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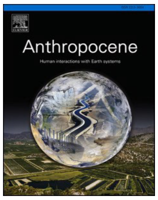

Reconstructing butterfly-pollen interaction networks through periods of anthropogenic drought in the Great Basin (USA) over the past century

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ABSTRACT

Interactions between plants and insects dominate terrestrial biomes and are altered in response to global human-environmental change. Documenting such changes in complex interactions is challenging, however, because traditional methods for describing plant-insect interactions at community scales are often based on relatively short sampling periods. This paper investigates quantitative networks of pollen-insect interactions gleaned from adult Lepidoptera from long-term museum collections that helped to overcome the challenge of limited temporal resolution. The paper reports how richness and frequency of butterfly-pollen associations have changed over a 100-year time series (1910–2020) in the Great Basin of Nevada and California, USA. First, we examined changes in pollen richness for 19 butterfly species over five consecutive 20-year time periods. We pooled interaction networks associated with specimens captured before and after the onset of drought in 2000. In doing so, we estimated variation in pollen-pollinator interactions under anthropogenic drought periods in the Great Basin in the last two decades. Overall, pollen richness associated with butterflies declined slightly over the study period. The details depend on the species, however, where a few species experienced moderate declines in richness and two species exhibited small increases in pollen richness. Butterfly-pollen networks indicated specialization in most pollen-butterfly species interactions. They are apparently more reticulate than observational networks. Interaction networks associated with specimens captured before and after the year 2000 revealed that, compared to previous decades, butterfly-pollen networks over the past 20 years had higher connectivity and diversity of interactions.

1. Introduction

Plant-pollinator interactions contribute to biodiversity and provide ecosystem stability in the face of climate change and human-environmental stress. To examine how global change may have affected these interactions, studies must quantify networks of plant-pollinator associations to estimate how pollinators' habitat, diet, migration, and impact on plant reproduction (Jones, 2012, 2014) are changing over time. This approach with butterfly pollinators is an important tool for identifying species that are both threatened and ecologically important, such that management efforts for imperiled species overlap with those for ecosystem function (Zografou et al., 2020). Long time series of such interactions are infrequently available, however, making it difficult to examine how quantitative networks respond to climate change or to other anthropogenic perturbations (Cuartas-Hernández and Medel, 2015; Ponisio et al., 2017). More generally, some recent declines of species diversity and interaction diversity in multi-trophic webs have apparently occurred within two decades in response to multiple global change parameters (e.g., Salcido et al., 2020; Wagner et al., 2021), but details of those declines were limited due to short time series. Examining network changes over long periods of time provide the best methods to inform how plant-insect interaction webs may have changed with global change and identifying taxa and interactions that are most susceptible to disturbances (Wagner et al., 2021). The current limitations of network studies attempting to quantify human impacts on interactions are a consequence of static, short-term, and spatially vague pollination interaction networks that are common for documenting plant-visitor interactions, pollen-insect interactions, and pollinator effectiveness (Ballantyne et al., 2015; Tur et al., 2016). These methods yield a plethora of metrics at both the network and species level that do not provide information about changes in fertilization efficacy. They can provide useful quantitative summaries of some key pollination network properties (Gibson et al., 2011; Jauker et al., 2018), however, particularly if the networks are

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connected to pollinator effectiveness (Ballantyne et al., 2015). Proper interpretations of selected network parameters can provide understanding of the pollinators' responses to short-term and small-scale habitat alteration and changes in diversity by comparing changes in interactions over time or across disturbance gradients. Responses to climate change, however, requires longer time series (Proulx et al., 2005; Ballantyne et al., 2015; Bohan and Dumbrell, 2017; Zografou et al., 2020). Analyzing network indices is also a useful tool for assessing the level of generalization or specialization for species on either side of the bipartite network. Published pollination networks have enabled estimation of diet breadths of pollinators on a continuum ranging from extreme specialists (one link between the pollinator and its host plant) to extreme generalists (many links between the pollinator and its host plants). Estimates are based, however, solely on network structure (Dormann et al., 2009; Bohan and Dumbrell, 2017; Novella-Fernandez et al., 2019). Estimates of specialization provide insight into conservation challenges and shifts in webs in response to global human-environmental change.

Traditional pollen analysis is an alternative to floristic surveys and ecological visitation networks. Existing visitation network methods in ecological studies typically yield short-term localized species richness and interaction diversity estimates. Alternatively, long time series over great spatial extents can be synthesized using pollen analysis, which is an approach commonly used in the reconstruction of past climate, vegetation history, historical ecology, and biodiversity (Burjachs and Julia, 1994; Matthias et al., 2015; Shennan et al., 2015; Gosling et al., 2018; Balmaki et al., 2019; Diaz et al., 2019). Analysis of pollen associated with insects has been used recently for addressing questions related to insect food sources and habitats (Jones, 2012; Silberbauer et al., 2004), but this approach has not been used to examine butterfly-pollen networks. For the pollen analysis approach, morphological and ecological characteristics of the insects determine pollen diversity on pollinators' bodies. Pollen persists on lepidopteran bodies long after collection and curation procedures (Courtney et al., 1982), so analyzing pollen grains on butterflies can provide information on insect-pollen interactions that is distinct from existing pollination network approaches.

