



Imperfect Replacement of Native Species by Non-Native Species as Pollinators of Endemic Hawaiian Plants

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Abstract: *Native plant species that have lost their mutualist partners may require non-native pollinators or seed dispersers to maintain reproduction. When natives are highly specialized, however, it appears doubtful that introduced generalists will partner effectively with them. We used visitation observations and pollination treatments (experimental manipulations of pollen transfer) to examine relationships between the introduced, generalist Japanese White-eye (*Zosterops japonicus*) and 3 endemic Hawaiian plant species (*Clermontia parviflora*, *C. montis-loa*, and *C. hawaiiensis*). These plants are characterized by curved, tubular flowers, apparently adapted for pollination by curve-billed Hawaiian honeycreepers. *Z. japonicus* were responsible for over 80% of visits to flowers of the small-flowered *C. parviflora* and the midsize-flowered *C. montis-loa*. *Z. japonicus* visited flowers set significantly more seed than did bagged flowers. *Z. japonicus* also demonstrated the potential to act as an occasional *Clermontia* seed disperser, although ground-based frugivory by non-native mammals likely dominates seed dispersal. The large-flowered *C. hawaiiensis* received no visitation by any birds during observations. Unmanipulated and bagged *C. hawaiiensis* flowers set similar numbers of seeds. Direct examination of *Z. japonicus* and *Clermontia* morphologies suggests a mismatch between *Z. japonicus* bill morphology and *C. hawaiiensis* flower morphology. In combination, our results suggest that *Z. japonicus* has established an effective pollination relationship with *C. parviflora* and *C. montis-loa* and that the large flowers of *C. hawaiiensis* preclude effective visitation by *Z. japonicus*.*

Keywords: biological invasions, *Clermontia*, mutualism disruption, novel mutualism, pollinator loss, *Zosterops japonicus*

Remplazo Imperfecto de Especies Nativas por Especies No-Nativas como Polinizadores de Plantas Endémicas de Hawaii

Resumen: *Las especies nativas de plantas que han perdido a sus compañeros mutualistas pueden requerir de polinizadores no-nativos o dispersores de semillas para mantener su reproducción. Sin embargo cuando las especies nativas están altamente especializadas, parece dudoso que especies generalistas introducidas se acoplarán efectivamente con ellas. Usamos observaciones de visita y tratamientos de polinización (manipulaciones experimentales de transferencia de polen) para examinar las relaciones entre la introducida y generalista *Zosterops japonicus* y tres especies de plantas endémicas de Hawaii (*Clermontia parviflora*, *C. montis-loa* y *C. hawaiiensis*). Estas plantas se caracterizan por sus flores curvadas y tubulares, adaptadas aparentemente a la polinización por mieleros hawaianos de pico curvo. *Zosterops japonicus* fue responsable de más del 80% de visitas a flores de *C. parviflora*, de flores pequeñas, y de *C. montis-loa*, de flores mediadas.*

Las flores visitadas por *Zosterops japonicus* establecieron significativamente más semillas que aquellas flores embolsadas. *Zosterops japonicus* también demostró el potencial para fungir como dispersor ocasional de semillas de *Clermontia*, aunque la frugivoría terrestre de mamíferos no-nativos probablemente domine la dispersión de semillas. *C. hawaiiensis*, de flores grandes, no fue visitada por aves durante las observaciones. Ejemplares de *C. hawaiiensis* sin manipular y embolsadas establecieron un número similar de semillas. La examinación directa de las morfologías de *Z. japonicus* y *Clermontia* sugieren un desajuste entre la morfología del pico de *Z. japonicus* y la morfología floral de *C. hawaiiensis*. Combinados, nuestros resultados sugieren que *Z. japonicus* ha establecido una relación efectiva de polinización con *C. parviflora* y *C. montis-loa* y que las flores grandes de *C. hawaiiensis* evitan las visitas efectivas de *Z. japonicus*.

Palabras Clave: *Clermontia*, disrupción del mutualismo, invasiones biológicas, mutualismo novel, pérdida de polinizadores, *Zosterops japonicus*

Introduction

Extinctions may disrupt mutualisms, potentially leaving extant species without their partners (Kiers et al. 2010). Partnerless species may demonstrate declines as a result (Terborgh et al. 2008). Increasing evidence suggests that mutualisms can be critical to participant persistence and that their disruption may impact substantial biodiversity (Kaiser-Bunbury et al. 2010).

Extinction rates are particularly high on oceanic islands, where, because of isolation and limited area, native species demonstrate small starting populations and high susceptibility to the impacts of biological invasion (Blackburn et al. 2004). Island mutualisms broken by extinction include avian pollination in New Zealand (Anderson et al. 2011), seed dispersal in Tonga (Meehan et al. 2002), and seed dispersal in the Balearic Islands (Traveset & Riera 2005).

Studies have identified novel mutualisms between non-native and native species (e.g., Aslan 2011). Mutualisms can boost non-native species performance and potentially drive biological invasions (Traveset & Richardson 2010). However, novel mutualisms may also bolster the fitness of native species (Cox 1983; Griffiths et al. 2011; Aslan 2012). Non-native ship rats (*Rattus rattus*) and Silvereyes (*Zosterops lateralis*) pollinate native plants in New Zealand, partially compensating for locally extinct native pollinators (Pattimore & Wilcove 2012). Other novel mutualisms include native Hawaiian plants dispersed by an assemblage of non-native birds (Foster & Robinson 2007), an endemic tree pollinated by non-native birds in New Zealand (Schmidt-Adam et al. 2000), and a Chilean shrub dispersed by introduced rabbits (Castro et al. 2008).

