2019

Non-native insects dominate daytime pollination in a high-elevation Hawaiian dryland ecosystem

Clare E. Aslan
Northern Arizona University, clare.aslan@nau.edu

Aaron B. Shiels
USDA National Wildlife Research Center

William Haines
University of Hawaii

Christina T. Liang
USDA Forest Service

American Journal of Botany

RESEARCH ARTICLE

Non-native insects dominate daytime pollination in a high-elevation Hawaiian dryland ecosystem

Clare E. Aslan[1,5], Aaron B. Shiels[2], William Haines[3], and Christina T. Liang[4]

Manuscript received 22 July 2018; revision accepted 13 November 2018.

[1] Landscape Conservation Initiative, Northern Arizona University, and Conservation Science Partners, Flagstaff, Arizona 86011, USA

[2] National Wildlife Research Center, USDA, Fort Collins, Colorado 80521, USA

[3] Center for Conservation Research and Training, University of Hawaii, Honolulu, Hawaii 96822, USA

[4] Institute of Pacific Islands Forestry, USDA Forest Service, Hilo, Hawaii 96720, USA

[5] Author for correspondence (e-mail: clare.aslan@nau.edu)

Citation: Aslan, C. E., A. B. Shiels, W. Haines, and C. T. Liang. 2019. Non-native insects dominate daytime pollination in a high-elevation Hawaiian dryland ecosystem. *American Journal of Botany* 106(2): 313–324.

doi:10.1002/ajb2.1233

PREMISE OF THE STUDY: Over one-third of the native flowering plant species in the Hawaiian Islands are listed as federally threatened or endangered. Lack of sufficient pollination could contribute to reductions in populations, reproduction, and genetic diversity among these species but has been little studied.

METHODS: We used systematic observations and manual flower treatments to quantify flower visitation and outcrossing dependency of eight native (including four endangered) plant species in a dryland ecosystem in Hawaii: *Argemone glauca*, *Bidens menziesii*, *Dubautia linearis*, *Haplostachys haplostachya*, *Sida fallax*, *Silene lanceolata*, *Stenogyne angustifolia*, and *Tetramolopium arenarium*.

KEY RESULTS: During 576.36 h of flower observations, only insects visited the flowers. Out of all recorded flower visits, 85% were performed by non-native species, particularly the honeybee (*Apis mellifera*) and flies in the family Syrphidae. Some plant species received little visitation (e.g., *S. angustifolia* received one visit in 120 h of observation), whereas others were visited by a wide diversity of insects. The endangered plant species were visited by fewer visitor taxa than were the common native plant species. For six of the focal plant species, bagging of flowers to exclude pollinators resulted in significant reductions in seed set.

CONCLUSIONS: The flower visitor community in this system, although heavily dominated by non-native insects, appears to be facilitating pollination for multiple plant species. Non-native insects may thus be sustaining biotic interactions otherwise threatened with disruption in this island ecosystem. This may be particularly important for the studied endangered plant species, which exhibit fewer partners than the more common plant species.

KEY WORDS *Apis mellifera*; Asteraceae; disrupted mutualism; flower pollination treatments; flower visitation observations; *Haplostachys haplostachya*; island endemics; *Silene lanceolata*; *Stenogyne angustifolia*; *Tetramolopium arenarium*.

Global change drivers including climate change, biological invasion, and habitat loss are transforming ecological communities (Barnosky et al., 2011; Wardle et al., 2011), resulting in novel species assemblages, altered ecological interactions, and shifts in ecological function (Koh et al., 2004; Dunn et al., 2009; Brodie et al., 2014). Essential ecosystem functions and services may depend on the presence of particular species or functional groups. If communities are altered sufficiently and these species or groups disappear, key functions and services could be lost. As a result, other species may be at risk of secondary extinctions (Koh et al., 2004; Colwell et al., 2012), and the composition of the affected community may be irreversibly altered (e.g., Clavel et al., 2010). To predict the biodiversity implications of environmental change, it is essential that critical ecological functions be examined.

Worldwide, oceanic islands have been heavily impacted by anthropogenic activities. As a result, they are considered among the most threatened systems on the planet (Blackburn et al., 2004; Sax and Gaines, 2008; Caujapé-Castells et al., 2010). Because of their isolation, remote oceanic islands often contain high numbers of endemic species, and the population sizes of these native species are often small as a result of limited land area and habitat extent (Loope et al., 1988). These factors alone make extinction risk high

on islands. Land-use changes to make way for human habitation and agriculture reduce native habitat still further, driving extinction rates still higher (Frankham, 1998; Cox and Elmqvist, 2000; Boyer, 2008). Other extinctions stem from non-native species introductions, particularly because island isolation can prevent certain functional groups from colonizing naturally and leave vacant ecological niches that further facilitate establishment of non-natives (Vitousek, 1988; Kueffer et al., 2009). Entire guilds, such as mammalian predators and herbivores, can be absent from islands, and endemic species often lack common defenses against predation, herbivory, and competition, making them vulnerable to extinction as a result of species introductions from continental ecosystems (e.g., Boyer, 2008). Over the past century, these combined processes have led to the formation of novel ecological communities on oceanic islands, comprising reduced sets of native species and high occurrence of introduced species (Kaiser-Bunbury et al., 2010a, b, 2011). The Hawaiian Islands are the most isolated archipelago on the planet and exhibit both high rates of endemism and absence of key functional guilds. Introduced species in the Hawaiian Islands bring with them novel diets, competition, and fire regimes and are therefore considered the primary threat to endemic biodiversity on the these islands (Loope et al., 1988). However, introduced species also have the potential to contribute key functions and services to ecological communities and to engage in positive interactions such as mutualisms with native species, and this phenomenon has been much less studied.

Pollination is a mutualistic interaction that can be critical to reproduction and/or population persistence and maintenance for plant species (Bond, 1994; Aslan et al., 2016). Although many plants exhibit some level of self-compatibility, pollinators can permit flowers to outcross, maintaining gene flow that can introduce new genetic variation into populations (Loveless and Hamrick, 1984; Ward et al., 2005). This, in turn, may maximize the adaptive capacity of a plant species by ensuring that the population contains a high diversity of genetic material (Kremer et al., 2012), a factor that may be important for individuals in heterogeneous environments such as those created by the varied topography and high elevational range of the Hawaiian Islands. For many plant species, outcrossing can result in more numerous or robust fruits and seeds than selfing (e.g., Waser and Price, 1989; Dudash, 1990). Meanwhile, flowering plants provide essential energy resources in the form of nectar and pollen for many animals (Rico-Gray, 1989; Wilson et al., 2010; McKinney et al., 2012).

We hypothesized that introduced insects are acting as flower visitors (and perhaps pollinators) in a highly disturbed island ecosystem and thus may play a role as replacement pollinators following native pollinator losses. To test this hypothesis, we determined the current flower visitors, outcrossing dependency, and pollen limitation for eight native plant species in a high-elevation dryland tropical ecosystem on Hawai'i Island. We also compared the role of introduced insects as flower visitors (and potential pollinators) for common vs. rare native plant species. Although lack of pollinators may not be a cause of plant rarity, the absence of potential replacement pollinators could affect future reproduction among rare species. We recorded flower visitation events and conducted manual experimental pollination treatments for four common native species and four federally endangered endemic plant species. Like much of the Hawaiian Islands, the study site has experienced widespread invasion by introduced browsers, grazers, predators, and plants. Our study examined current flower visitation and seed production within such transformed conditions.

MATERIALS AND METHODS

Study site

We evaluated ongoing flower visitation in a dryland ecosystem within the U.S. Department of Defense–managed Pōhakuloa Training Area (PTA) on Hawai'i Island. Dryland ecosystems, particularly tropical dry forests, are among the most threatened habitat types worldwide due to high rates of land-use conversion and fire-regime change resulting from anthropogenic activities (Janzen, 1988). Our research took place in two fenced tracts of mamane-naio (*Sophora chrysophylla–Myoporum sandwicense*) forest at 1500–1700 m in elevation (UTM 222105 × 2185212). The fencing protects remnant populations of several endangered plant species that lack adaptations to mammalian browsing and have therefore been heavily impacted by introduced ungulates such as sheep and goats. With the fencing in place, the PTA represents a protected refuge for many plant species, but a number of global change agents persist. The study site exhibits heavy infestations of invasive fountain grass (*Cenchrus setaceus*), which produces high levels of flammable biomass and has introduced a novel fire regime to the region. Introduced predators such as rodents, ants, and yellowjackets are abundant throughout the PTA and across the Hawaiian Islands and could impact pollinator communities (e.g., Hanna et al., 2013). At least some known native pollinators, such as honeycreepers, are completely absent in the study area, but introduced pollinators such as the honeybee (*Apis mellifera*) and flies in the family Syrphidae (especially *Allograpta exotica*) are abundant. Like the now excluded ungulates, these non-native animals have the potential to create novel interaction regimes that may impact native pollinators and plants alike.

