods (Hunter 2006).

Resurveys. Our resurveys (2013-2015) use the same transect routes as Morgan (R. Morgan, personal communication. Morgan surveyed 9 grasslands and 7 sandhill sites, but we selected those that had not been disturbed or converted to urban

landscapes in the interim. We surveyed each of nine sites at weekly intervals for a total of 6 surveys per site per year. The timing of surveys each year was based on site-specific spring blooming to capture as much of the active spring season as possible.

We collected bees contacting the stigmas or anthers via targeted net capture and recorded plant host species identity for each. We evaluated the identity of each bee specimen in the field using a hand lens after bees were relaxed with a mild dose of EtOH. If possible, we made a species-level identification in the field. However, in most cases, we collected the bee as a voucher specimen (one per species or morphotype) and identified it in the lab using species keys and the local reference collection (Appendix A). Species accumulation curves (raw data) comparing historical and resurveys (Supplemental figure 3). show that bee species richness was similar between historical and resurveys even though we observed fewer individuals overall during our resurveys.

Comparing historical and resurvey data: Prior to bootstrapping and analysis, we subset the *full* historical dataset to match our more focused spring resurvey window (Mar–July, when most pollinators are active) ('historical data' hereafter). For the purposes of comparison, we make the critical assumption that the historical dataset represents the full array and frequency of available interactions in each habitat of which resurveys are a subset (Supplemental figure 3).

Network Analysis

Bootstrapping historical data. To compare how network metrics differed over space and time, we used bootstrapping to standardize the historic plant-pollinator networks to the same number of floral visits as resurveyed networks. Bootstrapping was necessary because the limited site-by-year replication in the historical survey restricted direct statistical comparisons over time with resurvey data. This approach also allowed us to generate and standardize errors between surveys. We sampled directly from historical data to create bootstrapped, null response curves based on datasets equal in size to the resurvey data. The number of floral visits in the resurvey data defined the sample size for the 1000 bootstrapped datasets we ran for each habitat (grasslands = 823 observations; sandhills = 512 observations). We randomly sampled from the historical data, without replacement, because each data point represents a single plant-pollinator interaction (floral visit) in the field.

Plant-pollinator networks analysis. We calculated and compared standard network metrics for each habitat and survey period using packages 'bipartite', 'babar', 'nlme' and 'vegan' in R (Dormann, C.F., Gruber B., Frund 2008, Alston et al. 2010, Oksanen et al. 2011). **Nestedness (N)**, which has been related to network stability (Okuyama and Holland 2008, Thébault and Fontaine 2010, Bascompte and Jordano 2014a), is defined as the degree to which specialist species interact with generalist species, and generalist species interact with each other (values range from 0 –100, where N=100 indicates a perfectly nested, network (Rodríguez-Gironés and Santamaría 2006). **Links per species (*k*)** is the average number of interaction partners

across the network calculated as: $k = \frac{I}{S}$, where I is the number of pairwise interactions in an interaction matrix and S is the total number of bee and plant species in the matrix. Links per species is useful for understanding how the number of partners changed over time for each species, and across the network. **Network specialization** (H'_2) is interaction diversity, standardized to account for the total number of observations for each plant (i) and bee (j) species. Network specialization is calculated as: $H'_2 = -\sum_{i=1}^{i}\sum_{j=1}^{j}\left(\frac{a_{ij}}{m_i} \cdot ln\frac{a_{ij}}{m_j}\right)$; a is the number of pairwise interactions, m is the number of interaction records. Network specialization is useful for understanding the relative proportions of generalist to specialist species in the network. **Hub** species are those with more interactions than expected by chance, which we characterize as those with $k > \pm 2\sigma$ (σ = standard deviation) from the habitat-level mean for either bees or plants (separately) to remain consistent across methods (this approach is statistically equivalent to methods by Olesen et al. 2007; Supplimental table 2). **Degree distribution curves** describe the probability of finding species with at least k number of links in a network and help clarify the strength of highly connected species (hubs) relative to the rest of the species in a network (Supplemental table 3). In a mutualistic network, all species that are present have at least 1 link ($p(k) = 1.0$), while the probability of finding species with many interactions is low ($p(k) < 0.05$).

Network structure and stress in two habitats. To test our first hypothesis that plant-pollinator network structure would be least complex in communities with a

strong habitat filter (*a priori*: sandhills), we compared network metrics between habitats within each survey period. For the historical data, we take these response distributions (based on the 1000 bootstrapped networks) to represent the historical network mean and variation for each habitat, but now standardized to have an equal sample size (i.e., same number of floral visits observed) as our resurvey data. For each response distribution, we asked if grassland and sandhill community/pollination networks differed from one another, and if those differences were consistent with our expectations given *a priori* assumptions about habitat filtering and stress in each habitat. If habitat-level means were $> \pm 2\sigma$ distant from each other, we considered those metrics to be significantly distinct from one another (Zar 1999). In addition, we assess the species identity and number of plant and bee hub species in each habitat using the metrics outlined above (see: bootstrapping; $\pm 2\sigma$ from network-level mean *k*).

Network structure and stress over time. For each response distribution, we asked if historic and resurvey community/pollination networks differed from one another, and if those differences were consistent with our *a priori* assumptions about habitat filtering and stress in each habitat. We used the same criteria as above ($>\pm 2\sigma$ from habitat and survey mean) to determine if network structure was similar over time in each habitat.

We assessed hub species turn-over between surveys as above. We determined which plant and bee species were hubs during historical surveys and during resurveys, and compared whether the number of links (response variable) for

historical hub species differed between surveys and habitats (interaction term) using linear mixed-effects models with ANOVA. Links per species was the response variable, species identity, level (bee or plant) and survey (historical or resurvey) and habitat (grassland/sandhill) were fixed factors.

Species losses and host switching. To assess whether species losses were random due to sampling error, we used bootstrapping to calculate the percent likelihood that a given species was lost out of 1000 bootstrapped iterations. If a species was present in at least 90% of bootstrapped iterations, but was absent from resurveys, we considered that species to be truly lost (hereafter 'lost'). We used this list of lost species (in each habitat) to identify each bee and plant species that lost an interaction partner between surveys.

We assessed the likelihood that a species found alternative interaction partners after losing at least one partner. We first identified all plant and bee species that lost at least one interaction partner between surveys (using the criteria above) and the relative proportion in each of those species' partners in each habitat and survey, and then determined how many of those species were present during resurvey (hereafter 'active' species). We compared family-level classifications and origin for each active bee and plant species between historical bootstrapped and resurveys in each habitat. We used chi-squared tests to determine whether phylogeny or origin predicted host switching in either habitat compared to their relative proportions in the bootstrapped historical networks.

Results

Bee and plant species richness were both higher in grasslands relative to sandhills overall. We found 254 bee and 206 plant species across all surveys (84 of the bee morphotypes were unclassified; Appendix A). Grasslands had 12% more bee species (176 vs 155) and more than twice as many plant species (172 vs 80) as sandhills (Table 1). Nearly 33% of the bees (77 species) and 22% of plants (46 species) occurred in both habitats. Bootstrapped networks captured 70% of the numbers of species from the historical spring data and did not change the relative proportions of bees and plants in relation to the historical survey data.

Network structure and stress over in two habitats. Nestedness and network specialization were similar, but links per species and network specialization differed between habitats during historical surveys (bootstrapped data; Figure 1). In the historical surveys, nestedness (values 0–100) was similarly low in both grassland and sandhill networks ($4.21 \pm 0.35\sigma$ vs. $4.57 \pm 0.34\sigma$, respectively); grassland species were modestly more linked than sandhills ($1.77 \pm 0.04\sigma$ vs. $1.49 \pm 0.03\sigma$); and network specialization (H_2') was only slightly, though significantly lower in grasslands than in sandhills ($0.41 \pm 0.01\sigma$ vs.$0.48 \pm 0.01\sigma$). In the resurveys, nestedness was lower in grasslands relative to sandhills ($15.50 \pm 2.69\sigma$ vs. $20.63 \pm 3.90\sigma$, respectively). The number of links per species was similar in grasslands ($1.11 \pm 0.11\sigma$) and sandhills ($1.06 \pm 0.12\sigma$) as was network specialization ($0.27 \pm 0.06\ \sigma$ vs. $0.30 \pm 0.04\ \sigma$; grasslands and sandhills respectively).

Only 5% of species were hubs in each habitat and survey (Figure 3a & b). However, those species were involved in >50% of the total number of interactions in both habitats during historic surveys (55%) but in only 23% during resurveys. Plant hubs (n=11) in both surveys came from 6 plant families in grasslands and 3 in sandhills (Supplemental table 4) and were a mix of annual and perennial shrubs and herbs in both. The California poppy, *Eschscholzia californica* (Figure 2 a,b, node 'C'), is the only plant hub species common to both habitats. *Hypochaeris radicata*, (Figure 3a, node 'E') an introduced non-native species, was a hub in grasslands but not in sandhills, where it is also present.

Bee hubs (n=11) in both survey periods were primarily small solitary bees and social bees from just two families (Apidae and Halictidae; supplemental table 4). The introduced European honey bee, *Apis mellifera* (Figure 2, node '2') and a native bumblebee *Bombus vosnesenskii* (Figure 2, node '3') were hubs in both habitats and survey periods. The remaining four bee hubs during the historical survey were solitary and semi-solitary bees (Figure 2, nodes '1', '4'–'7'). In sandhills, a species from one additional family, Melittidae, (Figure 2, node '9') is also a hub species.

Network structure and stress over time. Plant-pollinator networks in stressful (sandhills) and in milder (grasslands) habitats changed similarly over time, even though plant species richness in grasslands declined overall. Bee species richness was 10% higher during resurveys compared to the historical mean from bootstrapped networks in both habitats (Table 1). This differs slightly from SAC curves comparing historical and resurvey data in each habitat directly (prior to

bootstrapping, see methods) that suggested that bee species richness was similar with total number of observations between surveys. Differences in bee species richness before and after bootstrapping suggests that very rare species in the full historical networks may not have been well represented in the bootstrapped networks. In contrast, plant species richness was stable over time in sandhill networks (at 54 species, within 1σ) but fell by 25% (43 species) in grasslands in the resurveys (Table 1).

Apis mellifera and *B. vosnesenskii*, both visited a disproportionately large number of plant species during both survey periods (20% of all plant species), and are the bees species with major departures from the degree distribution plots (Supplemental figure 4a-d) though more so during resurveys. In both survey times and habitats, these two bee species each visited > 20 plant species, with a 20% increase in plants pollinated during resurveys compared to historical surveys. This expansion of plant use by these two bee species skewed the degree distributions in both habitats toward higher average links per species (k) relative to the historical degree distributions and reflects their 'super-generalist' interaction pattern during resurveys.

Based on OFT, we predicted that nestedness would decrease as species became more generalized over time but that is not what we found. Nestedness increased over time, from $4.40 \pm 0.35\sigma$ on average in both habitats in historical surveys to $15.50 \pm 2.70\sigma$ and $20.63 \pm 3.90\sigma$ in grasslands and sandhills respectively (Figure 2a & b). Links per species declined in both habitats (k: $-0.5 \pm 0.11\sigma$) during

resurveys compared to bootstrapped historical data (Figure 2c & d). Grassland and sandhill plant-pollinator communities both became 35.5% less specialized overall between surveys (H'_2: $0.27 \pm 0.06\sigma$ and $0.30 \pm 0.04\sigma$ respectively; Figure 2e & f).

The proportion of species that were hubs in each habitat remained consistent over time (3–5%) but the proportion of total hub interactions declined by 50% in grasslands and 32% in sandhills. There were overall fewer plant hubs in both habitats spanning 3 plant families in grasslands and 2 in sandhills (Supplemental table 4). In contrast to the historical data, which had both annual and perennial hub species, resurvey hub species were all perennial species in both habitats. *Eschscholzia californica* (Figure 3, node 'B') remained a hub in both habitats while *H. radicata* (Figure 3, node 'A') remained a hub in grasslands and interacted with 12 new species in addition to historical bee partners. The total number of bee hub species declined over time in both habitats. In sandhill resurveys, only *A. mellifera* and *B. vosnesenskii* were bee hubs. However, none of the former hub species were lost from the networks; they were involved in fewer interactions per species and no longer considered *hubs* by our statistical definition.

Species losses and host switching. In the plant-pollinator networks, species losses were random and higher than expected by chance compared to bootstrapped data (Table 2). Bee and plant species across phylogenies and origin were lost in similar proportions to those groups' relative proportions in the historic networks. Overall, bee species losses were 47% higher than we expected from bootstrapping. Plant species losses were 60% higher in grasslands but only ~38% higher than

expected by chance in sandhills. After applying our <10% loss likelihood cut-off, neither phylogeny (family) nor origin (native v exotic) predicted species losses for bees or for plants (Tables 3 & 4). That is, the relative proportions of partner species and new host species (for those that switched) were similar to that of the historical bootstrapped dataset when grouped by phylogeny (Table 3) or by origin (Table 4). After applying our <10% species loss cut-off, there were fewer than 8 species in each plant and bee family and so we were unable to further analyze phylogenetic patterns of species loss. However, interaction turnover between surveys (net change) for active species (L'_2) differed between habitats (Table 5). Grassland bees'$_2$ lost 8 links (net) on average while sandhill bees'$_2$ only lost 2. Grassland plants gained 3 interactions (net) while sandhill plants lost 3 interactions on average and were more likely to have fewer interaction partners or to be absent from resurveys.

Discussion

Average minimum temperatures have steadily increased in central California effectively narrowing the range of temperatures in the region since at least the 1970's, since maximum temperatures have not changed. Increasing temperature and drought influence plant-pollinator interactions (Memmott et al. 2007, Tylianakis et al. 2008, Hegland et al. 2009) by altering plant and bee species phenologies (Inouye et al. 2003, Schwartz et al. 2006, Inouye 2008, Lambert et al. 2010, Bartomeus et al. 2011, Cleland et al. 2012, Kudo and Ida 2013, Parmesan and Hanley 2015), development times (Kemp and Bosch 2005, Jones et al. 2006, Hegland et al. 2009, Chamorro et al. 2013, Straka et al. 2014, Holland and Bourke 2015), and abundances. Drought also

negatively impacts germination, abundance, and survival in plants that lack the appropriate adaptations to tolerate it (Al-Ghzawi et al. 2009, Sedlacek et al. 2012, Ashbacher and Cleland 2015b, Carroll et al. 2015, Thomson 2016).

Responses to a shifting climate are not always synchronized between interacting species (Hegland et al. 2009, Bartomeus et al. 2011, Kudo and Ida 2013). In a community context, network rewiring (Arstensen et al. 2016), and not subsequent species loss (Bascompte and Stouffer 2009), were more likely as bee species found additional or alternative interaction partners when climate stress was high and partner availability become unreliable, consistent with optimal foraging theory.

Network structure and stress in two habitats. Across surveys, plant-pollinator networks were similarly structured in grassland (*a priori*: mild climate; weak habitat filter) and sandhill habitats (*a priori*: more extreme climate; strong habitat filter) despite different stress regimes. Our results suggest that habitats or conditions that are 'harsh' for plants, resulting in low plant species richness, as in sandhills, may not necessarily be harsh for pollinators since pollinators are mobile, and can modify foraging patterns or behaviors to avoid stressful conditions (Taíz and Zeiger 1982, Willmer and Stone 2004, Xu and James 2012).

The nestedness values in our historical surveys were low compared to other systems. Meta–analysis show nestedness values > 0.80 (scale 0 to 1; nested temperature > 20) are common in pollination systems (Bascompte et al. 2003) and this metric is likely not influenced by sample size (Nielsen and Bascompte 2007). Low nestedness in a mutualistic network can occur when many pollinator species

randomly forage on the plant species available within their foraging radius, of which there may only be a few, when stress is high. This is also consistent with finding fewer average links per species and low network specialization when species richness is also low and pollinators are foraging randomly (Bascompte and Jordano 2014b). This could occur if plants become scarce, or are patchily distributed within and across pollinators' foraging ranges. Despite generally low nestedness, a few plant and bee species crossed our threshold to be classified as hubs however these species were 'super generalist' in terms of foraging patterns, and skewed the community level mean *k* to be more generalized overall in both habitats over time (Supplemental figure 4).

In our system, and in grasslands in particular, non-native species occupy key positions in the interaction networks. This is consistent with simulations and space-for-time comparisons showing that mutualistic networks can accelerate species invasions (Olesen et al. 2002, Valdovinos et al. 2009). In sandhills, only native plant species were hubs, while in grasslands, plant hubs were a mix of native and non-native herbaceous species including *Hypochaeris radicata*, the common non-native cat's ear or 'false dandelion'. *Apis mellifera*, a non-native, super-generalist was a hub species in both sandhills and grasslands. Both *A. mellifera* and *H. radicata* were introduced in the early 1800's and are now largely naturalized across the landscape. The short and long-term impacts of non-native species on pollination network structure, and the community-level implications of controlling them (i.e., removal or mitigation) warrants further examination.

Plant-pollinator network structures in grassland and sandhill habitats were similar overall, which may imply similar sensitivities to increasing temperature stress with drought over time in these two habitats. Nestedness has been suggested as an indicator of mutualistic network stability in ecological networks (Bascompte et al. 2003, Okuyama and Holland 2008, Thébault and Fontaine 2010, Díaz-Castelazo et al. 2010). However, nestedness was not a good predictor of stability in terms of species losses in our study.

Network structure and stress over time. Plant-pollinator networks in an already stressful environment (i.e., sandhills) were less sensitive to increased temperature stress over time compared to networks in grasslands. Specifically, plant species richness was similar in sandhills but declined in grassland networks over time: only about half of grassland plant species in the historical network were also observed as part of an interaction during the resurvey. Although recent plant and arthropod species losses are widespread (Biernaskie and Cartar 2004, Potts et al. 2010), in our study, bee species richness was similar between the resurvey and bootstrapped historical data, suggesting that stress impacted species in each trophic level differently. In grasslands, plant species appeared to be more sensitive to stress than bees. This is the opposite of what most studies have found – that, higher trophic levels (like bees) are often more sensitive to perturbations than lower trophic levels (like plants; Voigt et al. 2003, Thackeray et al. 2016). Since both historical surveys and resurveys took place during droughts, the networks presented here may already reflect particularly low levels of species richness relative to the regional species pool;

many plant species expected for the region were never observed in our study (Neubauer 2013). Future data should be collected during average and high rainfall years at these sites (De la luz and Fox, *in prep*) to determine if the levels of plant and bee species richness we observed in our surveys (taken during drought) are low relative to that during periods of lower water stress (average and high rainfall years).

Non-native plant species invasions directly and indirectly negatively impacted native plant species in grasslands, likely via competition for resources and interaction partners. Non-native plant species often directly compete with native plant species for critical resources such as water, soil nutrients, and space (Daehler 2003, Graebner et al. 2012). Although non-native plant species were present in plant-pollinator networks in both habitats, we observed visits from (native and non-native) bee species with non-native plant species more frequently in grassland networks relative to sandhill networks, specifically with *H. radicata*. Because in our system these species were also pollinator generalists, they are also competing with native plant species for interaction partners. Pollination services are tied to fecundity, and population-level success for many plant species (Knight et al. 2005, Campbell and Halama 2015, Burd 2016). The indirect effects of competition for interaction partners (e.g., increased pollen limitation) has the potential to exacerbate plant species losses, particularly in areas where plant species invasions are pervasive.