In Hawaii, introductions of mosquitoes and avian diseases, coupled with habitat fragmentation, have led to dramatic native bird loss. Since human colonization, 71% of Hawaiian bird species have become extinct (Boyer 2008). Many non-native bird species have been introduced, resulting in a transformed avifauna (Foster & Robinson 2007). These changes in the bird community may impact native plant populations because 44% of

Hawaiian plants produce fleshy fruits adapted for bird-mediated dispersal and 18% are adapted for bird pollination (Sakai et al. 1995; Price & Wagner 2004).

The Hawaiian lobeliads (Lobelioideae: Campanulaceae) represent Hawaii's largest plant adaptive radiation (Givnish et al. 2009). The endemic genus *Clermontia* contains 22 of these species, all of which are adapted for bird pollination, with curved, tubular flowers (Lammers & Freeman 1986). For this study, we examined 3 focal *Clermontia* tree species from Hawaii Island (Supporting Information). All 3 are sufficiently common to allow field observations in multiple reproductive stands. *Clermontia parviflora* produces the smallest flower of the genus (perianth lobes 1.8–2.3 cm and strongly recurved) (Lammers 1991) and is the most abundant *Clermontia* species on the island. *Clermontia montis-loa* flowers are medium-sized (perianth lobes 3.8–5.0 cm long) (Lammers 1991). The species occupies cloud forest (approximately 950–1250 m) on Mauna Loa volcano. *Clermontia hawaiiensis* produces large flowers (perianth mean length 7.5 cm, personal observation) and is relatively rare, occurring as isolated trees and in small stands. All *Clermontia* flowers are hermaphroditic and protandrous. *Clermontia* trees produce a fairly small number of flowers per flowering season (range 6–102, personal observation), but each berry may contain many miniscule seeds (seed number range 20–2251, personal observation). Short-term field studies have confirmed visitation to *Clermontia* flowers and pollen transfer by birds (Spieth 1966; Lammers et al. 1987). We set out to determine whether non-native bird species may now be the primary pollinators (vs. nectar robbers) for *Clermontia* and which species may now be *Clermontia* seed dispersers.

The most common forest bird on Hawaii today is the Japanese White-eye (*Z. japonicus*) (Reynolds et al. 2003; Spiegel et al. 2006). Introduced from Japan in 1929 (Simberloff & Boecklen 1991), *Z. japonicus* is a generalist feeder and occupies an enormous range of habitats up to 3000 m elevation (Hawaii Audubon Society 1993). Adults are small (body mass < 15 g) (Work et al. 1999). Bills are nearly straight and average 14 mm in length (personal observation). *Z. japonicus* is known to visit the flowers of native species in Hawaii

(e.g., Carpenter 1976; Cox 1983; Lammers et al. 1987). However, *Z. japonicus* also acts as a nectar robber for some plant species, including lobeliads (Gardener & Daehler 2006; Akamatsu et al. 2011; Kagoshima 2011). The ability of the straight-billed *Z. japonicus* to serve as an effective pollinator of highly specialized lobeliad flowers has remained uncertain.

We used foraging observations and pollination treatments to determine whether *Z. japonicus* is serving as the effective pollinator and whether it acts as a likely seed disperser of the 3 focal *Clermontia* species.

Methods

Study Sites

Flower visitation observations, frugivory observations, and pollination treatments were conducted in 3 study sites per focal plant species on Hawaii Island. All sites were in wet forest (900–1300 m elevation) dominated by ohia (*Metrosideros polymorpha*). Total numbers of reproductive trees per site were as follows: *C. parviflora* site P1 = 12, site P2 = 55, site P3 = 13; *C. montis-loa* site M1 = 14, site M2 = 11, site M3 = 3; *C. hawaiiensis* site H1 = 6, site H2 = 6, site H3 = 11.

Most stands of the 3 focal species are below 1300 m in elevation and within the zone occupied by disease-transmitting mosquitoes and dominated by non-native birds (Atkinson & LaPointe 2009). The most common birds at our study sites included the non-native *Z. japonicus*, Northern Cardinals (*Cardinalis cardinalis*), Common Mynas (*Acridotheres tristis*), Spotted Doves (*Streptopelia chinensis*), and Zebra Doves (*Geopelia striata*). Common native birds at our study sites were the small honeycreepers Apapane (*Himatione sanguinea*) and Amakihi (*Hemignathus virens virens*) and the native thrush Omao (*Myadestes obscurus*). The Iiwi (*Vestiaria coccinea*), the largest remaining nectarivorous honeycreeper on Hawaii Island (and putative extant native *Clermontia* pollinator [Pimm 1996]) was present only rarely at higher elevation sites in our study (P1, M1, and M2).

Supplemental observations were conducted in Kilauea Forest at a high-elevation (1500 m) secondary forest site dominated by ohia and koa (*Acacia koa*). Kilauea Forest has a closed canopy, full secondary and understory layers, and low disturbance. The site contains an outplanted population of the endangered *Cyanea stictophylla*, a lobeliad in the sister genus of *Clermontia*. This site holds abundant *V. coccinea*, *H. sanguinea*, *H. v. virens*, and *Z. japonicus*. Observations at this site enabled comparison of visitation rates to similar plant species between areas with few or no *V. coccinea* and a site with abundant *V. coccinea*.