Study species

We performed pollination observations and flower treatments on all native shrub species that occur across the mamane-naio tract and produce pollinator-attractive flowers, with the exceptions of a few endangered species so limited in population that flower treatments were not possible for them. Our species list was shaped by planning for future restoration experiments in the system and included eight native Hawaiian plant species found within our study site: the common species pua kala (*Argemone glauca*; family Papaveraceae), kokolau (*Bidens menziesii*; family Asteraceae), shrubland dubautia (*Dubautia linearis*; family Asteraceae), and yellow 'ilima (*Sida fallax*; family Malvaceae); and the U.S. federally listed endangered species honohono (*Haplostachys haplostachya*; family Lamiaceae), lanceolate catchfly (*Silene lanceolata*; family Caryophyllaceae), narrowleaf stenogyne (*Stenogyne angustifolia*; family Lamiaceae), and Maui tetramolopium (*Tetramolopium arenarium*; family Asteraceae) (Fig. 1). Since these species span a diversity of plant families, we aimed to develop a snapshot understanding of ongoing pollination relevant to diverse flower morphologies and phenologies (Fig. 1). Flowers of *A. glauca* are large (>7 cm across), are produced singly on robust pedicels, and can be found in low numbers across the study site in most months of the year; flowers of *B. menziesii* are small yellow composites (~1.5 cm diameter), produced in clusters of >40 capitula, and again can be found in most months of the year; flowers of *D. linearis* are also small yellow composites (~1 cm diameter), produced in early winter in clusters of 8–90

FIGURE 1. Flower characteristics of the focal plant species. Species A–D are common native plant species; species E–H are endangered plant species. (A) *Argemone glauca* (Papaveraceae): flower width 8.5 cm. (B) *Bidens menziesii* (Asteraceae): flower width 1.4 cm. (C) *Dubautia linearis* (Asteraceae): flower width 0.8 cm. (D) *Sida fallax* (Malvaceae): flower width 2.3 cm. (E) *Haplostachys haplostachya* (Lamiaceae): flower width 1.8 cm. (F) *Silene lanceolata* (Caryophyllaceae): flower width 1.0 cm. (G) *Tetramolopium arenarium* (Asteraceae): flower width 1.0 cm. (H) *Stenogyne angustifolia* (Lamiaceae): flower width 0.7 cm.

capitula; flowers of *S. fallax* are pale yellow, ~2 cm across, produced singly or up to seven per node, and occur year-round (Wagner et al., 1999). Flowers of *H. haplostachya* are aromatic, bilaterally symmetrical, short, white tubes (~1.5 cm across) with enlarged lower corolla lobes, produced in a raceme with two flowers at each verticillaster, and plants produce flowers repeatedly throughout the year except in drought conditions; flowers of *S. lanceolata* are solitary short off-white tubes (~1 cm across), produced in spring, summer, and fall; flowers of *S. angustifolia* are ~2 cm long, bilaterally symmetrical tubes, with a reduced lip, ranging in color from red-orange to maroon and produced in pairs throughout the year except during drought conditions; flowers of *T. arenarium* are very small composites (<1 cm across) with white or pink corollas, borne on upright stems in clusters of 5–11 capitula, and senesce in drought conditions (Wagner et al., 1999; Fig. 1).

Flower visitation observations

The bulk of the data collection for this study involved systematic flower visitation observations, conducted from March 2015 to February 2016, to identify the primary flower visitors (i.e., the likely potential pollinators) for each focal plant species. There are no nectarivorous birds in the study area itself, although a nearby *Metrosideros polymorpha* woodland supports two native (ʻamakihi, *Hemignathus virens*, and ʻapapane, *Himatione sanguinea*) and one non-native (Japanese white-eye, *Zosterops japonicus*) nectarivorous bird species. As a result, our observations were tailored to insect visitation, placing us close enough to flowers to observe even very small visitors or fleeting visitation. When each plant species was in flower, it was observed approximately once per week throughout the study. Known local populations of each focal plant species were casually assessed for flowering on a weekly basis, and those populations that were actively flowering were observed in rotation so that as much spatial variation as possible was captured in observations. Because the plants of most of the focal species progress into and out of flowering repeatedly over the course of a year, the study encompassed multiple flowering events for all plants other than those of *D. linearis*, which flowers only once per year in the late fall/early winter. Each observation period lasted 3 h, and the dates on which each plant species was observed and start times of observations were randomly assigned so that all plant species were observed in early morning, mid-morning, midday, early afternoon,

late afternoon, and evening. Observation start times ranged from 0610 to 2135 hours. The large majority of observations took place during daytime hours, due to site access constraints, so we interpret our results with relevance to the daytime flower visitor suite in the focal system. (Note: for *S. lanceolata*, the flowers of which are open throughout the night and until early afternoon but then close for several hours and reopen at dusk, we performed a total of 40 h of nighttime observations using night-vision goggles to supplement the daytime observations.)

Each systematic observation period consisted of fifteen 10 min blocks, with a 10 min observer rest period every hour. Every 10 min, observers performed a 1 min scan of a focal stand of flowering plants, noting the total number of visible flowers (note that for Asteraceae, we treated each capitulum as a flower for analysis purposes) and the species and abundance of all insects that were interacting with those flowers in any way (note, other potential pollinators, such as birds, did not approach flowers of any species during any observation). If an insect could not be identified to species, the observer noted its functional group (e.g., small gray moth) and attempted to photograph it and/or capture it for identification. The data from these scans were used to calculate the average number of visitors of each visitor taxon (or functional group) to each plant species per visible open flower per unit time (see below).

Following each scan, the observer devoted the next 9 min to focal individual observations (Manson, 1997; Aslan et al., 2013b). During this time, the observer selected one flower visitor at a time to observe continuously, noting the number of plants of the target species and flowers visited and the behavior during visits (e.g., predation, herbivory, pollen collecting, nectar foraging via probing, nectar robbing). Flowers were considered "probed" if the visitor behavior carried the potential of contacting the flower reproductive parts. The focal individual observation continued until the visitor was lost from view (e.g., departed), until it had stopped interacting with the target species, or until 180 s had passed. Then the observer selected a new visitor to observe, repeating this until the 9 min period had elapsed. Observers selected visitors opportunistically: at the start of each 9 min block, the observer moved through the local population of plants as necessary to locate a visible visitor, and then began to observe and record data on that visitor. When switching to a new visitor, the observer selected, if possible, a visitor of another taxon. If this was not possible, the observer watched another individual of a previously observed taxon. Together, these focal individual observations were used to calculate the average number of flowers probed of the target plant species, per unit time, during visits by each insect taxon to each plant species (see below). All observations were conducted from a distance of approximately 1–2 m. Observers also noted time of day, weather conditions, and other flowering plants in the immediate vicinity.

Data analysis—To analyze flower visitation data for our focal plant species, we used observation data to calculate (1) the average number of individuals of each visiting taxon per open flower per minute for each target plant species, (2) the total richness of visitor taxa per plant species, and (3) the average number of flowers probed per minute by each visitor taxon. We multiplied the values for measures 1 and 3, and the product was our overall visitor importance value for each insect taxon/plant combination (after Renne et al., 2000; Aslan et al., 2013b). This analysis gave us a complete list of the observed visitors for each plant species, ranked by their relative importance so that the most important visitors could be identified and compared between plant species. Under this methodology, a taxon would have high importance if it visited the target plant frequently or probed a large number of flowers during each visit. To standardize importance values, we then set the importance value of the most important visitor for each target plant species equal to 1.0, and the importance values of all other visitors were scaled according to their value as a proportion of that visitor. For each plant species, we considered all visitors with scaled importance values ≥0.25 to be primary visitors.