Historically, flower visitation networks have served as the most common method for examining plant-pollinator interactions. For these networks, visitors to flowers of a particular plant are recorded and the interaction frequency provides a proxy for pollination. Visitation does not always correspond to successful pollen transfer, however, and this method can be ineffective in predicting the importance of particular pollinators or studying human impacts on pollinator communities (King et al., 2013). Some visitors do not pick up pollen, they simply remove pollen without transferring it to a conspecific flower, or they interfere with pollination by blocking the stigma of the flower (Ballantyne et al., 2015).

More advanced methods of studying plant-pollinator interactions have emerged in recent years. They present more precise alternatives to studying flower visitation (Garratt and Potts, 2011; O'Connor et al., 2019), including the approach of merging identification methods from palynology with samples collected from insects identified and curated. While butterflies are foraging for nectar with their proboscis, pollen grains stick to their eyes, proboscis, frons, antennae, wings, and legs, which renders them as pollen carriers and potential pollinators (Willmer, 2011). Identification and quantification of pollen grains found on the bodies of butterflies or other pollinators can provide information about the plants that an individual has visited. The grains are useful for creating plant-pollen networks for multiple inferences, assuming that this procedure estimates actual pollination networks (Butler and Johnson, 2020). Although the presence of pollen on insects imperfectly indicates which plants the insect provides pollination services to, it suggests an interaction with the reproductive floral parts of that species (Jennersten, 1984; Butler and Johnson, 2020). This method may provide an insightful alternative to the visitation networks that dominate the pollination network literature (Silberbauer et al., 2004; Kleijn and Raemaker, 2008; Jones, 2012; Scheper et al., 2014).

Because of this presence of pollen on curated insects, natural history museums are an important tool for recording taxonomic diversity. They can provide insight into native pollinator communities across spatial and temporal gradients (Colla et al., 2012; Titeux et al., 2017; Seltmann et al., 2017). Museums and their collections are also a key part of efforts to document and predict the consequences of habitat loss, fragmentation, invasive species, and climate change. The few studies that have used museum specimens to quantify insect movements and temporal changes in pollinators have mostly focused on the order Hymenoptera (Silberbauer et al., 2004; Wood et al., 2019). These studies have effectively documented striking spatial and temporal patterns. Wood et al. (2019) found that specimens from declining bumblebee species exhibited a one-third decrease in pollen richness, for example, from individuals collected before and after 2000.

The Sierra Nevada and Great Basin (USA) is an ideal location for investigating plant-insect interactions in the context of global change. Weather patterns are rapidly changing, and these changes have been especially severe in the last two decades (McEvoy et al., 2012; Hatchett et al., 2015). Substantial changes include decreasing snowpack and annual precipitation, and increased fire frequency (Belmecheri et al., 2016). The compounding effects of drought and warming due to anthropogenic change have exacerbated in last two decades (Mukherjee et al., 2020a, b), and evidence suggests anthropogenic warming in the western US accounts for some of the variability in recent drought events (Williams et al., 2015). Concurrently, invasions by exotic plants have increased, outcompeting the endemic species over the past 100 years. The proliferation of drought resistant and fire tolerant plants has contributed to a decline in the density of native plants, which are not adapted to these climate extremes (Rondeau, 2013). These changes in the composition of plants necessarily result in shifting pollinator communities that interact with them.

This study examines how richness and abundance of butterfly-pollen interactions have changed in the Great Basin and Eastern Sierra (USA) over the last century. It utilized data for plant-insect interaction available in collections for the order Lepidoptera at the University of Nevada Museum of Natural History (UNRMNH). The study addressed the general question of how butterfly-pollen interactions have changed over the period from 2010 to 2020, when climate change has occurred including droughts. We posed the following specific questions. First, how have butterfly-pollen network parameters changed in the period prior to, and following the year 2000, the latter of which saw increasing drought frequency and intensity. We hypothesized that the richness of pollen species associated with butterflies would exhibit negative impacts of climate change and found less commonly associated with butterflies. Second, we ask how has pollen species richness on Great Basin butterflies changed throughout the time period from 1910 to 2020. We hypothesized that increased frequency and intensity of drought in the Great Basin associated with climate change is partly responsible for these changes in pollen species richness associated with butterflies.

2. Methods

2.1. Data collection and pollen analysis

One hundred seventy-three Lepidopteran specimens from the most common species sampled over the past century were selected from the University of Nevada, Reno Museum of Natural History (UNRMNH) to collect pollen grains from their bodies. These specimens were collected between 1910 and 2020 in the Great Basin region of Nevada as well as the Sierra Nevada Mountain range in Nevada and California (Supplementary Material 1 Table 1A, B). One hundred nineteen of these specimens were collected before 2000 (the onset of severe drought in the region) and 54 specimens were collected after 2000. The samples represented three families in Lepidoptera and included 19 different species.

We rinsed the specimens with 95% ethyl alcohol (ETOH) to collect all pollen grains from their external tissues, including the proboscis, legs, and compound eyes (Fig. 1). Then, we used entomological pins to remove the remaining pollen grains from each insect's external tissue under a binocular microscope. We stored all recovered pollen grains in vials with 2000 cs silicone oil volume and stain (Safranin-O) to highlight morphological features (Jones, 2014). Representative pollen samples were mounted on glass slides by adding two drops of the sample solutions to a clean glass slide, securing with a coverslip, and sealing with clear nail polish. Light microscopy (LM) and a scanning electron microscope (SEM) enabled pollen identification. In addition, the Great Basin pollen database at the UNRMNH provided reference slides for pollen identification. All collected pollen grains were identified to the level of genus or species, with no unknowns.