Sandhill plants regularly experience high temperatures and chronically dry soils and are already specifically adapted to tolerate those stressors (Bowman and Estrada 1980, McGraw et al. 2004). Consequently, drought paired with higher

temperatures during the resurvey may not have impacted sandhill plants as severely as those in grasslands. Grassland plant-pollinator networks structure reflected the combined impacts of drought, increasing temperatures, and the presence of non-native species that were not always evident in sandhill networks. Nestedness increased in both habitats while plant species richness decreased in grasslands, but not in sandhills over time. Nestedness should increase when more species preferentially interact with the most generalist species (i.e., usually hubs; Bascompte et al. 2003). Interestingly, both links per species and network specialization declined in our study, which was inconsistent with any of our predictions. This unexpected pattern could be related to having fewer bee and plant hub species in each habitat during the resurveys. All of the hub species from historical surveys were present in each network, but in grasslands many of those species had fewer interactions overall, in some cases declining by more than 15 links between surveys, and were no longer hubs during the resurvey. However, hub species with the highest per capita links in each habitat during the historical survey, were also hubs during resurveys and more than half of those accumulated additional species interactions between surveys.

Some non-native species interacted with more native species during resurveys due to their role as hub species. Sandhill networks were supported by only two hub species in each trophic level, one of which was *Apis mellifera*. In grasslands, *H. radicata* interacts with more species than during historical surveys and was classified as a hub species during resurvey (but not historical survey). The presence of non-native species, and 'super-generalist' species in particular, as hubs has serious

implications for patterns of co-evolution in these, and other diverse systems where non-native species are present (Morales and Aizen 2006, Guimarães et al. 2011). Both historical and resurveys took place during droughts, suggesting that non-native and invasive plant species can be important alternative or additional resources during stressful periods (Marrero et al. 2017) when other native resources are absent. However, a long-term dependence on non-native species could disrupt patterns of co-evolution (Guimarães et al. 2011) and network function (Aizen et al. 2008, Bartomeus and Santamaría 2008, Palladini and Maron 2013).

Conclusions Plant-pollinator network structure was similar between habitats but varied between survey periods. Plant-pollinator network structure in already stressful environments (sandhills) lost fewer species over time relative to grasslands despite similar nestedness during historical surveys. This suggests that nestedness is not always a reliable metric for network stability. The most highly connected hub species (*A. mellifera, B. vosnesenskii, E. californica* in particular) were similar between habitats but other, less connected hub species changed between surveys. Species losses were random and were not predictable based on phylogeny or origin in either habitat or trophic level. However, after losing an interaction partner, species more often found novel partners in the community.

Figures

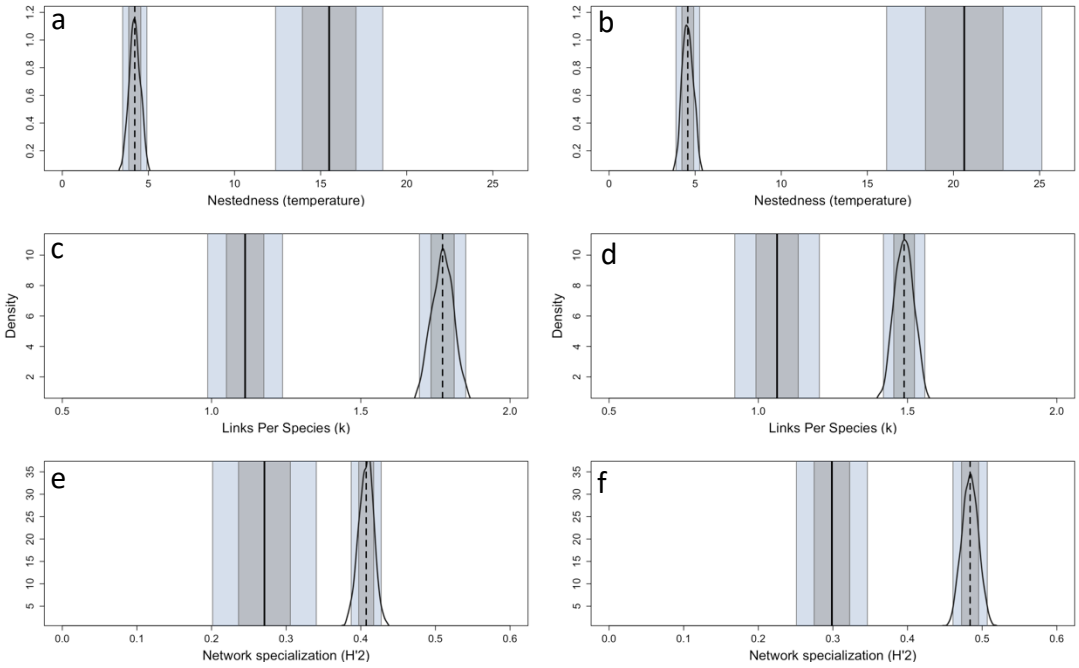


Figure 1. Density distributions (response curves) derived from bootstrapped historical survey data for grassland (left) and sandhill (right) habitats. In each panel, green solid line = Resurvey values; black dashed line = Bootstrapped historical survey mean; Grey box is $\pm$ 1SD, and blue box is $\pm$ 2SD from bootstrapped historical survey mean (black dotted line).

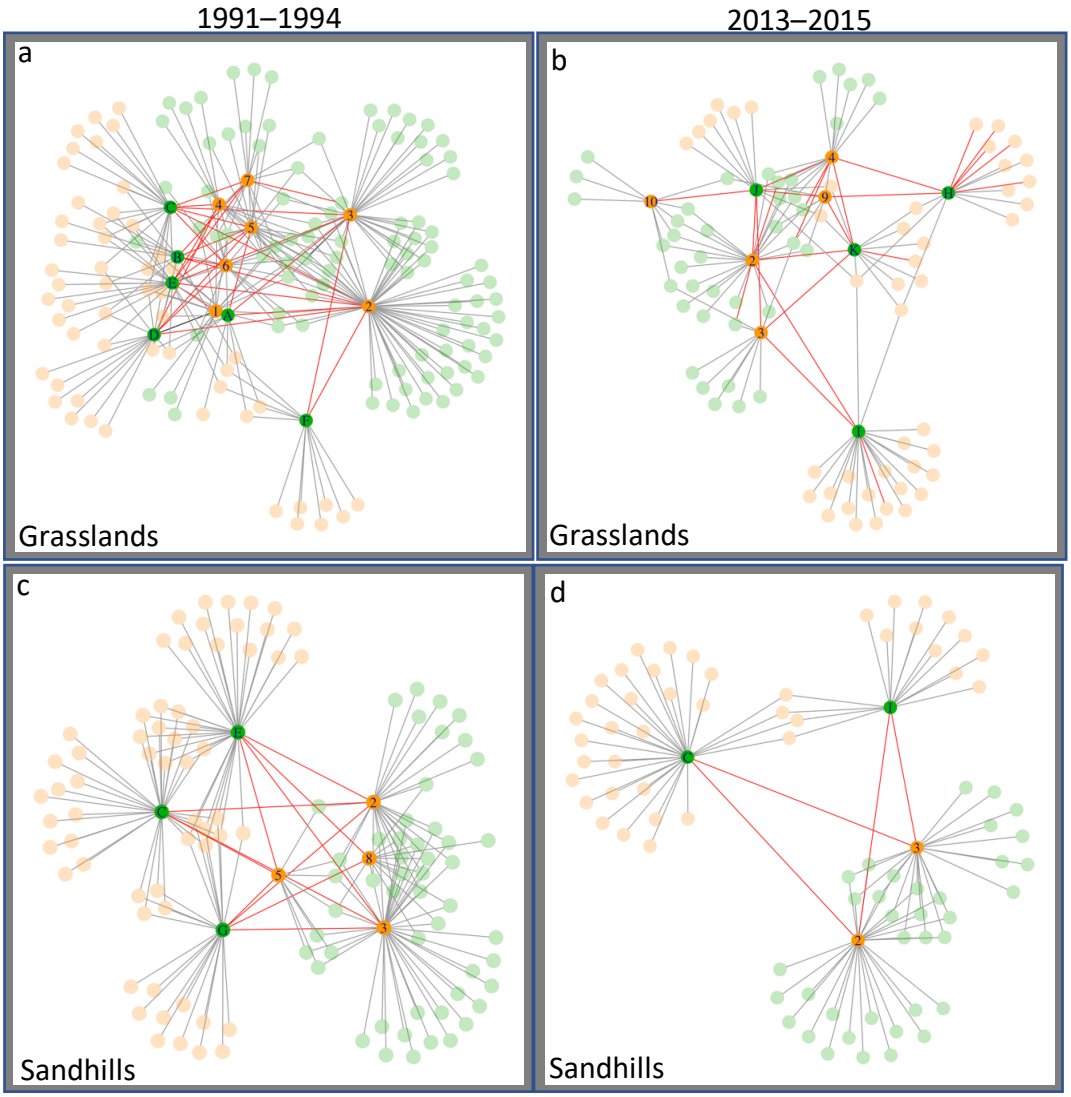
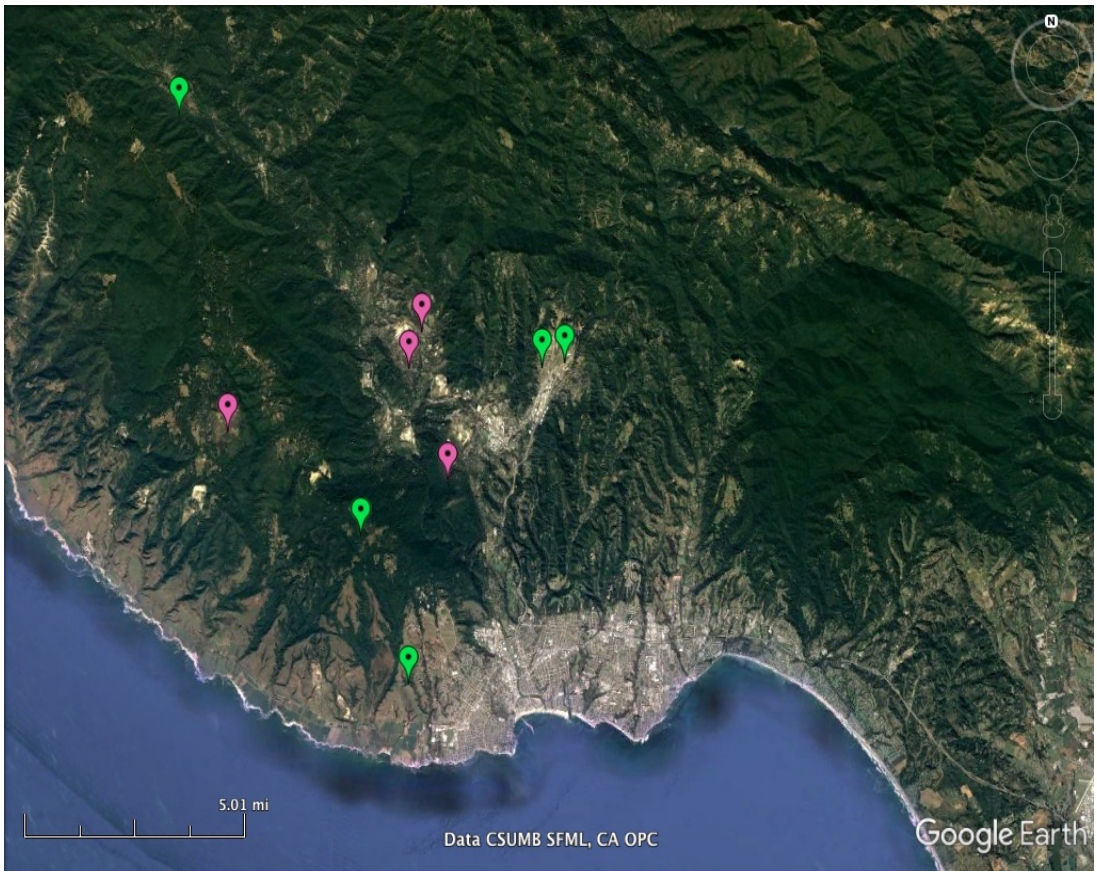
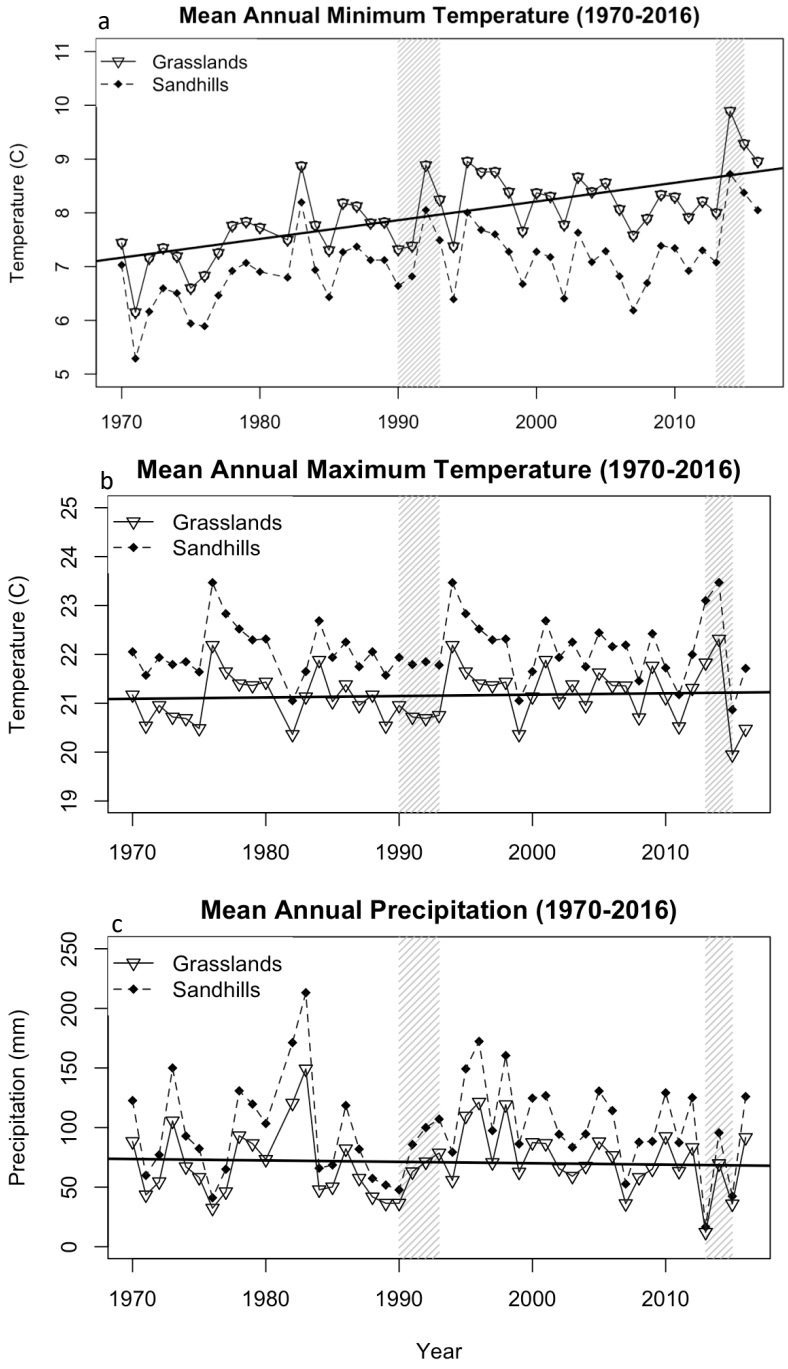


Figure 2. Core pollination network structure in grassland (left) sandhill (right) habitats during historical (top) and resurvey (bottom). Green circles = plants, orange circles = bees. Lines represent interactions. Red lines are redundant interactions, and the highlighted nodes represent hub species. Red links are redundant interactions in the network. Numbers (bees) and letters (plants) inside of each node correspond to species names, found in Supplemental table 4. Numbers and letters are consistent between panels and were assigned alphabetically. Hub species are those with more links thank expected by chance. Here, species with a network degree (k) greater than the trophic-level mean +2SD are considered hubs (hub-hub links in red).

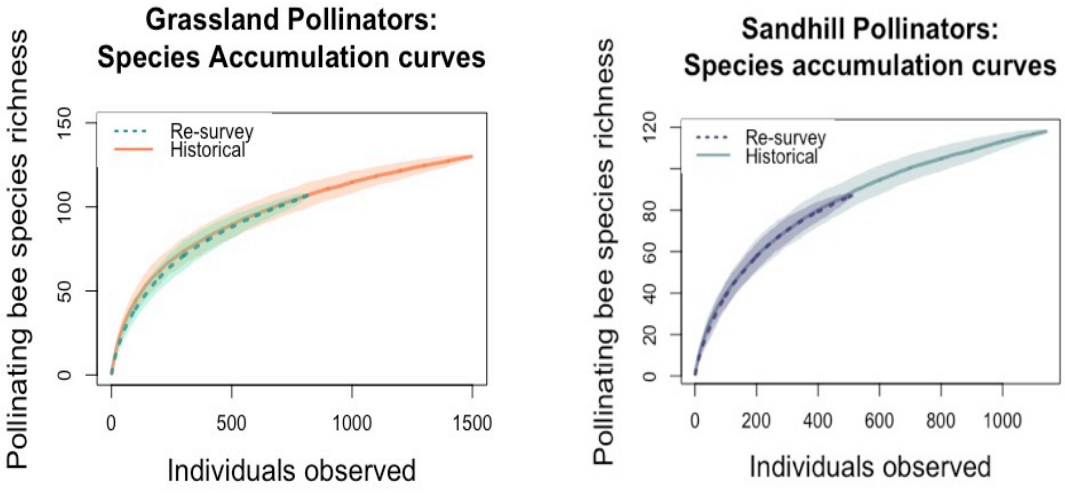
Supplemental Figures



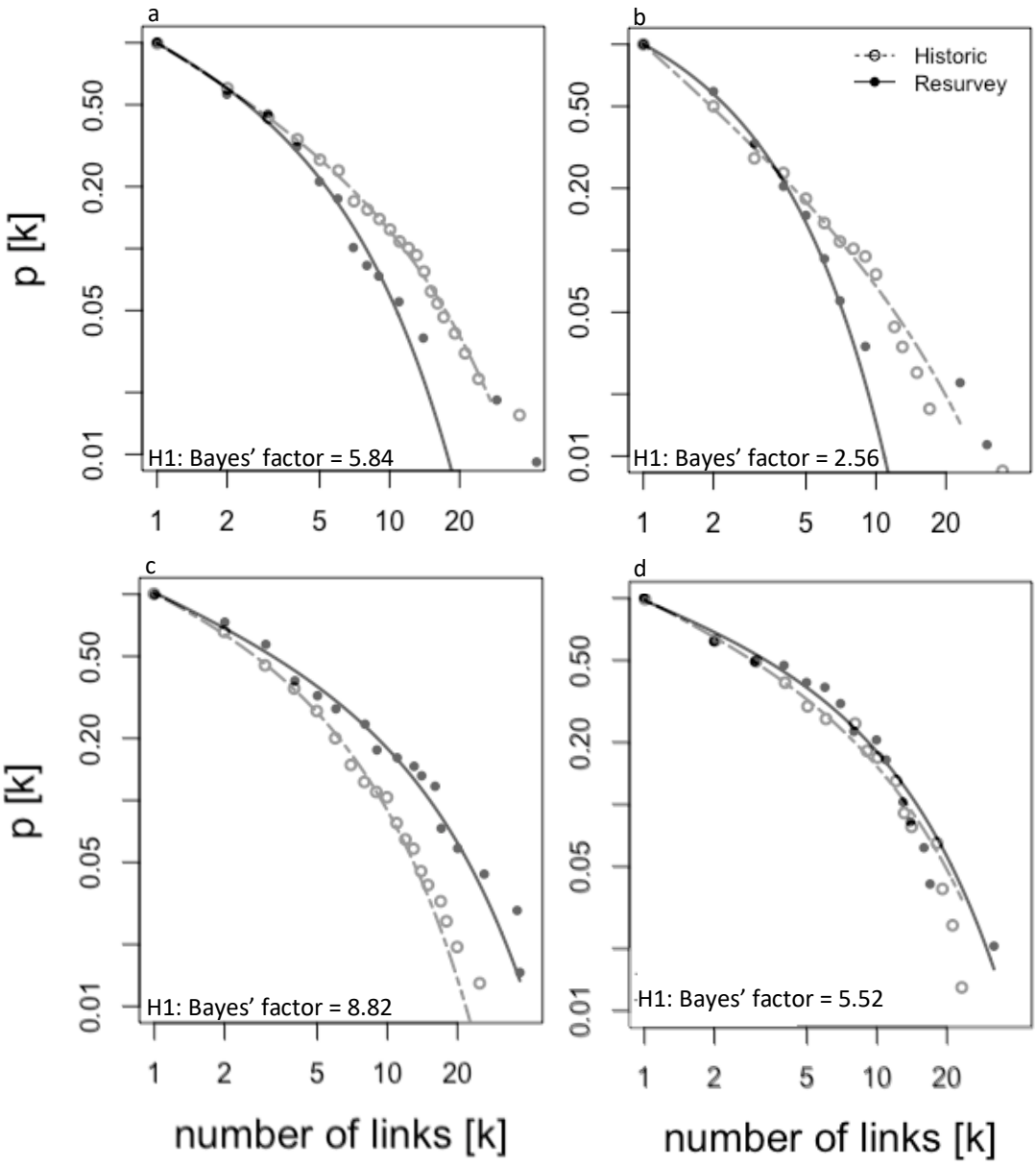
Supplemental Figure 1. Map of survey sites (2013–2015). Green flags are grassland sites. Pink flags are sandhill sites.