Pollination treatments

To quantify pollen limitation and assess the importance of outcrossing, we performed experimental flower treatments for each of our focal species. Treatments included bagging flowers in bud stage to prevent outcrossing; bagging flowers in bud stage and following with hand pollination when flowers were receptive as a bag treatment control; hand supplementation of pollen, taken from three conspecific individuals in the immediate population, to evaluate maximum seed set; and an unmanipulated flower control. We measured plant reproductive output under each treatment as seed set, defined as seeds per flower. Under the bagging treatment, all seeds produced are the results of self-fertilization, and a comparison between bagged-flower seed set and the seed set of unmanipulated, naturally pollinated flowers provides an assessment of rates of autogamy. Under the hand-supplementation treatment, seed-set values are indicative of natural pollination as well as manual pollen additions, and a comparison between hand-supplemented and unmanipulated flowers is indicative of pollen limitation under natural, unmanipulated conditions. We attempted to administer each treatment to a minimum of three flowers on each of at least six plants per species and continued to administer additional treatments opportunistically as plants flowered and we were able to access them, aiming for sufficient sample size to detect differences among treatments while minimizing the impact to plant reproduction across the site. This resulted in final treated flower numbers ranging from 38 (for *A. glauca*) to 129 (for *D. linearis*) (Table 1). Our sample sizes of successful treatments became unbalanced because of various factors, including low availability of pollen for hand supplementation (often, it was impossible to find the requisite three pollen donors within the local population), high wind events tearing bags from plants, and temporal constraints on our access to the study site (military base).

The structure of our "bags" for visitor exclusion varied by plant species. Many of the flowers we studied were difficult to bag because their buds were extremely small and their pedicels highly reduced, so it was a challenge to fashion a structure that could be firmly attached to the flower, fully exclude flower visitors, and remain in place in spite of the high winds characteristic of the study site without damaging the flower. For *T. arenarium*, we used small pieces of drinking straw, stapled closed at the ends and pierced with small pinholes for airflow. For *A. glauca*, we used bags made of nylon window screen (mesh openings <1 mm), sealed at the edges with staples and closed around the robust flower pedicel with plastic ties. For the remainder of our plants, we used either small (approximately 4 × 4 cm) rectangular bags made of nylon wedding tulle mesh (mesh openings <0.1 mm), sealed with fabric tape around all sides and with a small opening for the pedicel; or small bags of nylon wedding veil material (mesh openings <0.5 mm), cinched around the base of flowers with drawstrings or small plastic ties. All treatments excluded all

TABLE 1. Flower treatment results. Reproductive success was evaluated as seed set, defined as seeds produced per flower. Treatments included hand supplementation with conspecific pollen to evaluate pollination limitation, bagging to evaluate dependence on outcrossing, a bagged control treatment to evaluate the effect of the bag on seed production, and an unmanipulated control to assess seed production under ambient pollination conditions. Asterisks indicate endangered species.

Plant spp. (*n* treated flowers/ *n* plants)	Significant treatment (Kruskal-Wallis)	Significant contrasts (Dunn's test of multiple comparisons)	Treatment	Mean seed set (± SE)
Argemone glauca (38/12)	$\chi^2 = 9.53$; $P = 0.0230$	Unmanipulated vs. supplemented ($P = 0.033$)	Bagged Bagged control Hand-supplemented Unmanipulated	151.03 ± 36.79 253.89 ± 53.86 249.04 ± 41.23 138.28 ± 43.54
Bidens menziesii (91/12)	$\chi^2 = 30.86$; $P < 0.0001$	Unmanipulated vs. bagged control ($P = 0.029$) Unmanipulated vs. bagged ($P < 0.0001$) Supplemented vs. bagged ($P = 0.0002$)	Bagged Bagged control Hand-supplemented Unmanipulated	0.16 ± 0.24 1.00 ± 0.71 2.87 ± 0.47 3.61 ± 0.34
Dubautia linearis (149/14)	$\chi^2 = 25.69$; $P < 0.0001$	Unmanipulated vs. bagged ($P < 0.0001$) Bagged control vs. bagged ($P = 0.0023$) Supplemented vs. bagged ($P = 0.035$)	Bagged Bagged control Hand-supplemented Unmanipulated	0.56 ± 0.28 2.13 ± 0.36 1.78 ± 0.31 2.72 ± 0.33
Sida fallax (82/18)	$\chi^2 = 43.53$; $P < 0.0001$	Bagged vs. supplemented ($P < 0.0001$) Unmanipulated vs. bagged ($P < 0.0001$) Bagged control vs. bagged ($P = 0.011$) Supplemented vs. bagged control ($P = 0.040$)	Bagged Bagged control Hand-supplemented Unmanipulated	3.63 ± 0.48 5.73 ± 0.11 6.42 ± 0.14 5.89 ± 0.20
*Haplostachys haplostachya** (105/23)	$\chi^2 = 76.97$; $P < 0.0001$	Unmanipulated vs. bagged ($P < 0.0001$) Unmanipulated vs. bagged control ($P = 0.015$) Unmanipulated vs. supplemented ($P < 0.0001$) Supplemented vs. bagged control ($P < 0.0001$) Supplemented vs. bagged ($P < 0.0001$)	Bagged Bagged control Hand-supplemented Unmanipulated	0.23 ± 0.16 0.50 ± 0.29 2.84 ± 0.19 1.22 ± 0.28
*Silene lanceolata** (102/11)	None	None	Bagged Bagged control Hand-supplemented Unmanipulated	51.20 ± 2.17 30.00 ± 3.29 59.49 ± 5.50 53.14 ± 2.19
*Stenogyne angustifolia** (55/28)	$\chi^2 = 20.43$; $P = 0.0001$	Unmanipulated vs. bagged control ($P = 0.024$) Unmanipulated vs. bagged ($P = 0.0026$) Supplemented vs. bagged control ($P = 0.0072$) Supplemented vs. bagged ($P = 0.0008$)	Bagged Bagged control Hand-supplemented Unmanipulated	0.05 ± 0.34 0.41 ± 0.46 2.07 ± 0.25 1.14 ± 0.31
*Tetramolopium arenarium** (91/10)	$\chi^2 = 9.77$; $P < 0.021$	Unmanipulated vs. bagged ($P = 0.039$)	Bagged Bagged control Hand-supplemented Unmanipulated	11.84 ± 2.17 18.00 ± 3.29 10.24 ± 5.50 18.18 ± 2.19

insects from contacting flowers. All bags did permit airflow to maintain ambient temperatures within bags, so some wind transport of pollen could have occurred, but the pollen of our focal species is noticeably sticky and heavy and seems unlikely to move without the aid of an animal. Following the administration of each treatment, we marked each treated flower with an indicator color of embroidery thread and allowed flowers to develop on the plants. We removed bags when corollas had fully wilted, and we harvested treatment fruits for seed counts once they were mature.

Data analysis—For each plant species, we analyzed flower treatment effects on seed set, defined as number of seeds produced per flower. Data did not meet assumptions of normality (based on quantile-quantile plots, used to evaluate normality due to limitations in sample size; Wood, 2010), so we used nonparametric Kruskal-Wallis tests to examine differences among treatments and employed Dunn's multiple comparisons test to determine which pairs of treatments differed significantly. Multiple flowers receiving the same treatment on a given plant individual were treated as subsamples (= averaged) in these analyses.

We used seed-set data to calculate the pollen limitation index (PLI) for each focal plant species. PLI is calculated as $1 - (U/S)$, where U = the proportional fruit set of unmanipulated flowers and S = the proportional fruit set of hand-supplemented flowers (Larson and Barrett, 2000). PLI = 0 indicates no pollen limitation, and PLI = 1 indicates full pollen limitation.

Data were analyzed using the packages "nlme" (Pinheiro et al., 2018), "vegan" (Oksanen et al., 2017), and "FSA" (Ogle, 2016) in R version 2.14.1 (R Core Team, 2012), with significance accepted at $P \leq 0.05$.

TABLE 2. Flower visitation observation results (*n*). Plants were observed opportunistically as they came into flower, resulting in uneven effort across species. Unresolved visitors were those identified to a broad enough taxonomic group that their origin (native vs. non-native) could not be determined. Asterisks indicate endangered species.