2.2. Network and statistical methods

For each specimen, we quantified pollen species abundances and unique interaction frequencies for each available year from 1910 to 2020. These data enabled comparisons of patterns in diversity and abundance across time. Well-established protocols provided estimates of ecological networks and parameters used for statistical inferences (e.g., Pardikes et al., 2018; Dell et al., 2019; Salcido et al., 2020). We analyzed networks using the "bipartite" package in R (Fig. 2, Supplementary Material 2 Fig. 1), a commonly used approach for examining plant-pollinator interactions (e.g., Ballantyne et al., 2015). The nodes in the bipartite network represent the plant and pollinator (i.e. pollen-carrying) species. We determined the edges by quantifying pollen-insect associations present in museum specimens, with the width of edges representing the frequency of encounter of a given interaction. We calculated network indices including connectance, network specialization (H2'), and other commonly reported network metrics (Supplementary Material 1 Table 1B). Connectance represents the number of links between nodes over the number of species squared in a network. This parameter summarizes the number of realized possible connections (Martinez, 1992). Network specialization is a network-level index that summarizes the degree of specialization and is useful for comparisons across multiple networks. Values of network specialization range between zero and one, with zero representing complete generalization and one representing complete specialization (Blüthgen et al., 2006). We also calculated the Simpson's diversity entropy for pollen species found on each species of butterfly and converted this to effective number of species (Table 1) (Jost, 2006).

Two warm events with impacts in California and Nevada around the year 2000 (McEvoy et al., 2012; Hatchett et al., 2015; Belmecheri et al., 2016) guided selection of two distinct pollen-pollinator interaction networks. The first one utilized the data collected before 2000 (17 butterfly species from 120 specimens), and the second using the data collected after 2000 (14 butterfly species from 53 specimens) (Fig. 4, Supplementary Material 1; Table 1C, D). We also divided the data into 20-year periods (and lumped the first two 20-year periods, since the first 20 years only yielded 9 specimens) and created subsets of networks for those periods. We calculated network parameters for these temporal groups to compare the level of specialization for the selected butterflies over time, considering the changing climate before and after the onset of drought (2000), as well as across equally consecutive 20-year periods (Supplementary Material 1 Table 1A).

Hypothesis testing focused on pollen richness per butterfly species. We included these values as response variables in hierarchical Bayesian linear models to examine changes in butterfly associated pollen diversity over time (details are in the Supplementary material 2, Fig. 2). For pollen richness, butterfly species were treated as lower levels in the hierarchy. Parameters were estimated for the change in richness over time for each species as well as for overall pollen richness. Following Salcido et al. (2020) and Wagner et al. (2021), we used a conservative (i.e. less likely to make Type II error) 80% credibility interval range to compare parameter estimates of changes in richness to zero. The reasoning is that, for species changes, mistakes of inferring that a species, in our case pollen on butterflies, is declining (or increasing) when it is not (Type I error) are less serious than missing the possibility that a species is in trouble (Type II error).

To improve the relevance of results to insect pollination, data from wind-pollinated plant species were removed from the analysis (6 pollen species and 35 interactions were removed). Because of their prevalence in the air and soil, it is likely these pollen species inadvertently made contact with the insects' bodies. Most pollinators collect pollen grains from wind-pollinated plants while visiting the plants for a variety of functions, including feeding on pollen. A high ratio of pollen from wind-pollinated plants is common compared to insect-pollinated plants. Butterflies more likely carry airborne pollen due to their large body size and

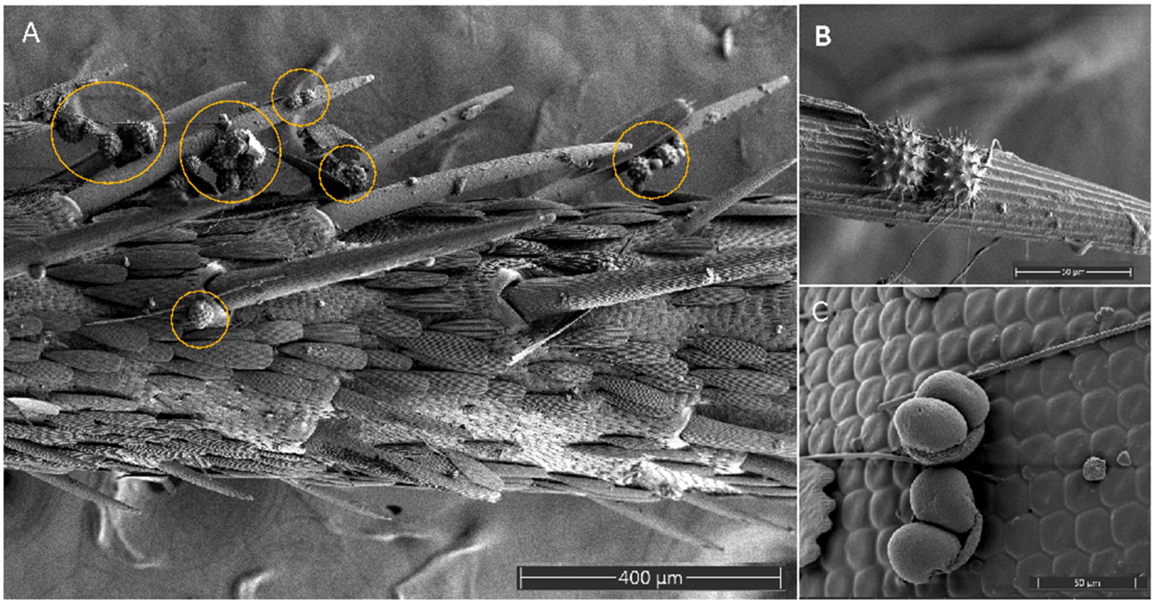

Fig. 1. A–C: Scanning electron micrograph of pollen grains on butterfly legs and eyes from the collection at the University of Nevada Reno Natural History Museum. A, B: Asteraceae pollen covering the leg of a nymphalid butterfly. C: Pine pollen on a butterfly eye.