Supplemental Figure 2. Tmin (a),Tmax (b),and precipitation (c) from 1970–2016 for all grassland and sandhill survey sites. Grey bands are historical (1991–1994) and Resurvey (2013–2015) periods.



Supplemental Figure 3. Pollinator species accumulation curves for historical(solid lines) and re-surveys (dashed lines) in A) grassland and B) Sandhill habitats. Clouds are 95% confidence intervals. These curves show similar levels of bee species richness in each survey per number of pollinators observed. Even though $Resurvey_{obs}$ < Historical $survey_{obs}$,, bee species richness is within 95% CI of that found during historical surveys. Similarly, sub-setting historical survey data = $Resurvey_{obs}$ (i.e., bootstrapped data sample size = Resurvey) is reasonable given the overlap in the 95% CI.



Supplemental figure 4. Degree distribution plots show the likelihood p[k] of finding species with at least k number of links for bees (a-b) and plants (c-d) in grassland (left) and sandhill (right) habitats. Historical data (o ---) are in grey, resurvey (, —) are shown in black. All degree distributions fit truncated power law models (*sensu* Bascompte 2014). Points are survey data (no bootstrap data), and curves are model fits.

Tables

Table 1. Number of bee and plant species in each habitat-level network during each of two survey periods. Bootstrapped means ± 1σ are calculated from i=1000 historic survey data subsets of samples size = N resurvey observations. **Bold values** indicate a resurvey value > ± 2SD from the bootstrapped historical and are non-random.

Habitat	Total	Historic	Bootstrapped	Resurvey
Grasslands				
Bees	176	129	97.3 ± 3.8	**109**
Plants	172	156	111.2 ± 3.9	**68**
Sandhills				
Bees	155	118	78.8 ± 3.6	**88**
Plants	80	76	53.7 ± 2.5	49

Table 2. Species loss and survey size. Total number of lost species in each habitat between surveys (Not accounting for species gains). Bootstrapped means and ± 2σ calculated from i=1000 data subset of samples size = N resurvey observations. **Bold** values indicate a resurvey value > ± 2SD from the bootstrapped historical mean. Lost species are those present in the historical survey but not resurvey (or bootstrapped data as a null-hypothesis).

Habitat	Bootstrapped	Resurvey
Grasslands		
Bees	23 ± 4	**69**
Plants	27 ± 4	**107**
Sandhills		
Bees	29 ± 4	**73**
Plants	16 ± 3	**35**

Table 3. Total number of plant partner species lost (to bees) in each bee family. Bees are those that lost at least one interaction partner between surveys L'$_2$). Predicted values are based on those plant species loss likelihood from bootstrapped data (i.e., $p<0.10$ = lost). X^2 tests = NS for number of partners lost compared to the predicted values.

Habitat		**# plant partners lost**	**Predicted lost**
Grasslands			
	Andrenedae'	3	0
	Apidae'	7	2
	Halictidae'	3	0
	Megachilidae'	3	0
Sandhills			
	Andrenedae'	4	0
	Apidae'	8	3
	Colletidae'	1	0
	Megachilidae'	5	2

Table 4. Total and predicted number of plant partner species lost (from bees that lost at least one interaction partner between surveys; L'_2) by origin. Predicted values are based on those plant species loss likelihood from bootstrapped data (i.e., $p<0.10$ = lost). χ^2 tests = NS for number of partners lost compared to the predicted values.

Habitat	**# lost**	**Predicted lost**
Grassland		
Native plants	32	26
Exotic plants	2	0
Sandhills		
Native plants	6	5
Exotic plants	0	0

Table 5. Interaction turn-over for active bee and plant species that lost at least one interaction partners between surveys (L'_2). Data are means and $\pm 1\sigma$.

Habitat		Partners Lost	Partners preserved	Partners gained
Grassland				
	Bees'	11.62 ± 11.56	2.96 ± 3.28	3.96 ± 7.35
	Plants'	7.29 ± 3.69	4.86 ± 4.02	10.29 ± 8.78
Sandhills				
	Bees'	10.17 ± 6.15	4.33 ± 5.47	7.17 ± 7.36
	Plants'	8.76 ± 4.32	3.12 ± 2.64	5.53 ± 5.61

Supplemental Tables

Supplemental Table 1. Climate comparison during the survey periods. Climate data are based on monthly PRISM data for each site/year averaged for each habitat. Tmin: $\chi^2_{df=1} = 8.39$, p=0.003; Tmax: $\chi^2_{df=1} = 5.94$, p=0.01; Precip: = NS

Habitat	Historic	Resurvey
Grasslands		
Tmin	7.96 ± 3.49	9.06 ± 3.49
Tmax	20.78 ± 5.56	21.36 ± 5.30
Precip	57.12 ± 95.21	50.23 ± 91.91
Sandhills		
Tmin	7.24 ± 3.39	8.05 ± 3.61
Tmax	21.84 ± 5.72	22.48 ± 5.74
Precip	78.46 ± 124.30	91.91 ± 124.34

Supplemental Table 2. Supplemental table 2. Number of hub species in each habitat and survey using two methods ($t_{(12.89)} = -1.0829$, p-value = 0.30)

Habitat	Olesen 2007 (z)	AA 2018 (+2σ)
Grasslands		
Bees		
Historic	3	4
Resurvey	2	2
Plants		
Historic	6	8
Resurvey	3	3
Sandhills		
Bees		
Historic	4	5
Resurvey	2	2
Plants		
Historic	2	5
Resurvey	1	2

Supplemental table 3. Degree distribution AIC model fits for grassland and sandhill networks and each trophic level (bees and plants).

Grasslands	**Historic survey (1991–1994)**					**Resurvey (2013–2015)**				
Bees	Estimate	Std. Error	Pr(>|t|)	R2	AIC	Estimate	Std. Error	Pr(>|t|)	R2	AIC
Exponential	0.28	0.02	<0.001	0.99	-59.65	NA	NA	NA	NA	NA
power law	0.90	0.02	<0.001	0.99	-76.89	0.98	0.06	<0.001	0.99	-37.92
Truncated power law	0.60	0.02	<0.001	0.99	-132.36	0.45	0.09	<0.001	0.99	-57.04
Plants										
Exponential	0.30	0.01	<0.001	0.99	-73.07	0.22	0.019	<0.001	0.98	-44.34072
power law	0.93	0.05	<0.001	0.98	-55.31	0.76	0.04	<0.001	0.98	-48.34888
Truncated power law	0.41	0.03	<0.001	0.99	-110.55	0.47	0.06	<0.001	0.99	-67.82473
Sandhills										
Bees										
Exponential	0.59	0.07	<0.001	0.98	-1.41	0.19	0.02	<0.001	0.97	-38.25
power law	1.24	0.03	<0.001	0.99	-66.77	0.72	0.05	<0.001	0.98	-36.41
Truncated power law	1.89	0.0	<0.001	0.99	-65.50	0.34	0.08	<0.001	0.99	-50.77
Plants										
Exponential	0.25	0.02	<0.001	0.98	-41.76	0.52	0.02	<0.001	0.99	-48.51
power law	0.84	0.05	<0.001	0.98	-41.52	1.12	0.09	<0.001	0.98	-25.56
Truncated power law	0.45	0.07	<0.001	0.99	-63.16	0.20	0.10	<0.001	0.99	-50.36

Supplemental Table 4. Hub plant and bee species in both habitats and total number of links in each survey. Numbers in bold indicate current day hub species. Number and letters in parenthesis ( ) relate to figure 2 (in main text).

Habitat	Genus	Species	Links	
Grasslands			*Historic*	*Resurvey*
Bees				
(2)	*Apis*	*mellifera*	72	**29**
(3)	*Bombus*	*vosnesenskii*	36	**43**
(8)	*Bombus*	*melanopygus*	6	**14**
(5)	*Halictus*	*tripartitus*	24	**11**
(6)	*Lasioglossum*	*incana*	21	3
(4)	*Ceratina*	*nanula*	19	2
(7)	*Lasioglousum*	*titusi*	17	9
(1)	*Agopostemon*	*texanus*	16	6
Plants				
(E)	*Hypocharis*	*radicata*	27	**37**
(C)	*Eschscholzia*	*californica*	25	**36**
(D)	*Grindelia*	*hirsuta*	20	8
(G)	*Ranunculus*	*californica*	18	**26**
(A)	*Calochortus*	*luteus*	17	16
(F)	*Lupinus*	*nanus*	15	16
(B)	*Deinandra*	*corymbosa*	14	14
(H)	*Salvia*	*mellifera*	13	NA
Sandhills				

Bees

(2)	*Apis*	*mellifera*	35	**30**
(4)	*Ceratina*	*arizonensis*	17	6
(3)	*Bombus*	*vosnesenskii*	15	**23**
(9)	*Hesperapis*	*pellucida*	13	6
(10)	*Lasioglossum*	*dialictus 'A'*	12	5

Plants

(I)	*Eriodictyon*	*californicum*	23	**17**
(J)	*Malocothrix*	*floccifera*	19	7
(C)	*Eschscholzia*	*californica*	18	**32**
(H)	*Corethrogyne*	*filaginifolia*	18	7

Supplemental Table 5. Species lost from historical networks in both habitats with $F_{sp} < 10\%$. (calculated from i=1000 bootstrapped datasets (samples size = N resurvey observations).

Habitat	Genus	Species	% failure$_{i=1000}$
Grassland			
Bees			
	Andrena	*plana*	0.002
	Habropoda	*depressa* (Fowler)	0.002
	Xylocopa	*tabaniformis*	0.003
	Lasioglossum	*sisymbrii*	0.005
	Megachile	*'B'*	0.005
	Melissodes	*subillata*	0.007
	Andrena	*w-scripta* (Viereck)	0.016
	Anthopohra	*pacifcia* (Cresson)	0.018
	Osmia	*"J"*	0.018
	Andrena	*astragali*	0.024
	Bombus	*sitkensis*	0.080
	Megachile	*parallela*	0.080
	Coelioxys	*rufitarsis* (Smith)	0.084
	Megachile	*fidelis*	0.088
	Melecta	*seperata* (Cockerell)	0.089
	Osmia	*"G"*	0.091
Plants			
	Frangula	*californica*	0.001

Persicaria	*punctata*	0.001
Triteleia	*laxa*	0.001
Calystegia	*occidentalis*	0.002
Lupinus	*variicolor*	0.002
Stachys	*ajugoides*	0.004
Holodiscus	*discolor*	0.005
Perideridia	*kelloggii*	0.007
Ranunculus	*repens*	0.012
Sanicula	*bipinnatifada*	0.012
Agoseria	*grandiflora*	0.013
Acmospon	*americanus*	0.015
Aesculus	*californica*	0.016
Cirsium	*occidentale*	0.016
Toxicodendron	*diversilobum*	0.017
Tragopogon	*porrifolius*	0.018
Plectritis	*congesta*	0.019
Toxicoscordion	*fremontii*	0.019
Trifolium	*microdon*	0.037
riophyllum	*confertiflorum*	0.044
Layia	*platyglossa*	0.046
Epilobium	*brachycarpum*	0.050
Arctostaphylos	*crustacea*	0.051
Rhus	*integrifolia*	0.051

	Heterotheca	*sessiliflora*	0.053
	Thermopsis	*californica*	0.078
	Ribes	*sanguineum*	0.087
	Vicia	*americana*	0.087
	Dudleya	*palmeri*	0.088
	Baccharis	*glutinosa*	0.092
	Monardella	*villosa*	0.093
	Lagophylla	*ramosissima*	0.097
	Lonicera	*hispidula*	0.097
Sandhills			
Bees			
	Anthophora	*urbana*	0.001
	Melecta	*edwardsii* (Cresson)	0.001
	Melissodes	*lupina*	0.001
	Andrena	"*R*"	0.006
	Andrena	"*J*"	0.007
	Melissodes	*subillata*	0.013
	Andrena	"*I*"	0.030
	Megachile	*fidelis*	0.032
	Colletes	*hyalinus*	0.055
	Melissodes	"*D*"	0.059
	Megachile	*perhirata* (Cockerell)	0.061
	Bombus	*Edwardsii*	0.080

	Ceratina	*michener* (Daly)	0.084
	Anthidium	*utahense* (Swenk)	0.087
	Bombus	*Flavifrons*	0.090
	Lasioglossum	*dialictus "J"*	0.093
	Osmia	*aglaia* (Sandhouse)	0.094
	Melissodes	*communis*	0.098
Plants			
	Eriogonum	*virgatum*	0.004
	Antirrhinum	*multiflorum*	0.007
	Frangula	*californica*	0.014
	Perideridia	*gairdneri*	0.049
	Notholithocarpus	*densiflorus*	0.079
	Nuttallanthus	*texanus*	0.096

Supplemental Table 6. Total proportion of native and exotic bee and plant species in each survey (discoverlife.org and calflora.org).

Habitat	Native		Exotic	
	Historical	Resurvey	Historical	Resurvey
Grassland				
Bees	92	90	3	2
Plants	81	77	19	23
Sandhills				
Bees	96	91	3	2
Plants	91	94	9	6

Supplemental Table 7. Total number of partners lost plant family . Plants are those that lost at least one interaction partner between surveys L'$_3$).

Habitat	Partners lost	Predicted
Grasslands		
Anacardiaceae'	2	0.04
Apiaceae'	2	0.10
Asteraceae'	8	1.44
Caprifoliaceae'	1	0.02
Convolvulaceae'	1	0.00
Crassulaceae'	1	0.00
Ericaceae'	1	3.00
Fabaceae'	5	0.90
Grossulariaceae'	1	0.00
Lamiaceae'	2	8.00
Melanthiaceae'	1	0.00
Onagraceae'	1	0.02
Polygonaceae'	1	0.03
Ranunculaceae'	1	0.01
Rhamnaceae'	1	0.03
Rosaceae'	1	0.03
Salicaceae'	1	0.00
Sapindaceae'	1	0.00
Themidaceae'	1	0.00
Valerianaceae'	1	0.00
Sandhills		
Apiaceae'	1	0.03
Fagaeae'	1	0.03
Plantaginaceae'	2	0.12
Polygonaceae'	1	0.06
Rhamnaceae'	1	0.04

Chapter 2: Temperature and water stress modify floral attraction traits and subsequent pollinator foraging.

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Abstract

Environmental stress strains ecologically critical species interactions, such as pollination, that support biodiversity worldwide. High temperature and water stress negatively affect flower production and nectar quality. However, it is unclear how climate stress impacts biotic pollination and subsequent plant reproduction via flower and the quality of nectar rewards. To address these questions, we first tested how flower attraction traits responded to temperature and water stress in three native California forb species. Next, we assessed how bumblebee (*Bombus impatiens)* workers responded to an array of experimental plants in a foraging choice experiment, and then measured seed production in those plants. High temperature and high or low water stress both negatively impacted floral attraction traits, including nectar volume, proportion of nectar-filled flowers, and flower size in all three plant

species. Bumblebees generally spent most time foraging at the least stressed plants. However, larger flowers and high nectar volume only predicted seed output in one plant species, *Collinsia heterophylla*. Our results highlight how climate stress could decouple pollination interactions via reduced floral attraction and pollinator foraging in three native California forb species.

Introduction

Abiotic environments affect an individual's survival, growth, and reproduction. Climate change may test limits of individual performance and important species interactions as environments become more extreme. Life-history theory predicts species' responses to low and high climatic stress. When abiotic stress is low, species are expected to allocate more resources toward reproduction; when stress is high, resources are allocated toward survival (Stearns 1989, Qiu and Qian 1999, Mazer et al. 2004, Vargas et al. 2004, Schultner et al. 2013, Ivey et al. 2016, Mangel 2016).

In flowering plants, a life-history trade-off means producing large showy flowers, with rewards to encourage biotic pollination (Darwin 1862, Clements and Long 1923, Willmer 2011a) when the environment is benign: this facilitates plant reproduction and outcrossing while also providing pollinators with nectar, nitrogen-rich pollen, and other plant resources (Heinrich 1979, Underwood 1991, Nieh and Sánchez 2005, Willmer 2011c, McCallum et al. 2013). In contrast, when abiotic stress is high, plants are expected to lower their physiological investment in floral

attraction traits (Bell 1980, Pleasants and Chaplin 1983, Stearns 1989, Galen 2000, Carroll et al. 2001, Karl et al. 2011, Liu et al. 2012, Gusmao et al. 2012) resulting in less attractive floral rewards for pollinators, and thus limiting biotic pollination.

Pollinator responses to declining flower quality in the context of physiological climate stress are not well studied (Scaven and Rafferty 2013). However, when abiotic stress is low, bee foraging patterns are consistent with an optimal foraging model (Heinrich 1979, Willmer 2011c): bees selectively forage in ways that balance the energy they gain from visiting a flower with the energy required to find, land upon, handle, collect, and return resources to their nests (Charnov 1976, Zimmerman and Url 1981). When abiotic stress is high, bees should avoid flowers with a poor energetic return in favor of those with the best energetic pay-off.