Plant spp.	Hours observed	Native visitor taxa	Non-native visitor taxa	Unresolved visitor taxa	Total visitor taxa
Argemone glauca	55.67	1	6	2	9
Bidens menziesii	70.67	2	6	3	11
Dubautia linearis	57.67	2	4	3	9
Sida fallax	59.17	2	6	2	10
*Haplostachys haplostachya**	60.67	0	5	1	6
*Silene lanceolata**	116.67	0	4	0	4
*Stenogyne angustifolia**	120.67	0	1	0	1
*Tetramolopium arenarium**	35.17	2	3	2	7

Pollen load analysis

Flower visitation is a necessary component of pollination, but it does not ensure successful pollen transfer or outcrossing. Species of flower visitors may vary widely in effectiveness, with some transporting large numbers of pollen between conspecific plants while others rarely carry pollen on their bodies, withhold it in pollen sacs from receptive stigmas, or frequently transfer heterospecific pollen. Both the anatomy and behavior of visitor species may be important. Once the set of primary flower visitors for all focal plant species was determined, we used sweep nets and vials to opportunistically collect up to five individuals of those visitors interacting with the focal plants. We swabbed the visitors' bodies and probosci with a small cube of fuchsin jelly and used a handheld lighter to melt the jelly onto a microscope slide with a coverslip. We allowed these samples to cool and returned them to a lab. We then used a Reichert Microstar IV microscope (Reichert Technologies, Depew, New York, USA) at 200× magnification to examine each slide for stained pollen grains (after Kearns and Inouye, 1993). We compared pollen morphotypes to voucher specimens of stained pollen taken directly from the anthers of each of our focal plant species. For each flower-visitor slide, we recorded the number of different pollen morphotypes on the slide as well as the approximate number of pollen grains (by classifying into logarithmic bins 0, 1–10, 11–100, 101–1000, 1001–10,000, and >10,000) in each morphotype.

RESULTS

Flower visitation observations

We performed 35–120 h of flower visitation observations per focal plant species (Table 2). The variation was due to seasonality of plants; some species flowered almost continually, whereas others flowered only during discrete periods within the year. Observations took place opportunistically when flowering individuals were found. Across all observations, the large majority of flower visitors either were non-native insects or were recorded at the order or family level and insufficiently resolved to determine origin (i.e., native vs. non-native; Fig. 2A). The most common visitor across the study was the non-native *A. mellifera*. Among native species, the most common visitor was *Hylaeus* (note that both native and non-native bees in the genus *Hylaeus* occur at the PTA, but natives are more common and likely account for most *Hylaeus* observations in our dataset). The only remaining known native flower visitors that we observed in systematic observations (total = 576.36 h of observation for all eight species) were *Orthomecyna* sp. (a crambid moth) and *Udara blackburni* (the Hawaiian blue butterfly) (Fig. 2A). Taxa with uncertain origin included unidentified moths (Lepidoptera), unidentified wasps (Hymenoptera), and unidentified beetles (Coleoptera). All other taxa were known non-natives (Fig. 2A).

The common native plant species were visited by a higher diversity of insects than were the endangered plant species (Table 3). Common natives *A. glauca*, *B. menziesii*, *D. linearis*, and *S. fallax* interacted with 9, 11, 9, and 10 flower-visitor taxa, respectively (Table 3). Endangered plants *H. haplostachya*, *S. lanceolata*, *S. angustifolia*, and *T. arenarium* interacted with 6, 4, 1, and 7 flower-visitor taxa, respectively (Table 3). The endangered species *H. haplostachya*, *S. lanceolata*, and *S. angustifolia* received no visits from known native insects (Table 2). In fact, *S. angustifolia* received just a single visit, in January 2016, from the non-native bee *Lasioglossum impavidum* (Table 2). By scaled importance value, only two primary visitor interactions involved known native insects: the native crambid moth *Orthomecyna* sp. was the most important visitor for *T. arenarium*, and native *Hylaeus* bees were among the primary visitors for *B. menziesii* (Table 3). No flower visitation was observed during the 40 h of nighttime observations we conducted for *S. lanceolata*, although we are continuing to explore options for further assessment of nocturnal flower visitation.

Pollination treatments

All plant species produced some seed when bagged to exclude outcrossing, indicating self-compatibility for each species (Table 1). The common species *B. menziesii*, *D. linearis*, and *S. fallax* and the endangered species *H. haplostachya*, *S. angustifolia*, and *T. arenarium* produced significantly more seed when flowers were allowed to outcross (unmanipulated controls) than when they were bagged, indicating limited autogamy. The PLI results, which are positive when hand supplementation boosts fruit or seed production compared with unmanipulated controls, were 0.44 for *A. glauca*, 0.57 for *H. haplostachya*, 0.08 for *S. fallax*, 0.11 for *S. lanceolata*, and 0.45 for *S. angustifolia* (Table 1). The highest pollen-limitation values were exhibited by the endangered species *H. haplostachya* and *S. angustifolia* and the common native *A. glauca*. The endangered species *S. lanceolata*, which also demonstrated no significant decrease in seed set when outcrossing was prevented, exhibited a very low PLI of 0.11, suggesting that much of the seed production for this species may occur via autogamy. For all of the

