

FLORAL TRAIT EVOLUTION AND POLLINATION ECOLOGY IN THE
HAWAIIAN LOBELIAD GENUS, *CLERMONTIA* (CAMPANULACEAE)

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ABSTRACT

The Hawaiian lobeliad genus, *Clermontia*, contains 22 species and 9 subspecies of bird-pollinated shrubs and small trees endemic to wet and mesic forests on the main Hawaiian Islands. This dissertation summarizes four studies concerning the floral trait evolution and pollination ecology of the genus. First, the floral nectar sugar composition, concentration, and nectar standing crop of 21 *Clermontia* taxa were investigated. All taxa produced nectar with low sugar concentrations dominated by hexoses (glucose and fructose). These results support the assumption that the genus has evolved a bird pollination syndrome. Second, existing distribution projections for 25 *Clermontia* taxa and six extant nectarivorous passerine taxa, and comparisons between floral and bird bill dimensions, were used to predict interactions between the potential mutualists. Seventeen taxa have floral morphologies (eg., corolla tubes > 27 mm) that likely prevent short-billed nectarivores (five taxa) from acting as pollinators. As a consequence, these plant taxa are likely to be dependent upon ‘i‘iwi (*Vestiaria coccinea*) for pollination. Third, the phylogenetic relationships in *Clermontia* are inferred from six non-coding chloroplast gene regions. The study found that the genus is not monophyletic. *Clermontia pyrularia* belongs either in the closely related genus *Cyanea* or is an intergeneric hybrid. The genus appears to have evolved either on Kaua‘i or O‘ahu with subsequent inter-island colonization events to the younger islands. Petal-like sepals have evolved once, with five or more reversals back to the simple sepal phenotype. Fourth, pollination ecology studies were undertaken to assess if four endangered ornithophilous Hawaiian lobeliads are

pollinated by extant nectarivorous passerines at two separate restoration sites; Hakalau National Wildlife refuge on Hawai‘i Island and Kahanahāiki and Pahole Gulches on O‘ahu. Two honeycreeper species, ‘i‘iwi and Hawai‘i ‘amakihi (*Hemignathus virens*), were infrequent floral visitors to *Clermontia lindseyana* and *Cl. pyrularia* at Hakalau. However, both ‘i‘iwi and ‘amakihi were not effective pollinators of either lobeliad species. Introduced Japanese white-eye (*Zosterops japonicus*) were not effective pollinators of *Cyanea superba* subsp. *superba* and *Delissea waianaensis* at Kahanahāiki and Pahole. All four lobeliad species were significantly pollen limited, suggesting that none of the animals that visited the flowers were effective pollinators.

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CHAPTER ONE:

INTRODUCTION

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The breakdown of plant-pollinator mutualisms is a pressing global concern in both managed and natural environments (Kearns and Inouye 1997, Kearns et al. 1998, Klein et al. 2007). The disruption of highly co-evolved plant-pollinator mutualisms, where each mutualist is dependent on the other for resources or services, presents even greater challenges (Bond 1994). Ecological theory suggests that species in facultative mutualisms will be driven to extinction or endangerment if their mutualist partner becomes rare over sustained time periods (Bond 1994, Lennartsson 2002). From a plant perspective, the loss of a facultative pollinator may lead to pollen limitation, lowered reproductive output and lowered recruitment (Wilcock and Neiland 2002, Barrett and Kohn 1991, Knight et al. 2005, Courchamp et al. 1999). Additionally, if the plant species is incapable of full or partial autogamy (selfing), a total loss of reproductive output may be experienced, resulting in extinction or strong selection towards autogamy (Lloyd and Schoen 1992). However, autogamy is often thought of as an evolutionary dead-end due to the negative impacts of inbreeding depression (Lande and Schemske 1985, Charlesworth and Charlesworth 1987).

The Hawaiian Islands provide ideal situations to test the impacts of pollinator decline and extinction. While the archipelago's isolation has led to unique levels of adaptive radiation and endemism the flora has undergone equally extreme levels of species extinction and endangerment due to human impacts. Currently, 30% of the flora is endangered (Sakai et al. 2002). The Hawaiian lobeliads (Campanulaceae) epitomise this situation. This monophyletic lineage is composed of 6 genera: *Brighamia*, *Cyanea*, *Clermontia*, *Trematolobelia*, *Lobelia* and *Delissea*, collectively containing *ca.* 140 species (taxonomy follows Lammers 2007ab, Lammers 2009) of woody trees, shrubs and

caudiciforms endemic to the main Hawaiian Islands (Givnish et al. 2009).

Clermontia, the second largest genus of Hawaiian lobeliads, is composed of 22 species and nine subspecies of shrubs and small trees found in mesic and wet forest between 150 and 2100 meters (Lammers 1991). The taxa are endemic to the main Hawaiian Islands with increasing numbers of taxa on the younger islands: Kaua'i (1 taxon), O'ahu (five taxa) Moloka'i (five taxa), Lana'i (four taxa), Maui (14 taxa) and Hawai'i (13 taxa). *Clermontia* species have little interspecific variation in vegetative characters. However, floral characters vary widely among taxa, particularly for corolla size and calyx arrangement that may either be reduced (sepaloid) or petal-like (petaloid; Lammers 1991, 1995).

Pollination syndromes in Clermontia and other Hawaiian lobeliads

A plants pollination syndrome is defined as the evolution of similar floral traits in often unrelated plant taxa in response to selection pressures from the same guild of animal floral visitors (Fenster et al. 2004). Bird-pollinated (ornithophilous) plants typically have flowers that are brightly colored (usually red or orange), unscented, tubular, and that produce copious amounts of dilute nectar (Brown and Kodric-Brown 1979, Pyke and Waser 1981, Cronk and Ojeda 2008, Nicolson and Fleming 2003, Fenster et al. 2004).

The nectar sugar characteristics of bird pollinated plants are defined by the two functional groups of bird pollinators (Johnston and Nicolson 2008). Nectar specialists (hummingbirds, South African sunbirds) favor nectar with relatively smaller volumes, higher sugar concentrations and high proportions of sucrose (e.g., Stiles and Freeman

1993), while passerine generalist species (most other nectarivorous bird species) favor nectar that is more dilute and rich in hexose sugars (Baker and Baker 1982ab, Johnston and Nicolson 2008).

In an attempt to understand if Hawaiian lobeliads had evolved a bird pollination syndrome, Lammers and Freeman (1986) surveyed the floral nectar sugar composition of ten species and three interspecific hybrids from the three Hawaiian lobeliad genera, *Lobelia*, *Clermontia* and *Cyanea*. Their study found that all the taxa examined produced nectar rich in hexose sugars (fructose and glucose) that were comparable to other generalist passerine pollinated plant species. This result is further supported by the study of Drake and Morden (unpublished) who found that the nectar concentrations in three Hawaiian lobeliad species were comparable to other passerine pollinated plant species. By contrast, recent analysis of the nectar of *Brighamia insignis* (S. Walsh, unpublished data), a caudiciform, found it to be sucrose dominant, which provides supportive evidence for the hypothesized pollination syndrome of this species (Lammers 1989).

Phylogenetic studies in Clermontia

The Hawaiian lobeliads have become a focal lineage in understanding evolutionary changes in the Hawaiian flora (Lammers 1995, Givnish et al. 1994, Givnish et al. 1995, Givnish et al. 2009). Phylogenetic studies undertaken in the largest genus, *Cyanea*, by Givnish et al. (1995) resolved two clades based on fruit flesh color, orange and purple. In a parallel study investigation, Givnish et al. (1994) utilized this phylogeny to interpret evolution of stem prickles and heterophylly in *Cyanea*, showing that prickles

have evolved independently at least four times, while heterophylly arose in three of the four lineages that have prickles. These adaptations were interpreted by Givnish et al. (1994) as defenses to now extinct giant ducks whose species, like the species of prickle bearing and heterophyllus *Cyanea*, were most common on the younger islands. Givnish et al. (2009) aimed to identify evolutionary trends in all six genera of Hawaiian lobeliads using 23 representative taxa with outgroup species from postulated geographical origins. Despite earlier predictions of paraphyly (Carlquist 1970), the study found that Hawaiian lobeliads are monophyletic and that the original colonist arrived in Hawai'i approximately 13 million years ago.

Focussing specifically on *Clermontia*, Lammers (1991) used a cladistic analysis based on vegetative and floral characters and suggested that *Clermontia* evolved and subsequently dispersed from Hawai'i Island. This interpretation was based primarily on the greater number of species on the younger islands of Maui and Hawai'i compared to fewer species on the older islands of O'ahu and Kaua'i. Givnish et al. (2013) recently published a phylogenetic study based on five chloroplast DNA (cpDNA) regions and nuclear inter-simple sequence repeat polymorphisms (ISSRs). The study found that the genus evolved either on Kaua'i or an older island with subsequent dispersal to the younger islands, a result that was further supported by Hofer et al. (2013). Givnish et al. (2013) also found that *Clemontia* is not monophyletic as *Cl. pyrularia* nested within *Cyanea* and that six species included in the study may have arisen through hybridization and/or introgression.

Lobeliad pollination ecology

Almost all of the Hawaiian lobeliad species are believed to have been historically pollinated by nectarivorous endemic Hawaiian honeycreepers (Drepanidae) and Hawaiian Mohoidae (Mohoidae) (Lammers and Freeman 1986). The first of these putative pollinators, the Hawaiian Drepanidinae, underwent an impressive adaptive radiation following a single colonization <5 m.y.a., subsequently evolving into approximately 50 species, comprising 21 genera (Fleischer and McIntosh 2001, Gorresen et al. 2009). The radiation is believed to have resulted primarily from shifts in dietary preferences, leading to the evolution of species with bills adapted for different foraging niches (Banko and Banko 2009). Five specialist nectarivorous honeycreeper species were historically recorded (*see* Banko and Banko 2009 for a review). In addition, based on published observations of nectar foraging (Banko and Banko 2009), seven additional species (including four ‘amakihi, and two ‘akialoa species (*Hemignathus* spp.)) may have pollinated lobeliad taxa.

The second pollinator guild, the Mohoidae, are an endemic passerine family of five species, four in the genus *Moho* (Ō‘ō) (one species each on Kaua‘i, O‘ahu, Moloka‘i (and possibly also Maui (Sykes et al. 2000)) and Hawai‘i), and a single species of *Chaetopila* (Kioea), endemic to the Hawai‘i Island (Sykes et al. 2000, Gorresen et al. 2009). All five species are nectarivores, possessing brush-tipped tongues adapted for nectar foraging (Fleischer et al. 2008).

The congruence between the curved bills of nectarivorous honeycreeper species, particularly in the genera *Drepanis* and *Vestiaria*, and the flowers of lobeliad species, combined with casual observations of honeycreepers foraging from lobeliad flowers (Cassin 1858, Wilson 1890, Rothschild 1893-1900, Perkins 1895, 1903, Bryan 1908,

Amadon 1950, Munro 1960, Spieth 1966, Richards and Bock 1973, Berger 1981) has led to much speculation regarding co-evolution between Hawaiian honeycreepers and lobeliad species (Perkins 1903, Carlquist 1970, Lammers and Freeman 1986, Givnish et al. 1995). Interestingly, the role that Mohoidae may have played as lobeliad pollinators has received little attention, possibly because the species lack curved bills, despite published observations of several *Moho* species visiting the flowers of lobeliad species (Wilson 1890, Perkins 1903, Munro 1960).

Determining what role Hawaiian Drepanidinae and Mohoidae played as pollinators of lobeliad species is now difficult due to the drastic decline of these potential mutualists following the colonization of the Hawaiian Islands by humans (Wagner et al. 1999, Sakai et al. 2002, Gorresen et al. 2009). Approximately 76% of Hawaiian lobeliad taxa are of conservation concern (Sakai et al. 2002). Introduced, non-native herbivores, particularly ungulates, alien plants and wide-scale habitat modification have negatively affected lobeliad species (Wagner et al. 1999, Sakai et al. 2002, Joe and Daehler 2008, Pender et al. 2013). Similarly, Hawaiian Drepanidinae and Mohoidae have also declined due to habitat modification, predation from introduced mammalian predators and avian diseases (avian pox; *Poxvirus avium*) and avian malaria (*Plasmodium relictum*)) that are transmitted by introduced mosquitoes (primarily *Culex quinquefasciatus*) (Banko and Banko 2009). As a result, two of the five specialist nectarivorous honeycreeper species and all Mohoidae species are extinct (Banko and Banko 2009). Potential interactions between lobeliad species and the remaining nectarivorous honeycreeper species (particularly 'i'iwi (*Vestiaria coccinea*)) are mostly limited to higher elevation habitats (1250 m and higher) above the mosquito zone on the

main Hawaiian Islands (Warner 1968, Fancy and Ralph 1998, Pender, unpublished data).

Five studies, undertaken over the past *ca.* 30 years, have attempted to address what bird species are currently visiting the flowers of Hawaiian lobeliad species (Cory 1984, Lammers, Weller and Sakai 1987, Drake and Morden unpublished, Gardener and Daehler 2006, Aslan et al. 2013). Cory (1984) found that no birds visited the flowers of *Cl. kakeana* and *Cy. angustifolia* on O‘ahu. Lammers et al. (1987) found that Japanese white-eye, an introduced passerine, visited the flowers of *Clermontia arborescens* subsp. *waihia* at Waikamoi preserve, Maui. Drake and Morden (unpublished) undertook a study at the Alaka‘i wetland on Kaua‘i Island and found that two honeycreepers (Kaua‘i ‘amakihi and ‘i‘iwi) and Japanese white-eye visited the flowers of the four lobeliad species that were studied. Gardener and Daehler (2006) did not observe any birds visiting the flowers of *Cl. kakeana*. However, they did record Japanese white-eye nectar robbing the flowers of *Cyanea superba* subsp. *superba* and *Cy. pinnatifida*. Lastly, Aslan et al. (2013) studied the pollination ecology of *Cl. hawaiiensis*, *Cl. montis-loa* and *Cl. parviflora*. The study found that Japanese white-eye and ‘apapane were rare visitors to the flowers of *Cl. hawaiiensis*. The study also showed that Japanese white-eye were frequent visitors to the flowers of *Cl. parviflora* and *Cl. montis-loa*. ‘Apapane occasionally visited *Cl. parviflora* and nectar robbed the flowers of *Cl. montis-loa*, while ‘i‘iwi occasionally visited *Cl. montis-loa* flowers.

The following four chapters summarize research on floral trait evolution and the pollination ecology of the Hawaiian lobeliad genus, *Clermontia*. Chapter Two summarized a study of the floral nectar sugar characteristics of *Clermontia* taxa. Chapter Three assessed potential interactions between extant nectarivorous passerines and

Hawaiian lobeliad species in the genus, *Clermontia*. Chapter Four summarized a phylogenetic study of the Hawaiian lobeliad genus, *Clermontia*, with special emphasis on floral trait evolution. Chapter Five reported on a pollination ecology study of restoration plantings of four endangered Hawaiian lobeliad species. The final chapter, Chapter Six, provides conclusions to the four research chapters. Chapter summaries are provided as follows:

Chapter Two: Floral nectar sugar compositions in the Hawaiian lobeliad genus, *Clermontia* (Campanulaceae)

Project outline: Based on both floral morphology and previous studies of nectar sugars (Lammers and Freeman 1986, Lammers 1991), species of *Clermontia* are believed to have evolved for pollination by nectarivorous passerines. To address this assumption, the floral nectar sugar composition, concentration, and nectar standing crop of 21 *Clermontia* taxa were analyzed as a means to assess their pollination syndrome.

Hypothesis: The nectar sugar characteristics (sugar composition and concentration) of all 21 *Clermontia* taxa included in the study will be similar to other plant species with a generalist passerine pollination syndrome.

Chapter Three: Predicting potential interactions between extant nectarivorous passerines and Hawaiian lobeliad species in the genus, *Clermontia* (Campanulaceae)

Project outline: Hawai'i has undergone widescale bird pollinator declines and extinctions since the arrival of humans to the archipelago (Banko and Banko 2009). What animals are visiting the flowers of native bird pollinated plants in Hawai'i is largely unknown. This study used 1) geographical information systems (GIS), 2) comparisons between floral and bird head and bill dimensions, and 3) results of recent

pollination ecology studies as a first step to predict potential interactions between extant native and introduced nectarivorous bird species and 25 bird-pollinated

Clermontia taxa

Hypothesis: In extant communities, the majority of *Clermontia* species have no avian pollinators present that are capable of effective nectar foraging and pollen movement.

Chapter Four: A phylogenetic study of the Hawaiian lobeliad genus, *Clermontia* (Campanulaceae) with special emphasis on floral trait evolution

Project outline: *Clermontia*, contains 22 species and 9 subspecies of shrubs and small trees endemic to wet and mesic forests on the main Hawaiian Islands. The flowers are particularly notable for having sepals either reduced (sepaloid) or long and petal-like (petaloid sepals). Phylogenetic relationships for 28 taxa in the genus were inferred from cpDNA sequence variation in the *atpB-rbcL*, *ndhF-rpl32*, *rpl32-trnL*, *trnH-psbA*, *trnL-F* spacer regions and the *trnL intron*.

Hypothesis one: *Clermontia* evolved on Kaua‘i, geologically the oldest of the larger islands of the Hawaiian archipelago and subsequently dispersed eastward to the progressively younger islands of O‘ahu, the Maui-Nui island complex, and the island of Hawai‘i.

Hypothesis two: The petal-like sepal arrangement that occurs in more than half of the *Clermontia* species has evolved multiple times.

Chapter Five: Are restoration plantings of endangered ornithophilous Hawaiian lobeliads (Campanulaceae) being pollinated by nectarivorous passerines in Hawai‘i?

Project outline: Pollination ecology studies were undertaken to assess if four endangered ornithophilous Hawaiian lobeliads are pollinated by passerines (native or introduced) and diurnal insects at two separate restoration sites. Two species, *Clermontia lindseyana* and *Cl. pyrularia* were studied at Hakalau National Wildlife Refuge (Hawai‘i Island), a site

that contains the highest densities of nectarivorous honeycreepers in the archipelago. Two other species, *Cyanea superba* subsp. *superba* and *Delissea waianaensis* were studied at Kahanahāiki and Pahole Gulches (Wai‘anae Mountains, O‘ahu) where nectarivorous honeycreepers are functionally extinct.

Hypothesis one: The four lobeliad species are not being visited by effective animal pollinators. As a consequence, all four lobeliad species will be pollen limited

Hypothesis two: All four lobeliad species are self-compatible (capable of autogamy). This will be the primary means by which all four lobeliad species sexually reproduce.

CHAPTER TWO:
FLORAL NECTAR SUGAR COMPOSITIONS IN THE HAWAIIAN LOBELIAD
GENUS, *CLERMONTIA* (CAMPANULACEAE)

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Abstract

This study reports the floral nectar sugar composition, concentration, and nectar standing crop of 21 taxa in the Hawaiian lobeliad genus, *Clermontia*. All taxa produced nectar with low sugar concentrations (mean = 9.0 % w/v \pm 1.8 S.D.) dominated by hexoses (glucose and fructose) (mean sucrose/hexose ratio: 0.02 \pm 0.02). Nectar standing crop volumes varied widely among species, ranging from 9.7 μ l \pm 7.1 to 430.5 μ l \pm 401.8 (mean volume: 181.1 μ l \pm 116.0). Collectively, the nectar traits support the assumption that *Clermontia* species possess a generalist passerine pollination syndrome, likely in response to selective pressures from nectarivorous Hawaiian Drepanidinae and Hawaiian Mohoidae; the majority of which are either very rare or extinct.

Introduction

Floral nectar is the most important reward presented to animal pollinators by angiosperms (Cruden et al. 1983, Simpson and Neff 1983) and is composed of a variety of compounds (Nicolson and Thornberg 2007). One disaccharide (sucrose) and two monosaccharide (glucose and fructose) sugars are the dominant chemical constituents (Baker and Baker 1983, Gottsberger et al. 1984, Stiles and Freeman 1993). Nectar sugar composition studies have been used for several decades to predict the pollination syndrome of animal pollinated plant species (Percival 1974, Baker and Baker 1983, 1990, Freeman et al. 1991). Despite several shortcomings (e.g., several pollinator guilds sharing similar sugar ratios and phylogenetic constraints; *see* Baker and Baker 1983), this

strategy allows a plant species' putative pollinator guild to be predicted without undertaking often logistically challenging pollinator studies, particularly for species that are rare, geographically isolated, or where historic pollinators are now rare or extinct (e.g., Lammers and Freeman 1986, Freeman et al. 1991, Wolff 2006).

Bird-pollinated (ornithophilous) plants are characterized as having flowers that are brightly colored (typically red or orange), unscented, tubular, and that produce copious amounts of dilute nectar (Brown and Kodric-Brown 1979, Pyke and Waser 1981, Cronk and Ojeda 2008, Nicolson and Fleming 2003, Fenster et al. 2004). Until recently, it was believed that the nectar sugar compositions of ornithophilous plant species fell into two separate classes: hummingbirds and passerines (Baker and Baker 1983).

Hummingbird pollinated plants were categorized as having nectar with relatively smaller volumes, higher sugar concentrations and high proportions of sucrose (e.g., Stiles and Freeman 1993), while passerine pollinated plant species were believed to produce nectar that is more dilute and rich in hexose sugars (Baker and Baker 1982ab, Martinez del Rio et al. 1992, Stiles and Freeman 1993, Baker et al. 1998). However, Johnston and Nicolson (2008) reported that passerine sunbirds in South Africa favor sucrose rich nectars and suggest a more appropriate categorization that should be based on whether the plants produce nectar favored by a nectar specialist (sucrose rich) or generalist (hexose rich) bird species.

Clermontia is the second largest Hawaiian lobeliad genus, containing 22 species and nine subspecies of shrubs or small trees. The species typically occur in montane rain forest habitats between 150 and 2100 m on the main Hawaiian Islands (Lammers 1991,

Wagner et al. 1999). All *Clermontia* species produce flowers that morphologically conform to the bird pollination syndrome described above (Lammers 1991). *Clermontia* and four other Hawaiian lobeliad genera (*Lobelia*, *Cyanea*, *Trematolobelia* and *Delissea*) are believed to have evolved flowers adapted for pollination by Hawaiian Drepanidae (Hawaiian honeycreepers) and Hawaiian Mohoidae (previously known as Hawaiian honeyeaters, *see* Fleischer et al. 2008) species (Lammers and Freeman 1986). Both passerine lineages have undergone drastic declines and extinctions since the arrival of humans to the Hawaiian archipelago (Banko and Banko 2009), greatly limiting the potential study of pollinator interactions between the potential mutualists.

Two studies have used high performance liquid chromatography (HPLC) to analyze nectar sugars as a proxy to understand potential mutualisms between Hawaiian lobeliad species and rare or extinct putative pollinators (Cory 1984, Lammers and Freeman 1986). Cory (1984) analyzed the floral nectar sugar composition of two Hawaiian lobeliads (*Cyanea angustifolia* and *Clermontia kakeana*) and found (following Baker and Baker 1983) that both species produce hexose dominant nectar. Lammers and Freeman (1986) surveyed the floral nectar sugar composition of ten species and three interspecific hybrids from the three Hawaiian lobeliad genera, *Lobelia*, *Clermontia* and *Cyanea*, finding a similar result to that of Cory (1984). An additional unpublished study (Drake and Morden 2006) recorded sugar concentrations in three lobeliad species, *Clermontia fauriei*, *Cyanea leptostegia* and *Trematolobelia kauaiensis*. All three studies found that the species examined produced nectar with sugar compositions that were comparable to other passerine pollinated plant species. However, to date, no comprehensive study has been undertaken in any one Hawaiian lobeliad genus.

The small number of *Clermontia* taxa ($n = 6$) which have had their floral nectar analyzed, and the diversity of corolla morphology in *Clermontia* (Lammers 1991), that potentially indicates selection pressures from different animal pollinator guilds, warrants a thorough study of the genus. To assess if the genus has evolved a generalist-passerine pollination syndrome, the nectar characteristics (e.g., nectar sugar compositions and sugar concentrations) of 18 of the 22 *Clermontia* species were analyzed.

Methods

Field collection of nectar and flower samples

Samples from nectar standing crop (the quantity of nectar in a flower at a given time; Corbet 2003) samples were primarily collected from wild populations of plants with the exception of one taxon that was sampled from cultivated plants (Table 1). In almost all cases, ten or more nectar samples were collected from each taxon. Where feasible, taxa that occurred either on different mountain ranges on the same island or on multiple islands were sampled across their distributional ranges. Nectar was sampled from up to ten plants per population, although in some cases due to small population sizes, multiple samples were collected from different flowers from the same plant. Nectar was extracted from flowers using a Fisherbrand[®] polyethylene transfer pipet (model: 13-711-5A; ThermoFisher Scientific, Massachusetts, U.S.A) with a 50 mm long by 2 mm wide polyethylene tube (model: AAC00004; Saint-Gobain Performance Plastics, Paris, France) inserted into the tip to allow access to the nectar at the base of the corolla tubes. Nectar samples were transferred to 1.5 ml (model: MH 815EZ; Phenix Research Products, North Carolina, U.S.A) microcentrifuge tubes. Nectar samples were stored at -20 °C until the

analyses were performed.

Nectar volume and sucrose composition

Nectar samples were thawed and their volumes calculated by extracting samples from the microcentrifuge tubes using a calibrated micropipette (0-200 μ l) (model: PR 200; Mettler-Toledo, Ohio, U.S.A) that was adjusted to precisely calculate the nectar volume. In most cases, ten or more samples were analyzed per taxon. The concentration of sugars in each nectar sample [in sucrose equivalents: % weight/volume (% w/v)] was determined using a handheld refractometer (model: Eclipse 0-50 % w/v; Bellingham and Stanley, U.K.). Samples were subsequently stored at -20 °C.

HPLC analysis

Nectar samples were thawed and diluted using distilled water (1:4 ratio, nectar:water). In cases where the nectar exceeded concentrations that the HPLC was capable of recording, between 50 and 400 μ l of distilled water was added to the samples. Nectar sugars (sucrose, fructose and glucose) were separated and quantified by HPLC using a Shimadzu Model 20 HPLC with a CBM-20A controller, LC-20AT pump, SIL-20A automatic injector, CTO-20A column oven and a ELSD-LT-II Evaporative Light Scattering Detector. The analysis column (Fast Carbohydrate Analysis Column 100 mm \times 7.8 mm with a precolumn, Bio-Rad Laboratories, Hercules, CA) was run at 1 mL min⁻¹ at 80 °C with degassed, distilled, deionized water. The ELSD was at 40 °C. Retention

times of the sugars were compared with that of pure standards. Sugar concentration was calculated based on the area of the individual sugar peaks compared to pure standards.