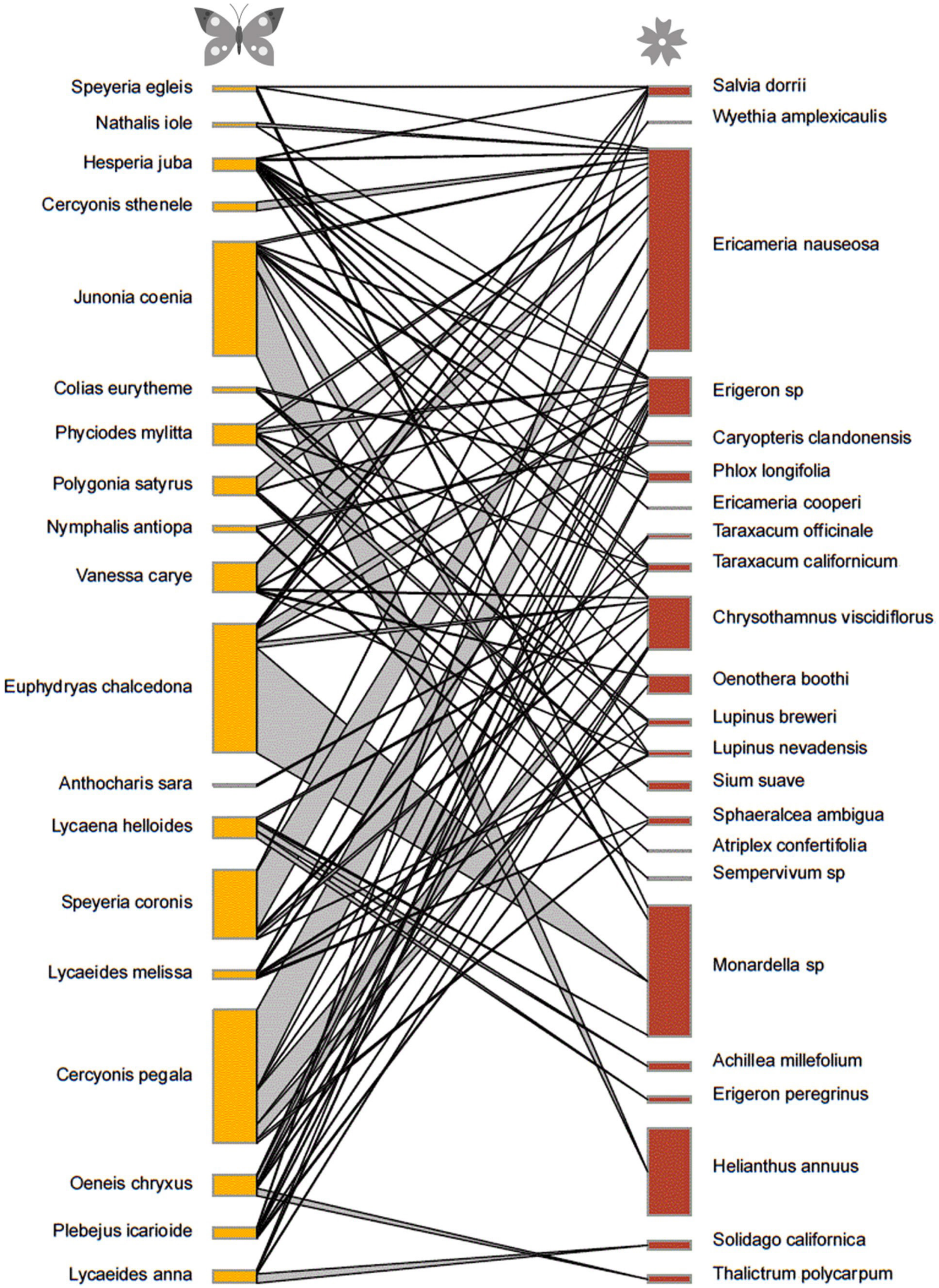

Fig. 2. Bipartite pollen-butterfly network of 19 butterfly species and pollen from 29 plant species obtained from museum collections of butterflies in the Great Basin Desert (USA). Links between plants and pollinators are represented with lines, whose width is proportional to the number of interactions, while the width of the nodes represents total abundance of that taxon across all its interactions.

Table 1
Butterfly taxa, pollen loads, pollen richness, estimated richness (based on rarefaction), and Simpson's diversity equivalents for butterflies collected in Nevada and California (USA) over the past 100 years. Rarefaction curves for each species are presented in the supplement.