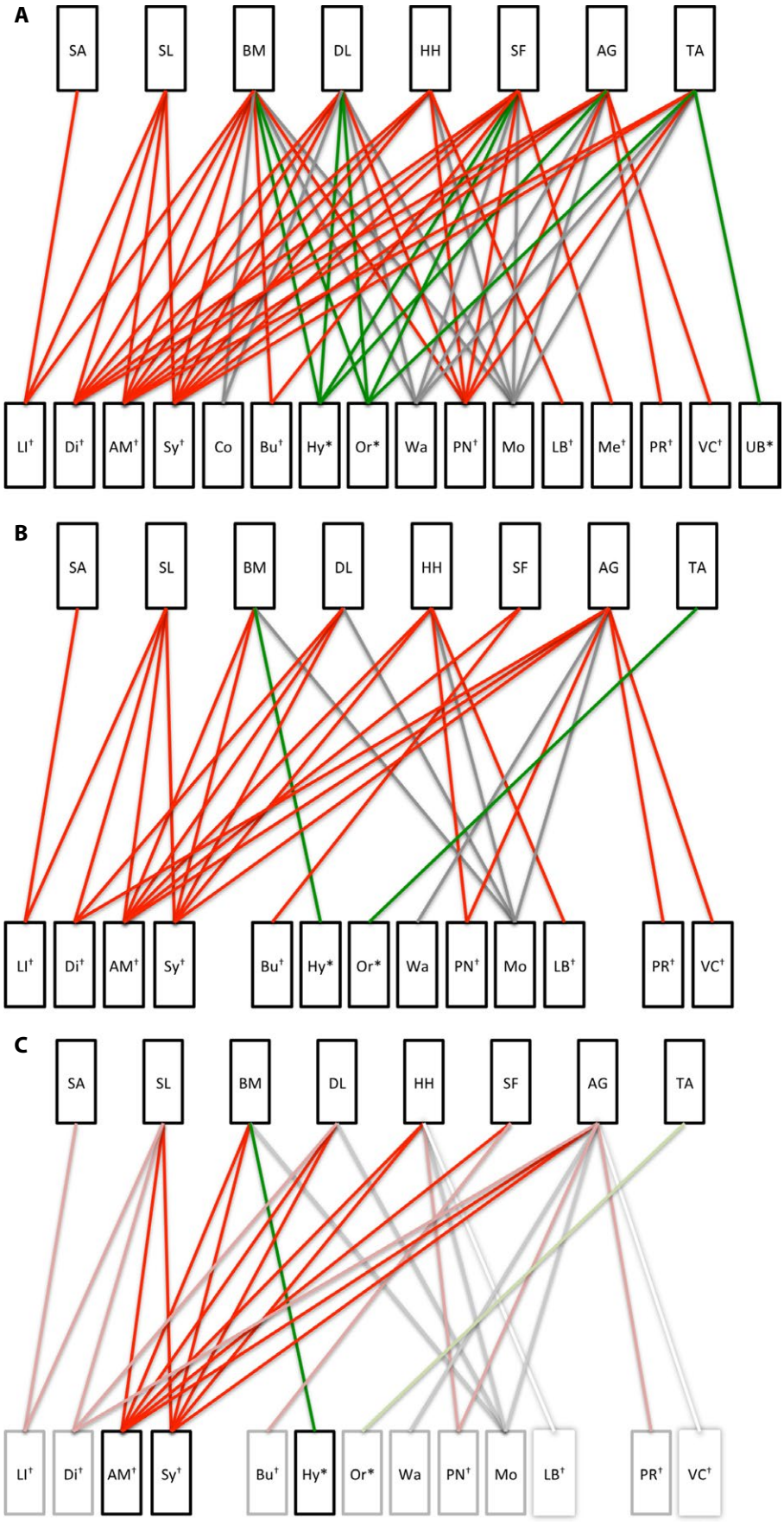

FIGURE 2. Networks displaying observed interactions between flower visitors and the eight focal native plant species in this study. Green connectors* = native flower visitors. Gray connectors = flower visitors of indeterminate nativity. Red connectors† = non-native flower visitors. Plants appear in the top row: SA = *Stenogyne angustifolia*, SL = *Silene lanceolata*, BM = *Bidens menziesii*, DL = *Dubautia linearis*, HH = *Haplostachys haplostachya*, SF = *Sida fallax*, AG = *Argemone glauca*, and TA = *Tetramolopium arenarium*. (A) Full network, containing all observed interactions. Flower visitors appear in the bottom row: LI = *Lasioglossum impavidum*, Di = Diptera (unspecified), AM = *Apis mellifera*, Sy = Syrphidae, Co = Coleoptera (unspecified), Bu = Butterfly (unspecified), Hy = *Hylaeus* sp. (unspecified), Or = *Orthomecyna* sp., Wa = Wasp (unspecified), PN = *Pachodynerus nasidens*, Mo = Moth (unspecified), LB = *Lampides boeticus*, Me = Megachilidae (unspecified), PR = *Pieris rapae*, VC = *Vanessa cardui*, and UB = *Udara blackburni*. (B) Primary network, containing just those interactions ≥25% as important as the most important interaction for each plant species. (C) Primary network with pollen transport information. Visitor taxa with white labels were not captured to determine pollen loads. Visitor taxa with gray labels were unreliable pollen transporters, with captured individuals frequently bearing no pollen. Visitor taxa with black labels reliably carried either small loads (Syrphidae and *Hylaeus*) or large loads (*Apis mellifera*) of pollen.

Asteraceae species we examined, PLI was negative (−0.78 for *T. arenarium*, −0.26 for *B. menziesii*, −0.53 for *D. linearis*), implying stigmatic damage during hand-supplementation treatments (see below; Young and Young, 1992).

Pollen-load confirmation

In all, we captured 119 flower visitors interacting with our focal plant species in order to swab their bodies and probosci for pollen transport. We targeted visitor taxa identified as "primary" visitors in our visitation observations (Fig. 2B). We failed to catch two of the taxa in our set of primary flower visitors: *V. cardui* and *L. boeticus*. All other primary visitors

TABLE 3. Quantitative interaction network displaying scaled importance values for each flower visitor observed to interact with focal flowers. Importance values are calculated as the number of flowers probed by each visitor per minute per visible open flower, multiplied by the number of visitor individuals present during each scan observation. Importance values were scaled such that the maximum importance value for each plant species was set equal to 1.0000 and the proportional values of all other visitors are in relation to that maximum. Bold indicates known non-native flower visitors. For quick reference, interactions with common native plant species are highlighted in blue and interactions with endangered plant species are highlighted in green; darker highlights indicate more important interactions. Asterisks indicate known native visitor taxa.

	Common native plant species				Endangered plant species				
Species	***Argemone glauca***	***Bidens menziesii***	***Dubautia linearis***	***Sida fallax***	***Haplostachys haplostachya***	***Silene lanceolata***	***Stenogyne angustifolia***	***Tetramolopium arenarium***	**Total plants visited**
Honeybee (*Apis mellifera*)	**1.0000**	**1.0000**	**0.9007**	**0.5442**	**0.4789**	**1.0000**	**0**	**0.0035**	**7**
Moth (unspec.) (Lepidoptera spp.)	0.6718	0.3685	0.3025	0.0080	0.6540	0	0	0.0078	6
Hoverfly (*Syrphid spp.*)	**0.2160**	**0.2318**	**1.0000**	**0.0033**	**0.4957**	**0.2658**	**0**	**0.0026**	**7**
Painted lady butterfly (*Vanessa cardui*)	**0.1932**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**
Fly (unspec.) (Diptera spp.)	**0.1907**	**0.1120**	**0.9014**	**0.0002**	**0.0111**	**0.3586**	**0**	**0**	**6**
Cabbage butterfly *(Pieris rapae)*	**0.1777**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**
Wasp (unspec.) (Hymenoptera spp.)	0.1362	0.0643	0.1466	0.0078	0	0	0	0.0082	5
Keyhole wasp *(Pachodynerus nasidens)*	**0.0299**	**0.0213**	**0.0006**	**0.0058**	**1.0000**	**0**	**0**	**0.0081**	**6**
Yellow-faced bee (*Hylaeus* spp.)*	0.0118	0.3505	0.0068	0.0502	0	0	0	0	4
Crambid moth (*Orthomecyna* sp.)*	0	0.0938	0.0275	0.0004	0	0	0	1.0000	4
Butterfly (unspec.) (Lepidoptera spp.)	0	0.0105	0	1.0000	0	0	0	0	2
Beetle (unspec.) (Coleoptera spp.)	0	0.0015	0.0048	0	0	0	0	0	2
Sweat bee (*Lasioglossum impavidum*)	**0**	**0.0136**	**0**	**0**	**0**	**0.3984**	**1.0000**	**0**	**3**
Leafcutting bee (Megachilidae sp.)	**0**	**0**	**0**	**0.0581**	**0**	**0**	**0**	**0**	**1**
Bean butterfly (*Lampides boeticus*)	**0**	**0**	**0**	**0**	**0.4722**	**0**	**0**	**0**	**1**
Hawaiian blue butterfly (*Udara blackburni*)*	0	0	0	0	0	0	0	0.0011	1
Total Visitor Taxa	9	11	9	10	6	4	1	7	

were captured. A total of six visitor taxa were determined to be unreliable pollen carriers because sampled individuals inconsistently carried pollen; that is, some individuals of each of these taxa carried no pollen, and the remaining individuals carried pollen in one of the smallest transport class (1–10 or 11–100 grains). These taxa included *L. impavidum*, Diptera (various), moths (various), *Pieris rapae*, butterflies (various), and *Orthomecyna* sp. The remaining primary visitors were reliable transporters, with each sampled individual carrying at least some pollen. Reliable transporters that carried low pollen loads (1–10 grains or 11–100 grains) included *A. exotica*, *Hylaeus* sp., wasps (various), and *P. nasidens*. The only reliable transporter that carried high pollen loads was *A. mellifera* (out of 19 captured *A. mellifera*, seven carried estimated pollen loads that far exceeded 10,000 grains); this was the case even when we excluded the pollen in *A. mellifera* corbiculae from examination and focused only on the pollen caught in body hairs (Fig. 2C). Among those visitors carrying pollen, approximately two-thirds (62%) carried only the pollen morphotype matching that of the

plant on which they were captured. Another 25% carried two morphotypes, and the remaining 13% carried three morphotypes.

DISCUSSION

The focal ecological community for this study has been heavily altered by non-native species introductions and native species losses, and our results indicate that the flower-visitor fauna interacting with focal native plant species, at least during the daytime, is overwhelmingly non-native. The only native taxon that visited our plants with regularity was *Hylaeus*, which visited four of our focal species and was a primary visitor to *B. menziesii*. *Orthomecyna* sp., an endemic moth, was one of the primary visitors to *T. arenarium*, but was otherwise rarely observed in our observations across the community. By contrast, the non-native *A. mellifera* and Syrphidae each visited seven of our focal species (all except *S. angustifolia*) and each was a primary visitor to five of these. As an example of known relevant declines among native flower visitors, the super-diverse genus *Hylaeus* has been found to be affected by environmental change in recent decades: at least 10 *Hylaeus* species are likely to have become extinct in Hawaii since European colonization (Magnacca, 2007), and seven species were recently federally listed as endangered (USFWS, 2016).

Our study detected important differences between common native plant species and endangered plant species. Fewer flower-visitor taxa overall, and fewer native flower visitors, interacted with the endangered species than with the common plant species. Indeed, we observed no native flower visitors interacting with three of our focal endangered plant species, and two of these endangered plants exhibited higher pollen-limitation values than other plants in the study. Although all the plant species examined here are self-compatible, outcrossing increased seed production significantly for six of the focal plant species, including three of the endangered species. Because non-native insects were the primary (or exclusive) flower visitors for most of these species, it is likely that their ability to transport pollen is particularly important for maintaining native plant reproductive output in this system.

