

Evaluating the Qualitative Effectiveness of a Novel Pollinator: a Case Study of Two Endemic Hawaiian Plants

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ABSTRACT

In situations where native mutualists have become extinct, non-native species may partner with remnant native species. However, non-native mutualists may differ behaviorally from extinct native mutualists. In the case of pollination, novel relationships between natives and non-natives could differ both quantitatively and qualitatively from native–native relationships. In Hawai'i, the non-native Japanese White-eye (*Zosterops japonicus*) has largely replaced endemic birds as pollinator of the endemic *Clermontia parviflora* and *C. montis-loa*. We surveyed *Clermontia* patches and found that they ranged from 106 to 1198 m in diameter. We performed manual pollination of flowers with pollen taken from plants at five distance categories, ranging from 0 (self-fertilization) to 20 km, and examined the germination of resulting seeds. We used radiotelemetry to estimate daily Japanese White-eye movement distances. Percent germination of seeds after short- to intermediate-distance pollination crosses (*i.e.*, 20–1200 m, or intra-patch pollen transfer distances) significantly exceeded germination of seeds from selfed trials for *C. parviflora*. No significant differences in germination rates among treatments were detected for *C. montis-loa*. The maximum daily movement distances of radio-tracked birds were generally <1 km. Together, these results suggest that this novel pollinator may be an effective mutualist for both *Clermontia* species. This study serves as an example of research examining qualitative components of novel mutualism, which are generally neglected relative to quantitative components.

Key words: *Clermontia montis-loa*; *Clermontia parviflora*; distance crosses; germination; radiotelemetry; *Zosterops japonicus*.

GLOBAL EXTINCTION RATES HAVE ACCELERATED IN RECENT YEARS, driven by anthropogenic environmental impacts (Butchart *et al.* 2010). The loss of native species from an ecosystem may result in the disruption of ecological functions, potentially impacting the taxa with which they historically interacted (Kaiser-Bunbury *et al.* 2010). At the same time, biological invasions of species into novel habitats are also increasing (McGeoch *et al.* 2010). Biological invasions carry the potential for novel interactions between native and non-native species.

Mutualistic relationships arise when species receive reciprocal fitness benefits from interacting with one another (Bronstein 2009). A 'widow' is a species that has lost all of the partner species that formerly provided a particular mutualistic service, such as a plant that has lost all of its pollinators (*sensu* Olesen & Valido 2004). Because mutualisms evolve through the reciprocation of fitness benefits (Foster & Wenseleers 2006), their disruption can reduce the fitness of widowed species (Kiers *et al.* 2010). Without an available replacement to provide the function, fitness declines could ultimately threaten a widow with extinction (Brodie *et al.* 2009). Pollination, for example, may require narrow circumstances to properly function (*e.g.*, pollen delivery in specific quantities and ways; Wilcock & Neiland 2002), and the functioning of

pollination networks is threatened by global change (Kearns *et al.* 1998). Pollination failure is becoming more common (Potts *et al.* 2010).

Novel mutualisms between native and non-native species can affect the reproductive output of widowed species or exert more complex, indirect, or subtle effects (Kiers *et al.* 2010). Non-native novel mutualists might provide a widowed mutualist with an essential ecological function, such as pollination, after it has been lost (Rezende *et al.* 2007). However, a novel mutualist may not be expected to perform this function in exactly the same way as the extinct mutualists (Aslan *et al.* 2012). The effectiveness of a mutualism can include both quantitative and qualitative components: for example, quantitative aspects of seed dispersal include the total number of seeds dispersed, whereas qualitative aspects of seed dispersal include the recruitment suitability of the seed deposition site (Schupp 1993). Quantitative aspects of pollination include the visitation rate of pollinators, whereas qualitative aspects include the age and stage of flowers that are visited and the movement patterns of pollinators (Herrera 1987, Aizen & Harder 2007). Qualitative aspects of pollination by a novel mutualist could alter the widowed species' distribution, phenology, or even evolutionary trajectory (Kiers *et al.* 2010). Quantitative aspects of pollination are more easily studied, involving direct observation of pollinator visitation (Aslan *et al.* 2012). These quantitative assessments allow researchers to ascertain whether a

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novel pollinator is interacting with focal plants. However, the more difficult-to-study qualitative aspects of pollination can help us understand how the novel pollinator is interacting with the plants, and what the implications of the novel relationship might be for the evolution and conservation of those plants. To accurately assess the effectiveness of such relationships, both qualitative and quantitative components of pollination must be examined.