In order to determine flower quality, bees use a combination of floral cues that operate at varying proximities to a focal plant (Essenberg et al. 2015). Floral scent, overall floral display, individual flower size and shape, pigmentation, pollen, and nectar quality are all important floral attraction traits, and are often closely linked (Biernaskie and Cartar 2004, Galliot et al. 2006, Tavares et al. 2016). This means that floral traits are usually honest indicators of resource quality (e.g., nectar and pollen). Climate stress is likely to have overall negative impacts on floral attraction cues which may, in turn, limit biotic pollination.

We hypothesize first, that floral attraction traits decrease with both increased temperature and water stress. Secondly, that pollinators will forage most often at plants with the highest realized rewards when given a choice, either switching away

from, or avoiding plants with lower rewards. Lastly, we assessed whether plants that receive longer pollinator visits, would produce larger seed sets relative to plants that are only rarely, or are not visited at all.

To test how temperature and water stress affect plant attraction traits, we allowed plants to flower in growth chambers with temperature and humidity controls and then measured attraction traits in those plants. In a separate, linked experiment, we assess how plant reward quality influences bee foraging decisions by allowing individual bumblebees to forage on the full array of experimental plants in a 'choice trial arena' while we monitored their activities. We replicated this design using three endemic California, herbaceous plant species that are frequently visited by bumblebees in the field.

Materials and Methods

We initially selected eight native California plant species that are represented in regional plant-pollinator networks (De la Luz 2018b) as candidates for our experiments. Seeds came from greenhouse and native nursery stocks. However only three plant species survived and produced enough flowers across all experimental treatments for data collection.

Study species *Collinsia heterophylla* (Buist), also called 'Purple Chinese houses', is an annual herb that blooms between April and July throughout California. It produces purple and white 'pea-like' flowers (Figure 1a), with anthers tucked into partially fused keel petals. When heavy pollinators, such as bumblebees, land on the

flower, the keel opens and pollen is exposed. *Co. heterophylla* has a mixed mating system; individuals are self-compatible with a delayed selfing mechanism. This species is found in shaded grassy fields, often associated with *Quercus* species.

Clarkia unguiculata (Lindley) or 'Elegant clarkia', is a California endemic annual species that blooms from April–August. Showy purple flowers offer pollinators both nectar and pollen rewards (Figure 1b). *Cl. unguiculata* also has a mixed-mating system with delayed selfing (Travers and Mazer 2000). *Cl. unguiculata* is most often found in open woods or grasslands where it is visited by a range of native bee species including bumblebees.

Scrophularia californica (Cham. & Schldl.) also known as 'California bee-plant' or 'Coast figwort', is a perennial herb that blooms from February through July. *S. californica* also has a mixed-mating system with delayed selfing (Ortega-Olivencia and Devesa Alcaraz 1993). Although usually found in wooded areas, it is also sometimes found in chaparral or coastal shrub. This species produces an urn shaped flower that yields high volumes of nectar and are frequently visited by native bumblebee species (Figure 1c).

We used lab-reared *Bombus impatiens* (Figure 1d; supplied by Koppert Biological Systems, Class C hive) for choice trials. Each hive arrived with a queen and ~30 workers and grew to over 100 workers over an 8-week period. We kept two hive boxes indoors inside a mesh flight cage (16.5" x 16.5" x 30") and under a single grow light. The hive boxes were initially shipped stocked with 1.8L of 60% sugar

solution and 27g of pollen in the form of 'bee bread', a mixture of pollen and nectar; these resources were not refreshed.

Experimental set-up To help ensure maximum seedling establishment and survival, we initiated all seedlings under common greenhouse conditions until budding, staggering two planting events by one week to extend the bloom window. For each species, we sowed four seeds into each of 1000 6-inch cone-tainers using a standard soil mix.

Once seedlings produced true leaves, we selected one individual per cone-tainer and removed the rest. We regularly rotated plants in the greenhouse to eliminate greenhouse-related effects and carefully monitored seedlings for early signs of budding (i.e. development of a floral meristem) at which point we introduced plants to experimental treatments.

We haphazardly assigned each plant to a temperature and water treatment combination for a total of n=20 replicates per plant species per treatment. We randomly assigned growth chambers to one of five daytime temperatures (T) ranging from 21ºC (lowest temperature stress) to 29ºC (highest temperature stress) in increments of 2ºC. Average spring temperature at our associated field sites is 25ºC (De la luz 2018a). Nighttime temperature (22ºC) was the same across all five growth chambers; thus plants experienced either smaller or greater temperature swings each day rather than a single, overall temperature. We confirmed growth chamber temperatures using an infrared temperature sensor (Ryobi® model: IR002, spot size = 5mm; Anderson, SC) throughout the course of the experiment.

We applied water treatments using blocks of floral foam (9 x 4 x 3 in^3, Oasis Floral Products, Fullerton, CA), each holding ten cone-tainers with one plant each, set within larger water reservoirs (6" x 16.25" x 23"; Sterilite Townsend, MA). Floral foam consistently and evenly distributes water to plants when available (Lambrecht et al. 2007). We set four blocks of floral foam into each water reservoir and applied water directly to the water reservoir at one of three levels: 1) Saturated; daily access to 2000ml, 2) intermediate; 2000ml water added to water reservoir every three days, or 3) dry; 2000 ml water added to water reservoir every six days.

We ran two separate growth chamber experiments, in different years, using four different plant species in each (Appendix B. Table 1). However, 5 plant species did not grow or flower successfully in the growth chambers, and these were not used for subsequent data collection or experiments (except to assess volumetric soil moisture). Growth chamber conditions were equivalent for each experimental run and temperature treatments were randomly assigned to growth chambers at the start of each run. Further, plants were rotated into new growth chambers half-way through each experimental run to minimize chamber–specific effects.

Plant response to climate stress We measured several plant and floral reward traits in half of the experimental plants (n=10 per treatment). Specifically, we measured plant height (cm) from the soil to the top of the plant, total number of open flowers, flower size (corolla length x width in mm), nectar volume (ul), nectar concentration (% brix), and the proportion of nectar-filled flowers for each plant. In testing for nectar quality, we haphazardly probed multiple open flowers per plant

using 3ul microcapillary tubes (Drummond Scientific Company, Broomall, PA) until we had collected three nectar measurements for each plant, or we ran out of flowers to probe. We used the number of probes required to reach three nectar measurements to calculate the proportion of nectar-filled flowers for each plant. We expressed each nectar sample onto a refractometer (Eclipse® Brix low volume; Xylem, Lawrenceville, GA) to measure sugar concentration. The plants used to assess floral traits were not used in foraging choice trials.

Foraging response We tested pollinator foraging responses to plants flowering under temperature and water stress for each species, separately. To do this, we set up a foraging choice arena (16.5"x16.5"x30") offering the full treatment array (n=15 plants of a given species) to a single bumblebee forager (per trial and plant species). We recorded each bee's behavior for twenty minutes (Appendix C; ethogram). Choice trials focused on one plant species at a time. For each trial, we randomly selected one blooming plant per treatment. We randomized positions of plants for each trial. (Supplemental figure 1).

We selected a single bee from the experimental hive for each choice trial. Any bee that did not begin foraging within five minutes was removed from the choice arena and returned to the hive; thus, that bee could have been used for subsequent trials. We began recording a bee's behavior using a digital voice recorder (Sony® model:ICD-PX333), once it began foraging (Appendix C; behavior ethogram). After each successful 20-minute choice trial, the bee was removed from the choice arena and kept separate from the hive until the end of the day. We painted each bee's thorax

using acrylic paint before returning them back to the hive so that each bee was only used in one choice trial.

Seed output We returned plants to their respective growth chamber and water treatment reservoir after choice trials, and monitored the plants until they either set seed or died. We collected and counted *Co. heterophylla* seeds by hand. However, *Cl. unguiculata* produces hundreds of small seeds per flower; we estimated number of seeds by measuring total seed weight and the average weight for 10 samples of 50 seeds to calculate the number of seeds produced by each plant. We could not collect seeds from *S. californica* because the experiment was lost to aphids before those plants produced seeds.

Analysis

Plant Responses We used principal components analysis (PCA) in R (R Foundation for Statistical Computing 2014) to understand how plant floral traits are related to each other within species. We used the combined PCA loadings (6 axes) as the response variables in separate Manova analyses for each plant species. Temperature and water treatment (as 1=dry, 2=intermediate, and 3=saturated) were continuous predictors; plant replicate was nested in water reservoir (fixed factors). We also used linear mixed effects models (nlme; Pinheiro J et al. 2016) to test how temperature and water (continuous) with replicate nested in water reservoir (random) impact PC1 in particular, which explains the most variation in each dataset.

Foraging response We used best fit linear models (after AIC model reduction) with Anova (type II ss) to test how floral attraction traits influenced bumblebee foraging. The response variable was the (log-transformed) proportion of time bumblebees foraged at each of 15 plants (15 treatments) in the choice trial array (response). We used the plant response models (Manova above) to determine the PC loadings for each choice trial plant based on their temperature and water treatment combination. Temperature, and water treatment were also included as continuous predictors. Trial was a fixed factor.

Seed output We used best fit linear models (after AIC model reduction) to test how temperature, water, floral attraction traits (PC loadings), and bumblebee foraging (proportion of time spent on a plant) impacts seed set for *Co. heterophylla* and *Cl. unguiculata* (but not for *S. californica* which were lost to aphids before setting seed). Seed set was the response variable, PC loadings (above), proportion of time bees spent per plant/trial, temperature, and water treatments were continuous predictors. Plant replicate was nested within species and included as a random factor. We used AIC model reduction to reduce the number explanatory variables in each species-specific model

Results

Plant response Floral traits, presumably related to pollinator attraction, show broad scatter across both PC1 and PC2 for all three species (Fig. 2) that reflect the impacts of the underlying temperature and water treatments. In general, flower size,

nectar volume, and proportion of nectar-filled flowers are collinear and load similarly on these axes; plant height is also correlated with these floral traits in *Co. heterophylla*, but not the other species. PC1 explains ~1/3 of variation in the data for all 3 species, while PC1 and PC2 together explain > 50% of this variation (Table 1). For both *Co. heterophylla* and *S. californica*, the PC1 axes primarily reflect flower size, nectar volume, and proportion of nectar-filled flowers (Sup Table 1). In contrast, nectar concentration, rather than flower size loads mainly on PC1 for *Cl. unguiculata*, and flower size loads mainly on PC3. Flower size and flower count also load on PC2 in all three plant species.

Plants with the highest loadings of 'attractiveness' for pollinators produced larger flowers at low temperatures (Manova, Table 2; Fig. 3). The loadings associated with more attractive trait values declined with increased temperature (Anova, Table 3). These floral attraction traits also declined with decreasing water availability in *Co. heterophylla* and *S. californica,* but not *Cl. unguiculata* (Figure 3).

Foraging Response *B. impatiens* spent 16.8 ± 9.3 (SD) minutes of each 20-minute trial actively visiting flowers (84% of the time). After AIC model reduction (Sup table 2), the temperature at which the plants flowered was the most consistent predictor of how long bumblebees foraged at *Co. heterophylla* and *Cl. unguiculata* and *S. californica;* Anovas, Table 4). Plant grown at the highest temperatures were visited 50% less frequently than those grown at the lowest temperatures (Figure 4). This is largely due to more stressed plants sometimes not being visited at all during choice trials. The temperature effect is lost upon removing non-visits (zeros) from

the dataset. Neither water stress nor any single PC axis (values predicted from Manova above) consistently predicted bumblebee foraging between plant species (Table 4).

Seed output Temperature stress led to smaller seed sets for *Co. heterophylla* but not *Cl. unguiculata* (Table 5). In *Co. heterophylla*, seed set was ~50% lower on average in plants grown at the highest, relative to the lowest temperature (Figure 5). However, both saturated and dry water treatments had negative impacts on seed set in both species, while plants grown at intermediate water levels produced the most seed overall. This pattern could be due to root rot, fungal growth, or waterlogging associated with high water stress over time. In our experiment, neither the proportion of time spent per plant by *B. impatiens,* nor any of the PC loadings predicted seed set in either plant species after AIC model reduction (Supplemental table 2)

Discussion

Temperature and water stress limit plant floral attraction traits, and subsequent foraging on those plants by bumblebees offered a choice among plants from different environments. Only a few other studies, most focusing on drought stress, address how physiological climate stress negatively impacts floral attraction traits (Halpern et al. 2010, Sedlacek et al. 2012, Scaven and Rafferty 2013, Jorgensen and Arathi 2013). However, stress comes in many forms that often act simultaneously over large spatial scales and will likely impact multiple plant species at the community level.

For plants, attracting effective pollinators is important for plant fitness because pollinators carry outcrossed pollen, increasing genetic diversity for plants at both the individual and population levels. This is particularly important for annual plant species that are unable to delay reproduction when stressed, as do many perennials (Primack and Stacy 2015). We included two annual and one perennial plant species in our study, but all showed similar negative responses to increased temperatures; and for two of our three focal plant species, to water stress.

Flower and nectar production is energetically costly for plants (Pyke 1991, Ashman et al. 1994, Pacini et al. 2003). When stressed, plants are expected to allocate resources toward survival (Bell 1980, Stearns 1989, Bond and Maze 1999), but can still maximize their fitness potential by producing at least a few flowers for outcrossing or self-pollination (Galen 2000, Carroll et al. 2001, Su et al. 2013). In our study, *Co. heterophylla* produced fewer, smaller flowers with low nectar volumes when growing under high temperature and low water relative to less stressed plants. Alternatively, under high temperature, *Cl. unguiculata* produced fewer, but larger flowers with low nectar volume and concentration. *S. californica* also produced fewer but not necessarily smaller flowers and nectar volume remained high, but became more dilute, with increasing temperature and water stress. This varied trait response indicates species-specific resource allocation strategies in plants (Tilman 1982, Lovett Doust 1989, Ashman et al. 1994, Karlsson and Méndez 2005, Berg and Ellers 2010).

Temperature stress was the most consistent predictor of bee foraging time across all three plant species in our controlled choice trials. Bumblebees spent

proportionally more time at plants grown at the lowest temperatures regardless of plant species. In general, temperature stressed (i.e., higher temperatures) plants had smaller flowers with either low nectar volumes or dilute nectar. This pattern is consistent with choice trial studies using artificial flowers where bumblebees used flower size to determine a flowers energetic value (Ishii 2006, Essenberg et al. 2015). In addition to temperature, at least one of the six PC axes also partially explained bumblebee foraging for each plant species.

Biotic pollination often leads to larger and more viable seed sets (Ollerton et al. 2011, Jorgensen and Arathi 2013, Campbell and Halama 2015), even for plants that can self-pollinate in the absence of biotic pollination (Travers and Mazer 2000)). However, in our study, plants that received longer and more frequent visits did not always produce more seeds. For instance, in experiments with *Cl. unguiculata* flower size mostly loads on PC3; nectar volume load in different directions on that same axis suggesting that flower size is not always indicative of nectar reward in this species. *Cl. unguiculata* has a range of alternative strategies such as self-fertilization, shortened bloom times, and dishonest floral cues that likely help this species compensate for the physiological constraints of growing under stress (Travers and Mazer 2000, Mazer et al. 2004, Ivey et al. 2016). Nonetheless, biotic pollination is closely tied to population-level success in plants despite these alternative strategies (Ollerton et al. 2011, Albrecht et al. 2012) .

Resource and energy mismatches between plants and pollinators may develop before range or phenological shifts more directly impact plant-pollinator interactions

(Inouye 2008, Van der Putten et al. 2010, Pyke et al. 2011, 2016, Bartomeus et al. 2011, Parmesan and Hanley 2015). Pollinators compensate for environmental stress best when they have access to high quality nectar rewards (De la Luz 2018c; Heinrich 1979; Nieh et al. 2006; May 1979). Unlike plants, pollinators have the advantage of mobility allowing them to make foraging choices when possible (Essenberg et al. 2015), and potentially switching to novel resources to maintain their energy balance (De la luz 2018a & c).

Conclusions Pollination supports high levels of biodiversity worldwide (Vamosi et al. 2006, Bascompte and Jordano 2007, Albrecht et al. 2012). However, environmental stress strains pollination and other critical species interactions. (Memmott et al. 2007, Tylianakis et al. 2008, Hegland et al. 2009, Potts et al. 2010, De la Luz 2018b). Tension between plant survival and reproduction and pollinator energetic balance may develop into a resource-energy mismatch as large-scale environmental stress tests the physiological limitations of important species interactions.

Figures

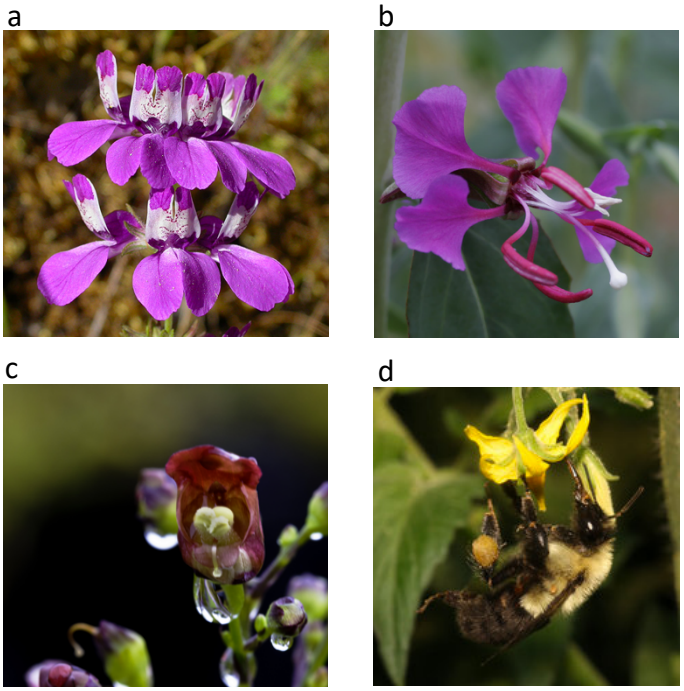


Figure 1. Experimental plant species a) Scrophularia californica (photo ©2003 Micheal Charters , b) Collensia heterophylla (photo ©2005 Christopher L. Charters) and c) Clarkia unguiculata (©2007 Lynne Watson). d) Pollinator species Bombus impatiens (Photo: ©2007 Lynne Watson) used in choice trials.