With new species participating in mutualistic interactions, the quality and quantity of ongoing pollination may differ substantially from historical conditions, but the community transformation is so profound that we have no way of knowing what those conditions may have been. Non-native species worldwide have caused declines in native populations and disruptions of key ecological functions (Sax and Gaines, 2008). On islands, these negative effects of non-native species can be particularly pronounced because so many endemic species are vulnerable to novel predators and competitors, and the Hawaiian Islands are an excellent example of this (Loope et al., 1988). Non-native species may also, however, form mutualisms with native species (Rodriguez, 2006; Pratt et al., 2012; Shay et al., 2016). Mutualisms are ubiquitous in nature (Bronstein, 1994). Most such interactions are relatively opportunistic and diffuse: the participating species can obtain mutualistic benefits from a diversity of partner species, and this can include non-native species exhibiting the needed traits and functions. If native species carrying out critical functions have been lost from the area of introduction, it may be that the non-native species can replace threatened functions (Aslan et al., 2012). In the case of our study system, the now dominant non-native flower visitors may enable outcrossing for endemic species when it appears likely that the relatively rare native fauna would be unable to do so. For the endangered *S. lanceolata* and *S. angustifolia*, both of which were visited exclusively by known non-natives during our year of data collection, the shift from native to non-native flower visitors may be particularly relevant, and future research on the implications of non-native pollinators for these two endangered species may be essential to support effective management. For *S. angustifolia*, moreover, the sole visitor was a non-native bee that was found to be an unreliable transporter of pollen, suggesting that outcrossing may be particularly rare for this species.

Our results must be interpreted with care because some of the flower-visitor taxa that we recorded were impossible to identify visually with sufficient taxonomic resolution to determine whether they were native or non-native. Additionally, all detected flower visits occurred during the daytime, but we were able to perform a much lower number of nighttime observation hours and thus cannot draw conclusions about potential nighttime visitation. Nevertheless, the primary visitors we recorded included many known non-natives, and the known natives were very few. Known non-natives were the most important visitors to *B. menziesii*, *D. linearis*, *S. lanceolata*, *H. haplostachya*, *S. angustifolia*, and *A. glauca*. Pollen was consistently found only on *Hylaeus* sp. bees, on wasps (a group comprising both native and non-native species in Hawaii), and on three non-native taxa (Syrphidae, *A. mellifera*, and *P. nasidens*). Only one of these species, *A. mellifera*, consistently carried large quantities of pollen—in the thousands of grains compared with dozens of grains for the other species. Although *A. mellifera* grooms itself to move pollen into its corbiculae (pollen-carrying sacs) and thus much transported pollen will be unavailable to stigmas of the next visited flower (Adler and Irwin, 2006), individuals may carry so many thousands of grains and their bodies and heads are often so coated with pollen that even incidental deposition of a very small percentage of those grains may make *A. mellifera* a reliable mover of pollen between flowers (as has been found in some other systems; e.g., Watts et al., 2012; Sun et al., 2013; but see Garibaldi et al., 2013; Mallinger and Gratton, 2015). Continued research examining stigmatic deposition is a necessary next step in this system to better understand the quantity of pollination provided by *A. mellifera* (e.g., Thomson and Goodell, 2001; King et al., 2013). We cannot know whether *A. mellifera* edged out native pollinators when it first became established in the system or is simply now carrying out a function that had been largely lost before it arrived; whether non-native pollinators in Hawaii have competitively displaced or are compensating for natives is, in general, a matter of uncertainty (e.g., Freed and Cann, 2009; Aslan et al., 2013b). Worldwide, honeybees are known to effectively transfer pollen for thousands of plant species (Moritz et al., 2005; Cayuela et al., 2011; Abrol, 2012), indicating that high visitation of *A. mellifera* to many of our plant species is likely indicative of some pollen transfer for those plants. Indeed, although *A. mellifera* may be less efficient per visit at transferring pollen, its high abundance can make it the most effective pollinator in a given system (Rader et al., 2009). At the same time, however, *A. mellifera* has also been shown to competitively displace native pollinators in some systems (e.g., Hudewenz and Klein, 2013; Lindström et al., 2016). *Apis mellifera* in Hawaii is facing some of the same threats as elsewhere, including parasitism by the varroa mite (Wilfert et al., 2016). Plant outcrossing in this system could perhaps be impacted if local honeybee populations decline, an event that would likely signify a second major pollinator change with unknown consequences for the full pollination network.

On remote oceanic islands, it may be difficult or impossible for the initial colonizing members of a plant species to encounter mates and establish reproductive populations. Colonization success may be significantly boosted by self-compatibility, or the potential of an individual plant to reproduce in the absence of any conspecifics (Baker, 1955). Self-compatible colonizers are likely to inbreed, and genetic disadvantages of this pattern may be alleviated if deleterious alleles were purged from a population during the process of successful colonization (Lande et al., 1994; Crnokrak and Barrett, 2002). Indeed, native species on islands exhibit high rates of self-compatibility compared to mainland communities (Barrett et al., 1996). At the same time, island species exhibit particularly high rates of dioecy, limiting inbreeding and hinting at the importance of outcrossing (Sakai et al., 1995). All the plant species studied here demonstrate self-compatibility, which may temporarily shield them from negative effects of pollinator loss by enabling them to continue to produce seed under uncertain pollination conditions. However, over time, the lack of gene flow among populations and the resulting increase in inbreeding could limit the adaptive capacity of plants relegated entirely or mostly to self-fertilization (Armbruster and Reed, 2005). In our study, open or unmanipulated flowers set more seed or fruit than bagged or self-fertilized flowers for most of our focal plant species, suggesting that autogamy is limited and effective outcrossing is indeed occurring in this system for species varying in flower morphology and phenology. At the same time, based on the PLI, three of our plants (*A. glauca*, *H. haplostachys*, and *S. angustifolia*) exhibited pollen limitation, implying that their reproductive output would be higher with increased pollen transfer.

Our treatments quantified current pollen limitation by comparing fruit and seed set between hand-supplemented and unmanipulated flowers. However, the variance in fruit and seed set exhibited by hand-supplemented flowers was extremely high, underscoring the difficulty inherent in adequate hand pollination of these flowers. In particular, the three Asteraceae species we examined, *B. menziesii*, *D. linearis*, and *T. arenarium*, exhibited slight numerical reductions in fruits and seeds when hand-pollinated vs. non-manipulated, although the treatment effect was nonsignificant; this resulted in negative PLI values for these species. Similar results have been found in other studies focused on flowers that exhibit precise timing requirements for pollination (Young and Young, 1992). Asteraceae produce protandrous flowers, and the transition from male to female occurs within each floret individually, such that for most of its life span a receptive flower head contains receptive florets, budding florets, and senescing florets simultaneously. To hand pollinate, it is necessary to gather pollen when it is most viable and to administer it when stigmas are most receptive. It may also be necessary to ensure that pollen transfer is occurring between non-sibling plants. These plants are naturally occurring, so we cannot know which individuals are closely related. Because the study site is a military base, we did not have constant access to any given plant for pollen administration. Within our restricted temporal window of access, we were unable to target each individual floret for pollination, and our treatments may have missed some receptive florets and damaged some senescing florets, which become only loosely attached to the receptacle as they age (Young and Young, 1992). As a result of these factors, we can only conclude that we have found no evidence of heavy pollen limitation for the Asteraceae in this study. In spite of these considerations, the ongoing transformation of the pollinator community in this system bears further scrutiny, particularly as restoration and conservation efforts continue. The isolation and small size of oceanic islands generally result in relatively small species richness within each ecological guild, including among pollinators and flowering plants; island pollination networks are therefore simplified in comparison to continental networks (Aslan et al., 2013a). High rates of extinction and species introduction can transform Hawaiian pollinator–plant communities, with losses of historical links that used to connect interacting partners (Cox and Elmqvist, 2000).

CONCLUSIONS

At least during daytime hours, non-native insects in this system appear to be facilitating outcrossing for most of the focal plants, particularly the endangered species, whereas native insects exhibit low diversity and low flower-visitation frequency. Restoration and conservation in this and similar systems (e.g., Shay et al., 2016) going forward represents a challenge (Seastedt et al., 2008). Without knowing what the historical native pollinators for these native plant species may have been, we are unable to attempt exact restoration of historical interactions. Thus, this ecological community may have entered a new stable state (*sensu* Holling, 1973). The long-term implications of new and transformed species interactions are unknown. There could be qualitative and quantitative ramifications of the shift from native to non-native pollinators (Herrera, 1987; Aizen and Harder, 2007; Aizen et al., 2008). Non-native species may carry pollen in different spatial, quantitative, and temporal patterns from those once exhibited by native pollinators, for example. We can speculate, in that case, that the relative abundance of different plant species in the community could change over time, as reproductive success shifts. As time goes on in this novel state, we may begin to observe new changes within the community as a result of the transformation of the pollinator functional guild.