Due to high numbers of endemic species, oceanic islands often exhibit specialized plant–pollinator interactions (Abe 2006). Although the effects of pollinator losses on plant reproductive output remain poorly understood on islands (Abe 2006), modifications could include altered quantity and quality of pollen transfer (e.g., Madjidian *et al.* 2008), and altered spatial flow of pollen (Adler & Irwin 2006). These changes may have conservation importance, because island species are unusually likely to be threatened with extinction (Aslan *et al.* 2013). A number of studies have identified novel mutualisms that have evolved on islands after accidental species introduction (Aslan *et al.* 2012). The qualitative components of these novel mutualisms must be explored to facilitate informed decisions regarding invasive species management.

Here, we report on the qualitative aspects of pollination by a non-native bird (the Japanese White-eye; *Zosterops japonicus*) on Hawai'i Island, U.S.A. A previous study evaluated the quantitative effectiveness of this bird as a pollinator of plant species in an endemic genus of angiosperm (*Clermontia*) (Aslan *et al.* 2014). In over 100 h of visitation observations per plant species, those authors found that the non-native bird accounted for 84 percent of legitimate pollinator visits to the small-flowered *C. parviflora* and 92 percent of visits to the larger-flowered *C. montis-loa* (Aslan *et al.* 2014). These species were probably visited by endemic Hawaiian honeycreepers in the past (further details in *Study Species*), but widespread extinction of native Hawaiian birds and introduction of non-native birds to the islands have transformed the local avifauna. In the quantitative study, native bird visitation was rare compared with the non-native visitor: the very small 'Apapane (*Himatione sanguinea*) performed 16 percent of visits to *C. parviflora*, whereas the medium-sized 'I'iwi (*Vestiaria coccinea*) performed just 2.5 percent of visits and the small-sized 'Amakihi (*Hemignathus virens virens*) performed 3.5 percent of visits to *C. montis-loa* (Aslan *et al.* 2014). The study also examined the plant breeding system and found that self-fertilized flowers produced significantly fewer seeds than did open-pollinated controls, but that flowers visited by the White-eye exhibited similar seed set to that of open-pollinated controls (Aslan *et al.* 2014).

Building from that previous quantitative assessment, we set out to examine the quality of pollination offered by this non-native pollinator. One way in which pollination may differ between the native and novel pollinators in this system is in the spatial pattern of pollen flow. Due to introduced mosquito-transmitted avian diseases ubiquitous at lower elevations and habitat fragmentation, native passerine pollinators are more elevation-restricted than Japanese White-eye (Hart *et al.* 2011). Japanese White-eye occurs in every vegetation type on Hawai'i Island

(Scott *et al.* 1986). Native nectarivorous honeycreepers, on the other hand, specialize on high nectar reward flowers and cluster near such species, with occasional long-distance movement (Pratt *et al.* 2001). As feeding specialists, they may be less likely than White-eyes to shift habitat types or to use disturbed areas (Pejchar *et al.* 2005). For this study, we hypothesized that: (1) *C. parviflora* and *C. montis-loa* would exhibit spatial structure, with optimal pollen flow distances evident in fruit set and germination as indicators of pollen quality (*sensu* Aizen & Harder 2007); and (2) generalist Japanese White-eyes would exhibit daily movements, on average, that exceeded the optimal pollen flow distances, as native birds are likely to cluster more than Japanese White-eyes. We performed pollination crosses between parents at different distances, in conjunction with radiotelemetry of wild Japanese White-eye individuals, to examine the potential impact of probable spatial pollen flow dynamics on reproductive fitness components (germination rate and time to germination) of two *Clermontia* species: *C. parviflora* and *C. montis-loa*. In so doing, we examine a qualitative aspect of pollination effectiveness in this system and build off of the existing quantitative understanding. Although small-scale, our examination serves as a case study of such qualitative assessments, which are lacking for many novel mutualisms.