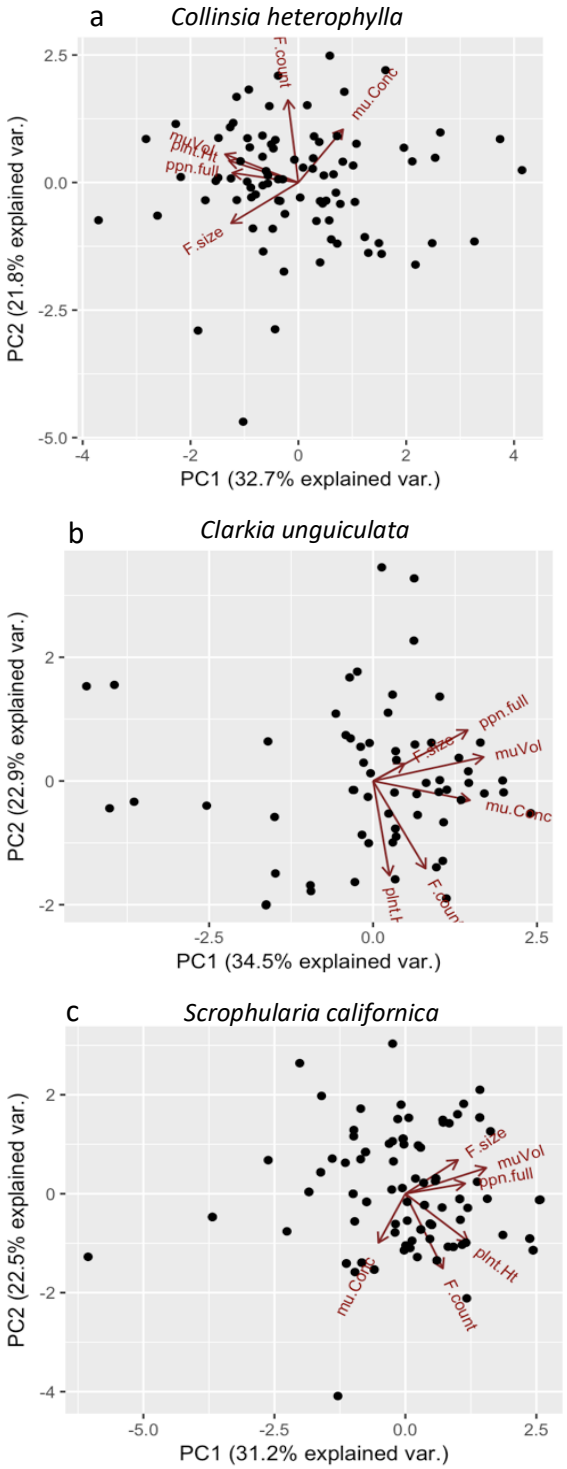


Figure 2. PCA axis 1 and axis 2 loadings for a) Co. heterophylla, b) Cl. unguiculata, and c) S. californica. See table 1 for PCA summary values. Variable loadings are found in Supplementary table 1.

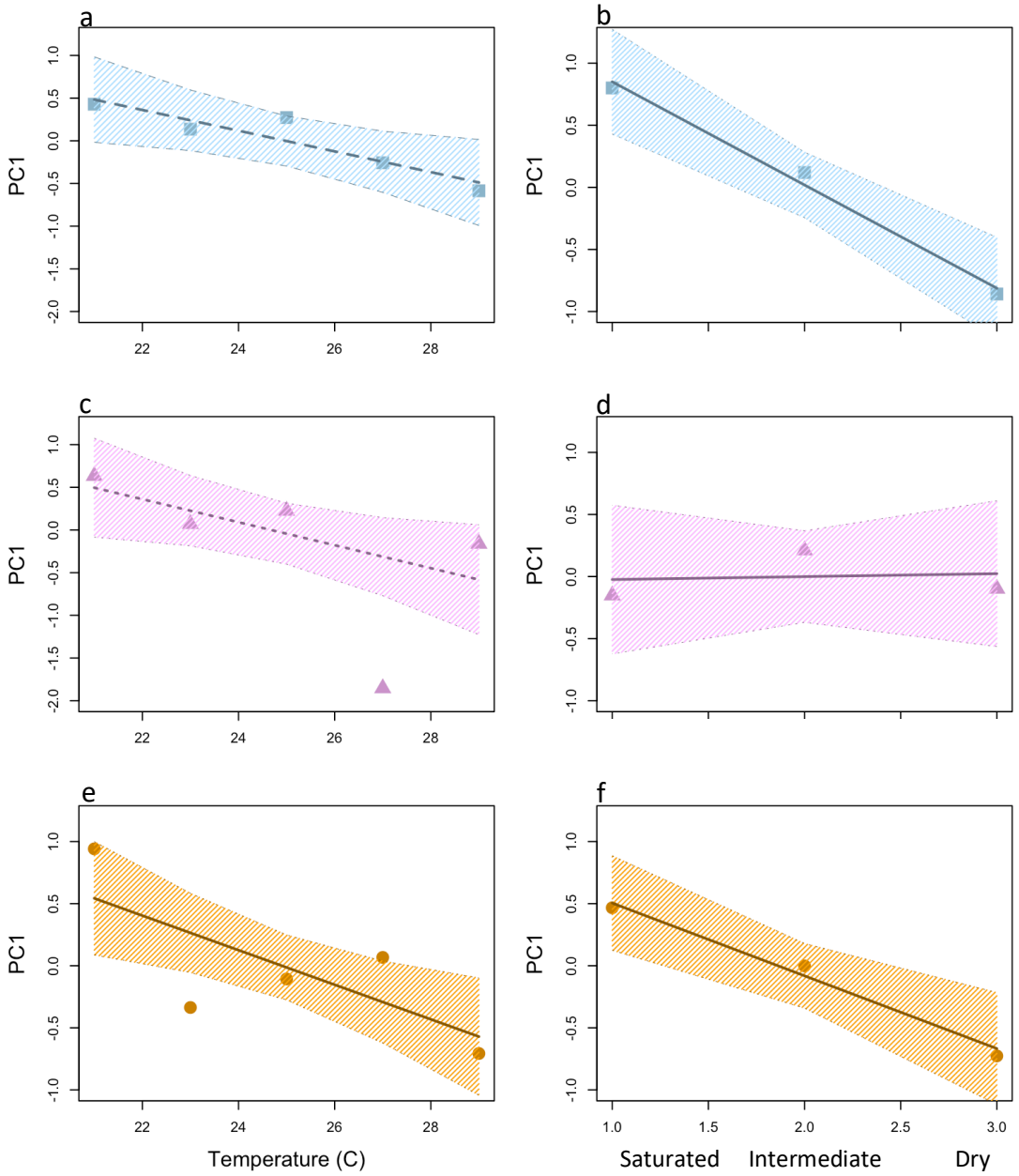


Figure 3. PC1 response (nectar volume, proportion of nectar filled flowers, and either: flower count(S. californica) and/or nectar concentration (Cl. unguiculata) to temperature (left) and water treatments (right) in a linear mixed effects models. Points are average PC1 values at each temperature and water treatment. Co. heterophylla (a-b), Cl. unguiculata (c-d), and S. californica were analyzed using separate species-specific models.

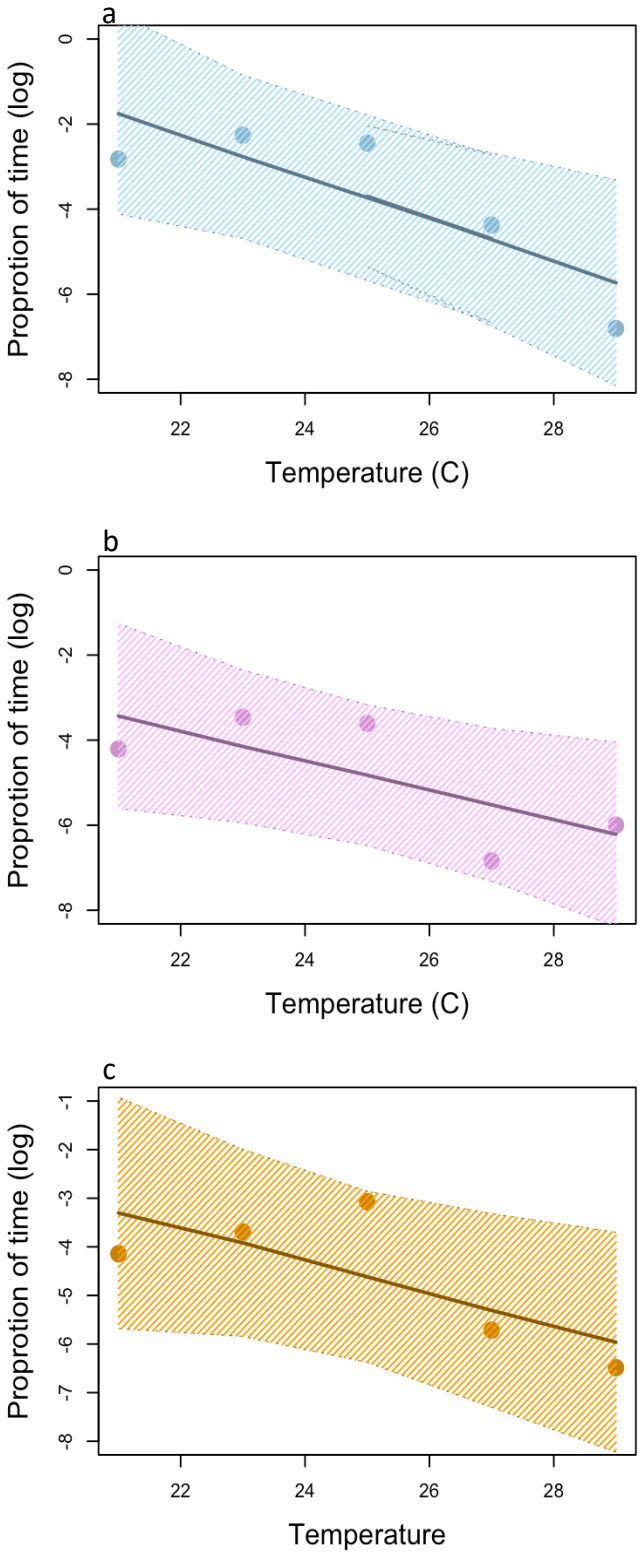


Figure 4. Choice trial data. (log) Proportion of time spent foraging at plants with increasing Temperature.Temperature consistently predicted foraging time (proportion) across plant species. Lines are model fits for each plant species. Blue squares are Co. heterophylla. Purple triangles are Cl. unguiculata, and orange circles are S. californica.

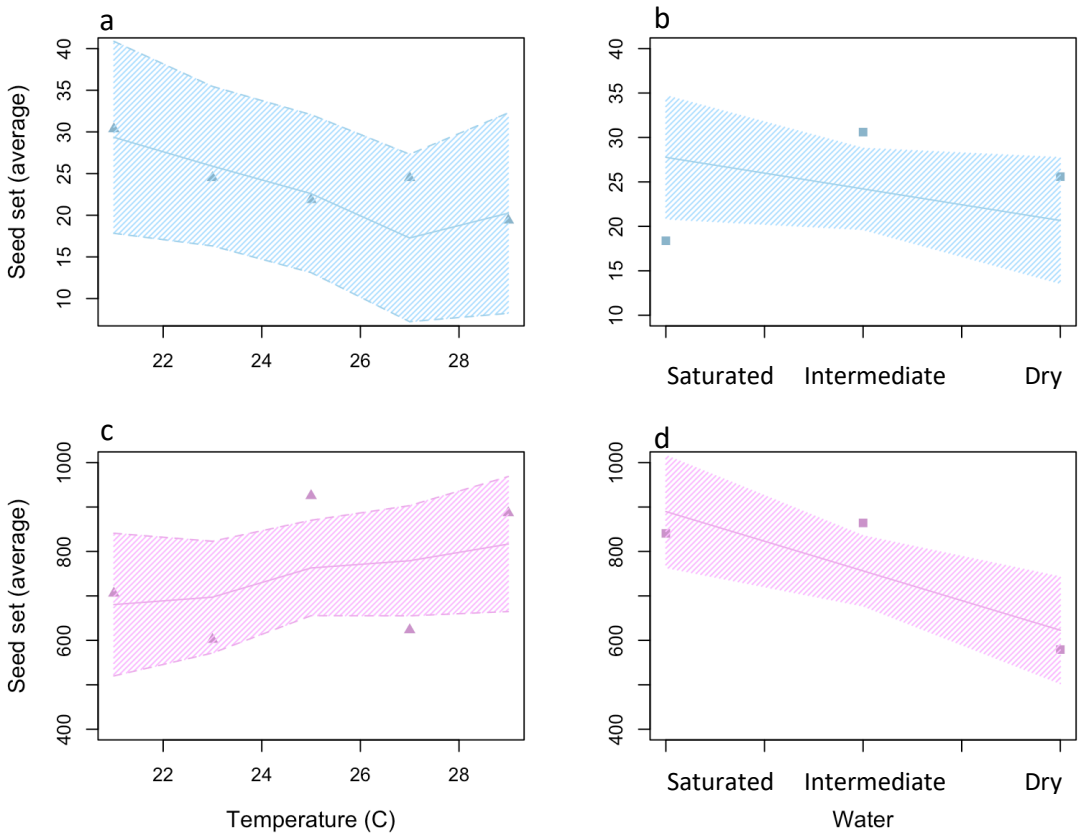
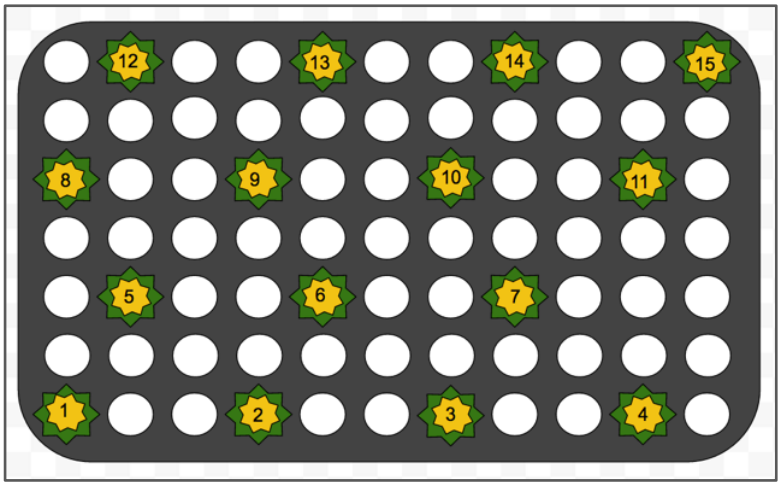


Figure 5. Seed response. Average seed set for a-b) Co. heterophylla and c-d) Cl. unguiculata over increasing Temperature (left) and water (right) stress. Lines are model fits for each plant species and shaded regions are 95% confidence intervals. Blue squares are Co. heterophylla. Purple triangles are Cl. unguiculata

Supplemental Figure



Supplemental Figure 1. Diagram showing how plants were arranged in a plant rack for choice trials. One plant from each temperature and water treatment combination (n=15) were randomly assigned a position prior to each choice trial.

Tables

Table 1. PCA results summary for a) Co. heterophylla, b) Cl. unguiculata, and c) S. californica. PC1 explained >30% of variation for each species. PC1 and PC2 together explained >50%.

	PC1	PC2	PC3	PC4	PC5	PC6
a. *Collinsia heterophylla*						
Standard deviation	1.40	1.14	1.01	0.83	0.72	0.71
Proportion of Variance	0.33	0.22	0.17	0.12	0.09	0.08
Cumulative Proportion	0.33	0.55	0.71	0.83	0.92	1.00
b. *Clarkia unguiculata*						
Standard deviation	1.44	1.17	0.99	0.85	0.75	0.54
Proportion of Variance	0.34	0.23	0.16	0.12	0.09	0.05
Cumulative Proportion	0.34	0.57	0.74	0.86	0.95	1.00
c. *Scrophularia californica*						
Standard deviation	1.37	1.16	0.98	0.94	0.72	0.65
Proportion of Variance	0.31	0.22	0.16	0.15	0.09	0.07
Cumulative Proportion	0.31	0.54	0.70	0.84	0.93	1.00

Table 2. MANOVA summary statistics for a) Co. heterophylla, b) Cl. unguiculata, and c) S. californica. The combined PC axis loadings (PC1–PC6) are the response variable.

	Df	Pillai	approx F	num Df	den Df	Pr(>F)
Collinsia heterophylla						
Temp	1	0.29	3.51	6	51	0.01
Water	1	0.43	6.47	6	51	0.00
Reservoir	1	0.10	0.97	6	51	0.45
Rep	26	2.10	1.16	156	336	0.13
Residuals	56					

Clarkia unguiculata	Df	Pillai	approx F	num Df	den Df	Pr(>F)
Temp	1	0.33	2.89	6	36	0.02
Water	1	0.34	3.15	6	36	0.01
Reservoir	1	0.32	2.79	6	36	0.02
Rep	17	1.75	0.99	102	246	0.52
Residuals	41					

Scrophularia californica	Df	Pillai	approx F	num Df	den Df	Pr(>F)
Temp	1	0.19	2.82	6	73	0.02
Water	1	0.45	10.04	6	73	0.00
Reservoir	1	0.10	1.32	6	73	0.26
Rep	19	1.47	1.33	114	468	0.02
Residuals	78					

Table 3. Anova summary statistics for floral attraction traits loading on PC1 (~30% variation explained) for each focal plant species. Each plant species was analyzed separately.

	Chisq	Df	Pr (>Chisq)
Collinsia heterophylla			
Temp	5.58	1	0.02
Water	25.30	1	0.00
Clarkia unguiculata			
Temp	4.61	1	0.03
Water	0.00	1	0.97
Scrophularia californica			
Temp	11.69	1	0
Water	19.90	1	0

Table 4. Anova summary statistics for bumblebee foraging time (proportion time spent per plant) during choice trials with a) *Co. heterophylla*, b) *Cl. unguiculata*, and c) *S. californica*. Anova results are from the best fit linear model determined via AIC model reduction (Sup Table 1).

	Sum Sq	Df	F value	Pr (>F)
Collinsia heterophylla				
pc1	79.10	1	2.41	0.12
pc2	72.56	1	2.21	0.14
pc5	162.59	1	4.94	0.03
pc6	69.92	1	2.13	0.15
Temp	192.11	1	5.84	0.02
Residuals	3649.81	111		

Clarkia unguiculata	Sum Sq	Df	F value	Pr (>F)
pc1	867.03	1	28.03	0
pc2	780.25	1	25.22	0
pc3	349.08	1	11.28	0
pc4	265.83	1	8.59	0
pc5	722.88	1	23.37	0
pc6	291.17	1	9.41	0
Temp	460.86	1	14.90	0
Residuals	4361.64	141		

Scrophularia californica	Sum Sq	Df	F value	Pr (>F)
pc1	60.31	1	2.71	0.10
pc2	93.28	1	4.19	0.04
pc4	148.19	1	6.65	0.01
Temp	210.86	1	9.46	0.00
Water	71.36	1	3.20	0.08
Trial	1113.59	1	49.97	0.00
Residuals	2518.42	113		

Table 5. Anova summary statistics for a) Co.heterophylla and b) Cl. unguiculata seed set. Results are from the best fit linear model after AIC model reduction (Sup Table 2). S. californica is not included due to plant loss.

	Sum Sq	Df	F value	Pr(>F)
Collinsia heterophylla				
Temperature	1786.77	1	6.20	0.02
Water_level	1616.86	1	5.61	0.02
pc2	854.63	1	2.97	0.09
pc3	850.94	1	2.95	0.09
pc4	661.42	1	2.30	0.14
Residuals	14983.60	52	*NA*	*NA*
Clarkia unguiculata	**Sum Sq**	**Df**	**F value**	**Pr(>F)**
Water level	1743070	1	7.82	0.01
Residuals	30758894	138		

Supplementary Tables

Supplemental Table 1. PCA variable loadings on each of six PC axes for Co. heterophylla, Cl. unguiculata, and Sc. California. Each plant species was analyzed separately. Data were log transformed prior to running PCA.