ACKNOWLEDGEMENTS

The authors thank H. Coad, M. Donofrio, J. Felt, J. Smith, and A. Wagner for tireless hours of field observations, as well as the PTA Natural Resources Office for essential logistical support and assistance. This work was funded by the Department of Defense Strategic Environmental Research and Development Program (grant no. RC-2432) and was performed under USFWS Native Endangered Species Recovery Permit: TE-28360B-0; Hawaii DLNR-DOFAW Permit for Threatened & Endangered Plant Species: P-201; and Hawaii DLNR-DOFAW Permit for Native Invertebrate Research: FHM17-428. The authors thank K. Magnacca for invaluable bee identification assistance and two anonymous reviewers for insightful and very helpful recommendations.

AUTHOR CONTRIBUTIONS

All authors conceived of and carried out the study jointly. C.A. led the analysis and writing, with substantial contributions, suggestions, and editing by A.S., W.H., and C.L.

DATA ACCESSIBILITY

Raw flower-visitation observation data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.tm575v4 (Aslan et al., 2018).

LITERATURE CITED

Abrol, D. P. 2012. Pollination biology. Springer, Dordrecht.

Adler, L. S., and R. E. Irwin. 2006. Comparison of pollen transfer dynamics by multiple floral visitors: Experiments with pollen and fluorescent dye. *Annals of Botany* 97: 141–150.

Aizen, M. A., and L. D. Harder. 2007. Expanding the limits of the pollen-limitation concept: Effects of pollen quantity and quality. *Ecology* 88: 271–281.

Aizen, M. A., C. L. Morales, and J. M. Morales. 2008. Invasive mutualists erode native pollination webs. *PLoS Biology* 6: e31.

Armbruster, P., and D. Reed. 2005. Inbreeding depression in benign and stressful environments. *Heredity* 95: 235–242.

Aslan, C. E., E. S. Zavaleta, D. Croll, and B. Tershy. 2012. Effects of native and non-native vertebrate mutualists on plants. *Conservation Biology* 26: 778–789.

Aslan, C. E., E. S. Zavaleta, B. Tershy, and D. Croll. 2013a. Mutualism disruption threatens global plant biodiversity: A systematic review. *PLoS ONE* 8: e66993.

Aslan, C. E., E. S. Zavaleta, B. Tershy, D. Croll, and R. H. Robichaux. 2013b. Imperfect replacement of native species by non-native species as pollinators of endemic Hawaiian plants. *Conservation Biology* 28: 478–488.

Aslan, C. E., J. L. Bronstein, H. S. Rogers, K. B. Gedan, J. Brodie, T. M. Palmer, and T. P. Young. 2016. Leveraging nature's backup plans to incorporate interspecific interactions and resilience into restoration. *Restoration Ecology* 24: 434–440.

Aslan, C. E., A. B. Shiels, W. Haines, and C. T. Liang. 2018. Data from: Non-native insects dominate daytime pollination in a high-elevation Hawaiian dryland ecosystem. Dryad Digital Repository. https://doi.org/10.5061/dryad.tm575v4.

Baker, H. G. 1955. Self-compatibility and establishment after "long-distance" dispersal. *Evolution* 9: 347–349.

Barnosky, A. D., N. Matzke, S. Tomiya, G. O. U. Wogan, B. Swartz, T. B. Quental, C. Marshall, et al. 2011. Has the Earth's sixth mass extinction already arrived? *Nature* 471: 51–57.

Barrett, S. C. H., B. Emerson, and J. Mallet. 1996. The reproductive biology and genetics of island plants [and discussion]. *Philosophical Transactions of the Royal Society B: Biological Sciences* 351: 725–753.

Blackburn, T. M., P. Cassey, R. P. Duncan, K. L. Evans, and K. J. Gaston. 2004. Avian extinction and mammalian introductions on oceanic islands. *Science* 305: 1955–1958.

Bond, W. J. 1994. Do mutualisms matter? Assessing the impact of pollinator and disperser disruption on plant extinction. *Philosophical Transactions of the Royal Society B* 344: 83–90.

Boyer, A. G. 2008. Extinction patterns in the avifauna of the Hawaiian islands. *Diversity and Distributions* 14: 509–517.

Brodie, J. F., C. E. Aslan, H. S. Rogers, K. H. Redford, J. L. Maron, J. L. Bronstein, and C. R. Groves. 2014. Secondary extinctions of biodiversity. *Trends in Ecology & Evolution* 29: 664–672.

Bronstein, J. L. 1994. Conditional outcomes in mutualistic interactions. *Trends in Ecology & Evolution* 9: 214–217.

Caujapé-Castells, J., A. Tye, D. J. Crawford, A. Santos-Guerra, A. Sakai, K. Beaver, W. Lobin, et al. 2010. Conservation of oceanic island floras: Present and future global challenges. *Perspectives in Plant Ecology, Evolution and Systematics* 12: 107–129.

Cayuela, L., S. Ruiz-Arriaga, and C. P. Ozers. 2011. Honeybees increase fruit set in native plant species important for wildlife conservation. *Environmental Management* 48: 910–919.

Clavel, J., R. Julliard, and V. Devictor. 2010. Worldwide decline of specialist species: Toward a global functional homogenization? *Frontiers in Ecology and the Environment* 9: 222–228.

Colwell, R. K., R. R. Dunn, and N. C. Harris. 2012. Coextinction and persistence of dependent species in a changing world. *Annual Review of Ecology, Evolution, and Systematics* 43: 183–203.

Cox, P. A., and T. Elmqvist. 2000. Pollinator extinction in the Pacific islands. *Conservation Biology* 14: 1237–1239.

Crnokrak, P., and S. C. H. Barrett. 2002. Purging the genetic load: A review of the experimental evidence. *Evolution* 56: 2347–2358.

Dudash, M. R. 1990. Relative fitness of selfed and outcrossed progeny in a self-compatible, protandrous species, *Sabatia angularis* L. (Gentianaceae): A comparison in three environments. *Evolution* 44: 1129–1139.

Dunn, R. R., N. C. Harris, R. K. Colwell, L. P. Koh, and N. S. Sodhi. 2009. The sixth mass coextinction: Are most endangered species parasites and mutualists? *Proceedings of the Royal Society B* 276: 3037–3045.

Frankham, R. 1998. Inbreeding and extinction: Island populations. *Conservation Biology* 12: 665–675.

Freed, L. A., and R. L. Cann. 2009. Negative effects of an introduced bird species on growth and survival in a native bird community. *Current Biology* 19: 1736–1740.

Garibaldi, L. A., I. Steffan-Dewenter, R. Winfree, M. A. Aizen, R. Bommarco, S. A. Cunningham, C. Kremen, et al. 2013. Wild pollinators enhance fruit set of crops regardless of honey bee abundance. *Science* 339: 1608–1611.

Hanna, C., D. Foote, and C. Kremen. 2013. Invasive species management restores a plant–pollinator mutualism in Hawaii. *Journal of Applied Ecology* 50: 147–155.

Herrera, C. M. 1987. Components of pollinator "quality": Comparative analysis of a diverse insect assemblage. *Oikos* 50: 79–90.

Holling, C. S. 1973. Resilience and stability of ecological systems. *Annual Review of Ecology and Systematics* 4: 1–23.

Hudewenz, A., and A. M. Klein. 2013. Competition between honey bees and wild bees and the role of nesting resources in a nature reserve. *Journal of Insect Conservation* 17: 1275–1283.

Janzen, D. H. 1988. Tropical dry forests: The most endangered major tropical ecosystem. *In* E. O. Wilson and F. M. Peter [eds.], Biodiversity, 130–137. National Academies Press, Washington, D.C.

Kaiser-Bunbury, C. N., S. Muff, J. Memmott, C. B. Müller, and A. Caflisch. 2010a. The robustness of pollination networks to the loss of species and interactions: A quantitative approach incorporating pollinator behaviour. *Ecology Letters* 13: 442–452.

Kaiser-Bunbury, C. N., A. Traveset, and D. M. Hansen. 2010b. Conservation and restoration of plant–animal mutualisms on oceanic islands. *Perspectives in Plant Ecology, Evolution and Systematics* 12: 131–143.

Kaiser-Bunbury, C. N., T. Valentin, J. Mougal, D. Matatiken, and J. Ghazoul. 2011. The tolerance of island plant–pollinator networks to alien plants. *Journal of Ecology* 99: 202–213.