Flower Visitation and Frugivory Observations

Visitation observations were conducted in 2 flowering seasons (June–October 2011; February–May 2012). Observations consisted of scan sampling and focal individual observations (after Aslan 2011). Each observation lasted 90 min, divided into 10-min intervals. During the first minute of each interval, all visible reproductive trees were scanned from a fixed point for bird activity. The species and numbers of individuals of any birds using the trees were recorded. During the next 9-min block, the observer moved slowly through the stand, conducting focal individual observations. One at a time, birds visiting flowers were observed through binoculars for up to 2 min or until the bird flew away from the tree. For each focal individual, the total number of flowers probed and pierced, the direction of probing (from the front, bottom, top, or side of the flower), and the total number of observation seconds were recorded. Following the first focal individual observation of the time block, the next individual was selected haphazardly. Approximately halfway through each flowering season, ripe fruits became available on focal trees, and thereafter number of fruits pecked was recorded simultaneously with flower visitation during focal individual observations. In all, 102 h of visitation observations were conducted for each of the 3 target *Clermontia* species. Because flowering season duration was shorter, only 40.5 h of supplemental visitation observations were conducted at the high-elevation *C. stictophylla* site. The start time of each observation was randomly selected from a set of 2-h intervals (0630, 0830, 1030, 1300, 1500, or 1700) but constrained so that all sites were observed at all time periods.

We also briefly examined ground-based fruit removal for the 3 *Clermontia* species. First, we marked a total of 10 fallen fruits per plant species per study stand by placing a 10-cm wooden dowel in the ground next to each fruit. We returned to the study stands after 24 h and determined the number of these fruits that had remained undisturbed overnight. These methods were repeated 3 times during the fruiting season. Removed fruit indicated ground foraging by either birds or mammals. To identify nocturnal frugivores, we placed 9 tracking tunnels at each of 2 study sites per species (H1, H2, P2, P3, M1, and M2) for 5 consecutive nights (90 sampling nights per plant species). Tracking tunnels consisted of 20 × 20 × 40 cm rectangular cardboard cartons placed beneath study trees and covered with fallen detritus as camouflage. Oil-based printer's ink was applied to each tunnel entrance in 10-cm-wide strips so that entering mammals walked on the ink (methods after Pender et al. 2013). Each tunnel was baited with 3 berries of the study site's focal plant species. Tunnels were checked daily, and missing berries were replaced.

Pollination Treatments

We compared fruit set, seed set, and seeds per flower (proportion fruit set * seed set) for flowers that received the following pollination treatments: bagged, bagged and hand-supplemented, hand-supplemented and not bagged, open, and White-eye pollinated. Bagged flowers were bagged in bud stage with mesh pollinator exclusion bags. Hand-supplemented flowers were open to normal pollinator visits and received additional pollen as follows. A male-phase flower was haphazardly selected, based on availability, from a plant located 50–150 m away from the focal flower. All pollen was collected from the male-phase flower and applied in a heavy coat with a small paintbrush to the focal female-phase flower until no further pollen would adhere to the stigma. Bagged and hand-supplemented flowers were bagged in bud stage and then given pollen according to the same procedure as for hand-supplemented flowers. Bags were then replaced on these flowers until the corollas and gynoecium had wilted. Open flowers were selected randomly during male phase but received no further treatment or manipulation. Finally, White-eye-pollinated flowers were those observed during focal individual observations to be visited by *Z. japonicus*. These flowers were marked with white thread but received no further manipulation.

The study plants produce flowers on a rolling basis, with a few flowers open and receptive at any given time. At the beginning of the flowering season, we selected plants for pollination treatments based on flower availability, aiming initially to administer all treatments to at least 6 plants per study site (2 flowers per treatment per plant) and to administer more treatments as additional flowers matured. Our final sample size was constrained, however, by high fruit set failure for all treatments, including unmanipulated flowers (across all treatments, an average of 61.1% of flowers failed to set fruit). When possible, we reapplied treatments when fruit set failed. This was impossible when no further flowers were available. For the White-eye-pollinated treatment, because that treatment relied on observed visitation, it was impossible to replace flowers that failed to set fruit. Furthermore, White-eye-pollinated flowers could be used only when flowers were in female phase at the time of the bird visit. Our final data set was therefore unbalanced across treatments; sample size was particularly small for the White-eye-pollinated treatments across all species and for *C. hawaiiensis*, which produces a smaller number (mean = 19.19 flowers [SE 8.06]) of flowers per plant per season and is available for study in smaller numbers of individuals than the other 2 focal species. Following maturation of fruits resulting from pollination treatments (approximately 2 months following treatment), seeds were extracted from fruits and counted to determine seed set.

No pollination treatments were conducted on *Cyanea stictophylla*, at the high-elevation comparison site, because the species is endangered.

Z. japonicus and *Clermontia* Flower Morphological Match

Since the vast majority of visits observed to *Clermontia* flowers were performed by *Z. japonicus* despite the bird's generalist morphology (small body size and short, straight bill), we evaluated the morphological match between the bird and flower. We obtained a disease-free *Z. japonicus* carcass from a U.S. Geological Survey disease testing facility. We inserted the carcass bill into 20 flowers (10 male and 10 female) on 5 plants of each of our target *Clermontia* species. We held the bird upright and inserted the bill straight into the flower to mimic the most common approach direction observed in the field. At each insertion, we recorded whether the bill contacted the nectar pool (i.e., emerged with visible nectar on its surface) and whether the bird's head contacted the flower's reproductive parts.