Results

Mean nectar standing crop varied widely between the species sampled, ranging from 9.7 μL (± 7.1) in the small flowered *C. micrantha*, to 430.5 μL (± 401.8) in the large flowered, *C. arborescens* subsp. *waihia* (Table 1). The overall mean standing nectar crop volume for all taxa was 181.1 μL \pm 116.0. The mean concentration of sugars (in % w/v) present in the nectar ranged between 6.3% (± 1.7) in *C. micrantha* to 12.9 % (± 1.4) in *C. fauriei*. The mean concentration for all taxa was 9.03 % (± 1.8) (Table 1). The nectar samples of the 21 *Clermontia* taxa predominantly contained hexoses (glucose and fructose). The sucrose-to-hexose ratio (% sucrose)/(% fructose + % glucose) of the samples ranged from 0.00 to 0.08 (0.02 ± 0). Sucrose was present in low amounts in the nectar of 17 taxa, with a mean range from 0.2% to 7.2%, while four taxa had no detectable sucrose (mean for all taxa: 2.0 ± 2.0) (Table 1). Mean glucose compositions ranged from 30.3% to 55.4% (mean for all taxa: 48.7 ± 5.2) (Table 1), while mean fructose compositions ranged from 43.9% to 65.7% (mean for all taxa: 49.2 ± 5.0) (Table 1). Four taxa had essentially equivalent mean percentages of fructose and glucose (less than *ca.* 2% difference) present in their nectar. Six taxa had mean percentages that were higher in fructose, while 10 taxa had nectar with greater mean glucose percentages (Table 1).

Table 1. Mean standing crop volume, sugar concentration, and percentage fructose, glucose and sucrose in the nectar samples of 21 *Clermontia* taxa. The localities where flowers and nectar samples were collected are provided. S/H is the sucrose/hexose (fructose + glucose) ratio. Means are presented \pm 1 S.D. Statistical analyses were undertaken in Minitab 16 (Minitab 16 Statistical Software 2010).

Taxon	Locality	n	Volume (μ L)	Sugar conc.	n	% Fructose	% Glucose	% Sucrose	S/H
<i>C. arborescens</i> (H. Mann) Hillebr. subsp. <i>arborescens</i>	West Maui, Wailuku, Kapilau Ridge Trail, (ca. 1023 m)	7	86.77 (\pm 96.5)	10.8 (\pm 1.9)	5	48.4 (\pm 2.2)	46.4 (\pm 1.5)	5.3 (\pm 3.1)	0.05
<i>C. arborescens</i> (H. Mann) Hillebr. subsp. <i>waihia</i> (Wawra) Lammers	West Maui, Puu Kukui Boardwalk Trail (ca. 1089 m).	11	430.5 (\pm 401.8)	11.6 (\pm 1.1)	5	47.0 (\pm 1.3)	50.1 (\pm 1.6)	1.2 (\pm 0.5)	0.01
<i>C. arborescens</i> (H. Mann) Hillebr. subsp. <i>waikoluensis</i> (H. St. John)	Molokai, TNC Kamakou Preserve, Puu Kolekole Trail (ca. 1187 m)	4	201.6 (\pm 125.8)	8.1 (\pm 1.8)	4	48.1 (\pm 0.85)	50.7 (\pm 0.7)	1.3 (\pm 1.0)	0.01
<i>C. calophylla</i> F. Wimmer	Hawaii, Kohala Mountain. Puu O Umi NAR. Near Kilohana Exclosure. (ca. 1439 m)	10	177.7 (\pm 111.6)	9.4 (\pm 0.8)	5	46.3 (\pm 2.3)	52.5 (\pm 3.4)	1.3 (\pm 1.2)	0.01
<i>C. clermontioides</i> subsp. <i>clermontioides</i> (Gaud.) A Heller	Hawaii, South Kona, Kona Hema TNC Preserve (ca. 1329 m)	18	125.4 (\pm 142.2)	8.5 (\pm 1.4)	5	49.3 (\pm 7.2)	47.3 (\pm 6.0)	3.5 (\pm 3.6)	0.03
<i>C. drepanomorpha</i> Rock	Hawaii, Kohala Mountain. Puu O Umi NAR. Kilohana exclosure. (ca. 1460 m)	5	276.8 (\pm 67.8)	8.8 (\pm 0.9)	5	47.0 (\pm 0.3)	51.4 (\pm 0.4)	1.6 (\pm 0.7)	0.02
<i>C. fauriei</i> H. Lév.	Kauai, Kokee Boardwalk Trail (ca. 1234 m)	13	278 (\pm 156.7)	12.9 (\pm 1.4)	5	44.6 (\pm 1.3)	48.2 (\pm 2.9)	7.2 (\pm 2.7)	0.08
<i>C. grandiflora</i> Gaud. subsp. <i>grandiflora</i>	West Maui, Puu Kukui Boardwalk Trail (ca. 1544 m)	12	119.4 (\pm 106.7)	8.0 (\pm 1.5)	5	47.2 (\pm 0.7)	49.2 (\pm 0.8)	3.6 (\pm 1.4)	0.04
<i>C. grandiflora</i> Gaud. subsp. <i>munroi</i> (H. St. John)	Multiple locations: West Maui, Puu Kukui Boardwalk Trail (ca. 995 m). West Maui, Wailuku Kapilau Ridge, (ca. 989 m). East Maui, TNC Waikamoi Preserve (ca. 1629 m). Lanai, Munro Trail, near summit (ca. 1033 m).	19	105.2 (\pm 90.5)	10.2 (\pm 1.6)	5	46.9 (\pm 1.3)	51.4 (\pm 1.5)	1.7 (\pm 1.3)	0.02

<i>C. hawaiiensis</i> (Hillebr.) Rock	Multiple locations: Hawaii, Puu Makala Natural Area Reserve and Greenwood, Kaleponi Road (ca. 1330 m. and 840 m, respectively)	10	384 (± 182.3)	8.9 (± 1.9)	5	51.6 (± 4.9)	48.2 (± 4.7)	0.2 (± 0.4)	0.002
<i>C. kakeana</i> Meyen	Multiple locations: Oahu, Mt Tantalus, Manoa Cliff Trail (ca. 560 m). West Maui, Puu Kukui Boardwalk Trail (ca. 919 m). East Maui, Makawao Forest Preserve (ca. 904 m).	37	161.8 (± 152.8)	8.6 (± 2.8)	5	54.2 (± 4.6)	45.9 (± 4.6)	0	0
<i>C. kohalae</i> Rock	Hawaii, Kohala Mountain. Near Mt Eke (ca. 1563 m)	10	210.1 (± 189)	9.9 (± 1.1)	5	45.7 (± 4.2)	49.5 (± 4.9)	4.9 (± 8.2)	0.05
<i>C. lindseyana</i> Rock	Hawaii, Hakalau National Wildlife Refuge, Maulua Tract (ca. 1707 m)	10	184.08 (± 190.41)	7.8 (± 1.9)	5	55.7 (± 2.7)	43.7 (± 2.9)	0.5 (± 0.7)	0.005
<i>C. micrantha</i> (Hillebr.) Rock	Maui, West Maui, Puu Kukui Boardwalk Trail (ca. 1335 m)	12	9.7 (± 7.1)	6.3 (± 1.7)	2	65.7 (± 17.7)	30.3 (± 12.0)	4.0 (± 5.7)	0.04
<i>C. montis-loa</i> Rock	Hawaii, Forest Plantation Road near Saddle Road (ca. 1260 m)	11	85.1 (± 32.3)	9.4 (± 1.1)	5	47.0 (± 1.8)	50.7 (± 1.1)	2.1 (± 1.9)	0.02
<i>C. pallida</i> Hillebr.	Molokai, TNC Kamakou Preserve, Puu Kolekole Trail (ca. 1073 m)	10	141 (± 72.85)	8.8 (± 1.7)	5	45.2 (± 1.7)	54.2 (± 1.2)	0.6 (± 0.7)	0.006
<i>C. parviflora</i> Gaud. ex A. Gray	Hawaii. Wright Road adjacent to Hawaii Volcanoes National Park Olaa Forest Preserve (ca. 1169 m).	13	19.85 (± 8.74)	7.7 (± 2.6)	5	52.1 (± 5.3)	47.9 (± 5.3)	0	0
<i>C. pyrularia</i> Hillebr.	Hawaii, Hakalau National Wildlife Refuge (ca. 1656 m)	10	145.24 (± 72.91)	11.4 (± 1.3)	5	49.9 (± 0.8)	49.0 (± 1.1)	1.2 (± 0.8)	0.013
<i>C. samuelii</i> C. Forbes subsp. <i>samuelii</i>	East Maui. Haleakala National Park Nursery (ca. 2070 m)	10	276.36 (± 128.36)	9.1 (± 0.8)	5	43.9 (± 0.8)	54.9 (± 1.1)	1.3 (± 1.1)	0.01
<i>C. tuberculata</i> C. Forbes	East Maui. TNC Waikamoi Preserve (ca. 1725 m)	8	27.04 (± 21.71)	5.0 (± 3.9)	4	53.3 (± 1.9)	46.7 (± 1.9)	0	0
<i>C. waimeae</i> Rock	Hawaii, Kohala Mountain. Puu O Umi NAR. Area surrounding Kilohana Exclosure. (ca. 1458 m)	10	333.05 (± 213.3)	8.5 (± 1.1)	3	44.6 (± 1.2)	55.4 (± 1.2)	0	0
Mean			181.08 (± 115.96)	9.0 (± 1.8)		49.2 (± 5.0)	48.7 (± 5.2)	2.0 (± 2.0)	0.02 (± 0.02)

Discussion

The nectar sugar compositions of all the *Clermontia* taxa that were sampled were hexose rich with low or non-detectible sucrose (Table 1). This finding supports the hypothesis that each of the *Clermontia* taxa included in the study has evolved a generalist passerine bird pollination syndrome. The sugar compositions were strikingly similar between almost all the taxa sampled. The only exception was *Clermontia micrantha* that had on average more than twice the amount of fructose compared to glucose (65.70% vs. 30.28% respectively; Table 1). This species has a small corolla tube (13 mm; Lammers 1991), making it difficult to obtain sufficient nectar volumes for HPLC analysis (Table 1). Whether this species has undergone selection from an alternative pollinator guild can only be fully addressed through further sampling and analysis.

Overall, the sugar compositions reported here are very similar to those of Lammers and Freeman (1986), who included six taxa (*C. arborescens* subsp. *waihia*, *C. hawaiiensis*, *C. kakeana*, *C. kohalae*, *C. montis-loa* and *C. parviflora*) that were also included in the current study. Like Lammers and Freeman (1986), the present study found that some taxa had higher levels of fructose compared to glucose and vice versa, although all species ultimately produced nectar rich in hexoses.

Plants that produce hexose rich nectar have been associated with other animal pollinator guilds (e.g., flies and New World bats; Baker and Baker 1983), including short-tongued bees (*Hylaeus* spp.) that are present in Hawai'i (Lammers and Freeman 1986). However, the floral morphology of *Clermontia* species (reviewed in Lammers

1991) and recorded observations of nectarivorous Hawaiian Drepanidinae and Mohoidae species visiting the flowers of *Clermontia* taxa (Lammers and Freeman 1986), suggests that these passerine species provided the selective pressures for the nectar traits recorded in the present study. However, whether these avian lineages strictly co-evolved with *Clermontia* taxa is now impossible to fully address given the wide-scale passerine extinctions that have taken place since the arrival of humans to Hawai‘i (Banko and Banko 2009).

The *Clermontia* species surveyed produced dilute nectar, falling within the range preferred by generalist passerine nectarivores (Johnson and Nicolson 2008). Lammers and Freeman (1986) did not measure sugar concentrations, but the sugar concentrations reported here are comparable to those reported for three Hawaiian lobeliad species (*Clermontia fauriei*, *Cyanea leptostegia* and *Trematolobelia kauaiensis* Drake and Morden 2006).

Drake and Morden (2006) sampled the nectar standing crop of 173 *C. fauriei* flowers on Kaua‘i and found that nectar volume was highly variable, ranging from 0 to 944 μ l. Despite this variability, the authors found that there was no significant difference in nectar volume produced by early (male) and mid to late (female) phase flowers. The present study also found that nectar standing crop varied widely both within and among the *Clermontia* taxa sampled (Table 1), but did not take the age of the flower into consideration.

To date, no studies have been published that quantify the composition of amino acids in the nectar of bird-pollinated plants in Hawai‘i. Such studies are an important

avenue for future research. Nectar has been found to contain between 2 and 24 amino acids (Baker 1978). Their overall concentrations tend to be low in bird-pollinated species (Baker and Baker 1986, Cronk and Ojeda 2008) and are generally not considered an important source of protein for nectarivorous birds (Gottsberger et al. 1984, Cronk and Ojeda 2008). As a result, nectarivorous birds primarily obtain alternative amino acid sources by foraging on insects (Cronk and Ojeda 2008).

Conclusions

The study supports the proposition that *Clermontia* species evolved floral nectar traits in response to selection pressures from passerine species in the Hawaiian Drepanidinae and Mohoidae. Given the species extinctions that have occurred in each passerine lineage, whether these potential mutualists were strictly coevolved is likely to continue to remain a topic of evolutionary speculation in the future.

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CHAPTER THREE:

PREDICTING POTENTIAL INTERACTIONS BETWEEN EXTANT
NECTARIVOROUS PASSERINES AND HAWAIIAN LOBELIAD
SPECIES IN THE GENUS, *CLERMONTIA* (CAMPANULACEAE)

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Abstract

Plant species that occur in ecosystems where pollinators have declined or are now extinct may be at risk of reproductive failure. In the Hawaiian archipelago, close to one-fifth of the native flora is believed to be bird-pollinated, yet 70% of the historically recorded specialist nectarivorous bird species are extinct. Little is currently known about the potential interactions between extant native and introduced nectarivorous bird species and native bird-pollinated plant species in Hawai‘i. The present study used 1) geographical information systems (GIS), 2) comparisons between floral and bird head and bill dimensions, and 3) results of recent pollination ecology studies to predict potential interactions between extant native and introduced nectarivorous bird species and 25 bird-pollinated plant taxa in the Hawaiian lobeliad genus *Clermontia*. The analysis suggests that eight *Clermontia* taxa have no potential pollinators present over their geographical ranges, while a further four taxa have potential pollinators present over only part of their geographical ranges. Seventeen taxa have floral morphologies (eg., corolla tubes > 27 mm) that likely prevent short-billed nectarivores (five taxa) from acting as pollinators. As a consequence, these plant taxa are likely to be dependent upon ‘i‘iwi (*Vestiaria coccinea*) for pollination, the sole extant long-billed nectarivorous species. The study highlights 1) the need for conservation measures that protect and enhance populations of ‘i‘iwi across the widest possible range of habitat, and 2) the need for detailed studies to assess the role that extant nectarivorous passerines play as pollinators of these *Clermontia* taxa.

Introduction

Animals are important agents of pollination for an estimated 87.5% of angiosperm species (Ollerton et al. 2011). A growing body of research evidence indicates that anthropogenic perturbations (e.g., habitat modification, invasive species introductions) threaten plant-pollinator mutualisms globally (Kearns and Inouye 1997, Allen-Wardell et al. 1998, Kearns et al. 1998, Kremen and Ricketts 2000, Spira 2001, Potts et al. 2010). The decline or extinction of plant species or their pollinators can, in theory, lead to the reciprocal decline of their mutualists (Bond 1994, Anderson et al. 2011). The degree to which a plant species is threatened by pollinator mutualism decline depends on a number of factors such as the plant's breeding system (Bond 1994), degree of pollinator specialization (Aizen et al. 2012, Pauw and Bond 2011), ability to reproduce asexually (Bond 1994), and resilience to anthropogenic habitat fragmentation or disturbance (Geerts and Pauw 2012, Pauw and Louw 2012). Potentially, other native or introduced animals may act as substitute pollinators (Cox 1983, Aslan et al. 2012, Aslan et al. 2013). However, this is dependent on several factors, namely 1) that the potential mutualists share at least partially overlapping distributional ranges, 2) the morphology of the pollinators body and feeding apparatus (e.g., bill in nectarivorous birds) is of a size and shape to effectively obtain resources from the plant species while making contact with the plants reproductive organs, 3) the putative pollinator is active during the flowering cycle of the plant species, and 4) both mutualists are sufficiently common that a putative pollinator will frequently locate the focal plant species.

Given the selective pressures pollinators are known to exert on floral morphology (Darwin 1862, Grant 1949, Grant and Grant 1964, Herrera 1996, Fenster 1991), studies

which compare the morphology of pollinators and flowers have long been used to suggest interactions between potential mutualists (Darwin 1862, Snow and Snow 1980, Feinsinger 1983, Johnsgard 1983, Temeles 1996, Gibson et al. 2012, Hargreaves et al. 2012). However, few studies have utilized such measurements in cases where the presumed historic pollinator guild has declined or is threatened by extinction (but see Makrodimos et al. 2008 for an exception). Such studies are a useful first step in predicting interactions between putative pollinators, as well as potentially highlighting those plant species that may be undergoing reproductive failure and therefore may warrant conservation attention.

Owing to the high levels of endemism, small distributional ranges, and vulnerability to biological invasions, island ecosystems are particularly vulnerable to pollinator mutualism decline or loss (Cox and Elmqvist 2000, Kelly et al. 2010, Kaiser-Bunbury et al. 2010). This is particularly apparent in the bird-pollinated flora of the Hawaiian archipelago. Close to one-fifth (ca. 200 spp.) of the Hawaiian flowering plant flora is believed to have been pollinated by nectarivorous passerine species of Hawaiian Drepanidinae and Mohoidae (Sakai et al. 2002). Close to a quarter of these plant species are of conservation concern (Sakai et al. 2002). Concomitantly, all five Hawaiian Mohoidae species and two of the five nectarivorous honeycreeper species are extinct (Banko and Banko 2009). The seven remaining honeycreeper species that are either specialist nectarivores or consume nectar as part of a generalist diet have undergone wide-scale distributional range and population reductions due to habitat destruction, the introduction of invasive species, and/or avian diseases (see Banko and Banko 2009 for a review). Several introduced birds have a generalist diet that includes nectar. The most

common is the Japanese white-eye (*Zosterops japonicus*), a passerine species that was first introduced to O‘ahu in 1929 (Caum 1933). This species is now common on all the main Hawaiian Islands from sea level to the subalpine zone (Berger 1981).

The Hawaiian lobeliads (Campanulaceae), composed of six genera of herbs, shrubs, trees and stout caudiciforms, comprise the majority of the bird-pollinated flora in Hawai‘i (Sakai et al. 2002). All but two of the 140 species (taxonomy follows Lammers 2007 ab, Lammers 2009) are believed to have been pollinated by passerines (Lammers and Freeman 1986). The second largest lobeliad genus, *Clermontia*, contains 22 species and nine subspecies of shrubs and small trees that typically occur in montane rainforest habitats on the main Hawaiian Islands (Lammers 1991, Wagner et al. 1999). All *Clermontia* species possess floral traits congruent with an ornithophilous pollination syndrome (Lammers 1991, Lammers and Freeman 1986, R. Pender unpublished data).

Based on anecdotal observations and more detailed pollination studies conducted in recent decades (Lammers et al. 1987, Drake and Morden unpublished, Aslan et al. 2013, R. Pender unpublished data), there is growing evidence that extant native honeycreeper species and the introduced Japanese white-eye interact with the flowers of *Clermontia* species. However, our overall understanding of these potential interactions and what role these floral visitors play as pollinators of *Clermontia* taxa remains largely unknown.

This study combines several lines of evidence as a first step towards predicting potential interactions between extant nectarivorous passerines and *Clermontia* taxa. First, head and bill dimensions of extant nectarivorous passerine species were compared. Second, the geographical distributions of both potential mutualists were compared.

Lastly, to test the validity of the predictions, the results of recent pollination ecology studies of *Clermontia* taxa were reviewed.

Methods

Floral morphology of Clermontia species

All 31 *Clermontia* taxa possess flowers that have a tubular corolla that is curved in the majority of species (Figure 1). Based on the measurements provided in Lammers (1991), corollas range in length from 1.5-2.8 cm long in *C. parviflora* to 5.1-8.5 cm in *C. grandiflora* subsp. *grandiflora* (Figure 1). The filaments and anthers are fused forming a hollow cylinder that encloses the style and immature stigma. Upon anthesis, the flowers pass through a male (pollen presentation) and then a female (stigma receptive) phase. The reproductive organs are exerted, projecting from the corolla tube in all taxa, although the degree of exertion varies between taxa (Figure 1). Flowers are typically held in pairs in subumbellate racemes (Wimmer 1943). The only exception occurs in the extinct *C. multiflora* that produces flowers in 7-10 flowered inflorescences.

Flower collection and taxonomic representation

In total, 25 *Clermontia* taxa from all 21 extant species were included in this study (Table 2). In the majority of cases, two flowers were collected from five plants within a single population for each taxon. In cases where taxa occur on multiple islands, ten flowers (or as many as were available) were collected from representative populations from each island, from which a subsample were ultimately measured. Flowers were initially stored in ziplock bags and placed in coolers containing ice before subsequently

preserving them in 90% ethanol. Twenty taxa were measured from flowers preserved in 90% ethanol, while two taxa were measured from herbarium specimens at the Bernice P. Bishop Museum. Three taxa were measured from both alcohol preserved specimens and herbarium vouchers (Table 2). Several taxa were not included in the analysis; *Clermontia multiflora*, a species that was historically collected from O‘ahu and West Maui (Lammers 1991) was not included in the study as it is presumed to be extinct (Lammers 1991). In addition, the two subspecies of *C. peleana*, *C. oblongifolia* subsp. *mauiensis*, *C. oblongifolia* subsp. *brevipes* and *C. samuelii* subsp. *hanaensis* were not included in the analysis. All five taxa are endangered species with small extant geographical ranges (Wagner et al. 1999, USFWS 2013). The small number of herbarium vouchers possessing flowers in each taxon made it impractical to obtain adequate sample sizes for floral measurements.

Table 2. Conservation status and altitudinal and geographical distribution of the 25 *Clermontia* taxa included in the study. Altitudinal ranges follow Lammers (1991). These ranges are based on voucher collection sites. Conservation status follows USFWS 2013. Flower source: A = flowers measured from specimens preserved in alcohol; H = flowers measured from herbarium vouchers. Island abbreviations: K = Kaua‘i; O = O‘ahu; M = Moloka‘i; L = Lana‘i; WM = West Maui; EM = East Maui; H/K = Hawai‘i – Kohala Mt; H/M = Hawai‘i-Mauna Kea; H/L = Hawai‘i-Mauna Loa. Conservation status abbreviations: S = secure (i.e., not of conservation concern); E = endangered; I = Insufficient information.

Taxon	Alt. range (m elevation)	Cons status	Flower source	K	O	M	L	WM	EM	H/K	H/M	H/L
<i>C. arborescens</i> (H. Mann) Hillebr. subsp. <i>arborescens</i>	550-1,325	S	A					X				
<i>C. arborescens</i> (H. Mann) Hillebr. subsp. <i>waihia</i> (Wawra) Lammers	610-1,825	S	A					X	X			
<i>C. arborescens</i> (H. Mann) Hillebr. subsp. <i>waikoluensis</i> (H. St. John)	520-1,280	S	A/H			X	X					
<i>C. calophylla</i> F. Wimmer	885-1,460	S	A							X		
<i>C. clermontioides</i> (Gaud.) A Heller subsp. <i>clermontioides</i> (Gaud.) A Heller	670-1,525	S	A									X
<i>C. clermontioides</i> (Gaud.) A Heller subsp. <i>rockiana</i> (F.E. Wimmer)Lammers	840-1,825	S	H									X
<i>C. drepanomorpha</i> Rock	915-1,460	E	A/H							X		
<i>C. fauriei</i> H. Lév.	365-1,400	S	A	X	X							
<i>C. grandiflora</i> Gaud. subsp. <i>grandiflora</i>	610-1,750	S	A					X				
<i>C. grandiflora</i> Gaud. subsp. <i>maxima</i> Lammers	1,640	I.	H						X			
<i>C. grandiflora</i> Gaud. subsp. <i>munroi</i> (H. St. John)	525-1,975	S	A			X	X	X	X			

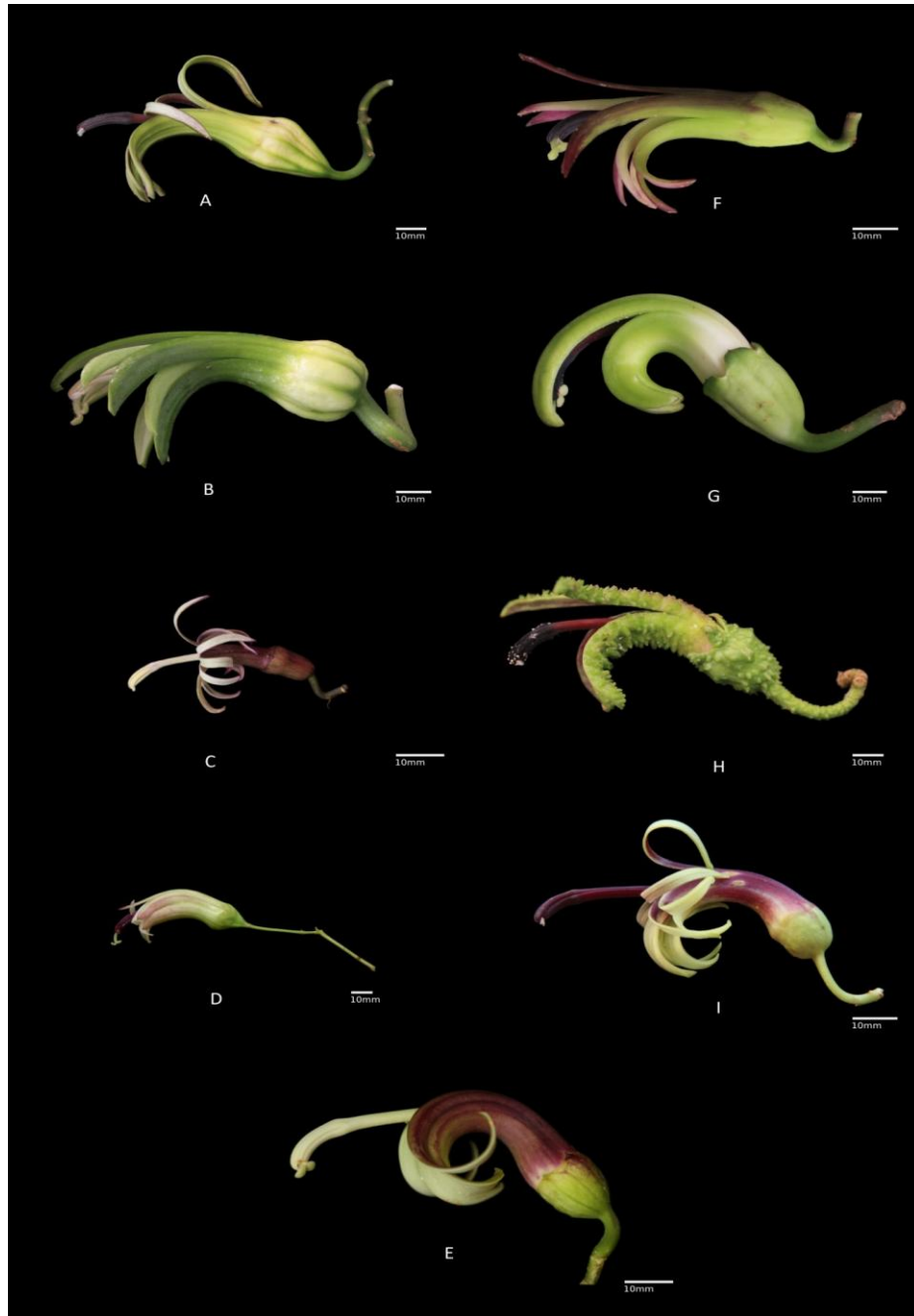


Figure 1. Flowers of selected *Clermontia* taxa. **A:** *Clermontia hawaiiensis*; **B:** *C. lindseyana*; **C:** *C. parviflora*, **D:** *C. grandiflora* subsp. *grandiflora* (note the length of the pedicel and peduncle); **E:** *C. fauriei*; **F:** *C. montis-loa*; **G:** *C. arborescens* subsp. *waihiaae*; **H:** *C. tuberculata*; **I:** *C. pallida*.