METHODS

STUDY SPECIES.—The *Clermontia* genus consists of 22 woody, endemic Hawaiian shrub species in the subfamily *Lobelioideae* (family *Campanulaceae*) (Lammers 1991). Each species is characterized by apparently self-compatible, protandrous, tubular, bird-pollinated inflorescences and large orange or yellow berries (Lammers 1991). We studied two species endemic to Hawai'i Island: *Clermontia parviflora* and *Clermontia montis-loa*. *Clermontia parviflora*'s range extends across four volcanoes on the eastern side of Hawai'i Island, whereas *C. montis-loa* is restricted to the eastern slopes of Mauna Loa volcano (Lammers 1991). Although they are uncommon in the ecosystem, both species are sufficiently abundant to allow study and experimental manipulation, whereas several other species in the genus are endangered and rigorously protected.

Hawaiian *Lobelioideae* were probably historically pollinated by endemic honeycreepers (Fringillidae) such as the Hawai'i Mamo (*Drepanis pacifica*) and endemic honeyeaters (Mohoidae) such as the 'O'o (Lammers *et al.* 1987). About half of these species are now extinct (including all honeyeater species) and others are endangered after undergoing large declines due to loss of habitat, reduced food supply, introduced avian diseases, introduced predators, and competition with introduced generalists (Scott *et al.* 1988). The largest non-endangered honeycreeper on Hawai'i Island is the 'I'iwi, but that bird is in decline and constrained to high elevation remnant forest patches, and its interactions with the focal *Clermontia* species are now extremely rare (Aslan *et al.* 2014).

Clermontia parviflora and *C. montis-loa* are now pollinated almost exclusively by the non-native Japanese White-eye (Aslan

et al. 2014). This bird is a habitat generalist (Scott *et al.* 1986) that was introduced to the island of O'ahu in 1929 (Waring *et al.* 1993) and has become the most abundant and widespread terrestrial bird in the Hawaiian Islands (Scott *et al.* 1986, Boelman *et al.* 2007). Occurring in high densities across a wide variety of habitat types throughout Hawai'i Island (Scott *et al.* 1986), the species feeds on insects, nectar, and fruit (Hart *et al.* 2011). Although the White-eye is now by far the most common visitor to *Clermontia* sp. flowers, the bird differs dramatically in morphology from the native honeycreepers. Both extinct and extant honeycreepers exhibit long, narrow, curved bills that are visibly suited to extracting nectar from the flowers of Lobelioideae. The White-eye, on the other hand, has a short, stubby, awl-shaped bill, a highly generalist morphology.

STUDY REGION AND SITES.—Manual pollination crosses were performed in two study sites per species (Fig. S1). All sites contained naturally occurring *Clermontia* populations in wet forest dominated by 'ohi'a (*Metrosideros polymorpha*) on the windward side of Hawai'i Island. *Clermontia montis-loa* occupies higher elevations in Mauna Loa cloud forest (ca 950–1250 m), whereas *C. parviflora* occurs on the windward sides of Mauna Loa, Mauna Kea, and Kilauea volcanoes and occupies a wider elevation range (ca 850–1250 m).

Bird communities at the four study sites were dominated by non-native species, probably due to the presence of disease-transmitting mosquitos, which carry avian malaria and avian pox and exclude susceptible native birds from low-elevation sites (Atkinson & LaPointe 2009). The most common birds in the study sites included the non-native Japanese White-eye, Northern Cardinal (*Cardinalis cardinalis*), Common Myna (*Acridotheres tristis*), Spotted Dove (*Streptopelia chinensis*), and Zebra Dove (*Geopelia striata*), all of which demonstrate disease resistance. Common native birds within the study area were 'Apapane and 'Amakihi, as well as the native thrush 'Oma'o (*Myadestes obscurus*).

STAND MEASUREMENTS.—We located five stands of each target *Clermontia* species and surveyed them for stand diameter. We first identified the likely extent of the stand by consulting with local native plants experts. This enabled us to identify a hypothesized stand perimeter using Google Earth. We then pushed back the edges of this perimeter by 40 m to establish a ring of 'unknown' area outside the area of the 'known' population. We surveyed this ring comprehensively using handheld GPS units, searching for additional adult plants of either *Clermontia* species. When such adults were detected, we marked them with the GPS unit and incorporated them into a revised perimeter delineation of the stand. At the conclusion of all such surveys, we calculated the maximum diameter of each stand (*i.e.*, the straight-line distance across the longest axis of the stand area) as well as the mean diameter of all five stands of each *Clermontia* species.