Data Analyses

To estimate visitation rate per bird species, we calculated the flock pollination importance (FPI) and the flock dispersal importance (FDI) (adapted from Renne et al. 2000; Aslan 2011) of each bird species for each focal plant species. The FPI and FDI are the product of the average number of individuals per bird species per tree across all periods, obtained during scan sampling, and the average number of flowers probed or fruits pecked per minute per individual bird. For each plant species, repeated observations of the same fruiting stand were treated as subsamples, and stands were pooled to calculate overall FPI and FDI. The FPI for each bird-plant combination was then multiplied by the average total number of trees per stand and converted to an hourly rate. We used a type 1 ANOVA to evaluate the effect of season and found that it was not a significant predictor of fruit removal, so data from the 2 study seasons were pooled to obtain final pollination and dispersal rates for each plant species.

We used a Spearman's rank correlation to compare the percentage of scans per observation in which *Clermontia* flower visitation by *Z. japonicus* occurred and the percentage of scans per observation in which *Z. japonicus* individuals were present in the immediate area. We then ran the same analysis for native honeycreepers.

We used a linear mixed model with split-plot design to compare seed set, fruit set, and seeds per fruit across pollination treatments. The model used site and individual tree as hierarchical blocking variables, treatment as a fixed explanatory variable, and seed set, fruit set, or seeds per flower as response variable. A power transformation was applied to meet assumptions of homoscedasticity and

normality, and response values were back transformed for results reporting. We additionally calculated pollen limitation index (PLI) for each *Clermontia* species as $1 - (P_o/P_s)$, where P_o = proportion fruit set of open flowers and P_s = proportion fruit set of hand-supplemented flowers (Larson & Barrett 2000). A PLI of 0 indicates no pollen limitation.

All data analyses were performed with the statistical software R version 2.14.1 (R Development Core Team 2012). Significance was accepted at $p \leq 0.05$.

Results

Z. japonicus performed the large majority of observed flower visitation for *C. parviflora* and *C. montis-loa*, 83.8% of 64 observed visits and 92.1% of 78 visits, respectively. Remaining visitation to *C. parviflora* flowers was performed by *H. sanguinea* (16.2%), whereas remaining visitation to *C. montis-loa* flowers was performed by *H. v. virens* (4.6%) and *V. coccinea* (3.3%). All *Z. japonicus* visits to *C. parviflora* and 98.9% of *Z. japonicus* visits to *C. montis-loa* appeared legitimate (entering flowers from the front with visible reproductive structure contact). Similarly, all visits by *H. sanguinea* to *C. parviflora* were legitimate. By contrast, no *H. v. virens* visit to *C. montis-loa* was legitimate. Over all observations combined, very few visits ($n = 10$) were observed to *C. hawaiiensis* flowers, and all appeared illegitimate. *Z. japonicus* individuals extracted nectar by inserting their bills between perianth lobes of 4 flowers (40.0%), and *H. v. virens* did the same for 6 flowers (60.0%), with neither species contacting the reproductive structures.

Across all study sites, *Z. japonicus* demonstrated the highest per-tree FPI for both *C. parviflora* and *C. montis-loa* (Figs. 1a and b). The average number of flowers visited per stand per hour by *Z. japonicus* far exceeded the average open flower availability, whereas native honeycreepers visited fewer flowers than were available across all 3 study species (Fig. 2). *Hemignathus virens virens* provided the most visits to *C. hawaiiensis*, but none of these visits were legitimate (Fig. 1c). Morphological match trials indicated high complementarity between *Z. japonicus* morphology and *C. parviflora*, with 100% of trials resulting in visible nectar on the carcass bill and contact between the bird's head feathers and the plant's reproductive structures. For *C. montis-loa*, 65% of trials indicated adequate morphological match, whereas for *C. hawaiiensis*, only 25% of trials indicated morphological match.

Only rare frugivory by *Z. japonicus* was observed. Per-tree FDI of *Z. japonicus* was 0.0026 (SE 0.0023) for *C. parviflora*, 0.1006 (0.0404) for *C. montis-loa*, and 0.0833 (0.0188) for *C. hawaiiensis*. No native birds were observed consuming *Clermontia* fruits during focal individual observations. Only 23% of marked fallen fruits

remained beneath focal trees overnight. Tracking tunnels recorded ground-based frugivory by non-native rats (*Rattus* sp.), mice (*Mus musculus*), and mongoose (*Herpestes javanicus*) (Table 1).

Across all observation periods, Spearman's rank correlation detected a strong relationship between the presence of *Z. japonicus* in the immediate area and visitation to focal flowers ($\rho = 0.8024$; $p = 0.0052$). For native honeycreepers, the Spearman's rank correlation between the presence in the immediate area and visitation to focal flowers was considerably weaker ($\rho = 0.3654$; $p = 0.2992$).

Most visits to *C. stictophylla* flowers were performed by *V. coccinea* (FPI = 0.03 [SE 0.01]); the remainder of visits were performed by *H. v. virens* (FPI = 0.005 [0.002]). All recorded visits to *C. stictophylla* were legitimate because the inflorescence structure largely shields the rear of flowers and thereby prevents flower piercing and nectar robbing.