Flower measurements

Where possible, ten flowers were measured in each taxon. Three measurements were made on each flower. In all cases, a flexible plastic ruler was gently bent to follow the curve of the corolla to record the measurements. Flowers were measured A) from the point that corolla lobes cleft at their bases to the ventral anther hairs (on male phase flowers) or the middle of the stigmatic surface (in female phase flowers), B) from the base of the corolla and the basal cleft of the corolla lobes, and C) from the base of the corolla to the ventral anther hairs (on male phase flowers) or the middle of the stigmatic surface (in female phase flowers) (Figure 2).

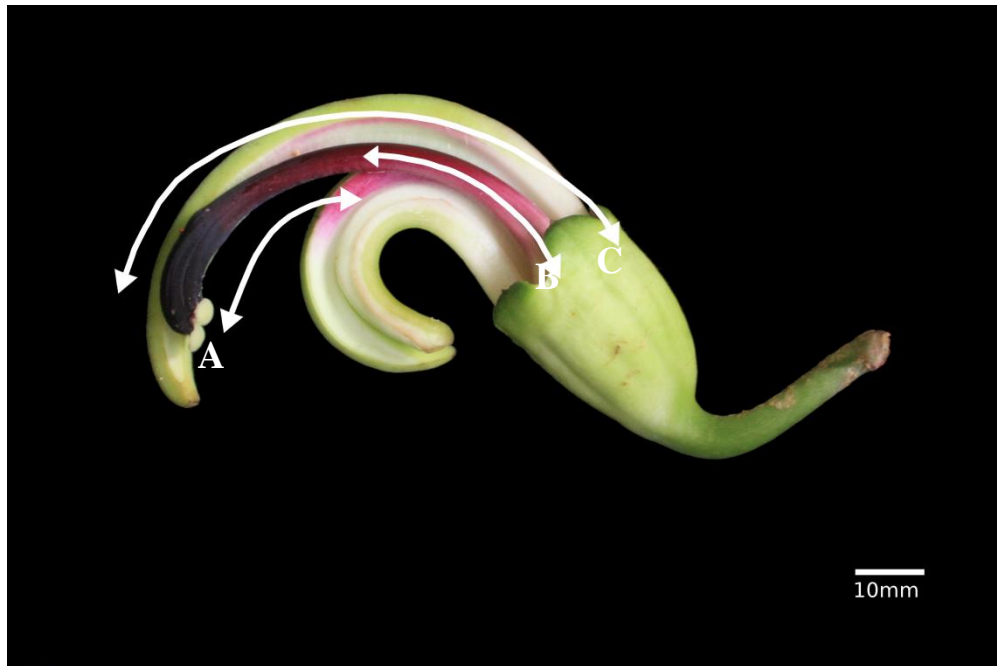


Figure 2. The *Clermontia* floral measurements recorded in the study. **A:** The distance from the base of the corolla and the ventral anther hairs (on male phase flowers), or the middle of the stigmatic surface (in female phase flowers). **B:** The distance from the base of the corolla lobes, where the corolla lobes cleft, and the ventral anther hairs (on male phase flowers) or the middle of the stigmatic surface (in female phase flowers). **C:** The distance from the base of the corolla and the base of the corolla lobes where they cleft. Note that this *C. arborescens* subsp. *waihia* flower has had half of its corolla lobes removed, allowing the reproductive organs to be seen.

Nectarivorous avian taxa included in the study

Recent literature (Berger 1981, Pratt 2005, Banko and Banko 2009) concerning both native and introduced birds was consulted to determine the nectarivorous passerine species to be included in the study. The two criteria for including a species in the analysis were the species diet that needed to be primarily (specialist) or partially (generalist) from floral nectar and that the species is sufficiently common that the birds might interact with *Clermontia* flowers. The opinion of local ornithologists and ecologists was subsequently sought to validate the inclusion of each species (Dr. D. Drake, M. Gorresen, pers.

comm.). Five native taxa were included: two nectar specialist species (‘i‘iwi and ‘apapane, *Himatione sanguinea*), and three species of the generalist ‘amakihī (*Hemignathus virens*, *H. kauaiensis*, *H. flavus*) (Table 3). Their selection was based on 1) direct evidence of their foraging from lobeliad flowers (Banko and Banko 2009) and 2) their being sufficiently common (Gorreson et al. 2009). Two subspecies of ‘amakihī (*H. virens* subsp. *virens* from Hawai‘i Island and *H. virens* subsp. *wilsonii* from Maui Nui) are here treated as one taxon. Two honeycreeper species, ‘akohekohe (*Palmeria dolei*) and ‘anianiau (*Magumma parva*), were not included in the analysis. ‘Akohekohe is restricted to forests on windward East Maui above 1100 m with a total population size of ca. 3800 birds (Berlin and VanGelder 1999). Although ‘akohekohe will forage on nectar from a number of plant species (Berlin and VanGelder 1999, Berlin et al. 2001, Vangelder and Smith 2001), it is believed to largely specialize on ‘ōhi‘a lehua flowers (Scott et al. 1986) and has never been observed foraging from the flowers of lobeliads. Similarly, ‘anianiau, a species that is endemic to Kaua‘i, has never been observed foraging from lobeliad flowers and, based on its small bill length (< 20 mm) and body size (10 cm) (Pratt 2005), this species is unlikely to forage from the flowers of *C. fauriei* (60-70 mm long), the only *Clermontia* species on Kaua‘i (Wagner et al. 1999). One introduced nectarivorous passerine, the Japanese white-eye (*Zosterops japonicus*), which is common throughout the main Hawaiian Islands between sea level and the tree line (Guest Van Riper 2000) was included in the analysis (Table 3). Three other introduced passerine species, the red vented bulbul (*Pycnonotus cafer*), red whiskered bulbul (*P. jocosus*) (both restricted to O‘ahu; Williams and Val Giddings (1984)) and the Japanese bush warbler (*Cettia diphone*), which was introduced to O‘ahu in 1929 (Berger 1981) and is now

present on all of the main Hawaiian islands (Pyle and Pyle 2009), occasionally obtain part of their diets from nectar (Berger 1981, Islam and Williams 2000, Carleton and Owre 1975, Olesen et al. 1998). However, none of the three are considered important pollinators in either their native or introduced ranges (Atluri et al. 2000, Islam and Williams 2000) and were therefore excluded from the analysis.

Bird dimension measurements

Preserved adult bird specimens, housed at the Bishop Museum, were measured using digital calipers (model 6942F, Neiko Tools, USA). In most cases, 30 birds (15 male/15 female) of each species were measured. Following the methods provided in Paton and Collins (1989), the A) distance from the middle of the rear side of the head and the tip of the culmen (bill), B) length of the exposed culmen, and C) true culmen (the point at which the bill is joined to the skull) were measured and rounded to the nearest millimeter (Figure 3).

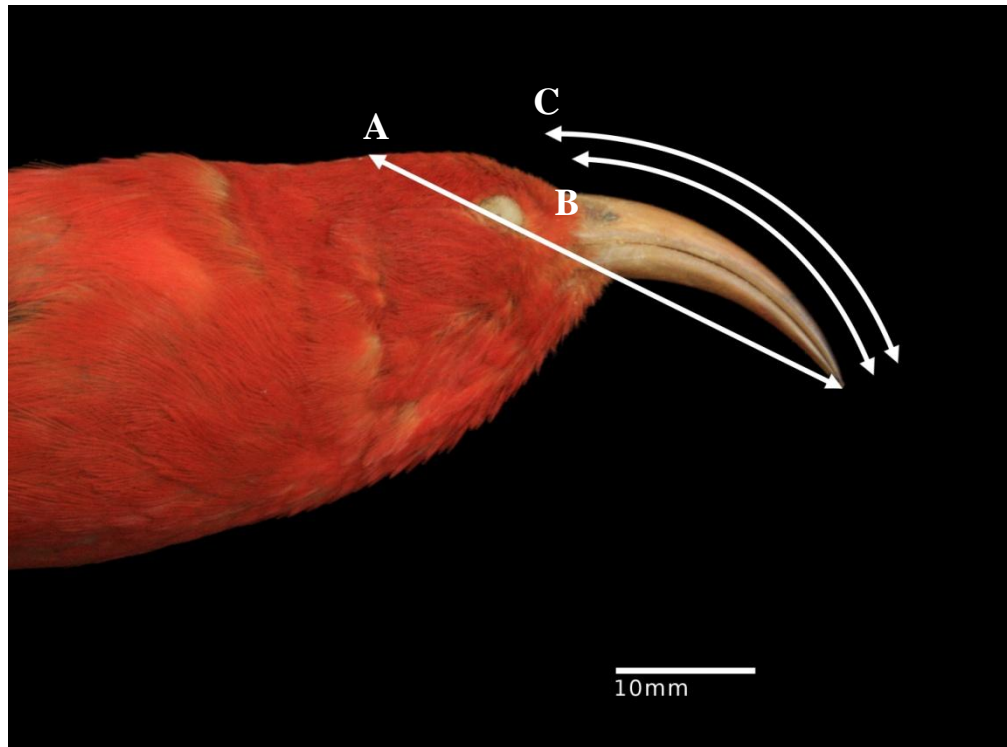


Figure 3. The head and bill (culmen) measurements recorded from each nectarivorous bird species in the study. **A**: distance from the middle of the back of the head and the tip of the culmen. **B**: Length of the exposed culmen. **C**: Length of the true culmen (including the base of the culmen).

Comparison of images of Clermontia flowers and the heads and bills of nectarivorous passerines

Half of the petals and sepals were manually removed from one fresh-collected flower of each *Clermontia* taxon thereby creating a longitudinal section of the flower (Figure 4). The flowers were photographed using a single reflex lens (SLR) camera (Canon XSi, Canon Corporation, Tokyo, Japan). Using the same equipment, photographs of representative specimens of each of the extant nectarivorous passerines were taken at the Bernice P. Bishop Museum. In cases where there were significant morphological differences between male or female birds of the same species, specimens of both sexes

were photographed. Photographs of each bird species were subsequently manipulated in Photoshop® (Adobe Corporation, San Jose, CA) so that only the bird was visible. If the extant distributional ranges of *Clermontia* taxa and any of the nectarivorous passerines overlapped (based on GIS analysis, addressed below), images of each bird species were overlaid over the respective *Clermontia* flower images to compare the dimensions of both potential mutualists (Figure 4). Bird species were considered potential pollinators if their bills projected sufficiently far enough within the corolla tube of each *Clermontia* taxon to successfully obtain nectar while at the same time also contacting the reproductive organs (anther or stigma) of the flower with their heads (Figure 4).



Figure 4. A comparison between the complementary bill and head dimensions of 'i'iwi and a flower of *C. fauriei*. This comparison indicates that 'i'iwi could legitimately forage from the flowers of *C. fauriei*.

Table 3. Distribution and population status of the six nectarivorous passerines that were measured in the study

Species	Kaua'i	O'ahu	Moloka'i	Lana'i	Maui	Hawai'i
'I'iwi	Secure* but declining. ^{1, 2} Mostly restricted to wet forest and scrub above 1250 m ¹	Functionally extinct† ^{1, 2}	Functionally extinct ^{1, 2}	Functionally extinct ^{1, 2}	Functionally extinct on West Maui, Secure in wet forests on windward East Maui, particularly above 1250 m. ^{1, 2}	Functionally extinct on Kohala Mt. Secure in forests on Mauna Kea and Mauna Loa, particularly above 1250 m. ^{1, 2}
'Apapane	Secure. Mostly restricted to wet forest and scrub above 1250 m ³	Functionally extinct in the Northern Waianae Mt. Secure in Southern Waianae Mt. Secure in Koolau Mts ^{2, 4}	Secure ^{2, 3}	Functionally extinct. ²	Secure ^{2, 3}	Secure ^{2, 3}
Hawai'i 'amakihi	Absent	Absent	Functionally extinct ^{2, 5}	Possibly Extinct., Functionally extinct if present ²	Secure on East Maui ^{2, 4} . Rare on West Maui ^{2, 5}	Secure ²
O'ahu 'amakihi	Absent	Secure in the Waianae and Koolau Mts., mostly above 500 m ⁵	Absent	Absent	Absent	Absent
Kaua'i 'amakihi	Secure in native forest above 600 m. ^{5, 6}	Absent	Absent	Absent	Absent	Absent
Japanese white-eye	Common ⁷	Common ⁷	Common ⁷	Common ⁷	Common ⁷	Common ⁷

* Secure: the taxon is relatively common and is not of conservation concern. † Functionally extinct: the taxon is rare. Because of this rarity, the taxon is unlikely to be a viable pollinator.

1. Fancy and Ralph (1998); 2. Gorreson et al. (2009); 3. Fancy and Ralph (1997); 4. Shallenberger and Vaughn (1978); 5. Camp et al. (2009); 6. Scott et al. (1986); 7. Guest Van Ripper (2000).

GIS analysis

Preexisting distribution layers for *Clermontia* taxa (Price et al. 2012) and the five native nectarivorous birds (USGS 2006) were overlaid on base maps of the Hawaiian Islands (State of Hawai‘i 2013) using ArcGIS version 10.0[®] (ESRI Corporation, Redlands, CA). The distribution layers of Price et al. (2012) and USGS (2006) are projections that do not strictly predict the actual distribution of a species but aim to provide the range that a taxon potentially could occur based on variables such as climate and habitat. In certain cases where either bird or *Clermontia* taxa had projected ranges that exceeded their known distributional range, or in the case of ‘i‘iwi where the species is likely to be functionally extinct (present but unlikely to serve an important role as a pollinator) over part of its range (e.g., on West Maui, but not East Maui), this section of the taxon’s projected distribution was removed from the map. The distribution for Japanese white-eye was adapted from the distribution map provided in Guest van Riper (2000). In addition, distribution points presented in maps for each *Clermontia* taxon in Lammers (1991) were incorporated into the maps to more accurately portray the current and historically known locations of each *Clermontia* taxon.

Statistical analysis

For flower measurements, the distance between the base of the filament and either the anther hairs in male phase flowers or the middle of the stigma lobes in female phase flowers were initially assessed to see if they conformed to parametric assumptions. The measurements were subsequently compared using a two-sample t-test in each taxon. The same approach was used to test for significant differences between the bill and head

measurements (outlined above) of female and male birds of the five separate taxa. The average measurements of both female and male birds were combined because the flowers of *Clermontia* taxa are potentially visited by both sexes in each bird species. Results are presented ± 1 S.D. All analyses were conducted in Minitab 15.

Results

Flower measurements

Corolla length ranged from 11.7 mm (± 0.7) in *C. parviflora* to 53.5 mm (± 2.1) in *C. grandiflora* subsp. *maxima* (mean for all taxa: 32.4 mm ± 10.1) (Table 4). There was no significant difference in reproductive organ length (filament and anther or stigma lobes) among male and female flowers in any of the taxa. Therefore, male and female reproductive organ measurements were pooled in each taxon. The distance between the anther hairs or middle of the stigma lobes and the base of the filament varied from 25 mm (± 3.2) in *C. micrantha* to 91 mm (± 0) in *C. grandiflora* subsp. *maxima* (mean for all taxa: 54.8 mm ± 15.3) (Table 4). The distance between the point at which the corolla tube expands and the anther hairs or stigma lobes varied from 10.4 mm (± 1.6 mm) in *C. micrantha* to 44.3 mm (± 3.3 mm) in *C. tuberculata* (mean for all taxa: 25.5 mm ± 8.3) (Table 4).

Bird dimension measurements

The dimensions of the nectarivorous passerine species measured fell into two distinct groups (Table 5). Firstly, 'i'iwi has a considerably larger combined head and bill (mean = 43.2 mm ± 1.6), exposed culmen (mean = 25.6 mm ± 1.7) and true culmen length

(mean = 29.2 mm \pm 1.9) measurements when compared with the other passerine taxa (Table 5). The second group of species, Hawai‘i ‘amakihi, O‘ahu ‘amakihi, Kaua‘i ‘amakihi, ‘apapane and Japanese white-eye, have similar head and bill dimensions (Table 5). The combined head and bill lengths of the species in this group ranged from 29.8 mm \pm 1.6 in Japanese white-eye to 35.2 mm \pm 1.2 in Kaua‘i ‘amakihi while the exposed culmen ranged from 10.7 mm \pm 0.7 in Japanese white-eye to 16.7 mm \pm 1.1 in Kaua‘i ‘amakihi. The true culmen ranged from 14.4 mm \pm 0.9 in Japanese white-eye to 19.3 mm \pm 1.0 in ‘apapane. The comparison of male and female measurements in each bird species is provided in Appendix A.

Comparisons between bird and floral dimensions

Of the 25 *Clermontia* taxa included in our analysis, eight taxa have either no potential pollinators present or the potential pollinators are functionally extinct over the entire geographical range of the *Clermontia* taxon (Table 6, Appendix B). Nine *Clermontia* taxa are potentially reliant on one bird species for pollination (in all cases: ‘i‘iwi). However, seven of these *Clermontia* taxa co-occur with ‘i‘iwi over only part of their potential distributional ranges (Appendix B). One taxon (*C. micrantha*) occurs in the presence of two potential bird pollinators (on Lana‘i only); five taxa occur with three potential pollinators over all or part of their distributional ranges, while four *Clermontia* taxa occur with 4 potential bird pollinators over all or parts of their distributional ranges (Table 6).

Owing to their long bills, ‘i‘iwi appear to be able to visit *Clermontia* taxa with

corolla tubes ranging from 11.7 to 51.5 mm. If 'i'iwi were still present in healthy populations on all of the main Hawaiian Islands they could potentially act as pollinators of 24 of the 25 (96%) *Clermontia* taxa included in this study. However, due to reduced distributions as a result of anthropogenic impacts, 'i'iwi are potential pollinators of only 13 (52%) of the *Clermontia* taxa. Owing to their similar bill lengths, 'apapane, Hawai'i 'amakihi and Japanese white-eye could potentially pollinate *Clermontia* taxa with corolla tubes ranging from 11.7 to ca. < 27 mm (in each case, eight *Clermontia* taxa) (Tables 3 and 4). Due to the corolla lengths of *Clermontia* taxa on O'ahu, which range, on average, from 30.54 mm in *C. persicifolia* to 48.9 mm in *C. oblongifolia* subsp. *oblongifolia*, O'ahu 'amakihi and the other two short-billed species ('apapane and Japanese white-eye) are not potential pollinators of any of the five *Clermontia* taxa on that island. Similarly, Kaua'i 'amakihi, 'apapane and Japanese white-eye have bill lengths (Table 6) that are too short to legitimately (making reproductive organ contact while foraging for nectar) visiting the long corolla tubes of *C. fauriei* (36.6 mm).

Table 4. Flower measurements of the 25 *Clermontia* taxa included in the study.

Taxon	n	Corolla tube length (mm)	Corolla + stamen or stigma length (mm)	Corolla tube to anther/stigma (mm)
<i>C. arborescens</i> subsp. <i>arborescens</i>	10	36.3 ± 7.2	55.5 ± 14	31 ± 2.6
<i>C. arborescens</i> subsp. <i>waihia</i>	10	34.8 ± 4.9	67.7 ± 5.1	33.1 ± 6.9
<i>C. arborescens</i> subsp. <i>waikoluensis</i>	15	33.4 ± 7.1	66.1 ± 5.6	31.9 ± 8.0
<i>C. calophylla</i>	10	23.4 ± 1.6	43.0 ± 1.9	20.7 ± 1.2
<i>C. clermontioides</i> subsp. <i>clermontioides</i>	10	26.4 ± 0.8	57 ± 4.4	35.6 ± 1.7
<i>C. clermontioides</i> subsp. <i>rockiana</i>	20	23.2 ± 2.6	52.5 ± 3.6	32.9 ± 3.3
<i>C. drepanomorpha</i>	10	25.6 ± 2.6	40.9 ± 2.9	17.9 ± 2.5
<i>C. fauriei</i>	10	36.6 ± 1.7	65.5 ± 3.2	32.5 ± 3.4
<i>C. grandiflora</i> subsp. <i>grandiflora</i>	10	42.4 ± 3.2	60.8 ± 5.6	20.8 ± 5.9
<i>C. grandiflora</i> subsp. <i>maxima</i>	1	53.5 ± 2.1	91 ± 0	39 ± 0
<i>C. grandiflora</i> subsp. <i>munroi</i>	10	51.5 ± 5.0	75.1 ± 3.1	25.3 ± 3.1
<i>C. hawaiiensis</i>	10	34.2 ± 1.7	52 ± 7.2	21.5 ± 4.4
<i>C. kakeana</i>	10	31.6 ± 2.5	41.3 ± 2.2	16.2 ± 1.9
<i>C. kohalae</i>	10	31.2 ± 2.9	52.4 ± 3.7	24.7 ± 2.3
<i>C. lindseyana</i>	10	34 ± 2.4	59.9 ± 3.3	26 ± 2.5
<i>C. micrantha</i>	16	14.8 ± 2.6	25 ± 3.2	10.4 ± 1.6
<i>C. montis-loa</i>	10	23.7 ± 1.1	40.2 ± 2.1	21.4 ± 1.3
<i>C. oblongifolia</i> subsp. <i>oblongifolia</i>	10	48.9 ± 4.2	66.2 ± 3.7	20.3 ± 1.1
<i>C. pallida</i>	10	32.7 ± 1.8	60.1 ± 2.7	27.9 ± 2.2
<i>C. parviflora</i>	10	11.7 ± 0.7	28.6 ± 2.1	17.1 ± 2.0
<i>C. persicifolia</i>	10	30.5 ± 2.9	54.9 ± 3.3	24.5 ± 4.7
<i>C. pyrularia</i>	10	29.8 ± 3.4	46.7 ± 3.6	18.5 ± 3.4
<i>C. samuelii</i> subsp. <i>samuelii</i>	8	33.9 ± 1.9	52.1 ± 2.5	19.4 ± 2.5
<i>C. tuberculata</i>	10	30.7 ± 2.1	75 ± 2.6	44.3 ± 3.3
<i>C. waimeae</i>	10	24.3 ± 3.5	39.5 ± 4.8	20.08 ± 4.0
Average		32.4 ± 10.1	54.8 ± 15.3	25.5 ± 8.3

Table 5. Mean bill and head measurements of the six nectarivorous passerine species and the potential *Clermontia* taxa that they appear morphologically capable of visiting. The two right hand columns predict potential interactions between the passerine species and the *Clermontia* taxa where 1) extant passerine distributions are not considered (excluding distributions), and 2) when extant passerine distributions are considered (including distributions).

Species	n	Bill and head length	Exposed culmen	True culmen length	Estimated range of accessible <i>Clermontia</i> corolla tube lengths	Potential interactions # <i>Clermontia</i> taxa (ex. distributions)	Potential interactions # <i>Clermontia</i> taxa (incl. distributions)
‘Iiwi	29	43.2 ± 1.6	25.6 ± 1.7	29.2 ± 1.9	<i>Clermontia</i> taxa with corollas ranging from 11.7-51.5mm. (see text).	24 (96%)	13 (52%)
‘Apapane	31	34.6 ± 1.5	16.1 ± 0.9	19.3 ± 1.0	<i>C. calophylla</i> , <i>C. clermontioides</i> subsp. <i>clermontioides</i> , <i>C. clermontioides</i> subsp. <i>rockiana</i> , <i>C. drepanomorpha</i> , <i>C. micrantha</i> , <i>C. montis-loa</i> , <i>C. parviflora</i> , <i>C. waimeae</i>	8 (32%)	8 (32%)
Hawai‘i ‘amakahi	30	30.7 ± 1.7	12.8 ± 1.0	16.3 ± 0.8	<i>C. calophylla</i> , <i>C. clermontioides</i> subsp. <i>clermontioides</i> , <i>C. clermontioides</i> subsp. <i>rockiana</i> , <i>C. drepanomorpha</i> , <i>C. micrantha</i> , <i>C. montis-loa</i> , <i>C. parviflora</i> , <i>C. waimeae</i>	8 (32%)	8 (32%)
O‘ahu ‘amakahi	30	31.0 ± 2.0	13.7 ± 1.0	15.7 ± 1.2	Bill too short to forage from the flowers of the three <i>Clermontia</i> taxa on O‘ahu	N/A	N/A
Kaua‘i ‘amakahi	14	35.2 ± 1.2	16.7 ± 1.1	18.5 ± 1.2	Bill too short to forage from the flowers of <i>C. fauriei</i>	N/A	N/A
Japanese White-eye	30	29.8 ± 1.6	10.7 ± 0.7	14.4 ± 0.9	<i>C. calophylla</i> , <i>C. clermontioides</i> subsp. <i>clermontioides</i> , <i>C. clermontioides</i> subsp. <i>rockiana</i> , <i>C. drepanomorpha</i> , <i>C. micrantha</i> , <i>C. montis-loa</i> , <i>C. parviflora</i> , <i>C. waimeae</i> ,	8 (32%)	8 (32%)

Table 6. Predicted potential nectarivorous passerine visitors to the flowers of the 25 *Clermontia* taxa included in the study (taking into account passerine distribution). Color codes: green = morphologically compatible; black = extinct bird species; grey = functionally extinct bird species; yellow = morphologically incompatible bird species; red = bird species did not naturally occur in geographical area.