Butterfly Species	Butterfly Family	Number of pollen grains (Insect- pollinated plants)	Number of pollen grains (Wind- pollinated plants)	Plant species richness (Insect- pollinated plants)	Number of butterfly specimens	Pollen richness, estimated (rarefaction)	Simpson richness
Hesperia juba	Hesperiidae	129	39	9	8	9	7.602
Anthocharis sara	Pieridae	18	78	1	4	1	1
Nathalis iole	Pieridae	39	9	2	3	4	1.707
Colias eurytheme	Pieridae	51	6	3	4	2	1.761
Lycaeides anna	Lycaenidae	138	7	4	7	–	–
Lycaeides melissa	Lycaenidae	85	40	5	14	3	2.768
Lycaena helloides	Lycaenidae	212	171	4	22	6	4.916
Plebejus icarioides	Lycaenidae	110	54	3	10	5	4.122
Speyeria egleis	Nymphalidae	53	71	3	3	7	4.266
Nymphalis antiopa	Nymphalidae	54	43	2	4	4	3.468
Cercyonis sthenele	Nymphalidae	89	21	1	6	2	1.447
Polygonia satyrus	Nymphalidae	190	159	4	7	7	3.940
Oeneis chryxus	Nymphalidae	220	66	6	11	8	5.152
Phyciodes mylitta	Nymphalidae	220	55	4	14	6	3.010
Vanessa carye	Nymphalidae	309	11	5	5	6	4.988
Speyeria coronis	Nymphalidae	726	21	5	10	5	1.262
Junonia coenia	Nymphalidae	1193	90	8	18	10	1.921
Euphydryas chalcedona	Nymphalidae	1349	14	7	8	9	1.367
Cercyonis pegala	Nymphalidae	1393	138	5	15	7	2.597

their high pilosity.

2.3. Pollinator observations

Short, targeted pollinator observations, combined with existing observational knowledge, provided qualitative comparisons to the pollen counts found on individuals. We selected two species, *Lycaeides anna* and *L. melissa*, due to familiarity with the natural history of these species as well as their high abundances at the selected sites. We observed a population of *L. anna* near Yuba Pass in eastern California and a population of *L. melissa* in Verdi, Nevada in August 2020, respectively. The host plant for *L. anna* larvae at this site is *Lotus nevadensis* and for *L. melissa* the host plant is *Medicago sativa*. Each time we encountered an individual, we recorded the sex of the individual and noted whether the individual was found on the host plant. In the case of *L. anna*, we recorded the identities of all other plants visited and observed all individuals found visiting the flowers of those plants. Due to the great abundance and activity of *L. melissa* at the other site, it was only possible to record whether individuals landed on their host plant or were observed flying or on another plant, visiting flowers or landing on stems or leaves. Then, we collected individuals opportunistically for pollen analysis. We captured a total of 13 individuals from *L. anna* and 20 individuals from *L. melissa* and froze them for pollen analysis, using the same methods described for the network assessment (Supplementary Material 2 Fig. 3).

3. Results

3.1. Pollen analysis of historic Lepidoptera

A diverse community of plant species was associated with the butterflies across the full time series (Table 1). Identification of 7686 individual pollen grains from 29 plant species on external tissues of the butterfly specimens Most of the pollen appeared on the eyes, proboscis, and legs of the butterfliesPollen from *Ambrosia dumosa* (Asteraceae), *Artemisia tridentata* (Asteraceae), *Atriplex confertifolia* (Amaranthaceae), *Alnus tenuifolia* (Betulaceae), *Leymus cinereus* (Poaceae), and Pinaceae appeared mostly on the butterflies' head and wings. As these species are not known as insect pollinated, they inadvertently picked up pollen from the environment, in the soil or from wind (Blauer et al., 1976). Overall, results indicated a varied range of pollen richness (r 2–11 per species) and abundance (average of 13.9 pollen grains per species) between butterfly species as well as the date captured.

Posteriors from the hierarchical Bayesian model (Fig. 3) indicated that overall pollen richness associated with butterflies has declined slightly during the study period. The mode of the posterior distribution for the full model was −0.2 (± 0.1 80% credibility interval), providing weak support for an estimated loss of.2 species of pollen per decade. Several species experienced moderate declines in richness (Fig. 3), however, with *Colias eurytheme* experiencing the greatest declines, losing.45 pollen species (± 0.1 80% credibility interval) per decade. In contrast, two species exhibited small pollen richness increases, with the largest increase in the addition of 0.35 (± 0.32 80% credibility interval) pollen species per decade for *Hesperia juba*.

3.2. Interaction networks

The quantitative bipartite networks using 19 pollinator (butterfly) and 29 plant taxa highlighted the interaction frequency for the time periods of interest (Supplementary Material 2 Fig. 4), as well as the entire time series. In these networks, the thickness of the bars represents pollen species abundance and linkage indicates the frequency of interaction (overall network is represented in Fig. 2). As with the subtle changes in pollen diversity associated with butterflies, network parameters also changed over time (Fig. 4, Supplementary Material 1 Table 2). Connectance values increased from 0.20 to 0.23, for example, when