POLLINATION TREATMENTS.—Crosses consisted of five interparent distance treatments: selfed (flowers that were bagged in bud stage, preventing outcrossing), nearest neighbor (20–50 m),

cross-patch (100–300 m), intermediate-distance (800–1200 m), and long-distance (10–15 km). We deliberately limited sample size because these plants are endemic species: in each of two study populations per species, each of six study trees were selected to receive all five treatments, for a total of 120 crosses. As described below, high flower failure rates forced us to expand the treatments to a few additional plants to bolster sample size. Across all treatments, a high proportion of flowers died after emasculation or failed to set fruit after pollination (discussed below), resulting in unbalanced treatments. When available, new flowers or new plants were selected and lost treatments were replaced. However, the small number of adult plants per site (range 6–55) and the small number of flowers per plant (range 3–102; with many plants no longer flowering when treatment failures became apparent) made it impossible to replace all lost treatments within the flowering season.

The experiment was repeated in two flowering seasons: June–October 2011 and February–May 2012. To perform crosses, we emasculated flowers early in male phase to prevent self-pollination, isolated flowers in mesh pollinator exclusion bags until they entered female phase, and then applied pollen to stigmas, which became receptive 24–48 h after emasculation. Pollen for treatments was collected in centrifuge tubes from paternal plants from preselected distance categories, but the pollen presentation system of these flowers made it impossible to standardize the amount of pollen collected: *Clermontia* flowers release the majority of their pollen in a single outpouring when the androecium is disturbed. If pollen release does not occur within 24 h, the stigma elongates through the center of the androecium, stimulating pollen release and coating the gynoecium with self-pollen. A gynoecium that does not receive self-pollination is receptive for a short time (ca 24 h). To perform our crosses, we searched for male-phase flowers located at the necessary distances from maternal plants; flowers in bud stage or female phase could not be used, and the generally low number of flowers in a patch on any given day often made it impossible to find a suitable flower when it was needed. If a flower was found but the major pollen dump had already happened for male-phase flowers, it was possible to gather only a small amount of pollen for crosses. We applied all collected pollen to each target flower, but this amount varied from a large clump to a dusting, influencing our fecundity metrics (see below). Following pollen application, treated flowers were isolated once again in mesh pollination bags and permitted to mature into fruits. Fruits were harvested when fully mature, 60–90 d after pollination. Following harvest, seeds were extracted from fruits using a series of sieves and dried on low-humidity, room temperature platforms prior to the commencement of germination trials. Seeds used in germination trials were visibly filled and plump and dark in color, in contrast to tiny, pale infertile seeds.

GERMINATION.—Because we were unable to standardize the amount of pollen delivered during treatments, we used two fitness indicators (germination rate and time to first germination, both metrics of seed robustness) (after Willis 1993, Verdú & Tra-

veset 2005) to compare distance crosses, rather than using seed set (which for this species reflects the number of pollen grains delivered). Percent germination of seeds of each fruit was determined by pooling two consecutive germination trials. During each trial, one subsample of seeds ($N = 25$) from each harvested fruit was plated on filter paper in a 150-mm-diameter Petri dish. Dishes were placed in haphazard order on a platform in natural light. Filter paper was moistened with distilled water, which was reapplied as needed throughout the germination trial. To ensure that all seeds were exposed to the same light and temperature conditions, dishes were rotated (moved two locations to the right along the platform and turned 90°) every other day throughout the trial. As the first seed of each subsample germinated, length of time to germination was recorded. Each trial ran for 35 d, after which the germination rate was calculated as the proportion of total seeds that had germinated.

Germination rates and days to germination statistics were then analyzed using R (v. 2.14.1; R Development Core Team 2011). Replicate treatments per plant as well as replicate germination trials per treatment were treated as subsamples and averaged prior to analysis. Data were analyzed using split-plot mixed effects linear models, available from R package 'nlme', with germination rate as the response variable and treatment as the fixed explanatory variable (Crawley 2007). These tests contrast seed robustness metrics of distance treatments against the selfed treatment; thus, poorer performance among seeds from self-fertilization is the *a priori* hypothesis in the test. Site, plant, and season were used as random effect blocking factors to account for inherent differences between sites and individuals. There was no significant effect of season either by itself or as an interaction term, so it was removed from the model during model simplification. The final model thus used site and plant as blocking factors. Separate models were developed and employed for proportion germinated and days to germination for each plant species. Assumptions of the linear model were examined using tests for homoscedasticity coupled with visual inspection of the data and variances, and no data transformations were necessary.