Taxon	Distribution	'I'iwi	'Apapane	Hawai'i 'amakihi	O'ahu 'amakihi	Kaua'i 'amakihi	J. white-eye
<i>C. arborescens</i> subsp. <i>arborescens</i>	West Maui	grey	yellow	yellow	red	red	yellow
<i>C. arborescens</i> subsp. <i>waihia</i>	West Maui	grey	yellow	yellow	red	red	yellow
	East Maui	green	yellow	yellow	red	red	yellow
<i>C. arborescens</i> subsp. <i>waikoluensis</i>	Moloka'i	grey	yellow	yellow	red	red	yellow
	Lana'i	black	yellow	red	red	red	yellow
<i>C. calophylla</i>	Kohala Mountain	grey	green	green	red	red	green
<i>C. clermontioides</i> subsp. <i>clermontioides</i>	Mauna Loa	green	green	green	red	red	green
<i>C. clermontioides</i> subsp. <i>rockiana</i>	Mauna Loa	green	green	green	red	red	green
<i>C. drepanomorpha</i>	Kohala Mountain	grey	green	green	red	red	green
<i>C. fauriei</i>	Kaua'i	green	yellow	red	red	yellow	yellow
<i>C. grandiflora</i> subsp. <i>grandiflora</i>	West Maui	grey	yellow	yellow	red	red	yellow
<i>C. grandiflora</i> subsp. <i>maxima</i>	East Maui	yellow	yellow	yellow	red	red	yellow
<i>C. grandiflora</i> subsp. <i>munroi</i>	West Maui	grey	yellow	yellow	red	red	yellow
	East Maui	green	yellow	yellow	red	red	yellow
	Moloka'i	grey	yellow	yellow	red	red	yellow
	Lana'i	grey	yellow	yellow	red	red	yellow
<i>C. hawaiiensis</i>	Mauna Loa	green	yellow	yellow	red	red	yellow
<i>C. kakeana</i>	O'ahu	grey	yellow	red	yellow	red	yellow
	Moloka'i	grey	yellow	red	red	red	yellow
	West Maui	grey	yellow	yellow	red	red	yellow

	East Maui	Green	Yellow	Yellow	Red	Red	Yellow
<i>C. kohalae</i>	Kohala Mountain	Grey	Yellow	Yellow	Red	Red	Yellow
<i>C. lindseyana</i>	Mauna Loa	Green	Yellow	Yellow	Red	Red	Yellow
	Mauna Kea	Green	Yellow	Yellow	Red	Red	Yellow
	East Maui	Grey	Yellow	Yellow	Red	Red	Yellow
<i>C. micrantha</i>	West Maui	Grey	Green	Green	Red	Red	Green
	Lana'i	Black	Green	Red	Red	Red	Green
<i>C. montis-loa</i>	Mauna Loa	Green	Green	Green	Red	Red	Green
	Mauna Kea	Green	Green	Green	Red	Red	Green
<i>C. oblongifolia</i> subsp. <i>oblongifolia</i>	O'ahu	Grey	Yellow	Red	Yellow	Red	Yellow
<i>C. pallida</i>	Moloka'i	Grey	Yellow	Yellow	Red	Red	Yellow
<i>C. parviflora</i>	Kohala Mountain	Grey	Green	Green	Red	Red	Green
	Mauna Kea	Green	Green	Green	Red	Red	Green
	Mauna Loa	Green	Green	Green	Red	Red	Green
<i>C. persicifolia</i>	O'ahu	Grey	Yellow	Red	Yellow	Red	Yellow
<i>C. pyrularia</i>	Mauna Kea	Green	Yellow	Yellow	Red	Red	Yellow
<i>C. samuelii</i> subsp. <i>samuelii</i>	East Maui	Green	Yellow	Yellow	Red	Red	Yellow
<i>C. tuberculata</i>	East Maui	Green	Yellow	Yellow	Red	Red	Yellow
<i>C. waimeae</i>	Kohala Mountain	Grey	Green	Green	Red	Red	Green

Discussion

The results suggest that 'i'iwi is potentially the most important pollinator of *Clermontia* taxa included in the study. For 13 species of *Clermontia*, 'i'iwi is the only nectarivorous species with bill and head dimensions of a sufficient size to potentially allow simultaneous nectar foraging and contact of the reproductive organs to take place.

However, four of these *Clermontia* taxa share only part of their geographical ranges with ‘i‘iwi, and therefore may be receiving no bird assisted pollination where ‘i‘iwi are absent (Table 6). The historic extinction of long-billed specialist nectarivorous species (black mamō; *Drepanis. funera*, Hawai‘i mamō; *D. pacifica*) and the restriction of ‘i‘iwi distributions to high elevation habitats has possibly resulted in the total loss of animal aided pollination for eight of the *Clermontia* taxa included in this study (Table 6). The five remaining short-billed bird species are unlikely to function as pollinators of these large-flowered *Clermontia* species (taxa with corolla tubes > 27mm) as the floral dimensions are likely to physically exclude these bird species from accessing floral nectar. By contrast, the five short-billed bird species appear to be potential pollinators for eight *Clermontia* taxa included in this study where corolla tube length is less than *ca.* 27 mm long (Tables 4 and 5).

Potential interactions on individual islands

Kaua‘i

Clermontia fauriei is the only *Clermontia* species on Kaua‘i, occurring between 365 and 1400 m (Lammers 1991). Based on the analysis, ‘i‘iwi appear to be the only avian nectarivores capable of visiting the flowers of *C. fauriei*. However, only *C. fauriei* populations that occur above 1,100 m elevation (principally on the Alaka‘i Plateau) are likely to co-occur with ‘i‘iwi (Appendix B: 1A, Camp et al. 2009). Populations of ‘i‘iwi are believed to be in decline on the island (Camp et al. 2009), further reducing the chances of mutualistic interactions. Drake and Morden (unpublished) undertook a pollination ecology study of *C. fauriei* at Koke‘e State Park and the Alaka‘i Plateau. In

contrast to our predictions, they recorded Japanese white-eye legitimately visiting the flowers, potentially contacting the reproductive organs. It is feasible that the strongly curved corolla tube of *C. fauriei* may allow the smaller-billed bird species, such as Japanese white-eye, to access nectar in the corolla tube. However, whether small-billed birds are effective pollinators of *C. fauriei* is unknown. Drake and Morden (unpublished) also anecdotally observed 'i'iwi legitimately visiting *C. fauriei*, but found that Kaua'i 'amakihī nectar robbed the flowers by inserting their bills into the cleft in the upper surface of the corolla near the ovary and did not appear to be effective pollinators.

O'ahu

Five species of *Clermontia* were historically recorded on O'ahu, of which two (*C. kakeana* and *C. oblongifolia* subsp. *oblongifolia*) remain relatively common, while one species (*C. persicifolia*), is believed to be in decline (M. Spörck pers. comm.).

Clermontia fauriei has been collected on O'ahu twice (both prior to 1970), once each from the Wai'anae and Ko'olau Ranges. The species may still occur on O'ahu, although personal communications with local botanists (J. Lau, M. Kier, pers. comm.) suggest that the species may either be very rare or possibly extirpated on this island.

Based on our analyses, 'i'iwi is likely to be the only nectarivorous passerine capable of pollinating the three common species of *Clermontia* (*C. kakeana*, *C. oblongifolia* subsp. *oblongifolia* and *C. persicifolia*). The bill and head dimensions of O'ahu 'amakihī, 'apapane and Japanese white-eye suggest that they are too small to function as adequate pollinators of these species. Although 'i'iwi technically still persist on O'ahu, they are extremely rare (Camp et al. 2009 and references therein) and are likely to be functionally extinct as pollinators. The only pollination study of *Clermontia*

to have been undertaken on O‘ahu (Cory 1984) found that no birds visited the flowers of *C. kakeaena* on Mt. Tantalus during observations made between 1981 and 1984.

Moloka‘i

Four species of *Clermontia* that were included in the analysis occur on Moloka‘i: *C. arborescens* subsp. *waikoluensis*, *C. grandiflora* subsp. *munroi*, *C. kakeana* and *C. pallida*. Based on the analysis, ‘i‘iwi remains the only nectarivorous bird species capable of functioning as a pollinator of all four species. However, like O‘ahu, ‘i‘iwi populations on Moloka‘i are believed to have drastically declined since European colonization (Fancy and Ralph 1998). Recent surveys (Reynolds and Snetsinger 2001, Camp et al. 2009) suggest that ‘i‘iwi are extremely rare and are likely to be functionally extinct for all four *Clermontia* taxa.

Lana‘i

The island of Lana‘i historically contained three species that were included in the study: *C. arborescens* subsp. *waikoluensis*, *C. grandiflora* subsp. *munroi* and *C. micrantha*. Only two taxa, *C. grandiflora* subsp. *munroi* and *C. arborescens* subsp. *waikoluensis*, remain common. *Clermontia micrantha* is either extremely rare or extinct on the island, having only been collected on Lana‘i three times before 1930 (Lammers 1991).

Given the corolla dimensions of *C. arborescens* subsp. *waikoluensis* and *C. grandiflora* subsp. *munroi* (Table 4), it is unlikely that these two species are effectively pollinated on Lana‘i. ‘i‘iwi, the only bird sufficiently large enough to contact the reproductive organs while foraging for nectar is extinct on the island (Munro 1960,

Gorreson et al. 2009). If *C. micrantha* does still occur on Lana‘i, there is a possibility that Japanese white-eye could act as pollinators of this species (Appendix B: 1B). Although morphologically compatible, ‘apapane are rare on Lana‘i. Based on the collection points for *C. micrantha* provided in Lammers (1991) and the projected distribution of ‘apapane (USGS 2006), it appears that the two species may not interact (Appendix B: 2A).

Maui

Ten taxa that were included in this study occur on Maui. Due to their present day ecological separation, the mountain ranges present on West and East Maui are here treated as separate geographical entities, summarized below.

West Maui

Six taxa that were included in the study occur on West Maui: *C. arborescens* subsp. *arborescens*, *C. arborescens* subsp. *waihia*, *C. grandiflora* subsp. *grandiflora*, *C. grandiflora* subsp. *munroi*, *C. kakeana* and *C. micrantha*. Subspecies of *C. arborescens* and *C. grandiflora* appear to be reliant upon ‘i‘iwi for pollination. However, ‘i‘iwi are very rare (< 200 individuals) on West Maui (Camp et al. 2009) and are likely to be functionally extinct as pollinators. The results suggest that these taxa are receiving no pollinator services on West Maui due to a lack of functional pollinators. By contrast, the small flowered *C. micrantha* has a corolla tube length that potentially would allow the three short-billed nectarivores, ‘apapane, Hawai‘i ‘amakihi and Japanese white-eye, to legitimately visit the flowers of this species. All three bird species occur across the range of *C. micrantha* on West Maui (Appendix B: 2B, 3A, 3B, respectively). Based on the distribution projections (USGS 2006, Price et al. 2012), Hawai‘i ‘amakihi and ‘apapane

occur at the highest density in the Northern (and wetter) half of *C. micrantha*'s West Maui range. These bird species may therefore be potentially variable in their effectiveness as pollinators for this *Clermontia* species on West Maui.

East Maui

Seven *Clermontia* taxa that occur on East Maui were included in the study: *C. arborescens* subsp. *waihia*, *C. grandiflora* subsp. *maxima*, *C. grandiflora* subsp. *munroi*, *C. kakeana*, *C. lindseyana*, *C. samuelii* subsp. *samuelii* and *C. tuberculata*. With the exception of *C. grandiflora* subsp. *maxima*, these taxa appear reliant on 'i'iwi for pollination on account of their relatively long corolla tubes (all are > 27 mm). 'I'iwi occurs only in the upper distributional range (above 1250 m; Fancy and Ralph 1998) of *C. arborescens* subsp. *waihia*, *C. grandiflora* subsp. *munroi*, *C. kakeana*, and *C. samuelii* subsp. *samuelii* (Appendix B: 4A, 4B, 5A, 5B, respectively). This suggests that all five taxa likely have no avian pollinators present over more than half of their potential distributional range. By contrast, *Clermontia tuberculata* has a projected distribution that closely mirrors that of 'i'iwi on East Maui (Appendix B: 6A). *Clermontia lindseyana* has only been collected twice on Maui, both from the south slopes of Haleakalā (in 1910 and 1920, respectively; Appendix B: 6B). If plants still occur on the south slopes of Haleakalā they are outside the current distributional range of 'i'iwi (Appendix B: 6B). Based on our measurements, *Clermontia grandiflora* subsp. *maxima* appears to have corolla lengths exceeding those from which 'i'iwi can realistically forage. *Clermontia grandiflora* subsp. *maxima* is a poorly known taxon (Wood 2012), collected once at 1645 m in the Makawao District of windward Haleakalā (Lammers 1991). Only two flowers of this taxon were measured in this study, both from the holotype. More recent collections attributed to this

taxon made by Ken Wood (NTBG) in 2007 were not measured as they appear to be specimens of *C. grandiflora* subsp. *munroi* (pers. obs.). *Clermontia grandiflora* subsp. *munroi* (corolla tube length: 91 mm) appears to have a corolla that is at the morphological limits that 'i'iwi can forage from. This, combined with the long pedicels and peduncles (up to 110 mm and 70 mm, respectively), which are likely to result in the flowers hanging potentially at some distance from the stems, raises uncertainty as to whether 'i'iwi could effectively pollinate this taxon.

Speith (1966) and Berger (1981) observed 'i'iwi legitimately visiting flowers of *C. arborescens* subsp. *waihia* on east Maui. Lammers et al. (1987) observed Hawai'i 'amakihi and Japanese white-eye visiting the flowers of *C. arborescens* subsp. *waihia* also on east Maui. However, the authors could not determine if the birds contacted the reproductive organs making it difficult to accept or refute our predictions that the small-billed bird species could not legitimately pollinate the flowers of this taxon.

Hawai'i Island

Eleven *Clermontia* taxa included in this study occur on the island of Hawai'i. Although Mauna Kea and Mauna Loa are connected ecologically via the saddle between the two mountains, for practical purposes, the island is here divided into the three prominent mountain ranges, summarized below.

Kohala Mountain

All five *Clermontia* taxa that occur on Kohala Mountain (*C. calophylla*, *C. drepanomorpha*, *C. kohalae*, *C. parviflora* and *C. waimeae*) were included in this study. It appears, based on the measurements, that *C. calophylla*, *C. drepanomorpha*, *C.*

parviflora and *C. waimeae* could potentially be pollinated by the three short-billed species (‘apapane, Hawai‘i ‘amakihi and Japanese white-eye) that have complementary distributional ranges on Kohala Mountain (Appendix B: 7A-12B, respectively). However, *C. drepanomorpha* has peduncles ranging from 5 to 12 cm long (Lammers 1991), potentially making them difficult for the small-billed bird species to access. Field-based pollination studies are needed to ascertain whether these bird species are capable of visiting this *Clermontia* species. The fifth *Clermontia* species, *C. kohalae*, has flowers which are too large for any of the small-billed birds to adequately forage from and appears to be reliant upon ‘i‘iwi for pollination. ‘i‘iwi are very rare on Kohala Mountain (Gorreson et al. 2009) and, based on a recent pollination study of *C. kohalae*, appear to be functionally extinct as pollinators for this species (R. Pender, unpublished data).

Mauna Loa

Six *Clermontia* taxa (*C. clermontioides* subsp. *clermontioides*, *C. clermontioides* subsp. *rockiana*, *C. hawaiiensis*, *C. lindseyana*, *C. montis-loa* and *C. parviflora*) included in this study occur on Mauna Loa or surrounding environs (e.g., *C. clermontioides* subsp. *rockiana* also occurs on Mt. Hualālai on leeward Hawai‘i). An additional species, *C. pyrularia*, was historically recorded from South Kona on the leeward slopes of Mauna Loa but is likely to be extinct at that site (Dr. T. Lammers, pers. comm.) and is not considered here (see Mauna Kea section, below). Based on our analysis, we predict that *C. clermontioides* subsp. *clermontioides*, *C. clermontioides* subsp. *rockiana*, *C. montis-loa* and *C. parviflora* could potentially be visited by ‘i‘iwi, ‘apapane, Hawai‘i ‘amakihi and Japanese white-eye (Appendix B: 10 A-B; 11 A; 13A-17A). ‘I‘iwi and *C. parviflora*

may only interact in this plant's upper distributional range (Appendix B: 17 A). In addition, *Clermontia parviflora* may have corollas that are too short for 'i'iwi to forage from. However, we have suggested that 'i'iwi may visit *C. parviflora* flowers as 'i'iwi have been observed visiting flowers with short corollas (e.g. *Lobelia grayana* on Maui, pers comm. P. Bily). A recent field-based pollination study (Aslan et al. 2013) has shown that Japanese white-eye are effective pollinators of *C. montis-loa* and *C. parviflora*. The study also showed that 'apapane occasionally legitimately visited *C. parviflora* and nectar rob the flowers of *C. montis-loa*, while 'i'iwi occasionally legitimately visited *C. montis-loa* flowers.

The remaining two species, *C. hawaiiensis* and *C. lindseyana*, possess flowers that only 'i'iwi appear capable of effectively visiting. As previously mentioned, 'i'iwi are largely limited to forested habitats above ca. 1100 elevation on the island of Hawai'i (Gorresen et al. 2009). Based on the projected distributions, 'i'iwi may potentially interact with the flowers of *C. lindseyana* over at least part of this species' geographical range (Appendices B: 17B and 18A respectively). *Clermontia hawaiiensis* ranges between 610-1,860 m elevation, but below 1100 m 'i'iwi are unlikely to be sufficiently common to act as pollinators of *C. hawaiiensis*. A recent study that included three populations of *C. hawaiiensis* on windward Mauna Loa found that Japanese white-eye and 'apapane were rare visitors to the flowers, nectar robbing in all cases (Aslan et al. 2013).

Mauna Kea

Four *Clermontia* taxa included in this study occur on Mauna Kea: *C. lindseyana*, *C. montis-loa*, *C. parviflora* and *C. pyrularia*. As previously mentioned, *C. montis-loa*

and *C. parviflora* may be potentially visited by ‘i‘iwi, ‘apapane, Hawai‘i ‘amakihi and Japanese white-eye. The three short-billed species occur over most of the range of *C. montis-loa* and *C. parviflora* on Mauna Kea (Appendices B: 15B, 16A and B; 10A and B, 11A). By contrast, any plants of either species occurring below 1,100 m are unlikely to be visited by ‘i‘iwi. The remaining two species, *C. lindseyana* and *C. pyrularia*, appear to be reliant upon ‘i‘iwi for pollination (Appendices B: 17B and 18B, respectively). Historical collections of *C. pyrularia* on Mauna Kea occur at the upper elevational limit of ‘i‘iwi. These collection points are disjunct from the predictions of Price et al. (2012). If this predicted distribution of *C. pyrularia* is correct, it appears from Appendix B: 18B that ‘i‘iwi may only interact with *C. pyrularia* at this species’ lower altitudinal distribution on Mauna Kea. The same trend also appears to be true for *C. lindseyana* (Appendix B: 17B). In a recent pollination ecology study of these species conducted at Hakalau National Wildlife Refuge (R. Pender, unpublished data), ‘i‘iwi visited both *Clermontia* species, however, they did not function as pollinators of either (R. Pender, unpublished data). Hawai‘i ‘amakihi also visited the flowers of *C. pyrularia* and *C. lindseyana*, but in almost all cases nectar robbed the flowers of both species (R. Pender, unpublished data).

Limitations to analysis and broader discussion

Despite the utility of such an analysis, this study has several overarching shortfalls that can only be fully addressed through more detailed studies. Firstly, the scale (island wide comparisons) at which this study was undertaken greatly limits the accuracy of the potential interactions predicted here. The distribution projections of both the *Clermontia* taxa (Price et al. 2012) and the native bird species (USGS 2006) likely

overestimate the true distributional range and potential interactions of both mutualistic groups. The density of potential mutualists often determines their potential interaction (Essenberg 2012, Nielsen et al. 2012). Such fine-scale population density data do not exist for native Hawaiian nectarivorous bird species or *Clermontia* taxa (M. Gorreson, pers. comm., pers. obs.). As previously discussed, population distribution and density of nectarivorous bird and *Clermontia* taxa are believed to have dramatically declined since the colonization of Hawai‘i by humans (Sakai et al. 2002, Banko and Banko 2009). Although the study provides predictions on the interactions between extant nectarivorous passerines and *Clermontia* taxa, Allee effects (a positive correlation between population size or density and the mean individual fitness) and, in most cases, dual Allee effects (rarity of both mutualists) are likely to be impacting these potential interactions.

Secondly, the floral morphology and physiology of the *Clermontia* taxa may contribute to the potential interaction between nectarivorous birds and the flowers of the *Clermontia* taxa. The peduncle length of *Clermontia* taxa ranges from 1 cm in *C. parviflora* to 12 cm in *C. drepanomorpha*. Although a bird may have a head and bill length compatible with the flowers of a particular *Clermontia* taxon, the distance that flowers are held from a stem (perching sites) may exclude bird species from reaching the floral nectar. In addition, the amount of nectar in the corolla and, by consequence, the distance that a bird may have to probe with its bill and tongue to forage, may be governed by a number of factors that may include the age of the flower and frequency of floral visitors (Jones et al. 1998, Canto et al. 2011, Nocentini et al. 2012). Given the small sample size of flowers utilized in this study, the distance between floral nectar and floral organs was not measured. However, this variable may be a factor that contributes to the

effectiveness of extant nectar-feeding birds as pollinators of the *Clermontia* taxa included in this study.

Third, the study only assessed bill dimensions. All five passerine species included in this study have brush-tipped tongues that the birds are known to project while foraging for nectar (Amadon 1950, Raikow 1977). However, tongue-projection lengths of these bird species have not been measured. This may have direct consequences for the range of corolla lengths that these passerine species can visit, potentially allowing legitimate visitation to *Clermontia* taxa with longer corollas than we have predicted.

Lastly, our study cannot predict how each bird species might potentially interact with the flowers of a given *Clermontia* taxon. For example, ‘apapane, although morphologically capable of visiting the smaller-flowered *Clermontia* taxa, appear to only rarely visit lobeliad species (Lammers and Freeman 1986, Aslan et al. 2013, R. Pender, unpublished data) and instead primarily feeds from ‘ōhi‘a lehua flowers (Carpenter and MacMillen 1976). Similarly, all the extant ‘amakihi species and Japanese white-eye (4 of the 6 passerine species in this study) obtain only part of their diets from nectar (Guest van Riper 2000, Banko and Banko 2009). This may potentially reduce their effectiveness as pollinators, as they are not solely reliant on nectar as a foraging resource. Predictions that a bird species may be morphologically compatible with the flowers of certain *Clermontia* taxa do not imply they will visit them legitimately. For example, the results suggest that ‘i‘iwi are potential pollinators of *C. lindseyana*. However, ‘i‘iwi nectar rob, on average, ca. 60% of the *C. lindseyana* flowers that they visit at Hakalau National Wildlife Refuge (R. Pender, unpublished data). Japanese white-eye and ‘amakihi species are also known to nectar rob the flowers of lobeliad species in cases where they cannot access the flowers

legitimately (Drake and Morden unpublished, Aslan et al. 2013, R. Pender, unpublished data).

Future studies

It is hoped that the present study will provide a foundation for more detailed investigations of bird-plant pollination interactions for *Clermontia* and other Hawaiian lobeliad taxa. Detailed, field-based pollination studies, similar to those of Aslan et al. (2013) would greatly improve our understanding of potential bird-plant interactions, particularly if such studies incorporate molecular analyses to assess rates of out-crossing and gene-flow (e.g., Byrne et al. 2007, Krauss et al. 2009). In addition, assessing viable pollinator and plant population densities (e.g., Anstett et al. 1997, Matthies et al. 2004, Brys et al. 2008, Klank et al. 2010) would have potential future benefits for the management of wild and restored populations of both mutualists. Lowland, avian-focused behavioral studies (e.g., floral handling, floral color and nectar preferences; *see* Gegear and Burns 2007, Campbell et al. 2012, Kaczorowski et al. 2012) could be undertaken relatively easily with the introduced Japanese white-eye, and possibly also with avian malaria and avian pox-tolerant individuals of ‘amakihi species (Foster et al. 2007). However, such studies are probably not realistic for ‘i‘iwi or ‘apapane that are susceptible to both diseases (Banko and Banko 2009). Lastly, the potential threat of climate change to nectarivorous bird species in Hawai‘i has received some attention (Benning et al. 2002, Hobbelen et al. 2012, Rock et al. 2012) but has not been addressed in detail for bird-pollinated plant species. Understanding climate change now may allow managers to implement mitigation strategies in advance of impacts in the future.

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CHAPTER FOUR:

A PHYLOGENETIC STUDY OF THE HAWAIIAN LOBELIAD GENUS,
CLERMONTIA (CAMPANULACEAE) WITH SPECIAL EMPHASIS ON
FLORAL TRAIT EVOLUTION

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Abstract

The Hawaiian lobeliad genus, *Clermontia*, contains 22 species and 9 subspecies of bird-pollinated shrubs and small trees endemic to wet and mesic forests on the main Hawaiian Islands. The flowers are particularly notable for having sepals either reduced (sepaloid) or long and petal-like (petaloid sepals). Phylogenetic relationships in the genus are inferred from cpDNA sequence variation in the *atpB-rbcL*, *ndhF-rpl32*, *rpl32-trnL*, *trnH-psbA*, *trnL-F* spacer regions and the *trnL* intron. The relationships for approximately half of the *Clermontia* taxa included in the study (n = 28) were resolved. The genus appears to have evolved on O‘ahu with subsequent inter-island colonization events, mostly from older to younger islands, followed by intra-island speciation events. The study suggests that the genus has evolved petaloid sepals once, with five or more reversals back to sepaloid sepals. The endangered *C. pyrularia* does not belong in *Clermontia* and either belongs in the purple-fruited clade of the closely related genus *Cyanea*, or is an intergeneric hybrid. *Clermontia oblongifolia* subsp. *brevipes* appears to warrant recognition as a distinct species. Further work is needed, principally to resolve the relationships within the most recently evolved Hawai‘i Island clade, before all the evolutionary patterns in the genus can be fully resolved.