Compared with open-pollinated flowers, hand-supplemented *C. parviflora* flowers set significantly more seed ($p = 0.008$), whereas significantly lower seed set was observed for bagged ($p = 0.001$) and bagged and hand-supplemented ($p = 0.011$) *C. parviflora* flowers (Fig. 3). There was no significant difference in seed set between White-eye-visited *C. parviflora* flowers and open-pollinated flowers. For *C. montis-loa*, bagged flowers set significantly less seed than open-pollinated flowers ($p = 0.003$), but the seed set of neither hand-supplemented nor White-eye-visited flowers differed significantly from open-pollinated flowers (Fig. 3). For *C. hawaiiensis*, no treatments differed significantly in seed set from open-pollinated flowers (Fig. 3), but low sample size limited the power of the test.

Fruit set failure as well as number of seeds per flower were relatively constant for all treatments; there were no significant differences among treatments or individual plants (Fig. 3; $p > 0.5$). PLI revealed almost no pollen limitation for *C. parviflora* but equivalent and detectable pollen limitation for *C. montis-loa* and *C. hawaiiensis* (Table 2).

Discussion

Several elements suggest that *Z. japonicus* is now the primary pollinator of *C. parviflora* and *C. montis-loa*. Open flowers set significantly more seed than bagged flowers, providing evidence that effective pollination is occurring (Fig. 3). The overwhelming majority of flower visits were from *Z. japonicus* (Fig. 1). On average, *Z. japonicus* visitation exceeded the quantity of flowers available at any given time (Fig. 2), an important requisite for effective pollination in these plant species because protandrous flowers require at least 2 visits for adequate gene transfer (one during male phase and one during

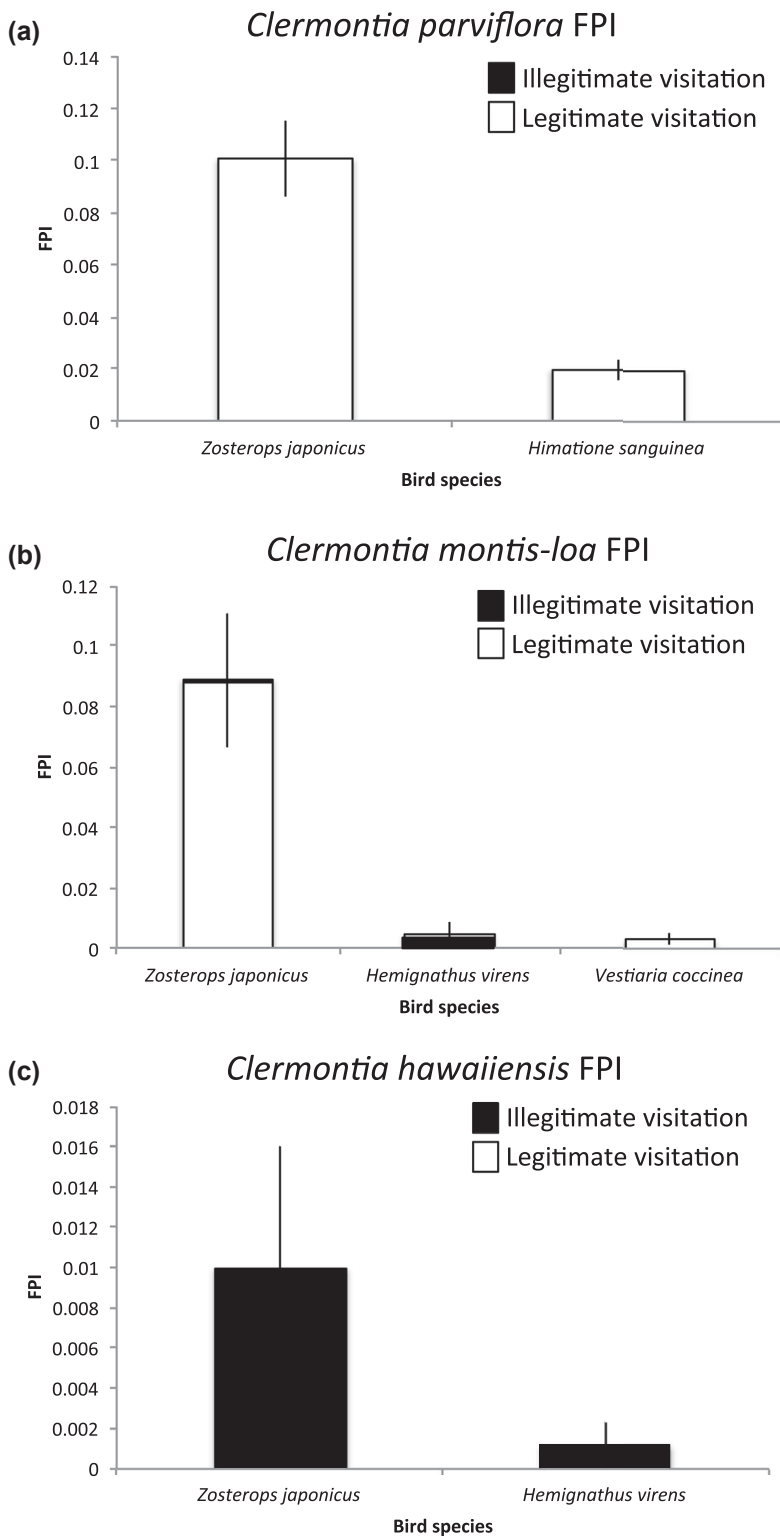


Figure 1. Per-tree flock-level pollination importance (FPI) (i.e., relative amount of pollination contributed by a flock, on average) of each bird species observed visiting the flowers of focal Clermontia species: (a) C. parviflora, (b) Clermontia montis-loa, and (c) Clermontia hawaiiensis. Legitimate visitation indicates a visit in which the bird's bill entered the floral opening and may have contacted the stigma or anthers. Illegitimate visitation indicates a visit in which the bird approached the flower from behind or the side and extracted nectar.

female phase) and these birds are generalist nectarivores and likely to deposit heterospecific pollen, so multiple visits to a particular flower elevate the likelihood that conspecific pollen will be transferred. Flowers visited by *Z. japonicus* set more seed than bagged flowers or the mean open (likely an average of bagged and visited

flowers) flower (Fig. 3). Finally, there was a visible morphological match between *Z. japonicus* and the flowers of the 2 species; pollen can be observed on bird foreheads when the nectar pool is accessed.