	PC1	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**
Co. heterophylla						
F.count	-0.07	0.73	-0.32	0.08	-0.28	0.52
F.size	-0.46	-0.36	0.04	0.63	-0.50	0.11
plnt.Ht	-0.48	0.19	-0.48	0.27	0.52	-0.40
muVol	-0.50	0.25	0.22	-0.50	-0.43	-0.45
mu.Conc	0.31	0.47	0.50	0.52	-0.02	-0.40
ppn.full	-0.45	0.09	0.61	-0.03	0.46	0.45
Cl. unguiculata	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**
F.count	0.28	-0.61	0.01	0.59	-0.22	-0.39
F.size	0.17	0.12	-0.96	0.01	0.11	-0.15
plnt.Ht	0.09	-0.66	-0.13	-0.68	-0.20	0.18
muVol	0.59	0.17	-0.02	0.20	-0.35	0.67
mu.Conc	0.52	-0.13	0.16	-0.07	0.82	0.03
ppn.full	0.51	0.36	0.19	-0.38	-0.31	-0.58
S. californica	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**
F.count	0.27	-0.68	0.08	-0.10	0.64	0.21
F.size	0.38	0.31	0.47	0.58	0.31	-0.34
plnt.Ht	0.46	-0.43	0.31	-0.17	-0.62	-0.30
muVol	0.59	0.23	-0.16	0.14	-0.20	0.72
mu.Conc	-0.20	-0.44	-0.34	0.77	-0.23	0.01
ppn.full	0.43	0.09	-0.73	-0.11	0.15	-0.49

Supplemental Table 2. Anova results for step AIC model reduction for choice trials with a) Co. heterophylla. b) Cl. unguiculata, and c) S. californica to determine the final linear models for analysis (Table 4).

Step	Df	Deviance	Resid. Df	Resid. Dev	AIC
Collinsia heterophylla	NA	NA	107	3562.60	419.68
- pc3	1	4.62	108	3567.22	417.83
- Water	1	2.55	109	3569.76	415.92
- pc4	1	27.65	110	3597.41	414.82
- Trial	1	52.41	111	3649.81	414.51
Clarkia unguiculata	NA	NA	139	4329.79	522.03
- Water	1	2.34	140	4332.13	520.11
- Trial	1	29.51	141	4361.64	519.12
Scrophularia californica			110	2477.49	383.30
- pc6	1	1.07	111	2478.57	381.35
- pc5	1	14.99	112	2493.55	380.08
- pc3	1	24.87	113	2518.42	379.27

Supplemental Table 3. Anova results for step AIC model reduction for a) Co heterophylla and b) Cl. umguiculata seed set.AIC model reduction was used to determine the final linear models for analysis (Table 4).

Step	Df	Deviance	Resid. Df	Resid. Dev	AIC
Collinsia heterophylla			130	29727195	1737.23
- pc3	1	130.73	131	29727326	1735.23
- pc5	1	157.51	132	29727483	1733.23
- pc1	1	24629.93	133	29752113	1731.35
- pc4	1	48544.64	134	29800658	1729.58
- pc2	1	111927.16	135	29912585	1728.10
- log(ppn.time)	1	216642.08	136	30129227	1727.11
- pc6	1	209589.66	137	30338817	1726.08
- Temperature	1	420076.92	138	30758894	1726.01
Clarkia unguiculata			48	14932.80	341.95
- pc1	1	0.11	49	14932.91	339.95
- pc5	1	5.12	50	14938.03	337.97
- log(ppn.time)	1	12.65	51	14950.69	336.02
- pc6	1	32.92	52	14983.60	334.15

Chapter 3: Nectar sugar concentration mediates cooling ability in three bumblebee species under acute heat stress

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Keywords: *Bombus*, bumblebee, climate, nectar, pollination, cooling, heat transfer

Abstract

Under heat stress, a bumblebee's ability to reduce body temperature via active cooling may depend on nectar sugar concentration. We tested whether sugar concentration of bumblebees' diet mediated cooling under acute heat stress and assessed this as heat transfer between an experimentally heated thorax and unheated abdomen in three bumblebee species: *Bombus impatiens, B. melanopygus,* and *B. vosnesenskii*. Heat was transferred from the thorax to the abdomen in the first 90 seconds and then temperatures in both segments remained at equilibrium. Heat transfer to the abdomen increased with sugar concentration in *B. melanopygus* but not in *B. vosnesenskii* or *B. impatiens*. Since plants may reduce flower output, size and/or nectar quality as climate warms, we suggest that bumblebees could become simultaneously constrained by the energetic demands of active cooling at the same time they face more limited high-quality nectar resources in their plants.

Introduction

Bumblebees are among a group of large-bodied insects with well-developed adaptations for controlling their body temperature. Many of these adaptations for thermal regulation require significant energy input from floral nectar to properly function (Heinrich 1979, May 1979, Stone and Willmer 1989, McCallum et al. 2013). Internal heat generation, counter-current heat exchange, and insulation allow bumblebees to persist in cold climates as far north as Alaska (Heinrich and Vogt 1993). Conversely, bumblebees use metabolic energy from nectar to quickly offload excess body heat via behavioral (e.g., wing-fanning), and physiological adaptations ('active cooling') in response to acute and high temperature stress.

Temperature regulation is energetically costly (Esch et al. 1991, Heinrich and Vogt 1993, McCallum et al. 2013). Bumblebees offset the high energetic costs of temperature control and flight by metabolizing carbohydrate rich floral nectar (Heinrich 1972, 1979, Pleasants 1981, McCallum et .al. 2013). However, both the amount and/or concentration of sugar in plant nectar are sensitive to environmental stressors such as temperature and drought (Wyatt et al. 1992, Carroll et al. 2001, Newman and Wagner 2013, Scaven and Rafferty 2013, De la Luz 2018a).

As temperatures or water stress increase, many plants reduce overall nectar output or sugar content in a life-history trade off that maximizes their reproduction and survival (Carroll et al. 2001; Newman and Wagner 2013; Jorgensen and Arathi 2013). Because of this response in plants, bumblebees gain less energy per flower visit when plants are stressed at the same time that bees' own energetic demands, and

risk of overheating, are also likely to increase (Hegland et al. 2009, Scaven and Rafferty 2013). This could develop into an energy mismatch, driving bumblebees toward their physiological limits as environmental stressors continue to surpass record levels. (Parmesan 2006, Memmott et al. 2007, IPCC 2013, 2014).

Studies relating nectar diet and temperature regulation in bumblebees have focused on the bee's ability to warm up at cooler temperatures. Bumblebees 'shiver' to warm flight muscles so they can forage longer and at cooler temperatures than smaller bees that remain in torpor when cold (Esch et al. 1991, McCallum et al. 2013). This active warming mechanism gives bumblebees a foraging advantage over smaller ectothermic bees, whose activities are largely limited by external temperature (Stone and Willmer 1989, Willmer 2011b). Bumblebees and other species in the Apidae family can increase thoracic temperatures with increased nectar concentrations: e.g., *Bombus wilmattae, Apis cerana, A. dorsata, A. laboriosa, and Melipona panamica* increased baseline thoracic temperatures after 30 minutes or more, as sugar concentrations in their nectar forage rose (Underwood 1991, Nieh and Sánchez 2005, Nieh et al. 2006). These studies demonstrate the importance of good food for temperature regulation in the group as a whole, but they do not address active cooling with temperature stress.

Although individual bumblebee activity increases at moderately high temperatures (~30°C), they can also easily overheat due to their high flight metabolism; more than 90% of nectar calories consumed are released as heat in the thorax (Heinrich 1975, 1979, 2009). Bumblebees stop foraging when temperatures

become too high for flight (36°C – 45°C; Heinrich 1977, Willmer 2011b). Instead, bumblebees may sit still, but pump their abdomens (and may also fan their wings), to maximize convective heat loss from active cooling: this is visible as slow (high amplitude) and strong (low frequency) abdominal pulsing when temperatures are too stressful for flight. These behaviors are visible in heat-stressed bees and likely represents a stress, or 'cooling' response (Heinrich 1976, May 1979, Willmer 2011c).

Active cooling in bumblebees is linked to their internal anatomy. When a bumblebee constricts the longitudinal muscle running through the petiole (ventral diaphragm, Figure 1), the heart vessel compresses, momentarily halting both blood flow (cool blood) from the abdomen to thorax and the internal counter-current heat exchange system (Figure 1a, Heinrich 1979). Blood from the insulated thorax does not exchange heat with cool abdominal blood returning to the abdomen (as would happen with warm-up) but is instead offloaded via either convection to the air, or conduction–through direct contact with a surface (as in brood incubation).

The impact of nectar diet on the ability of individual bumblebees to cool-down in response to heat stress has not been addressed. In this paper, we assess the effect of nectar diets on bumblebees' abilities to reduce their body temperature under heat stress. To do this, we experimentally tested the ability of three bumblebee species, *Bombus impatiens*, *B. melanopygus*, and *B. vosnesenskii*, to cool down after consuming sucrose solutions ranging from 0% sucrose (water) to 75% sucrose. Specifically, we hypothesized that bumblebees with a high sucrose diet transfer more

heat away from the thorax (cool-down) to the abdomen (warm up), than those with a low sucrose diet under acute heat stress.

Understanding how pollinators tolerate high temperature stress is of ecological and economic concern as average spring and summer temperatures continue to exceed record highs (Cayan et al. 2008, Rahmstorf et al. 2012, IPCC 2013). High temperatures due to climate change are linked to widespread losses in bumblebee abundance and diversity (National Research Councel 2007, Goulson et al. 2008, Potts et al. 2010, Cameron et al. 2011, Koch 2011, Hatfield et al. 2015a, Thomson 2016).

Materials and methods

Experimental Species

We studied three species of *Bombus* that are all generalists foraging on a wide variety of flowering species. *Bombus impatiens* Cresson, 1863, the 'common eastern bumblebee', is native to eastern North America, from Ontario to Maine and south through Florida (Koch et al. 2012). It is widely used in agriculture and commercially available. *Bombus melanopygus* Nylander, 1848, the 'black tailed bumblebee', is found throughout the western and southwestern United States, primarily in California and southern Oregon. though they have been observed as far north as the Arctic circle (Hatfield et al. 2015a). *Bombus vosnesenskii* Radoszkowski, 1862, the 'yellow faced bumblebee', is widespread throughout the western United states, from Baja California through British Columbia, with a limited range in Nevada (Koch et al. 2012). Spring

foragers of all three species have similar body lengths (head to end of abdomen 8-17mm) between and within species.

Acute heat stress and cooling response. Each trial day, we collected 10-12 *B. vosnesenskii* (2015) and *B. melanopygus* (2016) workers at the UCSC Arboretum (area = ~.10km^2 Santa Cruz, CA) and transported them to the lab for testing. Separately, we acquired a nest box of *B. impatiens* (2015; Class C research hive, Koppert Biological Systems, Howell, MI) and housed them in an indoor flight cage under a grow light. The nest box originally contained a queen and ~10 workers, and continuously produced new workers during the eight-week life of the hive. *Bombus impatiens* had constant access to a 60% sucrose solution in the nest box, and pollen in the form of "bee bread", a mixture of honey and pollen that nourishes growing larvae. We only used foragers between 13-20mm long (head to abdomen).

In the first of three experimental runs, individual wild caught *B. vosnesenskii*, were deprived of food in the lab for one hour. In the second and third experiments, *B. impatiens* and *B. melanopygus* were first misted with water and then deprived of food for one hour. These bees were kept in a glass holding jar with a small square of tissue (Kimwipes®) to help absorb excess water from the bees. Misting encouraged bees to move about and beat their wings to dry off. Bees became lethargic and eager to feed following this method.

After the starvation period, bees were moved individually into small glass chambers (haphazardly assigned) where they were offered a rudimentary "flower"

made of a (1.3 x 1.3 cm^3) sponge soaked in a known sucrose solution (Supplemental figure 1). The sucrose solutions were a mixture of deionized water and sucrose to give 0, 15, 35, 55 and 75% sucrose. These concentrations represent the range of nectar sugar concentrations bees might be exposed to in the field (Willmer 2011a), with water (0%) as a non-metabolized control. Each day, we created fresh solutions and confirmed all sugar concentrations with handheld refractometers (Eclipse® 0–50 brix and Eclipse® 45–80 brix low volume; Xylem, Lawrenceville, GA). Bees were allowed to feed freely at the artificial flower until they stopped on their own. Most of the bees readily foraged, even on deionized water. Bees that that did not feed were marked on the thorax with water-based acrylic paint and were not used in any experimental trials.

During the heating experiment, each bumblebee was restrained on a 1.2" thick styrofoam pad (to limit conductive cooling) with insect pins around the waist (petiole); we partitioned the abdomen and thorax with an aluminum heat-shield (Seely et al. 2012), wings were positioned in the front of the heat shield to eliminate "wing fanning" over the abdomen which would have aided convective heat loss (Holland and Bourke 2015). We recorded the baseline starting temperature of the thorax (IT-region) and abdomen (dorsal T3) before applying heat.

We applied an acute heat stress to each bee in the lab (~25ºC) with a 25-watt UV heat-lamp directed at the head and thorax. Every 30 seconds for five minutes, we removed the heat-lamp just long enough to measure the surface temperatures of the thorax and abdomen (~ 2 seconds total, same locations as above) using an infrared

temperature reader (Ryobi® model: IR002, spot size = 5mm at ~5 cm, ± 0.13ºC listed accuracy; Anderson, SC). This heat-stress treatment consistently induced a heat transfer response, visible as rhythmic low-frequency abdominal pumping, in live experimental bees. We ran 3 to 5 replicates for the entire range of sugar solutions each trial day, for a total of n=18 trials per treatment for *B. vosnesenskii*, n=16 for *B. melanopygus*, and n=10 for *B. impatiens*. After each trial, bees were marked with water-based acrylic paint and fed a sugar rich solution to encourage recovery before being returned to the field (*B. vosnesenskii, B. melanopygus*) or nest box (*B. impatiens*). Marked bees were not used in subsequent trials. As heat-stress controls, we applied the same methods described above to 15 starved and recently killed bumblebees (*B. melanopygus* (n=10) and *B. vosnesenskii* (n=5).

Statistical Analysis

We used linear-mixed effects models (Supplemental material) to determine if and how sugar concentration impacts temperature over time with acute heat stress. We considered the time course in 3 separate periods. 1) baseline temperatures, time=0 seconds; 2) initial warm-up, from 30–90 seconds; 3) equilibrium, from 120-–300 seconds.

We used inverse Gaussian distributions (log-link function), to assess the effects of % sugar on baseline temperatures, and initial warm-up (30-90 seconds).Fixed factors in these models were: time point (in 30 second intervals), species, % sugar treatment, and body segment, (i.e., 'part'; thorax or abdomen).

Random factors were observer (with % sugar treatments) and part nested within individual (with time point) to account for repeated measures. To calculate slopes (over time), we ran the same model but with % sugar as a continuous variable (AIC = +50 relative to model where % sugar is a fixed factor). Using model estimates, we calculated the average heat absorption rate of each species (per 30 second interval).

We used an inverse Gaussian model with an inverse mean squared link function to assess the effect of % sugar on average T_{th} and T_{ab} temperatures after bees reached equilibrium (120-300 seconds). Fixed factors in this model were part, species, and % sugar treatment. Random factors were observer and part nested within individual. We used the R statistical program (R core team 2014) with the 'lmerTest', 'lme4', and 'car' packages (Kuznetsova et al. 2015) for all analyses.

Results

Average baseline T_{th} and T_{ab} temperatures were similar between body segments within each species (Table 1) but differed among species with no interactions (Table 2). *Bombus melanopygus* had the lowest average baseline temperatures relative to either *B. impatiens* or *B. vosnesenskii*, which were similar.

Between baseline and initial heating (30-90 sec), *Bombus vosnesenskii* warmed up considerably less (by ~ 3.0ºC in T_{th}; ~ 0.30ºC for T_{ab}) than the other two species each of which warmed by ~5° for T_{th} and ~1.5° for T_{ab}. At equilibrium (120–300 seconds), *B. impatiens* had the highest average T_{th} followed by *B. melanopygus,* which had the highest average T_{ab}. Average T_{th} and T_{ab} were lowest for *B. vosnesenskii*

compared to the other two species; T_{th} only warmed to as much as average T_{ab} for *B. melanopygus*. In dead bee controls (Figure 2), T_{th} (but not T_{ab}) were higher for dead relative to living *B. impatiens* or *B. vosnesenskii* (X^2=35.9, df=1,p < 0.001; Table 1).

Both T_{th} and T_{ab} changed differentially from 30–90 seconds (Table 3), with bigger temperature increases in T_{th} than T_{ab} (Table 1) and differences in heat absorption rates among species (slope: Time*Species effect; Supplemental Table 1a). *B. melanopygus* had the highest heat absorption rate in either body segment (+1.40ºC/min) followed by *B. impatiens* (+1.02ºC/min), and then *B. vosnesenskii* (+0.36ºC/min). However, initial temperature increases only depended on % sugar for T_{ab} in *B. melanopygus* (Figure 3c) which increased by ~1.5ºC over % sugar treatments between 0 and 75% (Supplemental table 2). For *B. vosnesenskii* (Figure 3e), T_{ab} and T_{th} increased only at higher sugar concentrations (95% confidence intervals, 75%; Supplemental table 1 & 2). There was no relationship with % sugar and either T_{th} or T_{ab} for *B. impatiens* (Figure 3a).

After 2 minutes (120-300 seconds), T_{th} and T_{ab} were relatively constant. However, there was a significant part*species*sugar interaction (Table 4) due to higher average T_{ab} in *B. melanopygus* (Figure 3d) of ~1.9ºC increase with % sugar treatments (Supplemental tables 3 & 4). Sugar concentration did not impact either T_{th} or T_{ab} in *B. impatiens* (Figure 3b). However, T_{th} and T_{ab} highest in *B. vosnesenskii* (Figure 3f) only at the highest sugar concentrations.

Discussion

Bombus melanopygus was the only species that increased heat transfer between the body segments in response to acute heat stress with sugar concentration. In our experiment, abdominal warming, was consistent with heat transfer between the segments. Our experimental set-up limited both convective and conductive off-loading to the environment, allowing us to measure heat accumulation in the abdomen. However, since both T_{th} and T_{ab} reached equilibrium, and did not continue to rise after ~2 minutes of applying heat, that heat was likely being off-loaded from the bee to the surrounding environment. We observed slow rhythmic low-frequency abdominal pumping in live experimental bees, suggesting that they were using cooling responses.

Species-specific differences in our experiment could be partially related to differences the experimental design. There was a low but significant random effect between 'observers' (Supplemental table 1b). In addition, origin (lab reared vs. wild caught), experiment year (2015 vs 2016), and misting (*B. impatiens* and *B. melanopygus*).

Differences among bee species – particularly body color – could also have contributed to differential heating, since light colors reflect more heat compared to darker colors. During the first 90 seconds of heat trials, as T_{th} and T_{ab} increased, the overall rate of increase (i.e., heat absorption rate) differed among species. *Bombus vosnesenskii* has the lightest colored thorax, with a dense patch of light yellow hair on their face that extends partway along the thorax and also had the lowest heat absorption rate and body temperatures than the other two species. *B. melanopygus*,

has a mix of dark and yellow hairs on the face that extend into band of dark hair on the thorax and had the highest heat absorption rate overall. *B. impatiens* was intermediate in color pattern, with dark hairs on the face, an almost entirely yellow thorax, and an intermediate heat absorption rate. While body hairs help bumblebees retain body heat when ambient temperatures are low (Heinrich and Vogt 1993), hair color could increase reflectance (passive) and limit heat absorption when ambient temperatures are high.