Introduction

The development of new phylogenetic techniques in recent decades has revolutionized the study of plant evolution (Willis and McElwain 2002, Donoghue 2008, Endress 2011). In the field of plant reproductive biology, phylogenetics has begun to provide useful insights regarding breeding system evolution (Weiblen et al. 1999, Weller

and Sakai 1999, Truysers et al. 2005, Goldberg and Igic 2012, Kafer 2013) and floral trait evolution (Perez et al. 2006, DeWitt Smith 2010, Marten-Rodriguez et al. 2010, Schlumpberger and Renner 2012). The obvious benefit of this approach is the accurate identification of trait shifts (e.g., changes in corolla tube morphology) that may have arisen multiple times in a taxonomic group, compared to traditional morphological taxonomy that often groups taxa based on shared characters without taking convergence into consideration.

The flora of the Hawaiian Islands has proven particularly fruitful for understanding speciation tempo (Knape et al. 2012) and biogeographical patterns of dispersal and adaptive radiation (see Keeley and Funk 2011 for a review). The progressively older ages of the islands, isolation from continents, and habitat diversity have seen Hawai'i become a natural laboratory for plant evolutionary studies (see Keeley and Funk 2011 for a review). Despite Hawai'i's long association with phylogenetic and evolutionary studies, only a handful have looked at changes in angiosperm reproductive biology (e.g., breeding systems; Sakai et al. 1997, Sakai et al. 2006), floral traits (Costello and Motley 2001) and morphology due to evolutionary shifts in pollinators (Lindqvist et al. 2003, Givnish et al. 2009).

The Hawaiian lobeliads are composed of six genera and 140 species of herbs, shrubs, trees and stout caudiciforms (taxonomy follows Lammers 2007ab, Lammers 2009) that evolved following a single colonization event approximately 13 million years ago (Mya) (Givnish et al. 2009). *Clermontia*, the second largest genus of Hawaiian lobeliads, is composed of 22 species and nine subspecies of shrubs and small trees found in mesic and wet forests between 150 and 2100 m elevation (Lammers 1991). The genus

is endemic to the main Hawaiian Islands, with increasing numbers of taxa on the progressively younger islands: Kaua‘i (one taxon), O‘ahu (five taxa), Moloka‘i (five taxa), Lana‘i (four taxa), Maui (14 taxa) and the island of Hawai‘i (13 taxa). *Clermontia* species have little interspecific variation in vegetative characters. However, floral characters vary widely among taxa, particularly for corolla size and calyx arrangement that may either be reduced (sepaloid) or petal-like (petaloid; Lammers 1991, 1995). All *Clermontia* taxa produce flowers congruent with a passerine pollination syndrome (Lammers and Freeman 1986), and are believed to have been historically pollinated by now mostly endangered or extinct nectarivorous Hawaiian honeycreepers and Mohoidae (Lammers and Freeman 1986).

Lammers (1991), in the most recent taxonomic treatment of *Clermontia*, divided the genus into subgeneric classifications (2 sections, each containing 3 series) based on differences in the corolla and perianth morphology (Table 7). Lammers (1995), using a cladistic analysis based on vegetative and floral characters, predicted that *Clermontia* evolved and subsequently dispersed from Hawai‘i Island. This interpretation was based primarily on the greater number of species on the younger islands of Maui and Hawai‘i compared to fewer species on the older islands of O‘ahu and Kaua‘i. The recent work of Givnish et al. (2013), based on five chloroplast DNA (cpDNA) regions and nuclear inter-simple sequence repeat polymorphisms (ISSRs) found that the genus evolved either on Kaua‘i or an older island, with subsequent dispersal to the younger islands, a result that was further supported by Hofer et al. (2013). Givnish et al. (2013) also found that *Clemontia* is not monophyletic as *Cl. pyrularia* nested within *Cyanea* and that six species may have arisen through hybridization and/or introgression. Although a valuable step

towards understanding the evolution of the genus, many of the relationships between taxa in the study of Givnish et al. (2013) were poorly resolved, necessitating further phylogenetic studies of the genus.

The present study provides a phylogenetic analysis of chloroplast DNA (cpDNA) sequences from 28 *Clermontia* taxa; the most comprehensive analysis based on sequences from the plastid genome undertaken to date. The study specifically aims to address 1) the biogeographical patterns of dispersal that have taken place in the genus in Hawai‘i, 2) whether the flower forms (sepaloid vs. petaloid) evolved on single or multiple occasions, 3) the biogeographic patterns in corolla tube length and color, and 4) if Lammers (1991) subgeneric classification of *Clermontia* reflects the evolutionary history of the genus. This work is the first step in a larger collaborative study that includes nuclear gene regions that aims to comprehensively resolve the evolutionary relationships within the genus.

Methods

Clermontia flower sampling and floral trait analysis

Ten flowers (two each from five plants) were collected from the same populations that leaf samples were collected for each taxon (Table 8). Flowers were initially stored in ziplock bags and placed in coolers containing ice and subsequently preserved in 90% ethanol. Herbarium vouchers, collected from plants whose nectar and flower samples were collected, were deposited at the University of Hawai‘i Joseph F. Rock Herbarium (HAW). Twenty taxa were measured from ethanol preserved flowers; four taxa were measured from herbarium specimens at the Bernice P. Bishop Museum; and four taxa

from both ethanol preserved flowers and herbarium specimens. Corolla lengths were measured between the base of the corolla and the base of the corolla lobes in the preserved flowers and herbarium specimens of each taxon using a flexible plastic ruler by curving the ruler to follow the middle of the corolla tube along its longitudinal axis.

Taxonomic representation and DNA extraction

Leaf samples of 21 of the 22 species and seven additional subspecies (taxonomy follows Lammers 1991) were collected from an individual plant of each taxon (Table 8). All of Lammers (1991) subgeneric sections and series were represented in the analysis (summarized in Table 7). A currently undescribed taxon (Pender, unpublished data) was included in the analysis to assess its taxonomic placement (*Clermontia* sp. “X” in Table 8). One taxon (*Cl. samuelii* subsp. *hanaensis*) was sampled from a herbarium specimen. Several taxa that were either extinct (*Cl. multiflora* has not been seen since the 1920’s; Lammers 1991), critically endangered (*Cl. oblongiflora* subsp. *mauiensis* and *Cl. peleana* subsp. *singulariflora*), or has only been collected once (*Cl. grandiflora* subsp. *maxima*) were not collected and were not included in the phylogenetic analysis. Twelve outgroup taxa were included in the analysis, 11 taxa from the Hawaiian lobeliad genera *Brighamia*, *Cyanea*, *Delissea*, *Lobelia* and *Trematolobelia* and one non-Hawaiian lobeliad, *Lobelia tupa*, that is a species native to Chile (Table 8). The differences in taxa with either sepaloid or petaloid sepals is illustrated in Figure 5.

Total genomic DNA was extracted from 1.0 g fresh leaves or 0.2 g from recently collected herbarium specimens (< 5 yr old) using the CTAB extraction protocol (Doyle and Doyle 1987) with some modifications (Morden et al. 1996). DNA specimens were accessioned into the Hawaiian Plant DNA Library (Randell and Morden 1999).

Table 7. Taxonomic classification of *Clermontia* proposed by Lammers (1991). The island distribution, elevational range and sepal arrangement for each species are included for reference.

Lammers 1991 Section	Lammers 1991 Series	Series characteristics	Taxon	Island distrib.	Altitude range	Sepals petaloid/ sepaloid
Clermontioideae	Clermontioideae	<i>Corolla bilabiate, lobes = or > tube. Staminal column included or excluded</i>	<i>Cl. clermontioides</i> (2 subspecies)	Hawai'i	670-1,825	Sepaloid
			<i>Cl. pyrularia</i>	Hawai'i	1,585-2,130	
			<i>Cl. waimeae</i>	Hawai'i	1,070-1,520	
	Sarcanthae	<i>Corolla bilabiate, lobes fleshy; dorsal longer than the ventral</i>	<i>Cl. arborescens</i> (3 subspecies)	Maui, Moloka'i, Lana'i	520-1,825	
			<i>Cl. tuberculata</i>	Maui	1,650-1,825	
	Unilabiatae	<i>Corolla unilabiate. Lobes 1/5-1/4 as long as tube. Staminal column strongly exerted</i>	<i>Cl. fauriei</i>	Kaua'i	365-1,400	
			<i>Cl. peleana</i> (2 subspecies)	Maui, Hawai'i	530-1,150	
Clermontia	Clermontia	<i>Perianth tubular. Lobes 1/5-1/2 as long as the curved or arcuate tube. Staminal column included or slightly exerted</i>	<i>Cl. grandiflora</i> (3 subspecies)	Maui, Moloka'i, Lana'i	525-1,975	Petaloid
			<i>Cl. hawaiiensis</i>	Hawai'i	550-1,760	
			<i>Cl. oblongifolia</i> (3 subspecies)	O'ahu, Maui, Moloka'i, Lana'i	395-1,280	
			<i>Cl. samuelii</i> (2 subspecies)	Maui	610-2,100	
	Clermontia	<i>Periath bilabiate. Lobes as long or longer than the tube. Staminal column is included or slightly exerted</i>	<i>Cl. drepanomorpha</i>	Hawai'i	915-1,460	
			<i>Cl. kakeana</i>	O'ahu, Moloka'i, Maui	120-1,270	
			<i>Cl. kohalae</i>	Hawai'i	370-1,370	
			<i>Cl. lindseyana</i>	Maui, Hawai'i	1,220-1,825	
			<i>Cl. montis-loa</i>	Hawai'i	1,070-1,700	
			<i>Cl. pallida</i>	Moloka'i	915-1,390	
<i>Cl. persicifolia</i>	O'ahu	300-850				

	<i>Kakeanae</i>					Petaloid
	<i>Parviflorae</i>	<i>Perianth rotate, lobes strongly recurved, as long or longer than the tube. Staminal column strongly exerted.</i>	<i>Cl. calophylla</i>	<i>Hawai'i</i>	885-1,460	
			<i>Cl. micrantha</i>	<i>Maui, Lana'i</i>	670-1,460	
			<i>Cl. multiflora</i>	<i>O'ahu, Maui</i>	Unknown	
			<i>Cl. parviflora</i>	<i>Hawai'i</i>	120-1,460	

Table 8. Taxa included in the phylogenetic analysis. Included are the HPDL number, island that the sample was collected from, and the number of flowers that were collected

Taxon	HPDL #	Island distribution	Flowers measured (n)
<i>Cl. arborescens</i> subsp. <i>arborescens</i>	6829	Near Wailuku, West Maui	10
<i>Cl. arborescens</i> subsp. <i>waihia</i>	6803	Puu Kukui, West Maui	10
<i>Cl. arborescens</i> subsp. <i>waikoluensis</i>	6839	Kamakou Preserve, Moloka‘i	10
<i>Cl. calophylla</i>	6961	Kohala Mountain, Hawai‘i	6
<i>Cl. clermontioides</i> subsp. <i>clermontioides</i>	6859	Kona Hema Preserve, Hawai‘i	10
<i>Cl. clermontioides</i> subsp. <i>rockiana</i>	7012	Puu Waa Waa, Hawai‘i	10
<i>Cl. drepanomorpha</i>	7000	Kohala Mountain, Hawai‘i	10
<i>Cl. fauriei</i>	6786	Alakai Wetland, Kaua‘i	10
<i>Cl. grandiflora</i> subsp. <i>grandiflora</i>	6795	Puu Kukui, West Maui	10
<i>Cl. grandiflora</i> subsp. <i>munroi</i>	6805	Puu Kukui, West Maui	10
<i>Cl. hawaiiensis</i>	6886	Mauna Loa, Hawai‘i	10
<i>Cl. kakeana</i>	6801	Puu Kukui, West Maui	10
<i>Cl. kohalae</i>	6889	Kohala Mountain, Hawai‘i	10
<i>Cl. lindseyana</i>	6862	Hakalau National Wildlife Refuge, Hawai‘i	10
<i>Cl. micrantha</i>	6799	Puu Kukui, West Maui	9
<i>Cl. montis-loa</i>	6866	Mauna Loa, Hawai‘i	10
<i>Cl. oblongifolia</i> subsp. <i>brevipes</i>	6953	Moloka‘i. Collected by Steve Pearlman (NTBG). Exact location unknown	2
<i>Cl. oblongifolia</i> subsp. <i>oblongifolia</i>	6784	Mt Tantalus, O‘ahu	10
<i>Cl. pallida</i>	6841	Kamakou Preserve, Moloka‘i	10
<i>Cl. parviflora</i>	6885	Hawai‘i Volcanoes National Park, Hawai‘i	10
<i>Cl. peleana</i> subsp. <i>peleana</i>	7015	Volcano Native Plant Nursery	10
<i>Cl. persicifolia</i>	7011	Palikea, Waianae Mt, O‘ahu	13
<i>Cl. pyrularia</i>	6376	Hakalau National Wildlife Refuge, Hawai‘i	10
<i>Cl. samuelii</i> subsp. <i>hanaensis</i>	7211	Bishop Museum Specimen. Originally collected in Hana by H. Oppenheimer	2
<i>Cl. samuelii</i> subsp. <i>samuelii</i>	6794	Haleakala National Park Nursery, East Maui	10
<i>Cl. tuberculata</i>	6837	Waikamoi, East Maui	10
<i>Cl. waimeae</i>	7013	Kohala Mountain, Hawai‘i	10
<i>Cl. species “X”</i>	6890	Kohala Mountain, Hawai‘i	10
Outgroup taxa			
<i>Brighamia insignis</i>	4561	Lyon Arboretum. Collected by Dr. C. Morden	N/A
<i>Delissea subcordata</i>	3835	Collected by O‘ahu Army	N/A

		Natural Resources from Waianae Mt, O'ahu	
<i>Delissea rhytidosperma</i>	5416	National Tropical Botanic Garden, Limahuli, Kaua'i	N/A
<i>Cyanea angustifolia</i>	3061	Hawai'i Loa Ridge, O'ahu. Collected by C. Crooker	N/A
<i>Cyanea acuminata</i>	1754	Tracks Beach, O'ahu. Collected by Dr. C. Morden	N/A
<i>Cyanea shipmanii</i>	1763	Lyon Arboretum. Unknown collector	N/A
<i>Cyanea superba</i>	2452	Collected by O'ahu Army Natural Resources from Waianae Mt, O'ahu	N/A
<i>Trematolobelia macrostachys</i>	4764	Mt. Kaala, O'ahu	N/A
<i>Trematolobelia kauiensis</i>	4887	Alakai Wetland, Kaua'i	N/A
<i>Lobelia oahuensis</i>	7162	O'ahu. Exact location unknown. Collected by V. Caraway	N/A
<i>Lobelia villosa</i>	4097	Alakai Wetland, Kaua'i	N/A
<i>Lobelia tupa</i>	7210	Christchurch Botanic Gardens, New Zealand. Native to Chile.	N/A

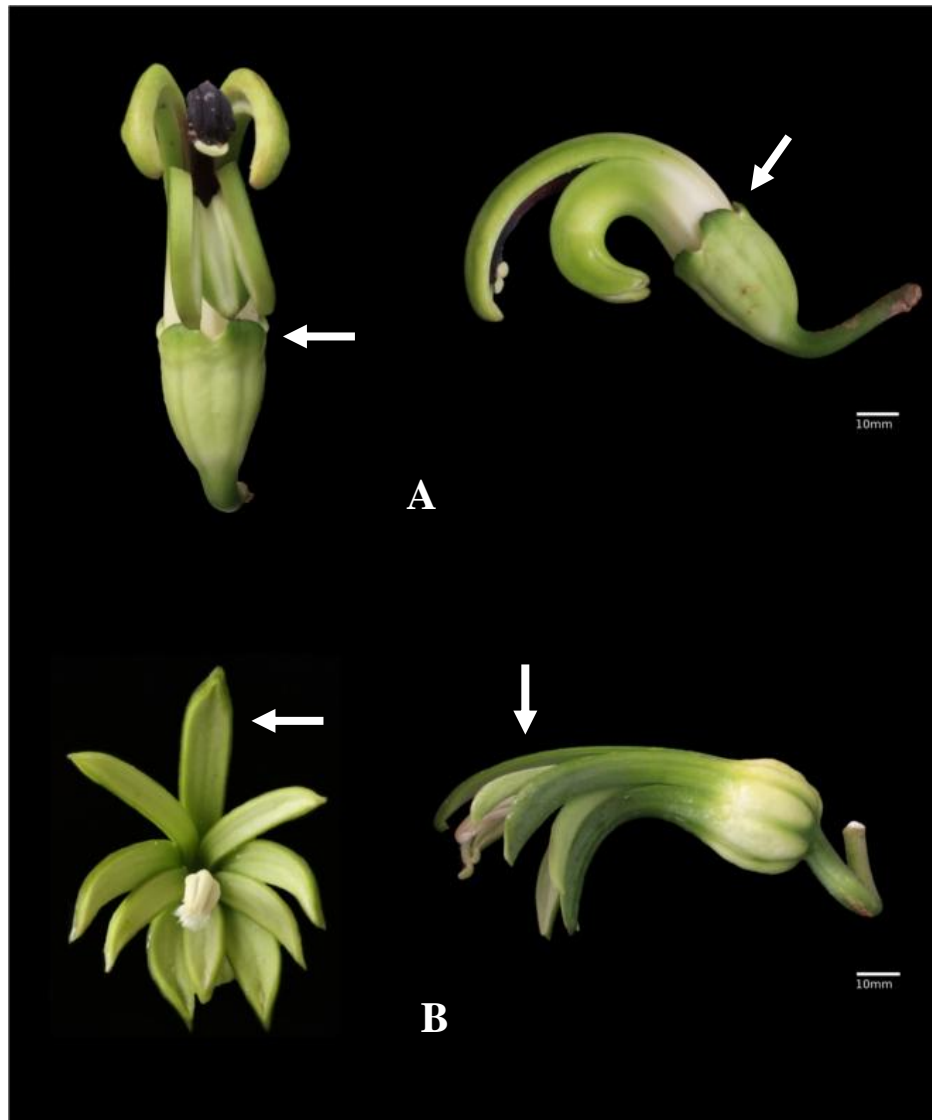


Figure 5. A comparison of *Clermontia* taxa that have either sepaloid or petaloid sepals. **A:** Sepaloid sepals. A *Cl. arborescens* subsp. *waiheae* flower viewed from the front of the flower (left) and from the side (right). Note the reduced sepal lobes that are morphologically distinct from the petals (indicated by the arrow). **B:** Petaloid sepals. A *Cl. lindseyana* flower viewed from the front of the flower (left) and from the side (right). Note the petal-like sepals that are morphologically similar to the petal lobes (indicated by the arrow).

Molecular markers, DNA amplification and DNA sequencing

Six chloroplast DNA (cpDNA) noncoding regions (*atpB-rbcL*, *ndhF-rpl32*, *trnH-psbA*, *rpl32-trnL* *trnL-trnF* and the *trnL* intron) were utilized for the analysis (Table 9).

All five gene regions were amplified and sequenced using standard primers found in the literature (Table 3). Polymerase chain reactions (PCR) were undertaken using a MJ Research thermal cycler (model PTC 200; GMI, Inc. Ramsey, Minnesota, USA). PCR reactions were performed in 25 μ L reaction mixtures containing: 1 \times GoTaq Flexi PCR buffer; 15 mM MgCl₂; 0.1% bovine serum albumin; 0.2 mM of each dNTP; 0.2 mM of each amplification primer, and 1 U GoTaq polymerase (Promega, Madison, Wisconsin). All five gene regions were amplified using the following PCR protocol: 2 min at 95°C; 1 min at 93°C; 1 min at 55°C; 1.5 min at 55°C; 29 cycles at 95°C; 3 min at 72°C. PCR amplicons were visualized on 1.5% agarose gels using electrophoresis.

PCR products were cleaned using 1 μ L of shrimp alkaline phosphatase and exonuclease (ExoSAP, USB Corp., Cleveland, Ohio, USA) using the following protocol: 37°C for 15 min followed by 80°C for 15 min. PCR amplicons were bidirectionally sequenced at the University of Hawai'i's Biotech Core (<http://core.biotech.hawaii.edu/>) using BigDye Terminator chemistry (Applied Biosystems, Foster City, California, USA) and visualized on an ABI 3130XL genetic analyzer (Applied Biosystems).

Table 9. The six cpDNA primer regions used in the study.

Gene	Primer sequences	Reference
<i>atpB-rbcL</i>	F: 5'-ACTTGCTTTAGTTTCTGTTGGTGA-3' R: 5'-ACTTGCTTTAGTTTCTGTTGGTGA-3'	Manen et al. 1994
<i>ndhF-rpl32</i>	F: 5'-GAAAGGTATCCAYGMATATT-3' R: 5'-CCAATATCCCTTYTNTTCCAA-3'	Shaw et al. 2007
<i>rpl32-trnL</i>	F: 5'-CAGTTCCAAAAAACGTA-3' R: 5'-CTGCTTCCTAAGAGCAGCGT-3'	Shaw et al. 2007
<i>trnH-psbA</i>	R: 5'-CGCGCATGGTGGATTCACAATCC-3' F: 5'-GTTATGCATGAACGTAATGCT-3'	Hamilton 1999, Shaw et al. 2005
<i>trnL</i> intron	(C) F: 5'-CGAAATCGGTAGACGCTACG-3' (D) R: 5'-GGGGATAGAGGGACTTGAAC-3'	Taberlet et al. 1991
<i>trnL-trnF</i>	(E) F: 5'-GGTTCAAGTCCCTCTATCCC-3' (F) R: 5'-ATTTGAACTGGTGACACGAG-3'	Taberlet et al. 1991

Sequence alignment and phylogenetic analysis

Sequences were edited and assembled using Sequencher 5.0 (Gene Codes Corporation, Ann Arbor, Michigan). Edited sequences were aligned using Muscle (Edger 2004) in MEGA 5 (Tamura et al. 2011). Contiguous strands were assembled and edited for all *atpB-rbcL*, *ndhF-rpl32*, *rpl32-trnL*, *trnH-psbA*, *trnL-trnF* and *trnL* intron sequences using MEGA 5 (Tamura et al. 2011). All sequences were aligned initially in ClustalW (ver. 2.1; Larkin et al. 2007) and Muscle (Edgar 2004) and then manually adjusted in MEGA 5 following the guidelines of Kelchner (2000) to minimize indels. Sequences from all six cpDNA regions were combined into one dataset.

For Maximum likelihood (ML) analysis, a model of sequence evolution was firstly selected using JModelTest (Darribo et al. 2012). Three different substitution schemes were employed for all alignments along with the options base frequency + F and variation rate + I + nCat = 4. The base tree used for maximum likelihood (ML) calculations was estimated by the program through the ML optimized option. The

selected model was that suggested by the AIC and BIC criteria. The ML analyses were run using RAxML v. 7.4.2 (Stamakis 2006, Silvestro 2012) utilizing 1000 reps and the non-Hawaiian endemic, *Lobelia tupa*, as an outgroup.

MrBayes version 3.1.2 (Huelsenbeck and Ronquist, 2001, Ronquist and Huelsenbeck 2003) was used to conduct Bayesian analyses using default model settings. Bayesian analyses were run using four incrementally heated chains for one million generations and a sampling frequency every 100 generations. Tree samples and parameter estimates from the first 25% of all trees (2500) were designated as the burn-in and discarded. The island distribution of taxa, Lammers' (1991) subgeneric classification, and the selected floral traits, sepal arrangement and corolla tube length, were manually added to combined ML and Bayesian phylogenetic trees using Mega 5.

Results

A total of 200 sequences were generated from the five gene regions of the 40 taxa examined. In total, 4199 aligned nucleotides were sequenced for each taxon, of which 556 were parsimony-uninformative and 243 were parsimony-informative. No indels were present among the *Clermontia* sequences for all genes. Accession gene sequences were concatenated for analysis. Table 10 summarizes the parsimony results for the individual gene regions. The 1000 ML searches using the base substitution model produced one tree with a score of -9959.3. The Bayesian analysis resulted in a single consensus tree with score $-\ln l = -10,049.195$. Because the two optimality criteria (ML and Bayesian) produced phylogenies that were concordant, only the Bayesian tree constructions are shown here, with ML bootstrap values added (Figure 6). Clade support within trees was

based on bootstrap values and posterior probabilities where high support was considered to be >90%, moderate support 70-90%, and low support <70%. (Hillis and Bull 1993).

Table 10. Sequence length and the number of variable and parsimony informative characters for the six cpDNA gene regions used in the analysis.

Gene	<i>atpB- rbcL</i>	<i>ndhF- rpl32</i>	<i>rpl32- trnL</i>	<i>trnH- psbA</i>	<i>trnL intron</i>	<i>trnL-F</i>	<i>Combined cpDNA</i>
Aligned length (bp)	914	1186	853	382	500	364	4199
Variable characters	78	211	132	52	33	50	556
Parsimony informative characters	30	82	66	24	17	24	243

Taxonomic evaluation

The combined ML and Bayesian analyses suggested that *Clermontia*, as it is currently circumscribed, is not monophyletic. *Clermontia pyrularia* nests within the purple-fruited clade of *Cyanea* and is possibly an intergeneric hybrid between *Clermontia* and *Cyanea*. The remaining taxa formed a monophyletic lineage that is sister to *Cyanea*. Within this lineage, at least one taxon appeared to be taxonomically misplaced. The two *Cl. oblongifolia* subspecies that were included in the study (*Cl. oblongifolia* subsp. *oblongifolia* and *Cl. oblongifolia* subsp. *brevipes*) occurred in separate clades, indicating that *Cl. oblongifolia* subsp. *brevipes* may represent a distinct species. Several other taxa (the two subspecies of *Cl. clermontioides*, *Cl. samuelii* and *Cl. grandiflora*) did not align as sister species in the analysis. Lammers' (1991) subgeneric classification does not

appear to adequately reflect the evolutionary history of the genus (Figure 6). Members of each of the sections and series proposed by Lammers (1991) occurred in different clades in the ML and Bayesian analyses. For example, the species in section *Clermontia* series *Kakeanae* occurred in four separate clades (Figure 6). In only one clade (containing *Cl. grandiflora* and *Cl. samuelii*) did the relationships approach Lammers' (1991) classification. However, even in this case, other members of section *Clermontia* series *Clermontia* occurred in different clades.