Kearns, C. A., and D. W. Inouye. 1993. Techniques for pollination biologists. University Press of Colorado, Niwot, Colorado, USA.

King, C., G. Ballantyne, and P. G. Willmer. 2013. Why flower visitation is a poor proxy for pollination: measuring single-visit pollen deposition, with implications for pollination networks and conservation. *Methods in Ecology and Evolution* 4: 811–818.

Koh, L. P., R. R. Dunn, N. S. Sodhi, R. K. Colwell, H. C. Proctor, and V. S. Smith. 2004. Species coextinctions and the biodiversity crisis. *Science* 305: 1632–1634.

Kremer, A., O. Ronce, J. J. Robledo-Arnuncio, F. Guillaume, G. Bohrer, R. Nathan, J. R. Bridle, et al. 2012. Long-distance gene flow and adaptation of forest trees to rapid climate change. *Ecology Letters* 15: 378–392.

Kueffer, C., L. Kronauer, and P. J. Edwards. 2009. Wider spectrum of fruit traits in invasive than native floras may increase the vulnerability of oceanic islands to plant invasions. *Oikos* 118: 1327–1334.

Lande, R., D. W. Schemske, and S. T. Schultz. 1994. High inbreeding depression, selective interference among loci, and the threshold selfing rate for purging recessive lethal mutations. *Evolution* 48: 965–978.

Larson, B. M., and S. C. Barrett. 2000. A comparative analysis of pollen limitation in flowering plants. *Biological Journal of the Linnean Society* 69: 503–520.

Lindström, S. A., L. Herbertsson, M. Rundlöf, R. Bommarco, and H. G. Smith. 2016. Experimental evidence that honeybees depress wild insect densities in a flowering crop. *Proceedings of the Royal Society B* 283: 20161641.

Loope, L. L., O. Hamann, and C. P. Stone. 1988. Comparative conservation biology of oceanic archipelagoes: Hawaii and the Galapagos. *BioScience* 38: 272–282.

Loveless, M. D., and J. L. Hamrick. 1984. Ecological determinants of genetic structure in plant populations. *Annual Review of Ecology and Systematics* 15: 65–95.

Magnacca, K. N. 2007. Conservation status of the endemic bees of Hawai'i, *Hylaeus* (Nesoprosopis) (Hymenoptera: Colletidae). *Pacific Science* 61: 173–190.

Mallinger, R. E., and E. Gratton. 2015. Species richness of wild bees, but not the use of managed honeybees, increases fruit set of a pollinator-dependent crop. *Journal of Applied Ecology* 52: 323–330.

Manson, J. H. 1997. Does female rank or age affect mate choice in free-ranging rhesus macaques? *Folia Primatologica* 68: 366–369.

McKinney, A. M., P. J. CaraDonna, D. W. Inouye, B. Barr, C. D. Bertelsen, and N. M. Waser. 2012. Asynchronous changes in phenology of migrating Broad-tailed Hummingbirds and their early-season nectar resources. *Ecology* 93: 1987–1993.

Moritz, R. F., S. Härtel, and P. Neumann. 2005. Global invasions of the western honeybee (*Apis mellifera*) and the consequences for biodiversity. *Ecoscience* 12: 289–301.

Ogle, D. H. 2016. FSA: Fisheries Stock Analysis. R package version 0.8.10.

Oksanen, J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P. R. Minchin, et al. 2017. vegan: Community Ecology Package. R package version 2.4-3, website: http://CRAN.R-project.org/package=vegan.

Pinheiro, J., D. Bates, S. Debroy, D. Sarkar, and R Core Team. 2018. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-137, website: https://CRAN.R-project.org/package=nlme.

Pratt, L. W., J. R. VanDeMark, and M. Euaparadorn. 2012. Status and limiting factors of two rare plant species in dry montane communities of Hawai'i Volcanoes National Park. USGS Technical Report HCSU-030.

R Core Team. 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rader, R., B. Howlett, S. Cunningham, D. Westcott, L. Newstrom-Lloyd, M. Walker, D. Teulon, and W. Edwards. 2009. Alternative pollinator taxa are equally efficient but not as effective as the honeybee in a mass flowering crop. *Journal of Applied Ecology* 46: 1080–1087.

Renne, I. J., S. A. Gauthreaux, Jr., and C. A. Gresham. 2000. Seed dispersal of the Chinese tallow tree (*Sapium sebiferum* (L.) Roxb.) by birds in coastal South Carolina. *The American Midland Naturalist* 144: 202–215.

Rico-Gray, V. 1989. The importance of floral and circum-floral nectar to ants inhabiting dry tropical lowlands. *Biological Journal of the Linnean Society* 38: 173–181.

Rodriguez, L. F. 2006. Can invasive species facilitate native species? Evidence of how, when, and why these impacts occur. *Biological Invasions* 8: 927–939.

Sakai, A. K., W. L. Wagner, D. M. Ferguson, and D. R. Herbst. 1995. Biogeographical and ecological correlates of dioecy in the Hawaiian flora. *Ecology* 76: 2530–2543.

Sax, D. F., and S. D. Gaines. 2008. Species invasions and extinction: The future of native biodiversity on islands. *Proceedings of the National Academy of Sciences USA* 105: 11490–11497.

Seastedt, T. R., R. J. Hobbs, and K. N. Suding. 2008. Management of novel ecosystems: Are novel approaches required? *Frontiers in Ecology and the Environment* 6: 547–553.

Shay, K., D. R. Drake, A. D. Taylor, H. F. Sahli, M. Euaparadorn, M. Akamine, J. Imamura, et al. 2016. Alien insects dominate the plant-pollinator network of a Hawaiian coastal ecosystem. *Pacific Science* 70: 409–429.

Sun, S. G., S. Q. Huang, and Y. H. Guo. 2013. Pollinator shift to managed honeybees enhances reproductive output in a bumblebee-pollinated plant. *Plant Systematics and Evolution* 299: 139–150.

Thomson, J. D., and K. Goodell. 2001. Pollen removal and deposition by honeybee and bumblebee visitors to apple and almond flowers. *Journal of Applied Ecology* 38: 1032–1044.

USFWS. 2016. Endangered and Threatened Wildlife and Plants; Endangered Status for 49 Species from the Hawaiian Islands. Federal Register vol. 81, no. 190. September 30, 2016. Rules and Regulations.

Vitousek, P. M. 1988. Diversity and biological invasions of oceanic islands. *In* E. O. Wilson and F. M. Peter [eds.], Biodiversity, 181–189. National Academies Press, Washington, DC.

Wagner, W. L., D. R. Herbst, and S. H. Sohmer. 1999. Manual of the flowering plants of Hawaii. Revised edition with supplement. University of Hawaii Press, Honolulu.

Ward, M., C. W. Dick, R. Gribel, and A. J. Lowe. 2005. To self, or not to self… A review of outcrossing and pollen-mediated gene flow in neotropical trees. *Heredity* 95: 246–254.

Wardle, D. A., R. D. Bardgett, R. M. Callaway, and W. H. V. der Putten. 2011. Terrestrial ecosystem responses to species gains and losses. *Science* 332: 1273–1277.

Waser, N. M., and M. V. Price. 1989. Optimal outcrossing in *Ipomopsis aggregata*: Seed set and offspring fitness. *Evolution* 43: 1097–1109.

Watts, S., D. H. Ovalle, M. M. Herrera, and J. Ollerton. 2012. Pollinator effectiveness of native and non-native flower visitors to an apparently generalist Andean shrub, *Duranta mandonii* (Verbenaceae). *Plant Species Biology* 27: 147–158.

Wilfert, L., G. Long, H. C. Leggett, P. Schmid-Hempel, R. Butlin, S. J. M. Martin, and M. Boots. 2016. Deformed wing virus is a recent global epidemic in honeybees driven by *Varroa* mites. *Science* 351: 594–597.

Wilson, E. E., C. S. Sidhu, K. E. LeVan, and D. A. Holway. 2010. Pollen foraging behaviour of solitary Hawaiian bees revealed through molecular pollen analysis. *Molecular Ecology* 19: 4823–4829.

Wood, S. N. 2010. Statistical inference for noisy nonlinear ecological dynamic systems. *Nature* 466: 1102–1104.

Young, H. J., and T. P. Young. 1992. Alternative outcomes of natural and experimental high pollen loads. *Ecology* 73: 639–647.