Sugar concentration did not generally predict heat transfer during the first 90 seconds of heat trials. Abdominal, but not thoracic, temperatures increased (more heat-transfer) linearly with higher sugar concentrations for *B. melanopygus*. Abdominal temperatures increased, and thoracic temperature decreased for *B. vosnesenskii* (making the difference between body parts smaller; also consistent with heat transfer), though only at the highest sugar concentrations. Nectar energy is important for metabolic warm-up (Corbet et al. 1993, Heinrich and Vogt 1993, Nieh and Sánchez 2005, Nieh et al. 2006) and fuels foraging and flight (Heinrich 1979, Nieh and Sánchez 2005, Willmer 2011c, McCallum et al. 2013). Although baseline temperatures or initial warm-up rates during the first 90 seconds did not reflect sugar concentration, at equilibrium, *B. melanopygus* with high sucrose diets had overall stronger cooling responses (i.e., heat transfer) than those with low sucrose diets. Our paper is the first to show that nectar resource quality, measured as concentration, helps some bumblebees avoid overheating via active cooling (Heinrich 1976) when ambient temperatures become stressful.

Bumblebees and other heterotherms have multiple adaptations to control body temperature. Active cooling combines metabolic heat transfer (e.g., from hot thorax to cooler abdomen or *visa-versa* if necessary (Heinrich 1976) with other adaptations such as 'wing-fanning' to help bees maximize convective and evaporative heat loss (Holland and Bourke 2015). In our study, we limited convective heat loss by positioning the wings in front of the heat shield so that wing movements would not fan the abdomen. We also limited (though likely not eliminated) conductive cooling (via styrofoam padding), which is another way that bees can quickly offload abdominal heat (Heinrich 1977). Controlling off-loading allowed us to measure heat accumulation in the bees' abdomens over time. Slow and strong (low frequency, high amplitude) abdominal pulsing was evident in all bumblebees during the heat stress trials, suggesting that they were actively attempting to regulate their body temperature (Figure 1: bypassing counter-current heat exchange; Heinrich 1976, May 1979, Willmer 2011b). A few individuals also regurgitated nectar, possibly an attempt to cool down (i.e., evaporative cooling; (Heinrich 1979)). These behaviors suggest that even though the external body temperatures that we measured in our experiments were lower than the thermal heat stress limit for some bumblebee species (< 45°C Heinrich 1977, 1975), bees were experiencing and responding to heat stress.

Bumblebees help to increase biodiversity in natural ecosystems worldwide. As highly generalist foragers, bumblebees often function as key pollinators in community-level interaction networks. However, bumblebee species diversity and abundance have declined in many areas (Colla et al. 2011, Koch et al. 2012), likely

due to anthropogenic and climatic stressors (Goulson et al. 2008, Grixti et al. 2009, Potts et al. 2010, Cameron et al. 2011, Hatfield et al. 2015b, Thomson 2016). As temperatures increase with climate change, bees will have increasing difficulty balancing their physiological need to cool down with their need to forage. Foraging for floral nectar will become more difficult as temperatures increase because stressed plants produce fewer, smaller, and emptier flowers to conserve critical resources (Pacini et al. 2003, Kim et al. 2011, Scaven and Rafferty 2013, Thomson 2016, De la Luz 2018a) This could lead bumblebees into a 'double-sided climate vice'; at the same time that they require more consumable energy to maintain functional body temperatures within a narrow range, their net energy gain per foraging attempt is lowered via poor quality nectar rewards.

Our results contribute to the limited (Scaven and Rafferty 2013), but growing body of research specifically addressing physiological responses to climate stress in pollination systems. Range and phenological shifts are one way pollinators have circumvented climate stress (Potts et al. 2010, Miller-Struttmann et al. 2015, Pyke et al. 2016), though at the expense of numerous plant species interactions and biodiversity (Seabloom et al. 2006, Memmott et al. 2007, Burkle et al. 2013). Pollinators, and the plants they visit, may be similarly negatively impacted by physiological constraints as high temperatures become the norm with climate change.

Figures

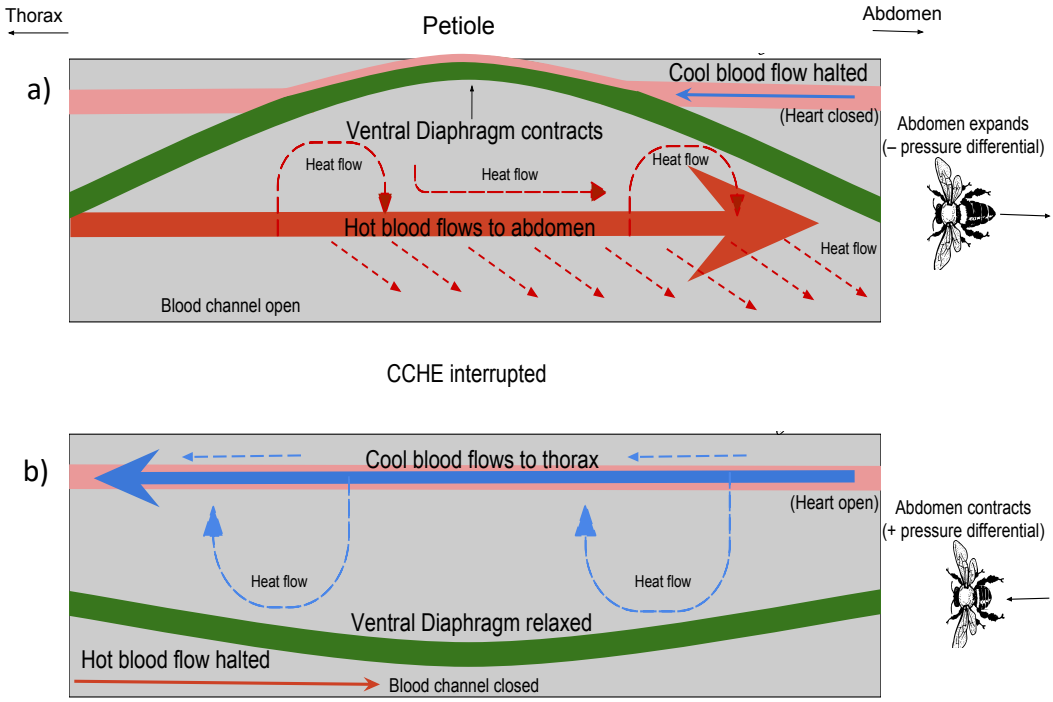

Figure 1. Diagram of counter current heat exchange (CCHE) bypass in bumblebees (adapted from: Heinrich 1993). Warm blood does not contact cool blood (as it does during warm-up for flight) therefor excess heat does not circulate back to the thorax but is instead released via convection/conduction through ventral surfaces (dashed arrows). a) Heat dissipation: abdomen expands creating a negative pressure differential. The ventral diaphragm (VD) muscle (green) contracts to momentarily compress the heart vessel (pink) and cool blood flow (blue) to the thorax is halted. Warm blood (red) passes to the abdomen through the now open blood channel below the VD. Excess heat is released from abdominal ventral surfaces, primarily the 'thermal window' and the (now) cooled blood is taken up by the heart vessel for recirculation. b) Cooling down: Abdomen contracts creating a positive pressure differential. The VD relaxes to momentarily shrink the blood channel and re-open the heart vessel. Warm blood flow to the abdomen is halted while cool blood is pushed into the thorax where it again picks up excess heat and returns to the abdomen as warm blood.

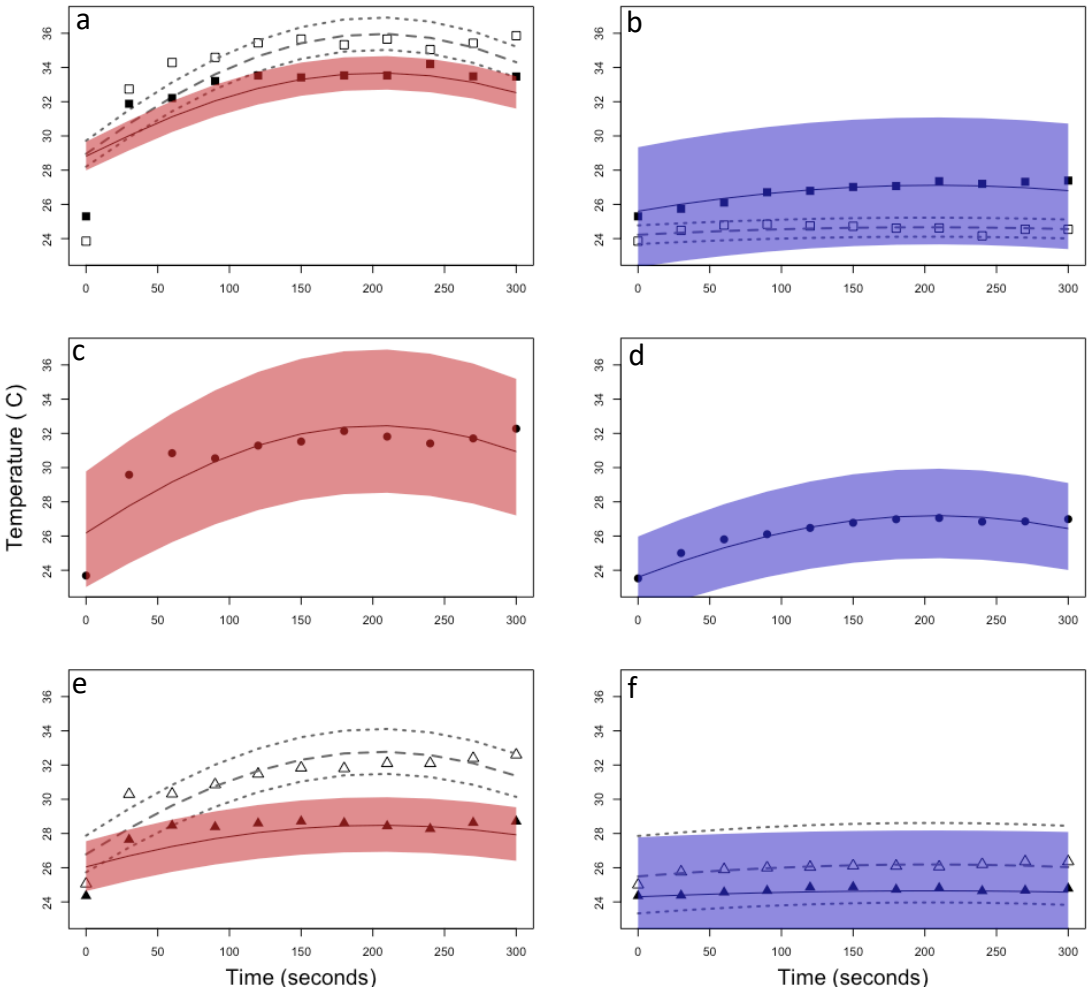

Figure 2. Thorax (red) and abdominal (blue) temperatures for *B. impatiens* (a & b; squares), *B. melanopygus* (c & d; circles), and *B. vosnesenskii* (e & f; triangles). Open points with dashed lines are dead bee controls (none for *B. melanopygus*). Lines are model fits using the initial temperature increase model (0–90 seconds), but over the full 5 minutes (not used for analyses) and clouds are 95% confidence intervals (values same models as 0-90 but for all time points).

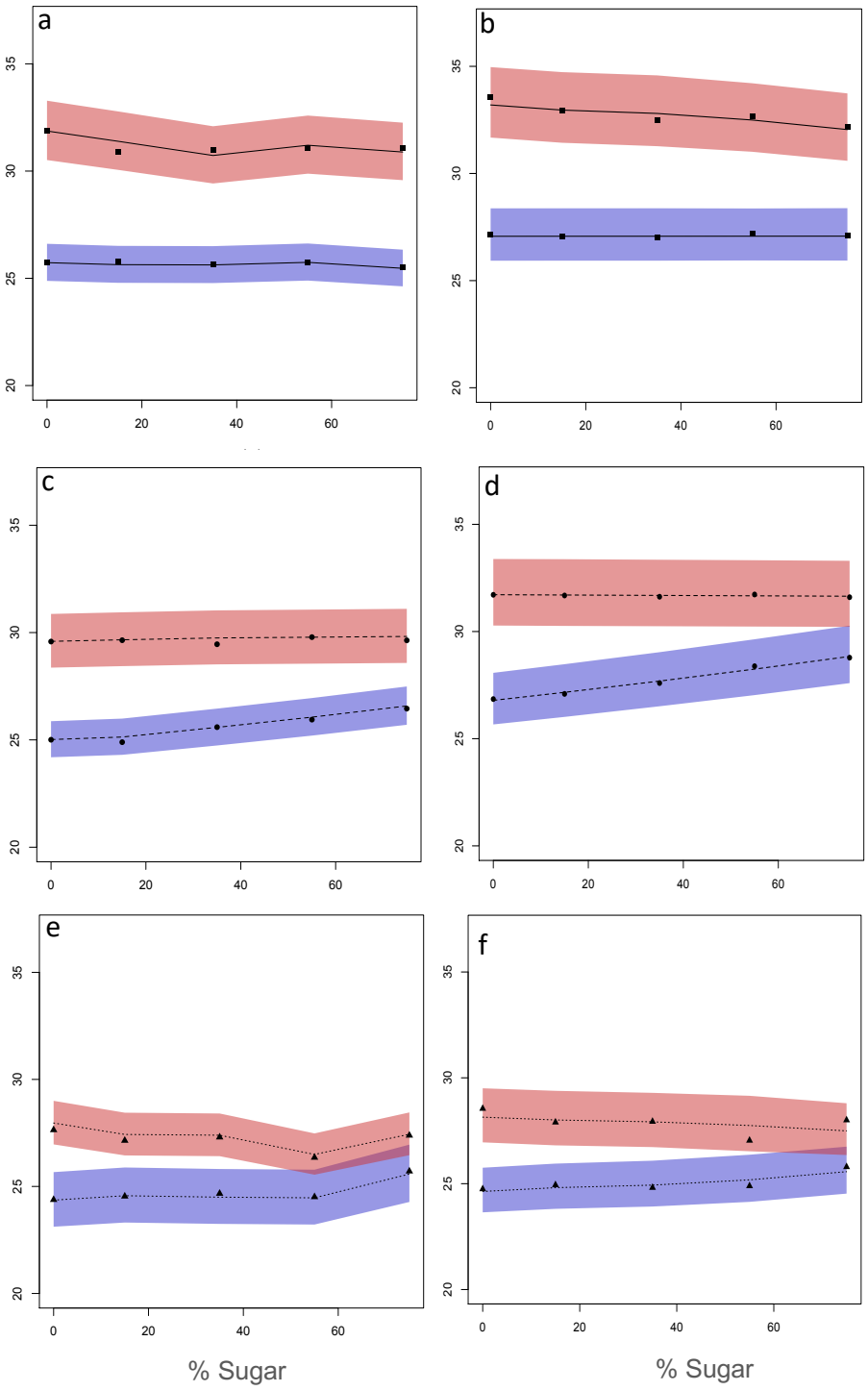

Figure 3. Average thoracic (red) and abdominal (blue) temperatures at each % sugar treatment between 30–90 seconds (left) and 120–300 seconds (right) for *B. impatiens*, (a & b; squares), *B. melanopygus* (c & d; circles), and *B. vosnesenskii* (e & f; triangles). Points are species averages. Lines are model fits. Clouds are 95% confidence intervals.

Tables

Table 1. Temperatures (°C) at time = 0 seconds (baseline), 30–90 seconds (initial increase), and 120-300 seconds (equilibrium) for live B. impatiens, B. melanopygus, and B. vosnesenskii, and for dead bee controls (B. impatiens and B. vosnesenskii). Data are means ±1 standard deviation.

	Baseline (seconds=0)	Initial Increase (seconds=30-90)	Equilibrium (seconds=120–300)
Live experimental bees			
Bombus impatiens			
T_{th}	24.45 ± 0.19	30.18 ± 0.63	32.70 ± 0.16
T_{ab}	24.45 ± 0.19	25.64 ± 0.42	27.07 ± 0.15
Bombus melanopygus			
T_{th}	23.69 ± 0.20	29.72 ± 0.70	31.68 ± 0.25
T_{ab}	23.61 ± 0.19	25.67 ± 0.73	27.74 ± 0.19
Bombus vosnesenskii			
T_{th}	24.41 ± 0.32	27.34 ± 0.11	27.86 ± 0.09
T_{ab}	24.41 ± 0.32	24.69 ± 0.32	25.03 ± 0.03
Dead controls			
Bombus impatiens			
T_{th}	23.85 ± 0.69	33.87 ± 1.00	35.50 ± 0.26
T_{ab}	23.85 ± 0.69	24.69 ± 0.20	24.53 ± 0.19
Bombus vosnesenskii			
T_{th}	25.06 ± 0.12	30.49 ± 0.32	32.14 ± 0.38
T_{ab}	25.00 ± 0.11	25.89 ± 0.12	26.01 ± 0.13

Table 2. ANOVA for baseline temperatures (at time=0) (type-III tests of fixed effects). Baseline temperatures were similar between body segments but varied between species

Fixed effects

Term	Chisq	Df	Pr(>Chisq)
(Intercept)	$2.54e^{5}$	1	< 0.0001
Species	68.40	2	< 0.0001
Sugar	1.98	1	0.159
Part	0.00	1	1.000
Species*Sugar	0.265	2	0.876
Sugar*Part	0.00	1	1.000
Species*Part	0.223	2	0.891
Species*Sugar*Part	0.117	2	0.943

Table 3. ANOVA for baseline temperatures the initial temperature increase (30-90 seconds), showing type-III tests of fixed effects and random effects. Observer and individual were random effects.

	Fixed effects		
Term	**Chisq**	**Df**	**Pr(>Chisq)**
(Intercept)	162790.00	1	<0.0001
Time	50.95	1	<0.0001
Species	20.48	2	<0.0001
Sugar	2.18	4	0.703
Part	507.19	1	<0.0001
Time	40.09	2	<0.0001
Species*Sugar	71.58	8	<0.0001
Part*Sugar	6.71	4	0.152
Species*Part	43.11	2	<0.0001
Species*Part*Sugar	20.23	8	0.009

Table 4. ANOVA for equilibrium temperatures (120-300 seconds). Since temperatures were relatively stable, time was not a factor. We tested for effects of species, and sugar in the different segments. Type-III tests of fixed effects.

	Fixed effects		
Term	**Chisq**	**Df**	**Pr(>Chisq)**
(Intercept)	11773.8731	1	<0.0001
Part	1026.6536	1	<0.0001
Species	389.3156	2	<0.0001
Sugar	0.001	1	0.975
Species*Part	16.0369	2	0.0003
Part*Sugar	7.0568	1	0.008
Species*Sugar	72.7848	2	<0.0001
Species*Part*Sugar	22.415	2	<0.0001

Supplemental Materials:

Supplement 1. Model code (in R) for each linear-mixed effects models at baseline (time=0seconds), initial temperature increase (0–90 seconds), and at equilibrium (120–300) in R with packages LMER and LME4. Time and sugar are fixed factors for tests of main effects and numeric (i.e., 'poly()' as a random variables) and to calculate slopes.