Remnant native honeycreepers may still be transferring a minority of pollen (*H. sanguinea* for *C. parviflora*; *V.*

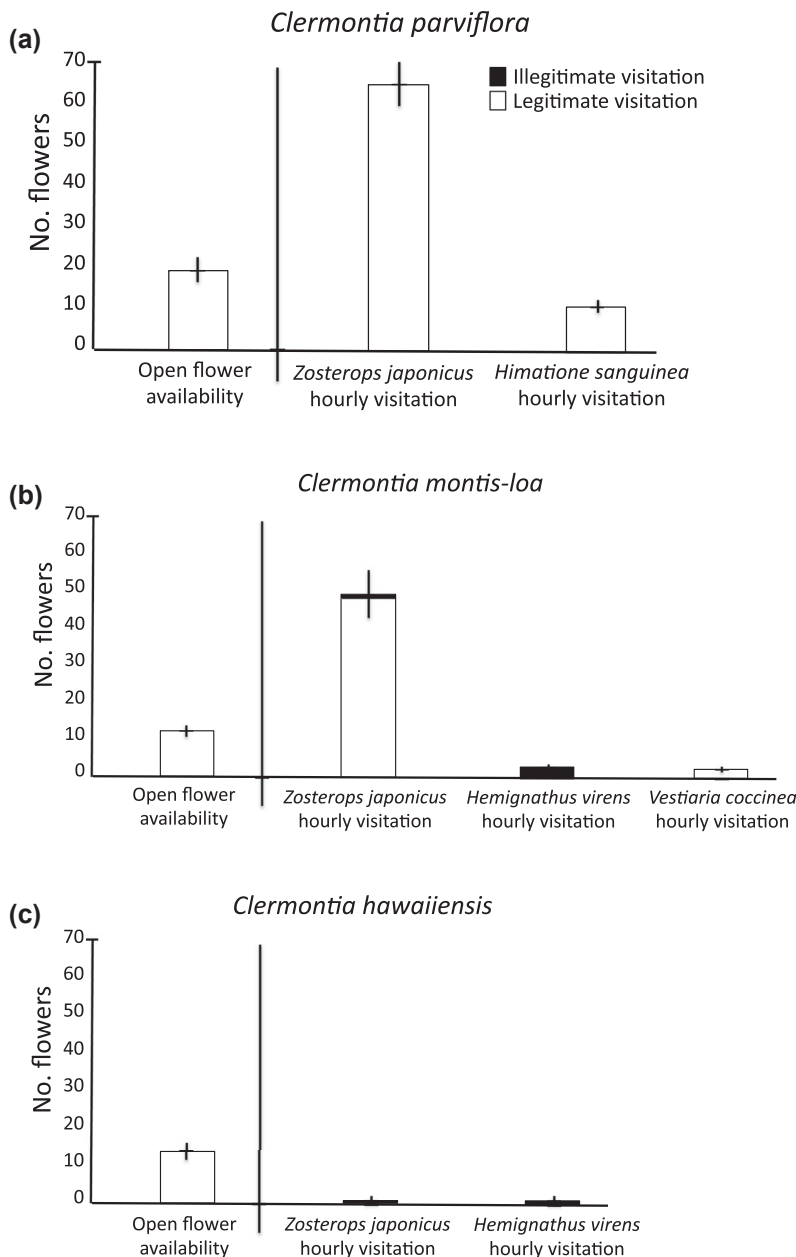


Figure 2. Number of flowers of 3 endemic Hawaiian plant species visited per study stand per hour by each bird species and average total number of open flowers per study stand. Legitimate visitation indicates a visit in which the bird's bill entered the floral opening and may have contacted the stigma or anthers. Illegitimate visitation indicates a visit in which the bird approached the flower from behind or the side and extracted nectar robbed.

coccinea for *C. montis-loa*). However, observed visits were constrained to 1 study site per plant species. The results of the Spearman's rank correlations further suggest that remnant honeycreepers are not very keyed into focal *Clermontia* species: native birds visit *Clermontia* occasionally, but we much more often observed them in ohia or olapa (*Cheirodendron trigynum*) overstory trees. By contrast, *Z. japonicus* more consistently visited the *Clermontia* when present, indicating that *Clermontia* species are a more important resource for these non-native birds than they are for the natives.