FLOWER FAILURE.—Approximately half of all maternal flowers used for *C. parviflora* treatments failed prior to fruit maturity. Failure rates were even higher for *C. montis-loa*, approaching two-thirds. One cause of these failures could have been our emasculations (*i.e.*, the stress of flower handling) or delivery of small pollen quantities. Anecdotally, however, flowers of *Clermontia* experience a high failure rate no matter how or if they are handled (68.5% for tracked *C. montis-loa* flowers and 56.5% for tracked *C. parviflora* flowers, C. Aslan, unpubl. data).

Because we observed such a high level of flower failure across treatments, we examined flower failure rate by treatment to test the null hypothesis that all treatments exhibited equivalent failure rates. We used a split-plot mixed effects model as above, to account for inherent differences between sites and plants, but now arcsine-transformed the proportion of treatment flowers that failed, to normalize the data. We again performed the analysis in R, with site and plant as blocking effects and treatment as a fixed

effect. The arcsine-transformed number of flower failures per treatment was the response variable.

RADIOTELEMETRY.—White-eye movement patterns were assessed in high elevation forest remnants (kīpuka) on the eastern slope of Hawai'i Island's Mauna Loa volcano. The study system was located at *ca* 19°40' N, 155°21' W, and ranged in elevation from 1480 to 1740 m. Kīpuka consisted primarily of stands dominated by native `ōhi`a trees (*M. polymorpha*), were located *ca* 10 km west of the northernmost *C. parviflora* and *C. montis-loa* study sites at which our pollination trials were performed, and offered superior conditions for the tracking of White-eye movement patterns (*i.e.*, more easily accessible forest patches, easier movement across lava flows for trackers).

To examine Japanese White-eye movement patterns, we fitted a total of nine White-eyes with radio transmitters and followed each of them on a per-day basis for up to 30 d or until transmitter signal was lost. Birds were captured in mist nets established within the study site kīpuka, fitted with radio transmitters, and released back into the source kīpuka. Radio tracking began 30 min after release to allow time for birds to adjust to the transmitter. Mist netting occurred between February 2012 and June 2012. For further mist-netting and radio tracking methodological details, see Wu *et al.* (2014).

Radiotelemetry analysis was designed to compare typical White-eye movements with spatial pollination patterns for *Clermontia*. Maximum movement distances within each tracking date were calculated because the average longevity of *Clermontia* pollen is probably <24 h (P. Moriyatsu, pers. comm.). Analysis was conducted using ArcView 10 (ESRI, Redlands, CA). GPS coordinates for each bird location for each day of tracking were entered into ArcView, and Geospatial Modeling Environment (GME; Spatial Ecology LLC) was used to determine the distances from each point to every other point, generating a range of straight-line distances over which each bird traveled during each tracking session (*i.e.*, within each date) (Wu *et al.* 2014). The maximum distance was calculated as the maximum total movement (within a single tracking date) for each bird.

POLLEN DETECTION ON CAPTIVE WHITE-EYES.—Pollen was removed from the faces and bills of mist-netted White-eyes while the birds were in hand. Clear Scotch tape was gently pressed across each bird's feathers and bill to lift any debris that might include pollen. The tape was placed on microscope slides for later examination. Nine slides from nine individual White-eyes were prepared in this manner. The slides were examined at 64× magnification and the presence or absence of pollen was recorded. Pollen was identified based on its morphology and comparison of its appearance with reference pollen samples.

RESULTS

Over the two seasons, a total of 157 flowers set fruit out of 519 pollination attempts administered to 36 plants in four sites (two

sites each for *C. parviflora* and *C. montis-loa*). A total of 88 *C. parviflora* flowers from 21 plants and 51 *C. montis-loa* flowers from 15 plants successfully set seed.

STAND MEASUREMENTS.—Our stand surveys found a mean maximum stand diameter (*i.e.*, straight-line distance across the longest axis of the stand area) for *C. parviflora* of 0.79 km (range 0.11–1.20, SE 0.25) and a mean maximum stand diameter for *C. montis-loa* of 0.59 km (range 0.24–1.20, SE 0.21).