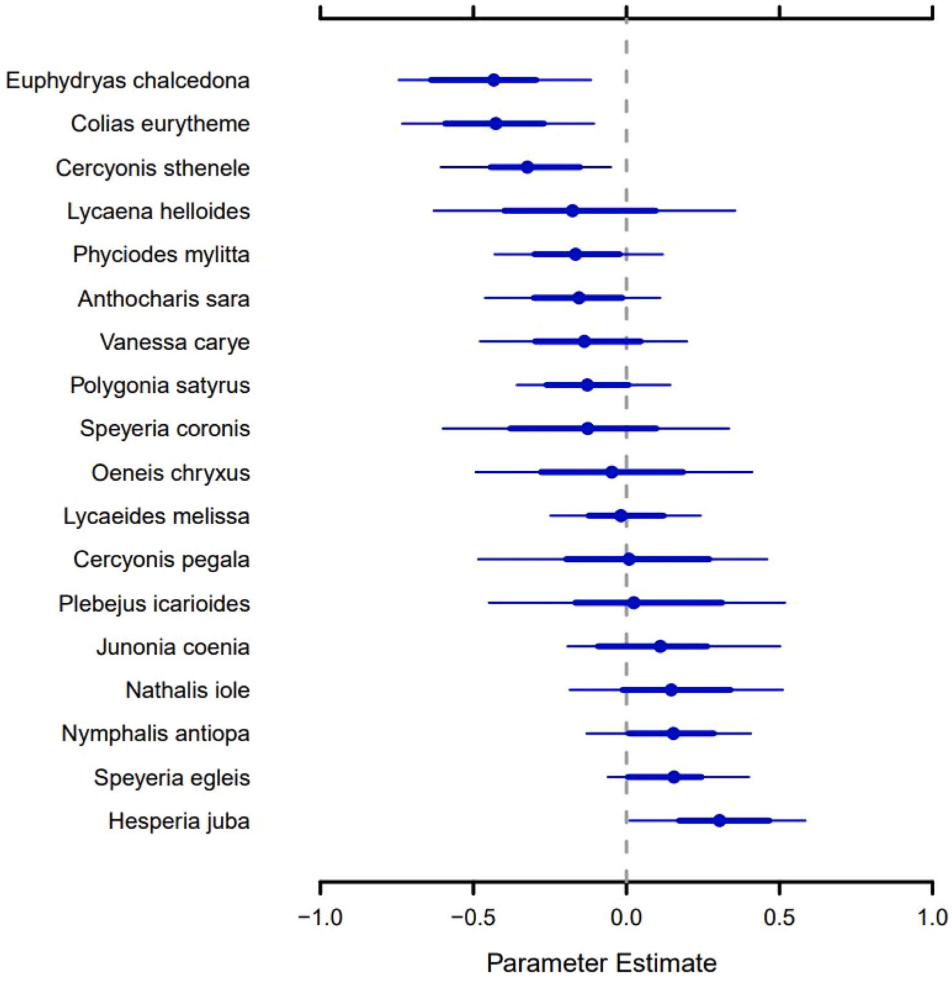

Fig. 3. Estimates of pollen richness change over the past 100 years for butterfly species collected in the Great Basin desert (USA). Estimates are from a Bayesian hierarchical model; posterior distribution medians are displayed with 50% (black bars) and 80% (gray bars) credibility intervals.

comparing networks before and after the onset of drought. High H2' values for both time periods indicate substantially specialized networks (0.68).

3.3. Focused comparison of field observations to pollen analysis

The pollen species and abundances found on the bodies of the specimens collected following the observation networks differed substantially from expected values when documenting visitation exclusively. For *Lycaeides anna*, 145 pollen grains representing five plant taxa were found on the specimens collected for pollen analysis. This result contrasts with field observations, where individuals appeared interacting with three different flower species, *Lotus nevadensis*, *Solidago californica*, and *Erigeron* sp., though two plant taxa, *Solidago californica* and *Erigeron sp*. (Asteraceae), from the different study types overlapped. For *L. melissa*, a total of 119 pollen grains were counted and a total of seven plant species were found on the collected specimens. Although we encountered many of the individuals while flying or landing on other plants during the observation period for this species, we only observed them interacting with the flowers of *Medicago sativa*, which was not one of the pollen species found in collected specimens. Flowers were available for the adult butterflies during the observations, and plant and butterfly phenologies overlapped for this focal study. Feral alfalfa flowers typically from June to September and *Lycaeides* fly from about May to October in the study area (personal observations).

4. Discussion

As plant-pollinator interactions are integral to the maintenance of terrestrial biodiversity long time series of changing insect-pollen networks improve understanding of changing biomes Constructing accurate pollination networks across variable habitats provides important estimates of interactions that can help understand pollinator responses to habitat reduction or fragmentation, climate change, invasions by exotic species, and changing communities generally. These data add to a growing consensus (e.g., references) that collecting pollen from museum specimens provides an effective method for characterizing and studying pollination interactions and niche breadth in light of changing environmental pressures over time.

The primary results from this study of butterfly-pollen interaction networks in the Great Basin of the USA suggested that niche breadth and pollen interactions for these species have changed very little in these species over the last 100 years. Among the butterfly species showing declines in pollen richness over time, *Euphydryas chalcedona*, and *Cercyonis sthenele* (Nymphalidae) and *Colias eurytheme* (Pieridae) had the greatest estimated declines in pollen richness (more than 80% of the posterior distribution for slope of richness over time is less than zero; Fig. 3) across the time series. The butterfly species *E. chalcendona* and *C. sthenele* are known to nectar at several flowers throughout the Great Basin and Sierra Nevada mountains (Pyle and LaBar, 2018). In the networks reported in this paper, the decline in pollen richness on *E. chalcedona* reflects a shift in visitation of several flowers to a single taxon, the genus *Erigeron*, which may indicate a decline in the diversity