Small mammals consume fruits after they have fallen. Tracking tunnels detected nearly equal fruit removal by rats and mice, as well as a small amount of frugivory by mongooses (Table 1). A study of frugivory by *Rattus*

Table 1. Proportion ground-based fruit removal from tracking tunnels by mammal species across 6 sites.^a

Frugivore	Proportion of fruits removed		
	<i>C. parviflora</i>	<i>C. montis-loa</i>	<i>C. hawaiiensis</i>
<i>Rattus</i> sp.	0.22	0.45	0.13
<i>Mus musculus</i>	0.33	0.18	0.13
<i>Herpestes javanicus</i>	0.00	0.36	0.00

^aBecause some fruits remained untouched, columns do not sum to 1.0.

sp. in Hawaiian forests found that seeds smaller than 1.5 mm in diameter can survive ingestion by *Rattus* and be deposited in viable condition (Shiels 2011). *Clermontia* seed size is well within this range. Further study on the

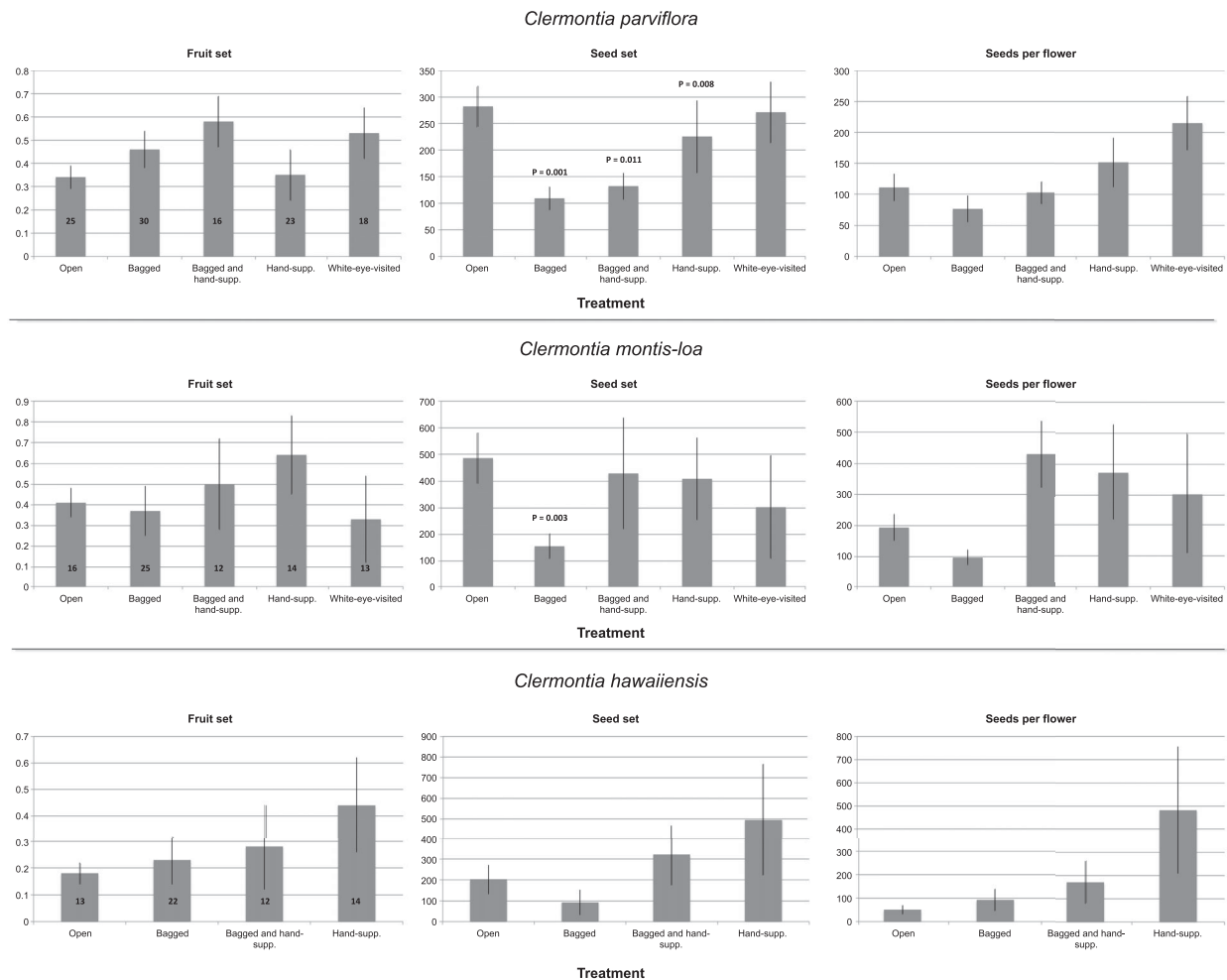


Figure 3. Mean (SE) fruit set, seed set, and seeds per flower resulting from each pollination treatment (bagged, bagged and hand-supplemented, hand-supplemented without bagging, and White-eye-visited) administered to *C. parviflora*, *C. montis-loa*, and *C. hawaiiensis*. A linear mixed-effects model with split plot design was used to contrast the results of each treatment against those of open, naturally pollinated flowers. Significant differences are indicated in the figure with a *p* value over the bar. Sample sizes for each treatment are displayed on the fruit set graphs.

effectiveness of mammals as dispersers of *Clermontia* would be necessary to investigate distances dispersed and germination following deposition.

We saw no evidence of ongoing pollination of *C. hawaiiensis*. Morphological trials implied that *Z. japonicus* is not an adequate match for *C. hawaiiensis*. The flowers were too deep to allow the bird to access nectar, and the bird's head was too small to contact the reproductive column during probing. *Clermontia hawaiiensis* occurs only at lower elevations and is now unlikely to overlap with more than the occasional *V. coccinea*, the sole remaining mid-sized honeycreeper. There is a chance that bees or other insects might perform a small amount of pollination. Other researchers have observed bees sitting on the anthers of other lobeliads (K. Mag-nacca, personal communication). Notably, however, the space between the nectar pool and reproductive column

for these lobeliads is quite a bit larger than the body of a bee: a bee accessing nectar would not contact the reproductive column. A bee collecting pollen would sit on the column itself. However, these are protandrous flowers, and thus such behavior would not be conducive to transfer of pollen to female flowers because only the male flowers are likely to hold attraction. Accidental landing on a stigma has been observed on at least 1 occasion (D. Drake, personal communication) but seems likely to occur only rarely.