GERMINATION TRIALS.—For *C. parviflora*, proportion germination ranged from 0.33 for selfed flowers to 0.60 for intermediate-distance crosses (Table 1). The split-plot mixed effects linear model revealed significantly higher germination rates for nearest neighbor (20–50 m), cross-patch (100–300 m), and intermediate (800–1200 m) distance treatments than for selfed flowers (Table 2). There was no significant difference in germination rate between long-distance treatments (10–20 km) and selfed flowers (Table 2). For *C. montis-loa*, no significant effects of treatment distance were detected (Table 2). Number of days to first germination ranged from 24.41 to 27.78, with no significant effect of treatment distance within either species (Table 1).

FLOWER FAILURE.—The rate of flower failure (*i.e.*, number of treated flowers that failed out of total attempts) for each species–treatment combination ranged from just under half to two-thirds (Table 1). The split-plot mixed effects model detected no significant effect of treatment distance on flower failure rate for either plant species ($P > 0.05$). Analysis with season as a blocking factor did not alter the results.

RADIOTELEMETRY.—The nine tracked *Z. japonicus* showed maximum daily movements ranging from 154.69 to 1053.23 m, with a mean of 392.72 m (Table 3).

POLLEN DETECTION ON CAPTIVE WHITE-EYES.—Of the nine slides examined for pollen, five contained at least one pollen grain fitting the morphotype of the study area's dominant tree species, *M. polymorpha* (E. Stacy, pers. comm.): equilateral, triangular pollen grains with rounded corners. No other pollen morphotypes were detected on any of the nine slides.

TABLE 2. Split-plot mixed effects linear model table of treatment (pollination distance) effects on percent germination for *Clermontia parviflora* and *C. montis-loa*. Pollination distance categories (= 'Treatment') were treated as fixed effects in the model, and the model contrasted all distance categories against selfed (bagged) flowers. Site was the whole plot treatment, and individual plant was the subplot.

Treatment	<i>Clermontia parviflora</i>		<i>Clermontia montis-loa</i>	
	$t_{(df)}$	P-value	$t_{(df)}$	P-value
Nearest neighbor (20–50 m)	2.25 ₍₆₇₎	0.0276	−0.65 ₍₂₁₎	0.5229
Cross-patch (100–300 m)	2.06 ₍₆₇₎	0.0431	−0.70 ₍₂₁₎	0.4916
Intermediate (800–1200 m)	3.29 ₍₆₇₎	0.0016	−0.24 ₍₂₁₎	0.8133
Long-distance (10–15 km)	1.36 ₍₆₇₎	0.1781	0.04 ₍₂₁₎	0.9721

DISCUSSION

We found no evidence that Japanese White-eye movement distances, as estimated by radiotelemetry, should hinder them from performing effective pollination of these two *Clermontia* species. Radio tracking results indicated that most tracked White-eyes moved <1 km/d, suggesting that pollen transported within a single date (*i.e.*, during maximum viability) will most likely be transported within populations or at most between adjacent populations (Table 3). The *C. parviflora* populations (patches) we have explored range from 106 m to 1.2 km in diameter. Adjacent populations of *Clermontia* spp. are separated by ≥ 1.6 km, and frequently much more. At the same time, maximum germination was obtained for crosses between 20 m and 1200 m for *C. parviflora* (Table 1). This suggests that intra-population pollen transfer may be optimal for *C. parviflora*. All germination treatments performed equally well for *C. montis-loa* (Table 2). While sample size was considerably lower for *C. montis-loa* than for *C. parviflora*, and the power to detect a statistical effect was therefore reduced, there was also no indication in the data of any trend toward spatial structure, making it seem unlikely that a larger sample size would qualitatively alter the results. The difference between the two *Clermontia* species may reflect their contrasting native distributions: whereas *C. parviflora* has a wide north–south distribution

TABLE 1. Overall mean proportion of treatment flowers that failed, proportion of treatment seeds germinating, and days to first germination. Values are means \pm SE. *Values differed significantly from selfed treatments.