Historical biogeography

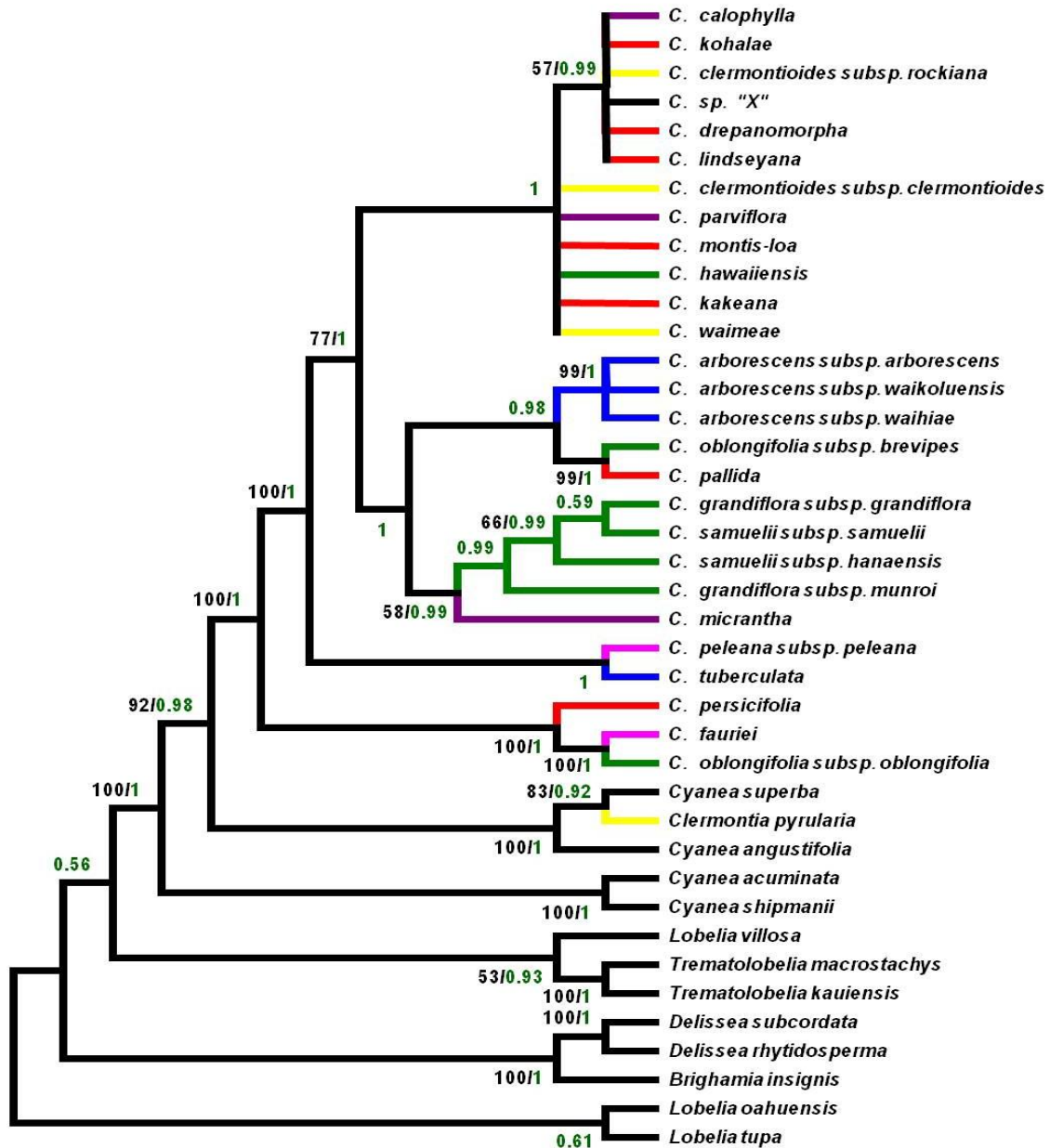
The most basal *Clermontia* clade in the ML and Bayesian analysis contained *Cl. fauriei* (Kaua'i and O'ahu), *Cl. oblongifolia* subsp. *oblongifolia* (O'ahu) and *Cl. persicifolia* (O'ahu), suggesting that the genus evolved either on Kaua'i (Kaua'i, 4.7 Mya) or O'ahu (3.0-2.6 My) (Figure 7). This O'ahu/Kaua'i clade was sister to three remaining clades that, with the exception of *Cl. kakeana*, were confined to the islands of the former Maui Nui island complex (1.2-2 Mya) and Hawai'i (< 0.7 Mya). The first clade, composed of *Cl. tuberculata* (East Maui) and *Cl. peleana* subsp. *peleana* (Hawai'i), were sister to a well supported larger Maui Nui clade, containing ten taxa (e.g., *Cl. arborescens*, *Cl. grandiflora* and *Cl. samuelii*, etc.). The last clade was primarily composed of Hawai'i Island endemic taxa. The only exception was *Cl. kakeana* that occurs on O'ahu, Moloka'i and Maui.

Floral trait evolution

In total, 18 *Clermontia* taxa included in the study had petaloid sepals and nine

taxa had sepaloid sepals (Table 7, Figure 8). The analysis suggested that petaloid sepals evolved at least once on O‘ahu (*Cl. oblongifolia* subsp. *oblongifolia*) with reversals back to sepaloid sepals occurring on five (*Cl. fauriei*, *Cl. peleana/Cl. tuberculata*, *Cl. arborescens*, *Cl. clermontioides*) or six (depending on the taxonomic placement of the *Cl. clermontioides* subspecies) separate occasions.

The majority of *Clermontia* taxa had corolla tubes that ranged from either 16 to 30 mm or 31 to 45 mm in length (Figure 9). There was a preponderance of taxa with long corolla tubes (> 31 mm) on islands in the Maui-Nui island complex, owing to the large-flowered taxa in *Cl. arborescens*, *Cl. grandiflora* and *Cl. samuelii*. Only four taxa had corolla lengths that were either larger or smaller than these two ranges. *Clermontia micrantha* and *Cl. parviflora* both had corolla tubes smaller than 15 mm long, while *Cl. oblongifolia* subsp. *oblongifolia* and *Cl. grandiflora* subsp. *munroi* had corolla tubes longer than 46 mm.



Legend

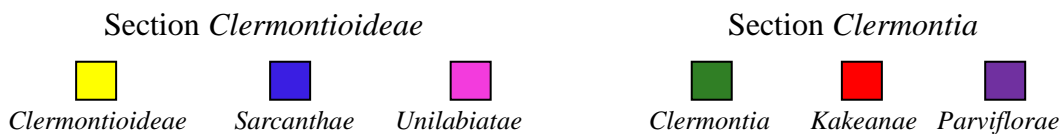
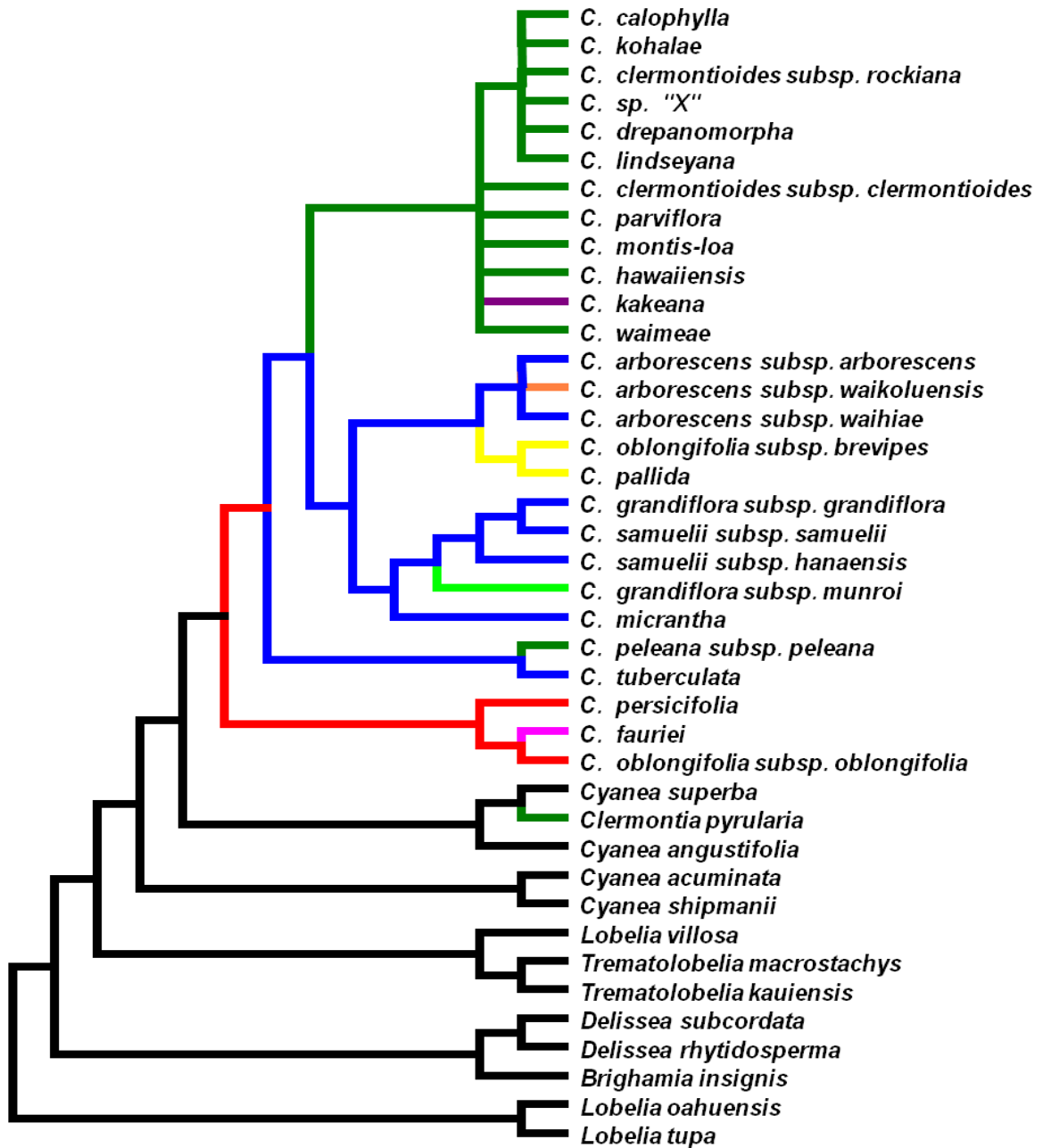


Figure 6. Combined ML and Bayesian tree of the phylogenetic relationships of *Clermontia*. The distribution of taxa following the classification of Lambers (1991) overlaid on the Bayesian analysis. ML bootstrap values are in black. Bayesian posterior probability values are in green.



Legend

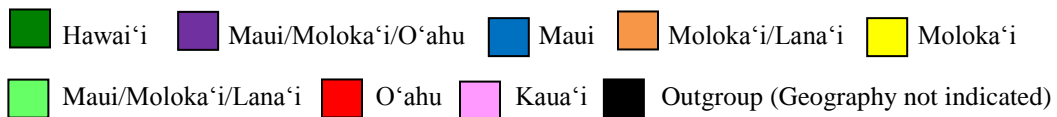
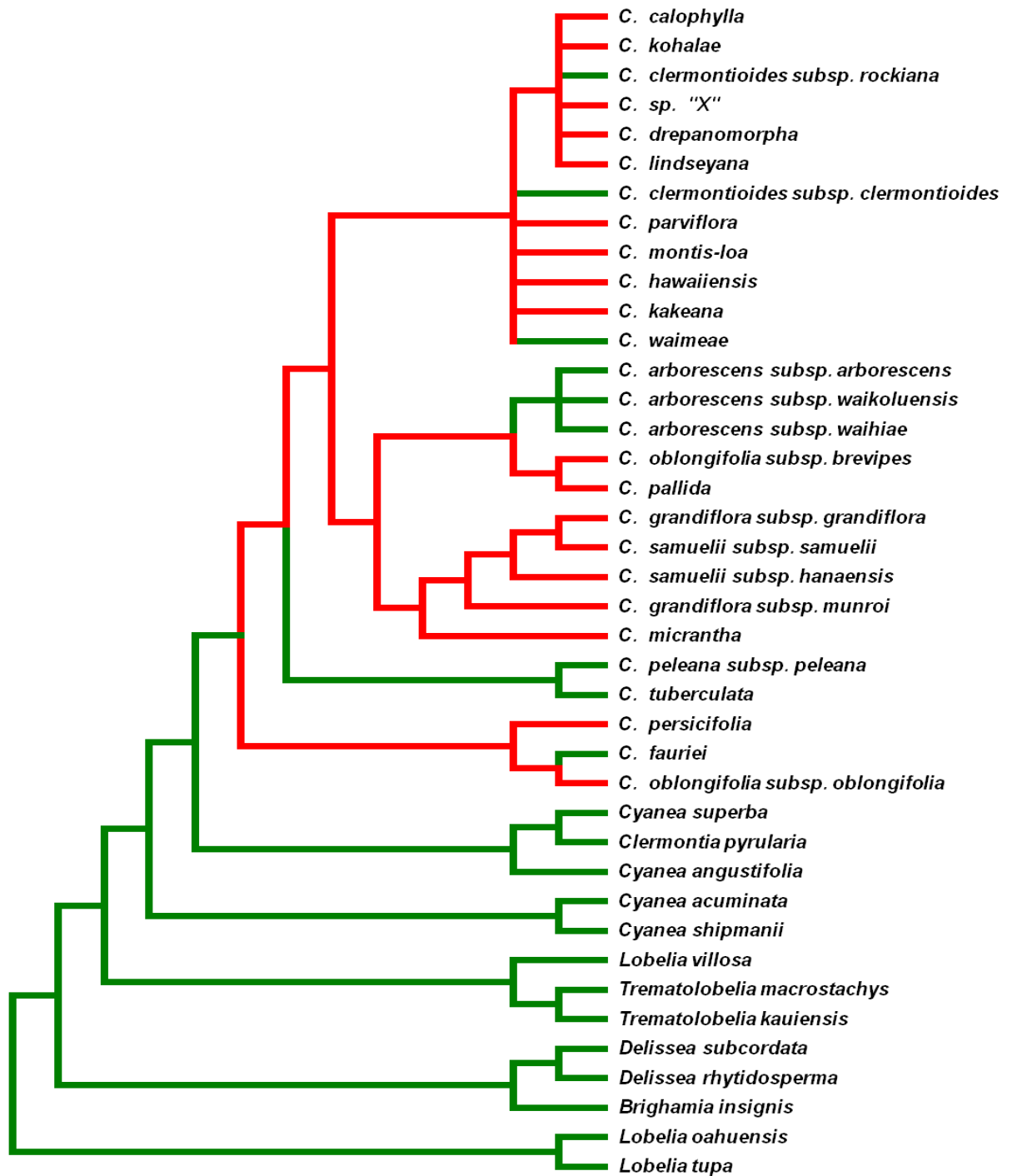


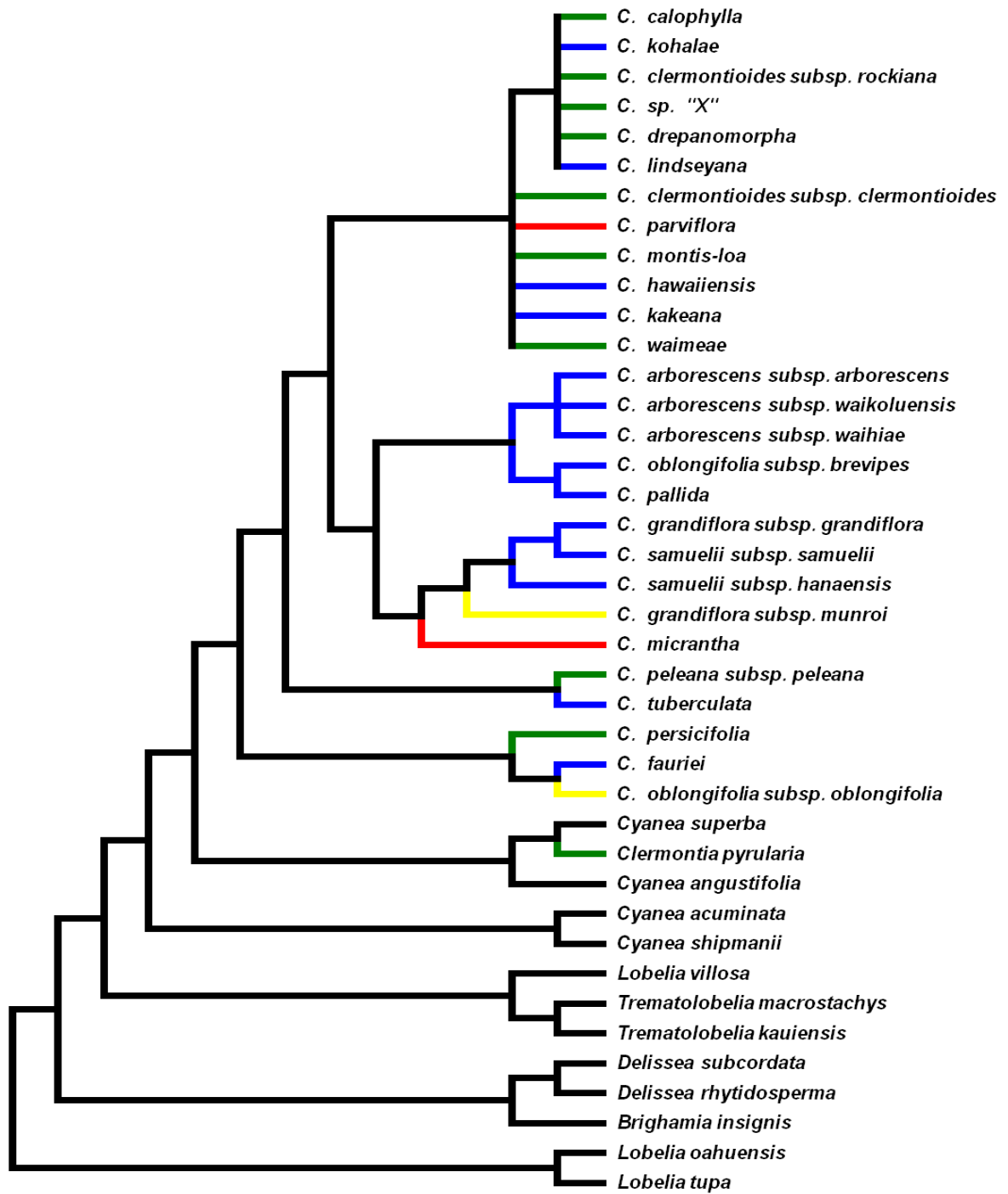
Figure 7. Biogeography and hypothesized patterns of dispersal of *Clermontia* taxa. The in-group taxa are indicated by branch color. Outgroup taxa indicated by black branches.



Legend

■ Petaloid sepals ■ Sepaloid sepals

Figure 8. Evolutionary shifts in sepaloid and petaloid flowers in the genus *Clermontia*. The characters were manually overlaid on the Bayesian analysis tree.



Legend

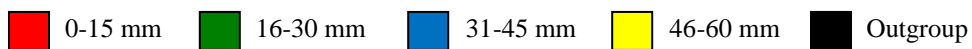


Figure 9. Differences in corolla tube length between *Clermontia* taxa included in the analysis overlaid on the Bayesian analysis.

Discussion

Potential taxonomic changes

The current analysis suggests that the subgeneric classification of *Clermontia* (two sections, each containing three series) utilized by Lammers (1991) does not adequately reflect the evolution of the genus. Lammers (1991) classification was based upon floral characters, principally the arrangement and morphology of the calyx and corolla lobes. As previously addressed above, these floral traits appear to have evolved separately and on multiple occasions. Only in one case, the existence of a clade containing *Cl. grandiflora* and *Cl. samuelii* (Section *Clermontia* series *Clermontia*), do the results approach a relationship proposed by Lammers (1991). However, even in this case, other taxa (*Cl. hawaiiensis*, *Cl. oblongifolia*) in the series occur in widely divergent clades. The floral morphological traits utilized by Lammers (1991) therefore appear to be inadequate for the subgeneric classification of *Clermontia* taxa.

This study supports the results of Givnish et al. (2013) that found that *Cl. pyrularia* does not belong in *Clermontia*. This taxon is critically endangered (USFWS 1996). However, historically it occurred on both leeward Mauna Loa and windward Mauna Kea. Despite *Cl. pyrularia* having dorsally cleft corollas, a key character that distinguishes *Clermontia* from *Cyanea* taxa (Lammers 1991), the placement of *Cl. pyrularia* either in *Cyanea* or as an intergeneric hybrid is supported by several lines of evidence. Firstly, the seeds of *Cl. pyrularia* are > 2 mm long. The seeds of *Clermontia* taxa that have been measured are consistently smaller (i.e., less than 1mm, n = 21 species) (A. Shiels and R. Pender, unpublished data). Second, *Cl. pyrularia* flowers in a pulse with the main flowering occurring in March and April (Pender, unpublished data).

By contrast, *Clermontia* taxa tend to flower sporadically throughout the year (pers. obs.). Clearly, the exact placement of *Cl. pyrularia* within *Cyanea* warrants further and more detailed investigation.

The critically endangered Moloka‘i endemic, *Cl. oblongifolia* subsp. *brevipes*, appears to be misplaced as a subspecies of *Cl. oblongifolia* and likely warrants recognition as a distinct species (Figure 6). Although the flowers of this taxon and *Cl. oblongifolia* subsp. *oblongifolia* are falcate with sepals fused, forming a tube around the corolla tube, it appears these traits have evolved independently in both taxa. Based on our analysis, this taxon is most closely related to the Moloka‘i endemic, *Cl. pallida*. Both species appear to be sufficiently distinct to warrant recognition as distinct species (descriptions in Lammers 1991).

Based on our analysis, further work is needed to resolve the relationship between *Cl. grandiflora* and *Cl. samuelii*. Similarly, whether the undescribed taxon (*Cl. sp. “X”*) collected from Kohala Mountain on Hawai‘i Island warrants recognition as a distinct entity is unclear. The relationships of this taxon and other taxa (e.g. the relationship between the two subspecies of *Cl. clermontioides*) can only be fully resolved through analysis of additional gene regions.

Biogeographical patterns

The general trend for Hawaiian lineages is the initial colonization of Kaua‘i, the oldest of the high Hawaiian Islands, with subsequent colonization and speciation on the emerging younger islands (reviewed in Funk and Wagner 1995). With several exceptions, the present analysis further supports this “progression rule.” These findings are in

contrast to Lammers' (1995) cladistic analysis that suggested that the genus evolved on Hawai'i Island and dispersed to the older islands. The present study suggests the genus evolved either on O'ahu or Kaua'i, indicated by a basal clade containing *Cl. oblongifolia* subsp. *oblongifolia* and *Cl. persicifolia* that are endemic to O'ahu and *Cl. fauriei* that occurs on both O'ahu and Kaua'i. It is not clear which of these three species is the most basal species or whether *Cl. fauriei* either evolved on O'ahu and subsequently dispersed to Kaua'i or dispersed from Kaua'i and is therefore the progenitor of *Cl. oblongifolia* subsp. *oblongifolia* and *Cl. persicifolia*. Givnish et al. (2013) and Hofer et al. (2012) both found that *Cl. fauriei* is the basal species, having first evolved on Kaua'i with subsequent dispersal to O'ahu. Their findings seem intuitive, given the tendency for progressive dispersal from older to younger islands and the fact that *Cl. fauriei* is the only species of the three in this clade with sepaloid sepals.

A dispersal event from O'ahu to Maui Nui appears to have occurred, giving rise to the East Maui endemic, *Cl. tuberculata*. This taxon forms a clade with the Hawai'i Island endemic, *Cl. peleana* subsp. *peleana*, suggesting that this taxon is derived from an East Maui ancestor. A taxon that was not included in this analysis, *Cl. peleana* subsp. *singuliflora*, was historically recorded from East Maui and has recently been rediscovered growing on Kohala Mountain on Hawai'i Island. *Clermontia peleana* subsp. *singuliflora* may represent the intermediate taxon between *Cl. tuberculata* and *Cl. peleana* subsp. *peleana*, given this species' distribution on East Maui and Kohala Mountain (0.7 Mya), the oldest volcano on the Island of Hawai'i. *Clermontia peleana* subsp. *peleana*, which occurs only on windward Mauna Kea (0.2 Mya) and Mauna Loa (< 0.2 Mya), may have subsequently evolved following dispersal to these mountains. Givnish et al. (2013) found

a similar relationship between *Cl. peleana* and *Cl. tuberculata* based on cpDNA. However, when combining cpDNA and ISSR datasets, Givnish et al. (2013) suggest that both taxa have a hybrid origin involving *Cl. arborescens*: 1) *Cl. tuberculata* being the product of an intergeneric hybridization with *Cyanea aculeatiflora*, and 2) *Cl. peleana* being the product of a hybridization event with *Cl. montis-loa*.

Sister to the *Cl. peleana/Cl. tuberculata* clade is a large Maui Nui clade composed of two smaller clades. The first, comprising *Cl. micrantha* and the two subspecies of *Cl. grandiflora* and *Cl. samuelii*, is primarily from East (both *Cl. samuelii* subspecies) or West (*Cl. grandiflora* subsp. *grandiflora* and *Cl. micrantha*) Maui. The only exception is *Cl. grandiflora* subsp. *munroi*, which occurs throughout Maui Nui. The second Maui Nui clade is composed of two subclades: 1) two taxa that are endemic to Moloka'i (*Cl. pallida* and *Cl. oblongifolia* subsp. *brevipes*) which appear to have both evolved on the island, and 2) the three subspecies of *Cl. arborescens*, two of which are restricted to Maui (*Cl. arborescens* subsp. *arborescens* on West Maui and *Cl. arborescens* subsp. *waihia* on both East and West Maui) while *Cl. arborescens* subsp. *waikoluensis* occurs on Moloka'i and Lana'i. More work is needed (greater sampling and the use of molecular clock dating) to ascertain if the taxa that occur on Moloka'i and Lana'i (*Cl. arborescens* subsp. *waikoluensis*, *Cl. grandiflora* subsp. *munroi*) occur on these islands either as a result of dispersal after the formation of Moloka'i and Lana'i or vicariance following the erosion and subsequent geographical separation of the Maui Nui complex. The relationships in the Maui Nui clade largely concur with those found by Givnish et al. (2013), with the exception that *Cl. samuelii* formed a clade with *Cl. clermontioides* in their cpDNA phylogeny. Givnish et al. (2013) suggest that *Cl. samuelii* may have resulted

through hybridization between *Cl. grandiflora* (Maui) and *Cl. clermontioides* (Hawai‘i). In addition, the Moloka‘i endemic, *Cl. pallida*, places with *Cl. lindseyana* (East Maui and Hawai‘i) in the combined ML analysis of Givnish et al. (2013). However, many of the relationships in the combined cpDNA and ISSR phylogeny of Givnish et al. (2013) are poorly resolved.