It may be possible for *C. hawaiiensis* to persist for quite some time without outcrossing, particularly because these plants are products of adaptive radiation, deriving from a lineage that underwent extreme population bottlenecks in colonizing the Hawaiian Islands and dispersing between islands (Givnish et al. 2009). If deleterious alleles were purged in these bottlenecks, reductions

Table 2. Pollen limitation index values for *C. parviflora*, *C. montis-loa*, and *C. hawaiiensis*.

Plant species	Proportion fruit set in controls (SE)	Proportion fruit set band-supplemented ^a flowers (SE)	Pollen limitation index value ^b
<i>C. parviflora</i>	0.34 (0.05)	0.35 (0.11)	0.03
<i>C. montis-loa</i>	0.41 (0.07)	0.64 (0.19)	0.36
<i>C. hawaiiensis</i>	0.18 (0.04)	0.44 (0.18)	0.36

^aStigma experimentally coated with pollen to evaluate fruit set following maximum pollen transfer.

^bCalculated as $1 - (P_o/P_s)$, where P_o is proportion fruit set of naturally pollinated unmanipulated control flowers and P_s is proportion fruit set of hand-supplemented flowers (Larson & Barrett 2000).

in seed quantity may not have clearcut impacts on plant fitness for *Clermontia* (Crnokrak & Barrett 2002). However, *C. hawaiiensis* has been given a threat rating of vulnerable on the IUCN Red List (IUCN 2012), whereas both *C. parviflora* and *C. montis-loa* are considered “apparently secure” by the Smithsonian Institution (Wagner et al. 1999).

Despite lack of visitation to *C. hawaiiensis*, PLI results suggested that the plant is no more pollen limited than *C. montis-loa*, which receives obvious *Z. japonicus* visitation (Table 2). Both species demonstrated higher fruit set from hand-pollinated than open flowers. When seed set itself was considered, *C. parviflora* was the only species to exhibit greater seed set for hand-pollinated flowers than for open flowers, implying that open-pollinated flowers do not receive maximum pollination (Fig. 3). One interpretation of these results is that in contrast with *C. parviflora*, a higher proportion of *C. montis-loa* and *C. hawaiiensis* flowers may fail to receive any visitation, whereas visits to *C. parviflora* fail to deposit maximum pollen loads (i.e., a higher number of pollen grains was transferred during hand pollination than occurs during most open pollination events). This underscores the imperfect nature of pollination by *Z. japonicus* in this system. Whether due to morphological or behavioral mismatch, *Z. japonicus* may be a mediocre pollinator for those flowers it visits.

Our results have relevance to endangered *Clermontia* species, several of which have large flowers. *Clermontia peleana* is the most critically endangered congener on Hawaii Island, with only 7 plants in the remnant population. Flowers of *C. peleana* are larger than those of *C. montis-loa* and smaller than those of *C. hawaiiensis* (typical corolla length 5–7 cm; Lammers 1990). *C. peleana* flowers are likely either at or beyond the morphological limit for effective pollination by *Z. japonicus*. Historical collections place *C. peleana*'s elevational range below 1300 m (Lammers 1990), below the forests currently occupied by *V. coccinea*. Therefore, an informal public and private partnership implementing large-scale reintroduction efforts for endangered *Clermontia* and other lobeli-

ads on Hawaii Island has outplanted greenhouse-raised *C. peleana* above its historical elevation limit in protected locations, including Kilauea Forest (Robichaux 2012). Although the outplanted *C. peleana* seedlings are not yet reproductive, the active *V. coccinea* floral visitation to *C. stictophylla* in Kilauea Forest suggests the site may offer pollination services to *C. peleana* as well.

Other studies have identified negative impacts of *Z. japonicus* on Hawaii. As a generalist feeder, *Z. japonicus* disperses non-native and native plant species (Woodward et al. 1990). Furthermore, *Z. japonicus* may outcompete some native bird species (Freed et al. 2008; Freed & Cann 2009), perhaps, because the non-native's generalist habits and disturbance tolerance enable it to display high population growth rates under a range of conditions.

When non-native species form novel mutualisms with natives, native species may obtain the functions they require from the relationship (e.g., Cox 1983; Lord 1991; Griffiths et al. 2011). In our study system, *Z. japonicus* appears to imperfectly substitute for native honeycreepers—carrying out some, but not all, of their former functions. Its generalist morphology may block partnership with some plant species. Globally, generalists form a large component of non-native biota (Clavel et al. 2010). For *C. hawaiiensis* and similar plants with specialized pollination strategies, the conservation community faces difficult choices. Should such plants receive hand pollination ad infinitum? Should more specialized (i.e., long-billed) bird partners be deliberately introduced via taxon substitution (Griffiths et al. 2011)? Or should such plants be permitted to dwindle and perhaps become extinct? Selecting among unsatisfactory options is likely to be controversial and contentious.

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Supporting Information

Photos displaying the relative flower sizes of *C. parviflora*, *C. montis-loa*, and *C. hawaiiensis* (Appendix S1) are available online. The authors are solely responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author.

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