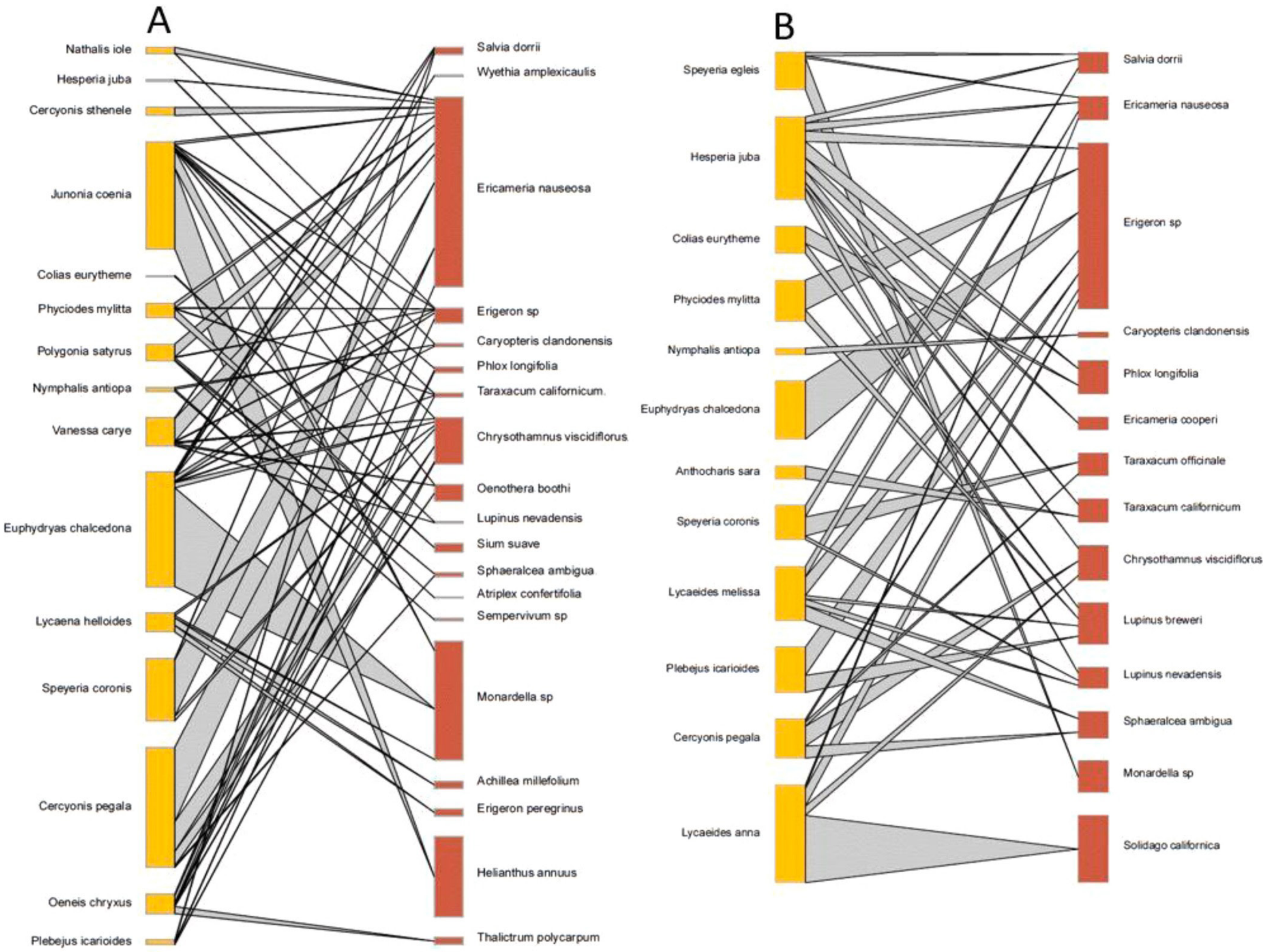

Fig. 4. Bipartite pollination networks for butterfly species collected in Nevada and California over the past 100 years. Networks include all data before 2000 (panel A) versus after 2000 (panel B).

of floral resources in the sites studied. *Cercyonis sthenele*, a known visitor of *Ericameria nauseosa* (Asteraceae) (Pyle and LaBar, 2018) experienced a decline in pollen richness across decades, and *E. nauseosa* pollen was present on specimens collected before 2000, but not after. Also known to nectar on yarrow and goldenrods, *C. sthenele*, is declining across the western United States (Forister et al., 2021). In contrast, *Colias eurytheme* is still abundant in its home range and occupies habitat ranging from alpine meadows to urban parks (Pyle and LaBar, 2018). These butterflies' frequent alfalfa as both a larval host plant and as a nectar plant, but adults' nectar at numerous other plants in addition to alfalfa. Findings from this study were consistent with these known visitation behaviors, as we observed pollen from lupines and *Phlox* on them. Pollen declines on *Colias* could reflect increases in alfalfa and declines in native plants across its range.