Treatment	<i>Clermontia parviflora</i>			<i>Clermontia montis-loa</i>		
	Prop. flower failure	Prop. germ.	Days	Prop. flower failure	Prop. germ.	Days
Selfed	0.54 \pm 0.08	0.33 \pm 0.07	25.86 \pm 1.05	0.63 \pm 0.09	0.41 \pm 0.07	26.56 \pm 0.67
Nearest Neighbor	0.44 \pm 0.10	0.53 \pm 0.07*	25.17 \pm 0.95	0.58 \pm 0.13	0.32 \pm 0.07	27.78 \pm 0.81
Cross-patch	0.55 \pm 0.08	0.51 \pm 0.07*	24.52 \pm 0.65	0.63 \pm 0.10	0.32 \pm 0.08	26.47 \pm 1.34
Intermediate	0.48 \pm 0.09	0.60 \pm 0.03*	24.41 \pm 0.65	0.66 \pm 0.09	0.36 \pm 0.10	26.14 \pm 1.37
Long-distance	0.46 \pm 0.10	0.45 \pm 0.07	25.23 \pm 0.90	0.63 \pm 0.09	0.36 \pm 0.06	27.27 \pm 1.55

TABLE 3. Daily movement patterns (the mean maximum distance moved across all days over which each bird was observed and the maximum distance moved in a single date) for each Japanese White-eye (*Zosterops japonicus*) as measured by radiotelemetry.

Bird ID	Number of individual days observed	Mean max. distance (m)	Max. one-day distance (m)
302	1	163.17*	163.17
302P	1	257.89*	257.89
317	21	783.99 ± 69.80	1053.23
609	23	155.94 ± 40.77	339.26
629	20	235.31 ± 66.65	444.14
640	19	109.66 ± 17.65	270.74
692	7	163.56 ± 57.71	331.00
720	7	149.22 ± 49.46	288.92
808	8	175.50 ± 79.07	472.41

*No variance, as bird was observed on only one date.

across multiple volcanoes on the island, *C. montis-loa* grows just on Mauna Loa volcano and occupies a smaller range. The wider diversity of environmental conditions in which *C. parviflora* occurs could promote the development of ecotypes, showing local adaptation, within the species.

Unfortunately, we know little about the movement behaviors of the historical Hawaiian avifauna, so we cannot determine whether extinct honeycreepers moved within spatial scales similar to the Japanese White-eye. Even extant honeycreeper movements are poorly understood. The most common honeycreepers today are the 'Apapane (*Himatione sanguinea*) (ca 13 g) and 'Amakihi (*Hemignathus virens virens*) (ca 11 g). These birds are on average just slightly larger than Japanese White-eye (ca 10 g) and occupy a similarly broad elevational range. Although large-scale movements of both 'Amakihi and 'Apapane in response to nectar availability have been documented (van Riper 1984, Ralph & Fancy 1995), no fine-scale information on their daily movements is available. However, a study of breeding season male 'I'iwi (*Vestiaria coccinea*) home range sizes provides a valuable comparison (Kuntz 2008). Because methodologies, analyses, and overall study objectives were different, statistical comparisons between Kuntz's (2008) data and ours are not applicable. Nevertheless, in examining Kuntz's (2008) adaptive kernel estimates and measuring straight-line average maximum movements (*i.e.*, from edge to edge of 90% utilization contours, measured along the longest axis), it appears that breeding season males' maximum movements ranged from ca 30–200 m. Because the goal of Kuntz's (2008) study was to assess home range size, movements were not evaluated on a daily basis. However, if males generally move within the home ranges delineated by Kuntz (2008), 'I'iwi demonstrate activities within spatial scales that are similar in order of magnitude to Japanese White-eye. As 'I'iwi is the sole remaining, non-endangered, mid-sized honeycreeper on Hawai'i Island, it is probably the best remaining native pollinator of larger-flowered species. Similarities between Japanese White-eye and 'I'iwi movements are therefore

further evidence that Japanese White-eye might be an adequate novel pollinator for at least the two *Clermontia* species examined here.