Baseline model (0 seconds):

Temperature ~ Inv. Gaussian(link=log)

temp ~ time*sp + as.factor(sugar)*sp*part + (0+poly(sugar, 1)|obs) + (0+poly(time, 2)|ind:part)

Initial temperature increase model (0–90 seconds):

Temperature ~ Inv. Gaussian(link=log)

temp ~ time*sp + as.factor(sugar)*sp*part + (0+poly(sugar, 1)|obs) + (0+poly(time, 2)|ind:part)

Equilibrium model (120–300 seconds):

Temperature ~ Inv. Gaussian(link=1/mu^2)

temp ~ part*sp*sugar + (1|obs) + (1|ind/part)

Supplemental Table 1a. Fixed effects summary table for the ANOVA of the initial temperature increase (30–90 seconds).

Effect	Term	Estimate	SE	t	P
(*B. imp;* abdom; sugar=0)	Intercept	3.23000	0.00801	403.467	<0.0001
Time	Time	0.00058	0.00008	7.138	<0.0001
Sp	*B. mel*	-0.03517	0.01043	-3.37	0.00075
Sp	*B. vos*	-0.04421	0.00992	-4.458	<0.0001
Sugar	Sugar=15	-0.00364	0.00878	-0.414	0.678697
Sugar	Sugar=35	-0.00411	0.00897	-0.458	0.64664
Sugar	Sugar=55	0.00056	0.00849	0.066	0.947454
Sugar	Sugar=75	-0.01033	0.00866	-1.193	0.233017
Part	Thorax	0.21390	0.00950	22.521	<0.0001
Time*Sp	Time*_B. mel_	0.00023	0.00011	2.165	0.030415
Time*Sp	Time*_B. vos_	-0.00035	0.00010	-3.424	0.000616
Sp*Sugar	_B. mel_*S15	0.00832	0.01147	0.726	0.468023
Sp*Sugar	_B. vos*S15_	0.01204	0.01101	1.094	0.273986
Sp*Sugar	_B. mel*S35_	0.02655	0.01163	2.282	0.022486
Sp*Sugar	_B. vos*S35_	0.00978	0.01110	0.881	0.378208
Sp*Sugar	_B. mel*S55_	0.04032	0.01129	3.572	0.000354
Sp*Sugar	_B. vos*S55_	0.00384	0.01061	0.362	0.717524
Sp*Sugar	_B. mel*S75_	0.07101	0.01144	6.206	<0.0001
Sp*Sugar	_B. vos*S75_	0.05914	0.01084	5.454	<0.0001
Part*Sugar	Thorax*S15	-0.01152	0.01312	-0.878	0.379991
Part*Sugar	Thorax*S35	-0.03234	0.01336	-2.42	0.015517
Part*Sugar	Thorax*S55	-0.02160	0.01266	-1.706	0.088089
Part*Sugar	Thorax*S75	-0.02091	0.01292	-1.618	0.105655
Sp*Part	_B. mel_*Thorax	-0.04599	0.01223	-3.762	0.000169
Sp*Part	_B. vos_*Thorax	-0.07601	0.01161	-6.545	<0.0001
Sp*Part*Sugar	_B. mel*S15*Thorax_	0.00929	0.01705	0.544	0.586102
Sp*Part*Sugar	_B. vos*S15*Thorax_	-0.01618	0.01631	-0.992	0.321335
Sp*Part*Sugar	_B. mel*S35*Thorax_	0.01526	0.01726	0.884	0.376621
Sp*Part*Sugar	_B. vos*S35*Thorax_	0.00607	0.01642	0.369	0.711768
Sp*Part*Sugar	_B. mel*S55*Thorax_	-0.01279	0.01674	-0.764	0.444762
Sp*Part*Sugar	_B. vos*S55*Thorax_	-0.03671	0.01570	-2.339	0.01933

Sp*Part*Sugar	*B. mel*S75*Thorax*	-0.03210	0.01695	-1.894	0.058243
Sp*Part*Sugar	*B. vos*S75*Thorax*	-0.04678	0.01602	-2.919	0.00351

Supplemental Table 1b. Random effects summary table for the ANOVA of the initial temperature increase (30–90 seconds). There was a small but significant observer effect. Time and sugar are continuous random variable (e.g., "poly()").

Groups	Name	Std.Dev.
Ind:Part	poly(time, 1)	0.00
	poly(time, 2)	$6.31e^{-7}$
Observer	poly(sugar, 1)	$1.37e^{-8}$
Residual		$7.13e^{-3}$

Supplemental Table 2. 95% Confidence intervals for the highest order effects in initial temperature increase model (30–90 seconds). Means that are not contained in another variable's confidence intervals are significantly different.

Component	Effect	Covariate	Species	Part	Sugar	LCL	Mu	UCL
Slope	Time*Sp	Time	*B. vos*			1.0001	1.00023	1.00004
Slope	Time*Sp	Time	*B. imp*			1.0004	1.0006	1.0007
Slope	Time*Sp	Time	*B. mel*			1.0007	1.0008	1.0010
Intercept	Sp*Sugar*Part		*B. imp*	Abdomen	0	24.89	25.28	25.68
Intercept	Sp*Sugar*Part		*B. imp*	Abdomen	15	24.81	25.20	25.58
Intercept	Sp*Sugar*Part		*B. imp*	Abdomen	35	24.79	25.18	25.58
Intercept	Sp*Sugar*Part		*B. imp*	Abdomen	55	24.93	25.30	25.67
Intercept	Sp*Sugar*Part		*B. imp*	Abdomen	75	24.65	25.03	25.40
Intercept	Sp*Sugar*Part		*B. imp*	Thorax	0	30.79	31.31	31.85
Intercept	Sp*Sugar*Part		*B. imp*	Thorax	15	30.35	30.85	31.35
Intercept	Sp*Sugar*Part		*B. imp*	Thorax	35	29.70	30.20	30.70
Intercept	Sp*Sugar*Part		*B. imp*	Thorax	55	30.19	30.66	31.14
Intercept	Sp*Sugar*Part		*B. imp*	Thorax	75	29.87	30.36	30.84
Intercept	Sp*Sugar*Part		*B. mel*	Abdomen	0	24.09	24.41	24.73
Intercept	Sp*Sugar*Part		*B. mel*	Abdomen	15	24.20	24.53	24.85
Intercept	Sp*Sugar*Part		*B. mel*	Abdomen	35	24.64	24.95	25.30
Intercept	Sp*Sugar*Part		*B. mel*	Abdomen	55	25.09	25.43	25.77
Intercept	Sp*Sugar*Part		*B. mel*	Abdomen	75	25.59	25.95	26.29
Intercept	Sp*Sugar*Part		*B. mel*	Thorax	0	28.48	28.88	29.28
Intercept	Sp*Sugar*Part		*B. mel*	Thorax	15	28.55	28.93	29.35
Intercept	Sp*Sugar*Part		*B. mel*	Thorax	35	28.63	29.02	29.43
Intercept	Sp*Sugar*Part		*B. mel*	Thorax	55	28.66	29.05	29.47
Intercept	Sp*Sugar*Part		*B. mel*	Thorax	75	28.70	29.11	29.50
Intercept	Sp*Sugar*Part		*B. vos*	Abdomen	0	23.91	24.19	24.47
Intercept	Sp*Sugar*Part		*B. vos*	Abdomen	15	24.11	24.39	24.69
Intercept	Sp*Sugar*Part		*B. vos*	Abdomen	35	24.05	24.34	24.61
Intercept	Sp*Sugar*Part		*B. vos*	Abdomen	55	24.02	24.29	24.57
Intercept	Sp*Sugar*Part		*B. vos*	Abdomen	75	25.11	25.41	25.70
Intercept	Sp*Sugar*Part		*B. vos*	Thorax	0	27.44	27.77	28.10
Intercept	Sp*Sugar*Part		*B. vos*	Thorax	15	26.90	27.25	27.57
Intercept	Sp*Sugar*Part		*B. vos*	Thorax	35	26.87	27.19	27.53
Intercept	Sp*Sugar*Part		*B. vos*	Thorax	55	26.01	26.31	26.62
Intercept	Sp*Sugar*Part		*B. vos*	Thorax	75	26.93	27.25	27.57

Supplemental Table 3. Fixed effects summary table for the equilibrium model (120-300 seconds).

Effect	Term	Estimate	SE	t	P
(*B. imp;* abdom; sugar=0)	Intercept	1.37E-03	1.26E-05	108.507	<0.0001
Part	Thorax	-4.58E-04	1.43E-05	-32.041	<0.0001
Sp	*B. mel*	2.88E-05	1.91E-05	1.505	0.132322
Sp	*B. vos*	2.83E-04	1.61E-05	17.639	<0.0001
Sugar	Sugar	-9.25E-09	2.96E-07	-0.031	0.975038
Sp*Part	*B. mel*Thorax*	5.79E-05	2.16E-05	2.677	0.007433
Sp*Part	*B. vos*Thorax*	7.21E-05	1.83E-05	3.93	<0.0001
Part*Sugar	Thorax*Sugar	8.93E-07	3.36E-07	2.656	<0.0001
Sp*Sugar	*B. mel*Sugar*	-2.54E-06	3.14E-07	-8.097	<0.0001
Sp*Sugar	*B. vos*Sugar*	-1.57E-06	3.79E-07	-4.131	<0.0001
Sp*Part*Sugar	*B. mel*Thorax*Sugar*	1.72E-06	3.62E-07	4.734	<0.0001
Sp*Part*Sugar	*B. vos*Thorax*Sugar*	1.49E-06	4.34E-07	3.423	0.000619

Supplemental Table 4. 95% Confidence intervals for the highest order effects in the equilibrium model (120–300 seconds). Means that are not contained in another variable's confidence intervals are significantly different.

Effect	Sp	Part	Sugar	LCL	Mu	UCL
Sp*Part	B. imp	Ab		1.34e-3	1.37e-3	1.39e-3
Sp*Part	B. imp	Th		8.84e-4	9.07e-4	9.31e-4
Sp*Part	B. mel	Ab		1.37e-3	1.39e-3	1.42e-3
Sp*Part	B. mel	Th		9.66e-4	9.94e-4	1.02e-3
Sp*Part	B. vos	Ab		1.63e-3	1.65e-3	1.67e-3
Sp*Part	B. vos	Th		1.24e-3	1.26e-3	1.28e-3
Sp*Part*Sugar	B. imp	Ab	Sugar	-5.89e-7	-9.25e-9	5.70e-7
Sp*Part*Sugar	B. imp	Th	Sugar	3.28e-7	8.83e-7	1.44e-6
Sp*Part*Sugar	B. mel	Ab	Sugar	-2.75e-6	-2.55e-6	-2.34e-6
Sp*Part*Sugar	B. mel	Th	Sugar	-1.09e-7	5.92e-8	2.27e-7
Sp*Part*Sugar	B. vos	Ab	Sugar	-2.04e-6	-1.58e-6	-1.11e-6
Sp*Part*Sugar	B. vos	Th	Sugar	3.51e-7	8.03e-7	1.25e-6

Synthesis

Climate change and other large-scale stressors such as habitat fragmentation and species invasions negatively impact plant-pollinator interactions, and likely other, important species-interactions (Tylianakis et al. 2008, Hegland et al. 2009, Burkle et al. 2013). Impacts of environmental stressors, including range shifts and phenological mismatches between plants and pollinators, have been shown for several different regions (Parmesan and Hanley 2015). Much less is understood about how changes in resource quality due to climate might de-couple species interactions (Memmott et al. 2007, Tylianakis et al. 2008, Hegland et al. 2009, Parmesan and Hanley 2015) . In areas where plant-pollinator phenologies have usually aligned, individual physiological responses to climate stress could induce mismatches that ultimately impact interaction network structure at the community-level.

In plants, physiological stress during reproductive growth elicits a trade-off that is at odds with the energetic needs of pollinators when they are also stressed. For instance, abiotic stress reduces nectar rewards in some plant species as they compensate for survival (Stearns 1989, Vargas et al. 2004, Schultner et al. 2013) but bees need high quality floral nectar to maintain energy balance at high temperatures. This tension sets up a resource-based mismatch between partners that is likely to impact pollinator foraging patterns and subsequently, population-level reproductive success for both trophic levels as average temperatures increase and rainfall becomes more variable.

My dissertation links divergent stress responses of plants and pollinators in the face of environmental stress and its impacts on plant-pollinator network structure. I applied what I learned about stress response mechanisms in lab experiments to explore implications for plant-pollinator network structure and stability in two distinct habitat types. I conducted a long-term, detailed study of plant-pollinator networks in the field (2013-2015) that I interpret in the context of environmental stress (mostly climate). Specifically, I tested 1) how nectar diet (% sugar) limits a bumblebees' ability to cool its body when heat stressed; and 2) how temperature and water stress limit floral resources and subsequent bumblebee foraging. I also 3) used historical and resurvey data in grassland and sandhill habitats to compare plant-pollinator network metrics related to stability, with respect to *a priori* hypotheses about habitat filtering and stress tolerance.

Bumblebee diet and heat stress: My study is among the first to explicitly examine the increased importance of nectar diet for bumblebees that need to quickly moderate body temperature when heat stressed. I found that 3 bumblebee species under acute heat stress were better able to transfer heat from their heated head/thorax regions to their (unheated) abdomens as nectar sugar concentration increased. These results are consistent with that of B. Heinrich's fundamental and in-depth study of bumblebee thermoregulation. However, much of Heinrichs work employed virgin queen bees rather than spring foragers that could have different energetic demands than queens. More recent work on bumblebees show that bumblebees increase baseline thoracic temperatures while feeding (Nieh et al. 2006), and that tropical

stingless bees extended their foraging ranges after eating higher sugar concentrations (Nieh and Sánchez 2005). We take this work further to indicate that a high quality nectar diets become increasingly important for bumblebee thermoregulation when foraging at high temperatures. Because of their already high metabolic rate, bumblebees should forage on the highest quality resources that they can access, within their foraging range, to avoid overheating when ambient temperatures are high.

Some bees may struggle to overcome physiological constraints of both cooling down and foraging as temperatures continue to increase and plant resources decrease over large spatial scales; this pattern will likely be exacerbated by water stress, which is also predicted to intensify (Cayan et al. 2008, IPCC 2013). These physiological dilemmas are likely to have similar negative impacts on the many other heterothermic bee and insect species other than bumblebees that depend on energetic resources from plants (Potts et al. 2010, Willmer 2011a). Next research steps would determine how nectar diet and heat stress interact to influence the combination of thermoregulation and foraging at high temperatures in native bees.

Plant floral rewards and bumblebee foraging: Temperature and water stress reduce nectar, flower quality, and subsequent foraging on those plants by bumblebees when they are given a choice. I measured a suite of floral traits (corolla size, flower count, nectar volume and concentration) in three native California plant species that I grew under a range of temperatures and water availability. All three

species showed reduced nectar reward (though not always other flower attraction traits) in response to increased stress. These results are consistent with similar studies that tested resource output in plants grown under stress (Carroll et al. 2001, Halpern et al. 2010, Newman and Wagner 2013). Additionally, bumblebees given choices among plants from all fifteen temperature and water treatments spent less time foraging on individuals that had fewer and smaller flowers and thus, lower predicted nectar reward. However, seed set was not always highest in plants with the highest nectar quality.

Bees use a combination of visual and olfactory cues to determine flower's foraging values. Those cues are usually, though not always, honest indicators of nectar quality (Milet-Pinheiro et al. 2015). For instance, in my experiments with *Clarkia unguiculata,* flower attraction traits were not as well correlated with nectar quality as in the other two species; but, this species has a range of alternative strategies such as self-fertilization, shortened bloom times, or dishonest floral cues that likely help compensate for the physiological constraints of growing under stress (Mazer et al. 2004, Ivey et al. 2016). Nonetheless, biotic pollination is closely tied to population-level success in plants despite these alternative strategies (Ollerton et al. 2011).

Plant-pollinator networks in two habitats over time: Plant-pollinator network structure was similar between grassland and sandhill habitats in surveys during 1991-1994 (Randall Morgan collection). At that time, grasslands had overall

higher plant and bee species richness, more hub species in each trophic level, and more links per species compared to sandhills. Also, network specialization was lower in grasslands relative to sandhills. However, nestedness – which has been used to imply network robustness (Okuyama and Holland 2008, Thébault and Fontaine 2010, Rohr et al. 2014) – was similarly low (< 3) in both habitats.

I tested the hypothesis that grassland bee and plant species richness would be more stable over time with increased environmental stress (particularly temperature) due to lower baseline levels of abiotic habitat stress relative to that of sandhills. However, my resurveys (2013-2015) of the same sites found unexpected differences in how each habitat changed over time. In contrast to the hypothesis, plant species richness fell in grassland networks but remained stable in sandhill networks, while bee species richness was stable in both networks over time. The networks in both habitats became much less specialized between surveys and show increased niche overlap for both trophic levels. Further, nestedness increased in grasslands but decreased in sandhills over time despite both habitats being similarly nested during historical surveys.

One potential explanation for these differential habitat responses is the higher abundance of non-native plant and bee species in grasslands relative to sandhills. Interactions with non-native species that compete for limiting resources might be amplified under climate stress (Esch et al. 2018). In our study, some non-native species in both habitats became increasingly integrated into key network positions

(e.g., hubs) over time. Nonetheless, former native hub species are still mostly present in each network, though they have been displaced as hubs. Spatial network comparisons and simulations on invasive species impacts on mutualistic networks show similar patterns (Memmott and Waser 2002, Aizen et al. 2008, Valdovinos et al. 2009). My results are the first to show this pattern occurring in multiple systems over time, and may relate to new, developing theories about how higher-level 'multi-plex' interactions, or between-network interactions (i.e., competition networks), influence within-network dynamics in ecological systems (P. Jordano, *personal communication)*. The importance of 'multi-plex' interactions cannot be discounted when studying ecological network changes over time.

Despite being used to assess robustness, nestedness could actually overestimate network robustness due to other species interactions, for instance with non-native species, that are simultaneously occurring within a community. Therefore, nestedness should be used with caution as an *a priori* diagnostic metric. Nonetheless, researchers and land managers could use certain mutualistic network metrics to strategically target ecologically important species. For example, identifying and reestablishing native hubs, while removing non-native hubs from areas where they are present could become a viable strategy to restore degraded communities. However, research should first focus on how removing non-native hub species will impact current levels of biodiversity in a community.

Conclusions

Temperature and water stress negatively impact plant-pollinator communities when physiological stress-responses of interacting species are misaligned. Bees are less able to thermoregulate when heat stressed after eating lower concentration nectar. This becomes a physiological problem for bees, since plants produce low quality flowers when grown under stress, making them generally less attractive to foragers. These disparate stress responses could be important in plant-pollinator network assembly and disassembly even if species continue to co-occur across landscapes. If fewer species are interacting, then plant-pollinator network structure becomes less complex. As a result, networks would be less stable against stress and further loss of species (i.e., extinction cascades).

Focused experiments for each trophic level help identify mechanisms that potentially scale-up to drive network assembly patterns and limit overall network stability in a more stressful landscape. Multi-year datasets that also capture high levels of spatial variation are critical to understand community-level dynamics of plant-pollinator systems. Resurveys over time are needed to assess how those systems have responded to changing climate and other stressors. Mutualistic network metrics are useful for monitoring –but possibly not diagnosing– ecosystem health and for pinpointing ecologically critical species for research and conservation.

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