Relationships among the Hawai‘i Island clade remain ambiguous. It is likely, given the young age of Hawai‘i Island, that these taxa are recently evolved, providing limited time for phylogenetically informative genetic variation to develop. One biogeographically anomalous species, *Cl. kakeana*, which grows on all of the mountain ranges of Maui, Moloka‘i and O‘ahu, also occurs in this clade. Hypothetically, this taxon either is sister to the large Hawaiian clade or it is nested within it. If the latter scenario is in fact correct, it suggests the species evolved following a recent (< 0.7 Mya) dispersal event (most probably to Maui) from the Island of Hawai‘i with subsequent dispersal events (potentially to Moloka‘i and then O‘ahu) taking place. More work is clearly needed to resolve biogeographical relationships within this species. With the exception of the placement of *Cl. pallida* in this clade (see previous paragraph), our results strongly concur with those of Givnish et al. (2013), i.e., the existence of a recently evolved Hawai‘i Island clade with the subsequent back dispersal of *Cl. kakeana* to Maui, Moloka‘i and O‘ahu.

Flower trait evolution

Both Givnish et al. (2013) and Hofer et al. (2012) suggest that the sepaloid species, *Cl. fauriei*, is the most basal in the genus. The present study was unable to

resolve the most basal species, making it difficult to determine whether the genus evolved from a species with a sepaloid or petaloid sepal arrangement. However, the present study suggests that petaloid sepals evolved once on O‘ahu (in *Cl. oblongifolia* subsp. *oblongifolia*) and were largely retained as the genus dispersed down the island chain. Five, possibly six, reversal (to the sepaloid form) events took place, depending on the actual evolutionary placement of the two subspecies of *Cl. clermontioides* and the inclusion of missing taxa. By contrast, Givnish et al. (2013) suggest that petaloid sepals arose two times, first in *Cl. persicifolia* from O‘ahu and once in *Cl. grandiflora* (Maui Nui) with three reversal events back to the sepaloid form.

The retention of the double flower form during the evolution of the genus suggests that this flower morphology has been evolutionarily successful. Unlike other ornithophilous Hawaiian lobeliad genera (*Cyanea*, *Delissea*, *Lobelia*, and *Trematolobelia*) that produce large amounts of flowers often over short time periods (pers. obs.), *Clermontia* taxa generally produce flowers in lower densities (typically in pairs), often sporadically throughout the year (pers. obs.). The petaloid sepals may therefore have been retained as they increase the visual acuity of sparsely dispersed flowers for avian pollinators.

The majority of the *Clermontia* taxa included in the study have corolla tube lengths that ranged from 16 to 45 mm. These taxa occur on each of the islands that *Clermontia* occupy, perhaps suggesting that there has been consistent selective forces to maintain this generalized range of corolla lengths. It is feasible that specialist nectarivorous honeycreepers with mid-length bills (e.g., ‘i‘iwi, *Vestiaria coccinea*) that were present on all of the main Hawaiian Islands before the arrival of humans (Banko

and Banko 2009) may have provided the stabilizing selection pressures to maintain these corolla tube lengths. In comparison to the other ornithophilous Hawaiian lobeliad genera, *Clermontia* taxa produce fewer, larger flowers. This may represent a trade-off, whereby there has been selection for flower sizes that are both more visible and capable of holding larger nectar rewards for passerine pollinators when compared to larger quantities of smaller flowers. By contrast, a small set of *Clermontia* taxa either have short (< 20 mm: *Cl. micrantha* and *Cl. parviflora*) or long corolla tubes (> 45 mm: *Cl. grandiflora* subsp. *munroi* and *Cl. oblongifolia* subsp. *oblongifolia*) (Figure 8). These taxa may represent cases of directional selection provided by short (e.g., ‘amakihi species (*Hemignathus* spp.)) or long-billed (e.g., mamo species (*Drepanis* spp.)) nectarivorous passerine species, respectively. Unfortunately, addressing hypotheses regarding niche partitioning by native passerine species is now very difficult due to wide-scale historic extinctions and species declines.

Future research

Further work is needed before the complete evolutionary relationships in *Clermontia* can be fully resolved. First, taxa that could not be included in the present analysis (e.g., the critically endangered *C. oblongifolia* subsp. *mauiensis*, *C. peleana* subsp. *singuliflora* and the extinct *C. multiflora*) should be included in future analyses to clarify their relationships with the remaining taxa. Second, the use of low-copy nuclear genes and/or next generation sequencing will likely clarify the ambiguous relationships that exist in the current analysis. Recently developed nrDNA primers, designed for DNA bar-coding *Clermontia* taxa (Pillon et al. 2013) and other recently utilized nrDNA regions

(Hofer et al. 2012) could be employed to resolve the relationships within the genus.

Ultimately, clarifying the taxonomic and biogeographical relationships between Hawaiian lobeliad taxa and those of other genera in the Lobeliodeae should remain a long-term goal of evolutionists working with the lineage.

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CHAPTER FIVE:

ARE RESTORATION PLANTINGS OF ENDANGERED
ORNITHOPHILOUS HAWAIIAN LOBELIADS (CAMPANULACEAE)
BEING POLLINATED BY NECTARIVOROUS PASSERINES IN
HAWAI'I?

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Abstract

Restoration is regularly used as part of recovery programs for plant species of conservation concern, yet few plant recovery programs assess if these efforts lead to the reestablishment of pollinator interactions in animal-pollinated plant species. Pollination ecology studies were undertaken to assess if four endangered ornithophilous Hawaiian lobeliads are pollinated by passerines (native or introduced) and diurnal insects at two separate restoration sites. Hakalau National Wildlife Refuge (Hawai‘i Island) contains the highest densities of nectarivorous honeycreepers in the archipelago. In contrast, Kahanahāiki and Pahole Gulches (Wai‘anae Mountains, O‘ahu) has had significant degradation of habitat and nectarivorous honeycreepers are functionally extinct. Two honeycreeper species, ‘i‘iwi (*Vestiaria coccinea*) and Hawai‘i ‘amakihi (*Hemignathus virens*), were infrequent floral visitors to *Cl. lindseyana* and *Cl. pyrularia* at Hakalau. ‘Amakihi nectar robbed 91% and 66% of flowers of both species, respectively, and are not effective pollinators of either lobeliad species. ‘I‘iwi also nectar robbed the flowers of both species (60% and 32%, respectively) although appeared to pollinate the flowers of *Cl. lindseyana* on rare occasions. At Kahanahāiki and Pahole, introduced Japanese white-eyes (*Zosterops japonicus*) are not effective pollinators of *Cy. superba* and *D. waianaeensis*, nectar robbing all of the flowers they visited of both species. However, the native yellow-faced bee, *Hylaeus connectens*, and the introduced hoverfly, *Allograpta obliqua*, may pollinate flowers on rare occasions. Pollination treatments indicate that all four species employ a mixed mating system, whereby both autogamy and animal-assisted pollination would likely occur naturally. However, under extant field conditions, all four species are likely to be reproducing almost exclusively by autogamy and are pollen

limited. The potential impacts of pollinator loss (including inbreeding depression and changes in plant population demographics) in these and other bird-pollinated species in Hawai‘i warrant future research attention.

Introduction

One of the fundamental goals of ecological restoration is to reestablish plant-animal interactions (e.g., plant-pollinator interactions) (Nabhan and Buchmann 1997, Ruiz-Jaen and Aide 2005, Davy 2002). Despite the pivotal role animals play as pollinators for approximately 90% of flowering plants (Ollerton et al. 2011), the reestablishment of pollinator interactions at the species or community level in restored ecosystems has received little research attention (Handel 1997, Allen-Wardell 1998, Dixon 2011, Menz et al. 2011). Investigating plant-pollinator interactions in restoration plantings has many potential benefits. For example, by providing insights into how pollinator community composition changes through time, how pollinators colonize and disperse within novel ecosystems and, in cases where pollinators (or the plant species) are now rare or extinct, an understanding of ecological interactions that may have shaped evolutionary traits in the mutualists. In this way, the information gained can be profitably utilized to improve the future restoration and knowledge of the species involved.

Compared to mainland ecosystems, islands tend to have high levels of species endemism, smaller distributional ranges of species and ecosystems that are highly vulnerable to biological invasions (Whittaker and Fernandez-Palacios 2007, Kaiser-Bunbury et al. 2010). Because of these factors, oceanic islands are particularly susceptible to parallel declines in plants and their pollinators (Cox and Elmqvist 2000,

Sekercioglu et al. 2004, Steadman 2006, Kaiser-Bunbury et al. 2010). The bird-pollinated component of the Hawaiian native flora highlights this situation. An estimated 18% of the flora (*ca.* 190 species) was historically pollinated by birds (Sakai et al. 2002). One quarter of these plant species are now endangered (Sakai et al. 2002). Similarly, two of the five specialist nectarivorous honeycreeper (Drepanidinae) species and all five Hawaiian Mohoidae species are extinct (Banko and Banko 2009). The remaining extant nectarivorous honeycreeper species are mostly restricted to fragmented populations in montane forests above the zone (<1250 m) of avian disease-transmitting mosquitoes (Warner 1968, Fancy and Ralph 1998). The impacts of these declines are largely unknown in Hawai'i (for exceptions, *see* Carpenter 1976, Aslan et al. 2013). However, in New Zealand, an ecologically similar archipelago, the decline or loss of bird-pollinated plant species has been attributed to pollen limitation (Kelly et al. 2010 and references therein, Pattermore and Wilcove 2012, Pattermore and Anderson 2013), inbreeding depression (Schmidt-Adams et al. 2000, Robertson et al. 2011) and the demographic decline of plant populations due to cascade effects (the reciprocal decline or extinction of mutualist species) (Anderson et al. 2011).

Hawaiian lobeliads (Campanulaceae) are a lineage of trees, shrubs, herbs and caudiciforms, contained in six genera (Wagner et al. 1999). All but two of the 140 endemic species of Hawaiian lobeliads are believed to have been historically pollinated by nectarivorous honeycreepers and Hawaiian Mohoideae species (Lammers and Freeman 1986, R. Pender, unpublished data). Therefore, lobeliads comprise a significant (*ca.* 70% of species) component of the bird-pollinated flora. Approximately one-third of lobeliad species are extinct, while three-quarters of extant species are threatened with

extinction (Wagner et al. 1999).

Currently, restoration is being widely utilized to reestablish populations of Hawaiian lobeliads that are of conservation concern. Restoration plantings are often logistically challenging to undertake and can be expensive to initiate and subsequently manage (Duffy and Krauss 2006, Goldstein et al. 2008). Despite the resources that are invested into restoration of lobeliad and other endangered bird-pollinated species in Hawai‘i, there has been little research undertaken to identify the animals (e.g., native or introduced birds, insects) that might pollinate these restoration plantings (however, *see* Gardener and Daehler 2006).

This paper summarizes the results from a two-year pollination ecology study in restoration sites of four endangered Hawaiian lobeliad species. Two species, *Cyanea superba* subsp. *superba* (hereafter, *Cy. superba*) and *Delissea waianaensis* are being restored on the Wai‘anae Range, O‘ahu, where nectarivorous honeycreepers are largely absent. The second pair of species, *Clermontia lindseyana* and *Clermontia pyrularia*, are being restored on Windward Mauna Kea, Island of Hawai‘i, where three nectarivorous honeycreeper species are still common (Camp et al. 2010). Specifically, this study aimed to address three questions. First, are nectarivorous passerines visiting the flowers of the four lobeliad species? Second, are passerine species that visit the flowers of the four lobeliad species effective pollinators? Third, can diurnal insects function as effective pollinators? Forth, what are the breeding systems of the four lobeliad species?

Methods

Study species and floral biology

The four lobeliad species (*Cy. superba*, *D. waianaeensis*, *Cl. pyrularia* and *Cl. lindseyana*) are arborescent shrubs or small trees with tubular flowers (Figure 10) (Wagner et al. 1999). The distribution, floral characteristics, and the natural and restored population status of each species are summarized in Table 11. Like all Hawaiian lobeliads, all four species are protandrous with anthers and filaments fused forming a tube around the stigma and style (Wagner et al. 1999). Anthers emit pollen into the fused anther tube that is subsequently expelled out of the anther apex by the immature stigma lobes as the style elongates. Short, stiff hairs on the ventral side of the tips of the anthers aid in collecting the pollen, allowing the pollen to be brushed onto pollinators (Lammers 1991). Flowers enter the female phase when the style emerges from the anther tube and the stigma lobes enlarge and spread.

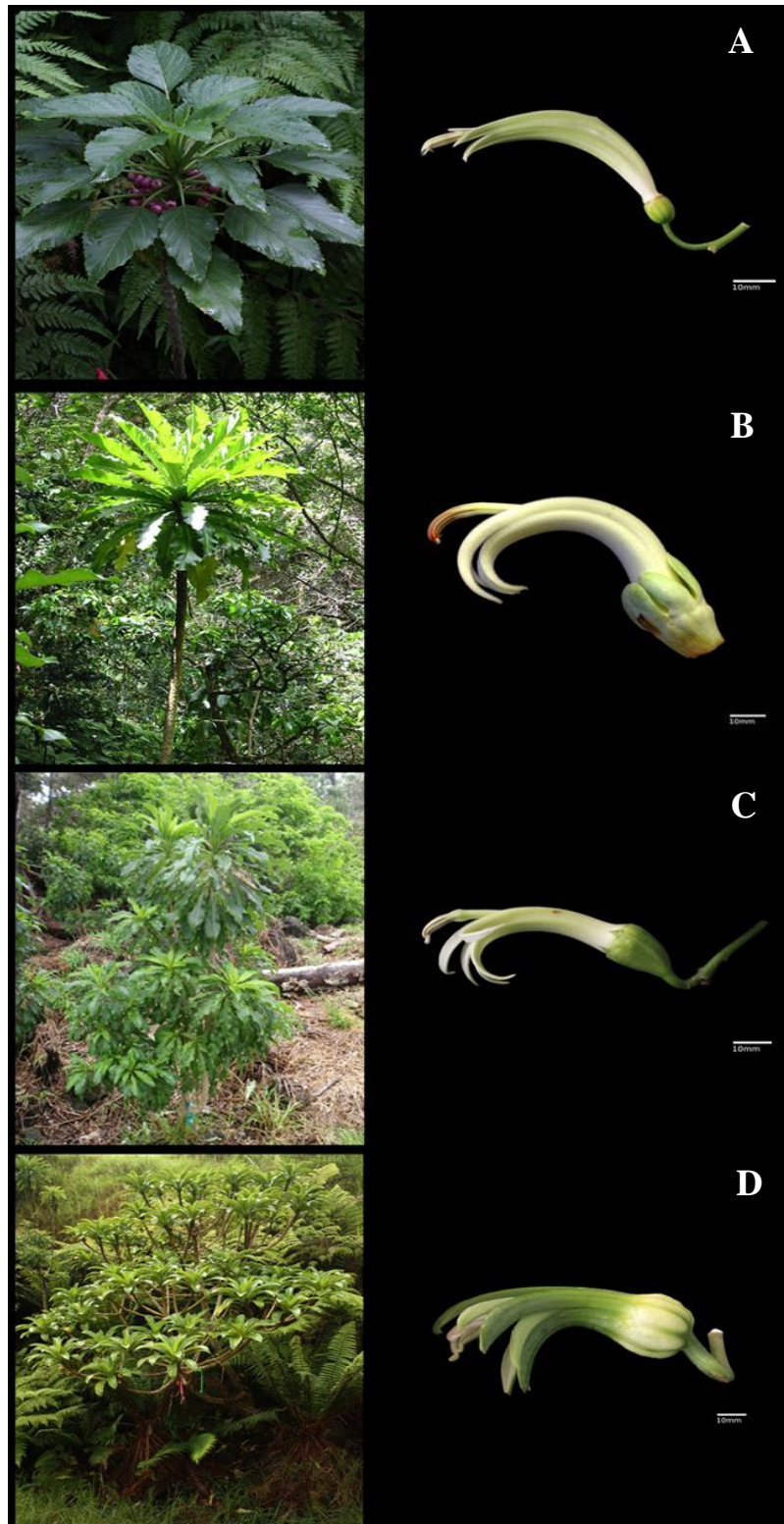


Figure 10. The four lobeliad species that were studied. **A:** *D. waianaeensis*, **B:** *Cy. superba*, **C:** *Cl. pyrularia*, **D:** *Cl. lindseyana*.

Table 11. Conservation status, floral and fruit characteristics and phenology of the four lobeliad species.

Taxon	Habitat/population status	Inflorescence type	Corolla dimensions	Corolla color	Fruit characteristics	Flowering and fruiting phenology
<i>Cl. lindseyana</i>	Small tree reaching six meters. Naturally occurs between 1220 and 1825 m on Haleakala, Maui and Mauna Loa and Mauna Kea, Hawai'i. ¹ Ca. 600 natural plants exist in wild. Restored plants: exact number unknown, however >1000 plants ²	2 flowered cymes ¹	Perianth: 55-65 mm long ¹	Corolla and sepal lobes of similar size. Perianth lobes green ¹	Berries, green to orange, 2.5-4 cm long and wide ¹	Flowering/fruiting: sporadically through year. Main flowering May-August ³
<i>Cl. pyrularia</i>	Small tree reaching 4 meters. Naturally occurs between 1585-2130 m on Windward Mauna Kea and Leeward Mauna Loa. ¹ 8 natural plants occur on Mauna Kea, Hawai'i. Ca. 2050 outplanted ²	2-5 flowered cymes ¹	Corolla curved, 40-45 mm long ¹	White, occasionally tinged green ¹	Berries, 1.5-2.4 cm, 1.8-2.8 cm wide. Green or orange ¹	Flowering: January-June. Fruiting: September-November ¹
<i>Cy. superba</i>	Monopodial stems reaching 6 m. Historically occurred in mid elevation forests in the Wai'anae Mountains. ⁴ Extinct in the wild since 2002. > 800 plants out-planted ⁵	Pendent, 5-15 flowered racemes. Peduncles 200-350 cm long ⁴	Corolla curved, 55-80 mm long ⁴	Corolla cream to white ⁴	Berries white/green, 16-22mm long, 10-16mm wide ⁴	Flowering: September to mid October. Fruiting: December and January ³
<i>D. waianaensis</i>	Small shrub reaching 3m. Occurs in mesic forests in the Wai'anae Mountains between 245 and 760 m. ⁴ Ca. 28 natural plants. Ca. 750 plants reintroduced. ²	5-12 flowered racemes ⁴	Corolla curved, 45-62 mm long ⁴	Corolla green with white streaks ⁴	Berries pink to purple, 7-16 mm long; 7-11 mm wide ⁴	Flowering: November to June. Fruiting between November and September ³

¹Lammers (1991), ² USFWS (2012), ³ R. Pender, unpublished data, ⁴ Wagner et al. (1999), ⁵ M. Keir, pers. comm. (O'ahu Army Natural Resources Program).

Study sites

This study was undertaken at individual study sites on two separate islands of the Hawaiian archipelago. Two study locations were utilized on O‘ahu. The Kahanahāiki Management Unit (21° 32' 29.05" N, 158° 11' 39.19" W; hereafter, Kahanahāiki), is a 36 hectare parcel in the northern Wai‘anae Mountains, O‘ahu that is managed by the O‘ahu Army Natural Resources Program (OANRP). A population of *D. waianaeensis* was also studied at the adjacent 266 hectare Pahole Natural Area Reserve (21° 32' 26.88" N, 158° 11' 26.97" W; hereafter Pahole), managed by the State of Hawai‘i, during the 2010 flowering season. Both sites occur at approximately 600 m elevation and have similar amounts of monthly rainfall (ca. 1200 mm; Giambelluca et al. 2013). The vegetation at both Kahanahāiki and Pahole is composed of mesic forest species. The native canopy species include *Metrosideros polymorpha* and *Acacia koa*, but alien trees are the canopy dominants and include *Psidium cattleianum*, *Psidium guajava*, *Aleurites moluccana*, *Schinus terebinthifolius* and *Grevillea robusta* (further site details are provided in Pender et al. 2013). Restoration plantings of *Cy. superba* and *D. waianaeensis* began in 1998 at Kahanahāiki and in 2000 at Pahole. Through the use of fencing and subsequent trapping, both sites have been ungulate free since 2000.

Hakalau National Wildlife Refuge (hereafter Hakalau) on Hawai‘i Island, is a 13,350 hectare montane wet forest located on the windward slopes of Mauna Kea, between 750 and 2,000 m elevation, managed by the U.S. Fish and Wildlife Service (USFWS 2013). The mean annual rainfall in the upper reaches of the refuge is ca. 2300 mm (Giambelluca et al. 2013). The lower section of the refuge is a natural *M. polymorpha* dominant forest. However, this study was undertaken in the upper reaches of

the refuge that were formerly ranchland and contain a sporadic canopy of old-growth *M. polymorpha* and *A. koa* trees under which grows a *Pennisetum clandestinum* dominated pasture containing regenerating native trees (*A. koa*, *M. polymorpha*, *Cheirodendron trigynum*) and shrubs (*Coprosma rhynchocarpa*, *Ilex anomala*, *Myrsine lessertiana*, *Rubus hawaiiensis* and *Vaccinium calycinum*). To supplement natural regeneration, wide-scale restoration of native canopy and understory forest species has been undertaken in this section of the refuge since the early 1990's (J. Jeffrey, pers. comm.). Ungulates were eradicated from the refuge between 1988 and 2002 (Hess et al. 2006), although occasional reinvasion episodes occur (J. Jeffrey, pers. comm.). Two restoration sites were used at the refuge for the duration of the study. The first was at a location in the Hakalau Unit of the Refuge known as Magnetic Hill (19° 48' 24.25" N, 155° 19' 38.98" W, 1897 m.) that contained an approximately 10-year-old restoration planting of mature *Cl. lindseyana* (J. Jeffrey, pers comm.). The second site was located in the Maulua Tract of the Refuge (19° 52' 05.07" N, 155 ° 18' 28.38" W, 1632 m) that contained mature restoration plantings (ca. 10 years old) of both *Cl. pyricularia* and *Cl. lindseyana*.

Bird pollinators present at the two study sites

Kahanahāiki and Pahole have very small populations of two honeycreepers, the O'ahu 'amakihi (*Hemignathus flavus*), that consume nectar as part of a generalist diet, and 'apapane (*Himatione sanguinea*), a specialist nectarivore (Banko and Banko 2009). Population size may be limited to one or a few individuals, thus both species are likely to be functionally extinct (present but unlikely to serve an important role as a pollinator) at both Kahanahāiki and Pahole (pers. obs). Also common at both sites is the alien Japanese

white-eye (*Zosterops japonica*) that consumes nectar as part of a generalist diet (Guest Van Ripper 2000).

Hakalau was primarily established for the protection of Hawaiian forest birds (Camp et al. 2010). Two nectar specialist honeycreepers, 'i'iwi and 'apapane, and a generalist that consumes nectar as part of its diet, the Hawaiian 'amakihi, are common at Hakalau (Camp et al. 2010). The alien Japanese white-eye is also common at the refuge (Camp et al. 2010).

Pollination studies

At Kahanahāiki, 36 and 32 plants of *Cy. superba* and 18 and 26 plants of *D. waianaeensis* were monitored during the 2009 and 2010 field seasons, respectively. To increase the sample size for *D. waianaeensis*, an additional 122 plants were monitored at Pahole during 2010. At Hakalau, 38 plants of *Cl. pyrularia* were monitored during the 2009 and 2010 flowering seasons. For *Cl. lindseyana*, 31 plants were monitored at Magnetic Hill during the 2009 season. Due to a summer drought in 2010, only nine plants flowered at Magnetic Hill. Therefore, an additional eight plants were monitored at the Maulua Tract at the same location as the *Cl. pyrularia* population.

Nine days (see below) of vertebrate and invertebrate floral visitor observations were undertaken in each of the two flowering seasons for each lobeliad species. These observation periods were further divided into three separate three-day periods each year, undertaken throughout the flowering season of each species. The number of open flowers on the plants that were being monitored for pollinator activity was recorded during each observation day.

Avian floral visitors were monitored between 7:00 am and 5:00 pm. Observations were conducted during 20-minute observation periods followed by short intervals to allow for travel between observation stations. Observations were conducted from no less than ten meters from each focal plant, with up to five plants observed from each monitoring station. Observers wore army camouflage clothing using binoculars to view flower visitors, record the total time a bird spent physically on the plant (both visiting flowers and otherwise), number of flowers visited, and whether a flower visitor nectar robbed.

Insect visitation was recorded between 8:00 am and 5:00 pm during ten-minute observation periods, followed by a short interval to travel between plants. Between three and five flowers were randomly selected on an individual plant at the beginning of each observation period and monitored by standing within two meters of the focal plant. The total duration of insect contact with anthers and stigmas was timed using a stopwatch. Observers also recorded the behavior of the insect while visiting sexual organs, (e.g., whether the insect harvested pollen). Insect movement on other floral organs was not recorded, except where visually confirmed cases of nectar robbing took place. Representative insect specimens were collected and later identified by staff at The University of Hawai‘i Insect Museum, College of Tropical Agriculture and Human Resources.

Pollination treatments

Manipulative pollination treatments were undertaken on each of the four species for each of the study seasons. Six pollination treatments were utilized (Table 12).

However, due to the limitation of available flowers, the application of treatments varied. For all four species, three flowers were randomly selected from each plant for each treatment during the course of the flowering season, from which a subsample of maturing fruit were randomly selected. Flowers in each treatment were identified using specific colors of craft wire for each pollination treatment.

Because not all plants that were monitored for pollinator visitation produced sufficient quantities of flowers for the pollination treatments, a subsample of plants were utilized. Twenty-one and 30 plants of *Cy. superba* at Kahanahāiki were used for the pollination treatments in 2009 and 2010, respectively. Twelve and 11 plants of *D. waianaeensis* at Kahanahāiki had pollination treatments applied to them in the 2009 and 2010 seasons, respectively. Because of the large number of *D. waianaeensis* plants at Pahole, a subsample of 15 plants was randomly selected for the pollination treatments in 2010. To test for pollen limitation, 10 plants of *D. waianaeensis* were utilized in 2012 at Kahanahāiki. At Hakalau, 30 plants of *Cl. pyrularia* were used in both field seasons. For *Cl. lindseyana*, 30 plants were used at Magnetic Hill during the 2009 season. Nine plants at Magnetic Hill and an additional eight plants at the Malua Tract were utilized for the pollination treatments in 2010.