Spatial structure such as we found for *C. parviflora* has been found to be important for a diversity of plant species. As a few examples, germination and seedling metrics were measured for bird-adapted *Grevillea mucronulata* (Proteaceae) plants, and both inbreeding depression and outbreeding depression were detected after crossing parents of different distances (Forrest *et al.* 2011). Seed viability, germination, and growth were measured for the naturalized weed *Silene vulgaris*; inbreeding depression was detected for all tested lineages, and some displayed outbreeding depression, as well (Bailey & McCauley 2006). Seed set, seed weight, germination, and early growth (1–2 mo) in a population of *Impatiens capensis* (Balsaminaceae), an annual herb with a mixed mating system, demonstrated that distance yielded different patterns depending on which parameter was measured (McCall *et al.* 1991).

If movement patterns of a novel pollinator lead to shorter pollen exchange distances relative to native mutualists, altered spatial dynamics of pollination could generate increased inbreeding relative to native mutualists, leading to reduced genetic diversity and perhaps lowered fitness (Robertson & Ulappa 2004, Seltmann *et al.* 2009). On the other hand, increased distances of pollen transfer could erode local adaptation, if present, and prove to be detrimental to population fitness (= outbreeding depression) (Waser & Price 1994). Finally, spatial dynamics may have no effect. Because the *Clermontia* lineage experienced narrow population bottlenecks when colonizing the Hawaiian Islands, detrimental alleles may have been purged from the population, making outcrossing less important than might otherwise be expected (Lande *et al.* 1994, Crnokrak & Barrett 2002).

One important aspect of this case study—and indeed of many known novel mutualisms, (Aslan *et al.* 2012)—is that the likely positive impact on pollination of the novel mutualist may be offset by negative impacts. As a non-native species, Japanese White-eye may compete with the 'I'iwi (Mountainspring & Scott 1985). Increased numbers of Japanese White-eye have also been blamed for the sharp collapse of the native Hawai'i Akepa (*Loxia coccinea*) in Hakalau Forest National Wildlife Refuge on Hawai'i Island's Mauna Kea volcano (Freed & Cann 2009).

Today, the Japanese White-eye is the most likely bird to come into contact with *Clermontia* (Aslan *et al.* 2014). However, *Clermontia* plants are relatively rare. Much more common species in the study region are overstory plants such as the dominant *M. polymorpha* tree, which is bird-pollinated and ubiquitously available as a nectar source. Because they are generalist feeders, a foraging Japanese White-eye is more likely to opportunistically encounter *M. polymorpha* than *Clermontia*, as the *M. polymorpha* pollen detected on mist-netted White-eyes indicated. Transfer of heterospecific pollen to *Clermontia* seems probable; furthermore, *C. parviflora* and *C. montis-loa* do hybridize in their zone of overlap (J. Johansen, pers. comm.) and the White-eye could facilitate such hybridization. A study of the impact of heterospecific pollen

transfer on *Clermontia* reproductive success could provide further understanding of the qualitative components of the White-eye's effectiveness as a novel pollinator in this system.

Understanding both quantitative and qualitative pollination components may be critical for reintroduction efforts for threatened species, such as the ongoing program to reintroduce the critically endangered *Clermontia peleana* on Hawai'i Island (Robichaux 2012). If novel pollinators are shown to be both quantitatively and qualitatively effective mutualists and their movement scales can be defined, outplantings can be planned to maximize likely pollen exchange under current pollination conditions.

To rescue species threatened by potential extinctions due to the disruption of mutualisms, the deliberate introduction of non-native species that are functional analogs—*i.e.*, taxon substitution (Atkinson 2001)—has been proposed as a conservation strategy (Griffiths & Harris 2010, Kaiser-Bunbury *et al.* 2010). In Mauritius, for example, non-native giant tortoises (*Aldabrachelys gigantea* and *Astrochelys radiata*) were recently introduced under controlled conditions to reestablish the seed dispersal function of the extinct Mascarene giant tortoise (*Cylindraspis* spp) (Griffiths & Harris 2010). Native plants have been shown to benefit from novel mutualisms in cases where native mutualists have become extinct (Cox 1983, Kaiser-Bunbury *et al.* 2010). However, the introduction of non-native species into new ecosystems has frequently proven to be highly detrimental to the environment and the deliberate introduction of mutualists is therefore highly controversial. In light of the growing interest in these restoration measures among researchers and managers, there is a clear need for more complete understanding of both the quantitative and qualitative aspects of novel relationships between native and non-native species.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

FIGURE S1. Map of Hawai'i Island with the relative locations of the four principal study sites.

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