For some butterfly species, pollen richness increased after anthropogenic drought in the Great Basin. More than 80% of the posterior distributions for the change in pollen richness over time is greater than zero for several species. The main species for which we estimated an increase in pollen richness was *Hesperia juba* (Hesperiidae), but moderate increases were also apparent for *Speyeria egleis* and *Nymphalis antiopa* (Nymphalidae) (Fig. 3). *Hesperia juba*, known commonly as the Juba skipper, is a bivoltine skipper that frequents several flowering plants during its first flight of the year. It often appears on rubber rabbitbrush during its flowering period in the fall season (Berkhouse and Shapiro, 1994). In this study, *H. juba* shifted from associations with pollen from only two plant species (*Ericameria* and *Erigeron*) to eight species when comparing before and after the onset of drought in year 2000. *Speyeria egleis*, or the Great Basin Fritillary, is another common visitor of *Ericameria* for which we also observed pollen from *Salvia* and *Monardella* in recent years. *Nymphalis antiopa* is also common and wide-ranging, with a holarctic distribution, and in the arid western United States tends to inhabit riparian areas. These butterflies are commonly seen nectaring at flowers, but are typically known to feed on sap, ripened fruit, and honeydew from aphids (Hall et al., 2014). In the museum specimens examined, *N. antiopa* was carrying *Caryopteris* and *Sempervivum* pollen. The increases for these species can indicate expansion in niche breadth in response to environmental change. Alternatively, plant communities could have shifted, such that available pollen richness has increased, and the specimens collected from butterflies reflects such shifts.

The results from the network analysis point to the ways in which butterfly-plant communities are influenced by corresponding anthropogenic climate events in the Sierra Nevada and Great Basin regions. Although these results indicate some mild increases in network specialization over time, most butterflies including specialized species may utilize novel plants when focal resources are scarce. Such opportunistic host range expansion may help stabilize ecosystem processes (Dunne et al., 2002; Tylianakis et al., 2010). The observed pollen-butterfly networks had connectance values near 0.20, which is higher than values common in the literature for visitation networks

(Dunne et al., 2002). This level of connectance may indicate that pollen-butterfly interactions comprise a higher degree of complexity than that suggested from visitation networks.

Shifts in Great Basin butterfly-pollen networks are part of overall community responses to anthropogenic drought in the Great Basin. A drying and warming climate in western North America has caused decreasing butterfly abundances, as well as decreasing floral abundance (Forister et al., 2018; Inouye, 2008). In 2015, the snowpack in the Sierra Nevada mountains was only five percent of its historical average. This clear indication of long-term drought in the region (Belmecheri et al., 2016) is part of an overall trend of increasing drought in the Sierra Nevada and Great Basin regions. Droughts affect pollinators negatively by decreasing the quantity and quality of floral rewards (Phillips et al., 2018). The networks from common specimens in the University of Nevada Museum of Natural in History indicated clear shifts in pollen-butterfly interactions before and after the onset of drought (Fig. 3). These butterfly-pollen networks were more specialized after 2000, possibly due to lower resource availability. The increased drought and other anthropogenic changes are likely impact plant communities with which pollinators interact, often priming them for invasion by exotic species and a decrease in abundance of native plants. While butterflies may confront a narrower nectar and pollen niche breadth, some may form novel interactions with new plant resources, increasing numbers of interactions over time (Forister et al., 2018). Related to these patterns, the most common pollen species represented from museum butterflies, *E. nauseosa* (Rubber rabbitbrush) and *Monardella* sp. sharply declined in abundance in the last two decades compared to the years before 2000. This drop may result from habitat loss and ecosystem alteration in the last two decades. Finally, the focused observations of two butterfly species, *L. Melissa* and *L. anna*, along with decades of natural history observations, provided a snapshot of the differences between interactions observed in the field. This approach was designed to mirror widely used visitation network methods, and to complement interactions inferred from pollen found on the specimens' bodies. Although observations overlapped with the pollen composition found on *L. anna*, no pollen from the larval hostplant (*Medicago sativa*) were found on the bodies of *L. Melissa* collected in the field in spite of frequent observed interactions between butterflies and flowers for these species. This result highlights potential shortcomings of visitation networks, as well as the method in this study. The visitation networks clearly underestimated diversity of pollen picked up by butterflies. In the case of the study method, pollen abundances associated with frequent visitation of a focal flower did not result. These observations were very limited in temporal and spatial extent, but they underscore the importance of combining multiple methods for estimating true networks.

5. Conclusion

Findings from this study provide insight into the research questions posed in this paper pertaining to how butterfly-pollen interactions have changed over the past century during a time of rapid global change. First, estimates from the hierarchical Bayesian linear models demonstrate a moderate overall decrease in pollen richness over time on butterfly species, with the majority of species showing declines in pollen richness, and a few showing increasing richness. These results support the hypothesis that the richness of pollen species associated with butterflies has declined over time for most species. Second, changes in network parameters reflected in the analysis examining pre-2000 and post-2000 interaction networks, may correspond to environmental changes that took place between these time periods. This supports the hypothesis that recent droughts are partly responsible for changes to species interaction networks, although other concomitant factors relating to global change are likely contributing.

In a time of growing concern about declines in insects globally owing to global human-environmental change, these conclusions provide a view of how interactions between insects and plants are also changing. Much media coverage has focused on the declines in pollinators in recent decades (Biesmeijer et al., 2006; Colla and Packer, 2008; Wood et al., 2019), and long-term data sets have been used to validate these trends (Colla et al., 2012; Bartomeus et al., 2013; Wood et al., 2019). Combining multiple measures of such changes is preferable, such as with the approach using specimens from museums. Combined with the more popular visitation networks and studies of pollinator effectiveness (Ballantyne et al., 2015), such approaches offer much promise toward a more thorough understanding of global changes in pollination interaction networks in response to disturbance gradients.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ancene.2022.100325.

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