Table 12. Manipulative pollination treatments utilized in the study.

Treatment name	Purpose	Description of treatment
Control	To assess how the plants reproduce naturally	Flowers were selected and left unmanipulated throughout the flowering period
Self	To test if the plants are self-compatible	Flowers were bagged using bridal-veil pollinator exclusion bags (1 × 1 mm mesh) held in place by small zip-ties. Bags were removed following flower senescence
Emasculation open	To assess if floral visitors are effective pollinators	Flowers were emasculated shortly after bud break using a razor blade. Flowers were left to pass through to the female phase to allow pollinators to visit
Emasculation Bagged	To assess levels of self pollen contamination following emasculation	Flowers were emasculated shortly after bud break using a razor blade. Flowers bagged to prevent pollinator access to the flowers.
Plus pollen-self	To assess pollen limitation using self pollen	Mature stigmas of female-phase flowers were supplemented with pollen collected from male-phase flowers from the same plant. Pollen was collected in a 1.5 ml micro-centrifuge tube and applied to the stigma by dipping the stigma in the pollen cocktail
Plus pollen-outcross	To assess pollen limitation using out-cross pollen	The same procedure as the plus self pollen treatment, except that pollen was collected from male-phase flowers from three or more alternative plants

The control, self, and both emasculation treatments were applied to flowers of *Cl. pyrularia*, *Cy. superba* and *D. waianaensis* during both field seasons and *Cl. lindseyana* during the 2009 flowering season. Both pollen supplementation treatments were applied to *Cl. pyrularia* and *Cy. superba* during the 2010 field season. Due to a drought that limited flowering of *Cl. lindseyana* in 2010, only the control, emasculation open, and plus self pollination treatments could be applied. To test if pollen limitation occurs in *D. waianaensis*, the control and the two pollen supplementation treatments were undertaken during the 2012 flowering season at Kahanahāiki.

Fruit harvesting and seed processing

Immature fruits were checked within one month of applying the pollination treatment to assess the abortion rate. As fruit matured, a subsample of one fruit from each treatment was collected from each plant that had been selected for the pollination treatments. Seeds were extracted from fruits of *Cy. superba*, *Cl. lindseyana* and *Cl. pyrularia* by smearing the fruit pulp onto paper to expose the seeds followed by seed collection. Fruits of *D. waianaeensis* were initially cut open using a razor blade and their seeds extracted using a dental pick. Seeds of all species were stored in coin packets and initially dried at room temperature for at least 3 days before being weighed and counted. In all cases only well-formed, plump seeds, an indicator of seed viability in lobeliad species (Cory 1984), were counted. The seeds were subsequently returned to their respective collection sites for dispersal.

Statistical analysis

Seed count data from the pollination treatments were first tested to assess if the distributions meet parametric assumptions. Two sample *t* tests were subsequently performed to assess if significant differences existed between years in each pollination treatment in each lobeliad species. Data from both years was combined in cases where no significant differences were detected. In addition, because there were no significant differences in the mean seeds per fruit in the two pollen addition treatments (plus pollen-self and plus pollen out-cross) the data from the two treatments were combined and are hereafter identified as one treatment (plus pollen) in each species. ANOVA's followed by Tukey's Honest Significant tests were conducted for each species. To assess if

differences existed in fruit abortion rates, between-year fruit formation rates were compared in each treatment in each species using Mann-Whitney *U* tests. If no significant differences were detected, the data were subsequently pooled in each treatment. Because the distributions for the pollination treatments in each species did not conform to parametric assumptions, Kruskal-Wallis tests followed by pair-wise Mann-Whitney *U* tests were used to assess differences between the pollination treatments. Unless stated otherwise, all means are presented \pm 1 S.D. All analyses were conducted in Minitab 16.

Results

Bird visitation

O‘AHU – In total, 67 and 51 hours of observations were undertaken for *Cy. superba* during 2009 and 2010, respectively. For *D. waianaeensis*, 82 and 54 hours of observations were undertaken at Kahanahāiki in 2009 and 2010, respectively, and 51 hours at Pahole during 2010. At both restoration sites, Japanese white-eyes were the only birds to visit the flowers of each lobeliad species, nectar robbing 100% of *Cy. superba* flowers and 99% of *D. waianaeensis* flowers (Figure 11). The flowers of *Cy. superba* were accessed at their bases via the cleft in the upper part of the corolla. In *D. waianaeensis*, the birds wedged their bills in the upper side of the corolla, which resulted in distinctive tears in the corolla tube in older, female-phase flowers (Figure 12). Japanese white-eye made fewer visits to *Cy. superba* flowers in 2009 compared to the 2010 flowering season ($n = 21$ and 68 , respectively). During both years, the mean number of flowers contacted per visit on each plant was 5 ± 4 (1 S.D). The mean time spent visiting each plant was 49 ± 48 seconds. Birds made no visits to the *D. waianaeensis* plants in 2009 at Kahanahāiki. Birds made only four visits to the plants at Kahanahāiki

(only one visit resulted in flower contact) and 30 visits to the plants at Pahole in 2010. On average 4 ± 2 flowers were visited per plant per visit. The mean duration of time spent visiting each plant was 66 ± 75 seconds.

Hakalau – A total of 57 hours of observations for *Cl. lindseyana* were conducted during 2009. In the 2010 field season, 145 and 125 hours of observations were undertaken at the Magnetic Hill and Maulua sites, respectively. For *Cl. pyrularia*, 51 and 77 observation hours were conducted in 2009 and 2010, respectively. ‘I‘iwi and Hawai‘i ‘amakihi (hereafter ‘amakihi) were the most frequent visitors to both *Cl. lindseyana* and *Cl. pyrularia*.

‘I‘iwi made approximately twice as many visits as Hawai‘i ‘amakihi to the flowers of *Cl. lindseyana*. However, both bird species were infrequent visitors (Figure 13). Both species primarily nectar robbed the flowers (‘i‘iwi: 60%, ‘amakihi: 91%). ‘I‘iwi did so by either drilling a hole at the base of the perianth or by wedging the perianth lobes apart using their bills (Figure 12). ‘Amakihi almost always nectar robbed the flowers by inserting their bills through the base of the perianth from either the upper surface or the side (Figure 12). During both years, on average, ‘i‘iwi visited 4 ± 3 flowers while ‘amakihi visited 3 ± 3 flowers per plant. The mean duration of visits to each plant lasted 86 ± 63 seconds for ‘i‘iwi and 72 ± 72 seconds for ‘amakihi.

For *Cl. pyrularia*, 52% and 47% of the total flowers visited by birds were by ‘i‘iwi and ‘amakihi, respectively. A single ‘apapane made one visit (< 1% of all flowers visited). Figure 13 illustrates the mean hourly visitation rate of ‘i‘iwi and ‘amakihi to the flowers of *Cl. pyrularia*. Both species also nectar robbed the flowers of *Cl. pyrularia* (‘i‘iwi: 32%; ‘amakihi: 66%), primarily by inserting their bills into the base of the cleft

on the upper surface of the corolla. During both years, on average, ‘i‘iwi visited 7 ± 4 flowers, while ‘amakihi visited 7 ± 6 flowers per plant during each visit. The mean duration of visits to each plant lasted 33 ± 33 seconds for ‘i‘iwi and 50 ± 72 seconds for ‘amakihi.

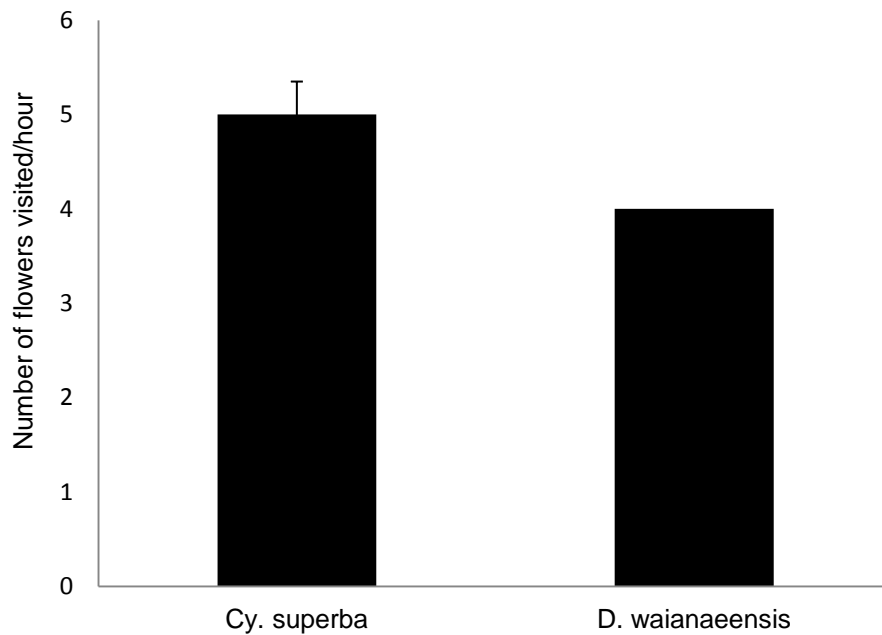


Figure 11. Mean (± 1 S.E.) *Cy. superba* and *D. waianaensis* flowers visited by Japanese white-eye per observation hour. The mean (± 1 . S.D.) number of *Cy. superba* flowers available to visit per observation hour was 79 ± 76 . The mean (± 1 . S.D.) number of *D. waianaensis* flowers available to visit per observation hour was 75 ± 78 .



Figure 12. Damage to the flowers by nectar robbing birds. (A) *Delissea waianaeensis* flowers damaged by Japanese white-eye. Note the tear made in the upper corolla tube to access the nectar. (B) *Clermontia lindseyana* flowers damaged by 'amakihi and/or 'i'iwi. Note the hole made by their bills at the base of the perianth (arrow). 'I'iwi also wedge their bills between the perianth lobes. In many cases, the sepals may fall off from the repeated visitation (as has happened here).

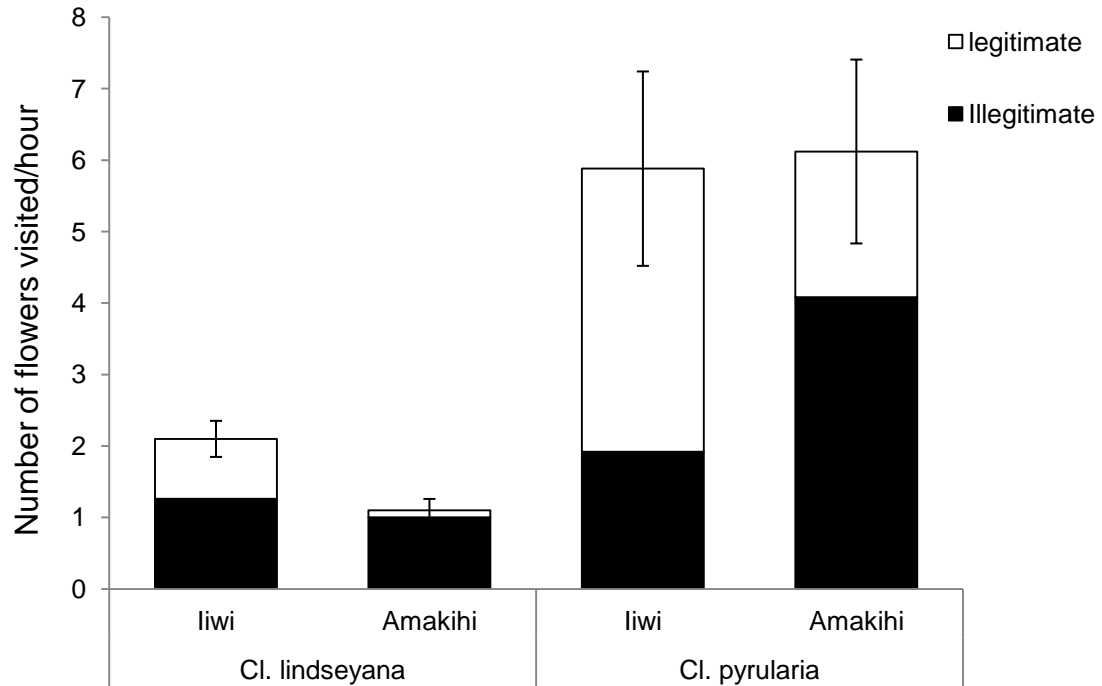


Figure 13. Mean (± 1 S.E.) *Cl. lindseyana* and *Cl. pyrularia* flowers visited by ‘i‘iwi and ‘amakahi per observation hour. The mean (± 1 S.D.) number of *Cl. lindseyana* flowers available to visit per observation hour was 28 ± 20 . The mean (± 1 S.D.) number of *Cl. pyrularia* flowers available to visit per observation hour was 119 ± 46 .

Insect visitation

Observations of insect visitations were made for *Cy. superba* for 41 and 51 hours during 2009 and 2010, respectively. For *D. waianaeensis*, 69 and 56.5 hours of observations were undertaken at Kahanahāiki in 2009 and 2010, respectively, and 54.5 hours at Pahole during 2010. Of the three insect visitors that visited *Cy. superba* flowers for floral (pollen or nectar) rewards, the native yellow-faced bee, *Hylaues connectens*, was the most frequent visitor ($n = 121$ flowers visited, 93% of all flowers contacted by insects), predominantly visiting the anthers (92% of flower visits) to harvest pollen. The bees primarily contacted one flower per visit (98% of visits). The average time spent during each flower visit was 52 ± 107 seconds. The remaining visits were made by the

introduced hoverfly (*Allograpta obliqua*; 7 flowers, 5% of all flowers contacted by insects) and carpenter bees (*Xylocopa sonora*; 2 flowers, 2% of all flowers contacted by insects) (Figure 14). These same three insects and honeybees (*Apis mellifera*) visited the flowers of *D. waianaeensis*. *Hylaeus connectens* was the most frequent visitor (27 flowers; 59% of all flowers contacted by insects). Again, as for *Cy. superba*, the bees predominantly visited anthers (78% of all flowers) to forage for pollen. *Allograpta obliqua* (10 flowers, 22% of all flowers contacted by insects) was the next most common visitor followed by *Xylocopa sonora* (n = 5, 11% of all flowers contacted by insects) and *Apis mellifera* (n = 4, 9% of all flowers contacted by insects) (Figure 14).

At Hakalau, 46 hours of observations for *Cl. lindseyana* were conducted during 2009. In the 2010 field season, 50 and 53 hours of observations were undertaken at the Magnetic Hill and Maulua sites, respectively. For *Cl. pyrularia*, 44 observation hours were undertaken in 2009 and 53 hours in 2010. Insect visitors to the flowers of *Cl. lindseyana* and *Cl. pyrularia* were extremely rare. Two unidentified *Hylaeus* species visited the flowers of *Cl. lindseyana*; the first contacting the reproductive organs on two flowers and the second contacting five flowers. The only insects to actively visit the reproductive organs of *Cl. pyrularia* flowers were honeybees, who visited five flowers during the two years of observations.

Pollination treatments

Generally, all four species had similar trends in the mean quantity of seeds produced within each of the six treatments (Table 13). All four species were significantly pollen limited; the plus pollen treatments (both self and outcross) had greater mean seeds

per fruit than the other treatments in all four species (p values are reported in Figures 15-18). There were no significant differences in mean seeds per fruit between the control and self treatments in each of the four lobeliad species. Similarly, there were no significant differences in mean seeds per fruit when comparing the emasculation open and bagged treatments in *D. waianaeensis*, *Cl. lindseyana* and *Cl. pyrularia*, although there was a significant difference in mean seeds per fruit between these two treatments in *Cy. superba*. Lastly, there were no significant differences in mean seeds per fruit when comparing control/self treatments and the two emasculation (open and bagged) treatments in *Cy. superba*, *Cl. lindseyana* and *Cl. pyrularia*. However, there was a significant difference in mean seeds per fruit when comparing these treatments in *D. waianaeensis*.

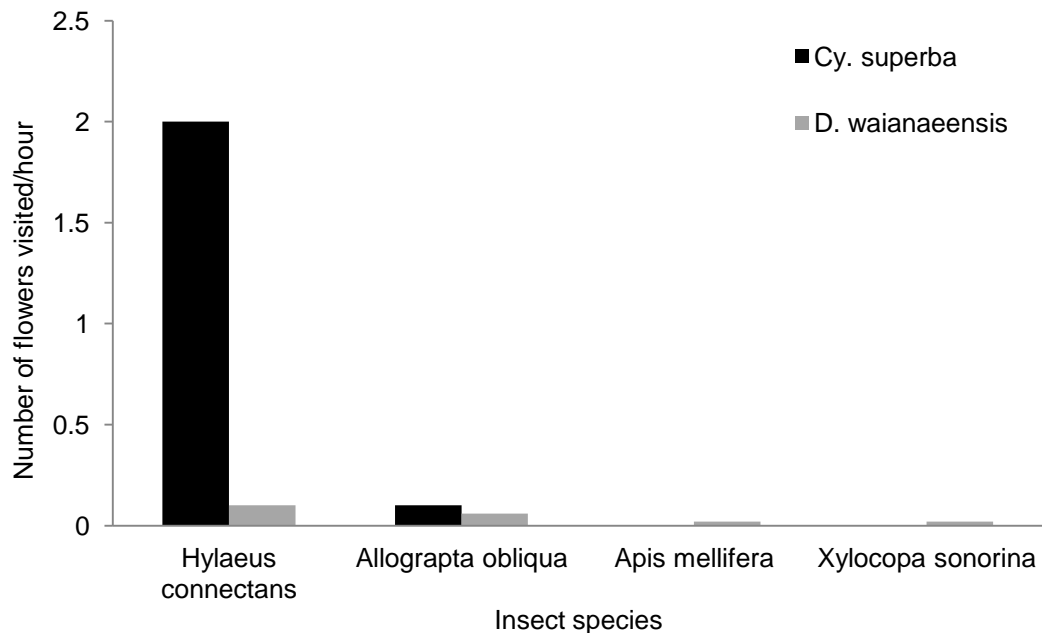


Figure 14. The mean (± 1 S.E.) number of *Cy. superba* and *D. waianaeensis* flowers visited per observation hour by insect species. The mean (± 1 S.D) number of *Cy. superba* flowers that were available to visit per observation hour was 36 ± 12 . The mean (± 1 S.D) number of *D. waianaeensis* flowers that were available to visit per observation hour was 30 ± 4 .

Table 13. Mean (\pm 1 S.D.) seeds per fruit in the five treatments for the four lobeliad taxa. Seed data was combined from both field seasons.

Taxon	Control	Self	Emas. open	Emas. bagged	Plus Pollen
<i>D. waianaeensis</i>	133 \pm 109 n = 41	134 \pm 114 n = 35	38 \pm 55 n = 33	32 \pm 14 n = 8	217 \pm 101 n = 11
<i>Cy. superba</i>	153 \pm 92 n = 52	163 \pm 103 n = 42	48 \pm 76 n = 43	12 \pm 21 n = 35	255 \pm 80 n = 40
<i>Cl. lindseyana</i>	1046 \pm 322 n = 47	1108 \pm 534 n = 30	1125 \pm 973 n = 17	840 \pm 420 n = 12	2635 \pm 1162 n = 17
<i>Cl. pyrularia</i>	147 \pm 92 n = 60	195 \pm 136 n = 60	81 \pm 44 n = 22	41 \pm 25 n = 14	291 \pm 140 n = 60

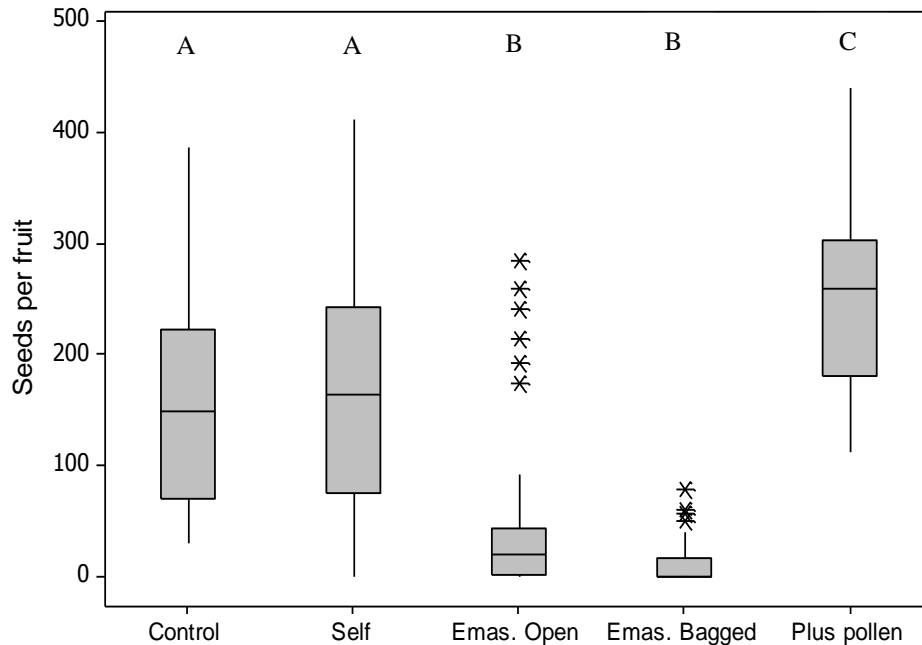


Figure 15. The distribution of *Cy. superba* seed counts resulting from five pollination treatments. Each outlier symbol represents an individual fruit. There were no significant differences in the seed counts between the control and self treatments or between the emasculatation open and emasculatation bagged treatments. However, there were significant differences when comparing the self/control treatments and the two emasculatation treatments ($F = 36.25$, $P = <0.001$). Fruits in the plus pollen treatment produced significantly ($F = 55.08$, $P = <0.001$) more seeds when compared to all other pollination treatments. Letters A-C indicate cases of statistical significance between the treatments.

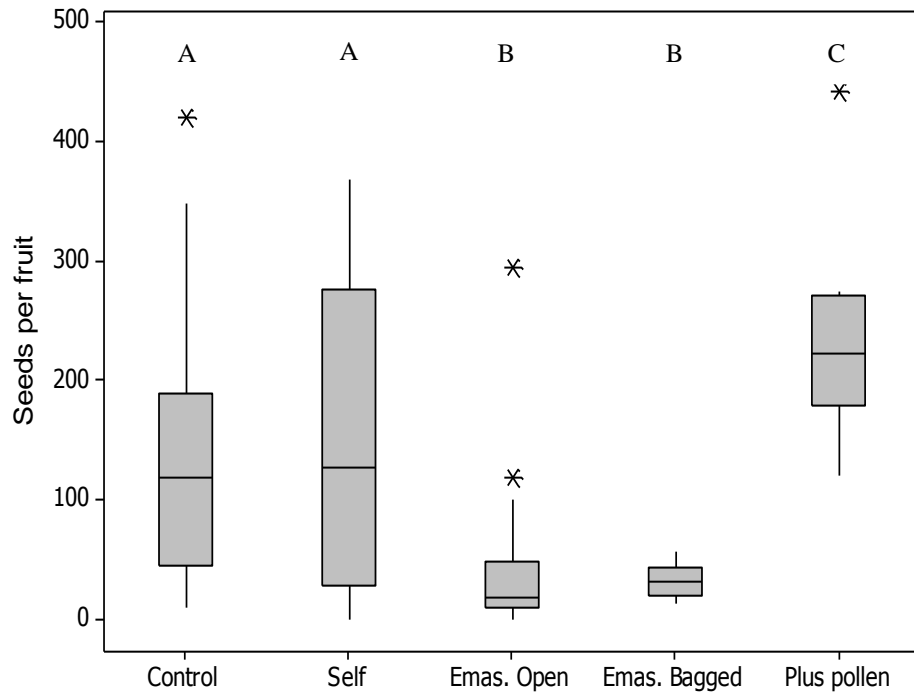


Figure 16. The distribution of *D. waianaensis* seed counts resulting from five pollination treatments. Each outlier symbol represents an individual fruit. There were no significant differences in the seed counts between the control and self treatments or between the emasculaton open and emasculaton bagged treatments. However, there were significant differences when comparing the self/control treatments and the two emasculaton treatments ($F = 10.27$, $P = <0.000$). Fruits in the plus pollen treatment produced significantly ($F = 12.42$, $P = <0.000$) more seeds when compared to all other pollination treatments. Letters A-C indicate cases of statistical significance between the treatments.

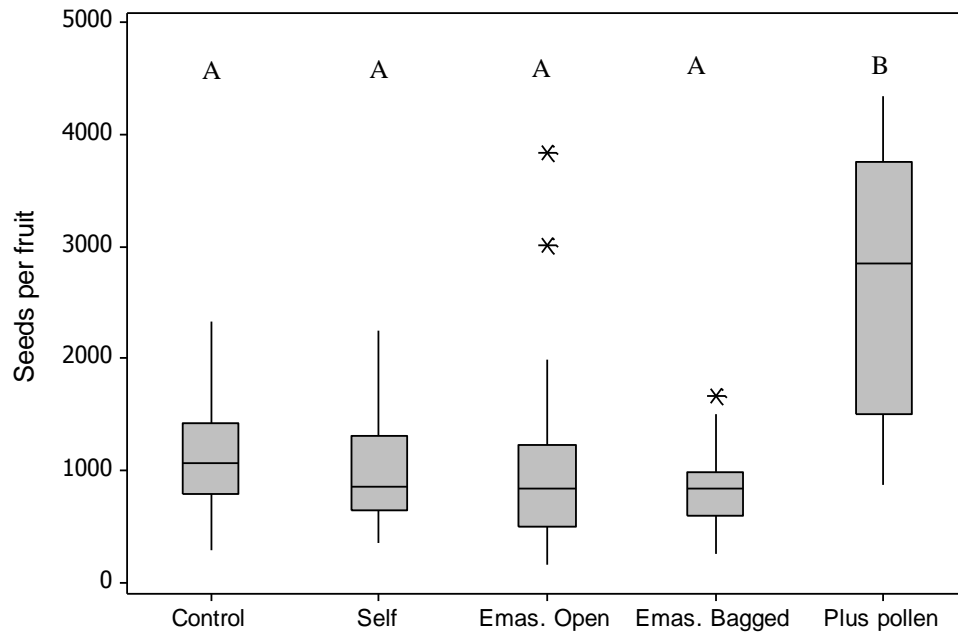


Figure 17. The distribution of *Cl. lindseyana* seed counts resulting from five pollination treatments. Each outlier symbol represents an individual fruit. Fruits in the plus pollen treatment produced significantly ($F = 18.84$, $P = <0.000$) more seeds when compared to all other pollination treatments. Letters A and B indicate cases of statistical significance between the treatments.

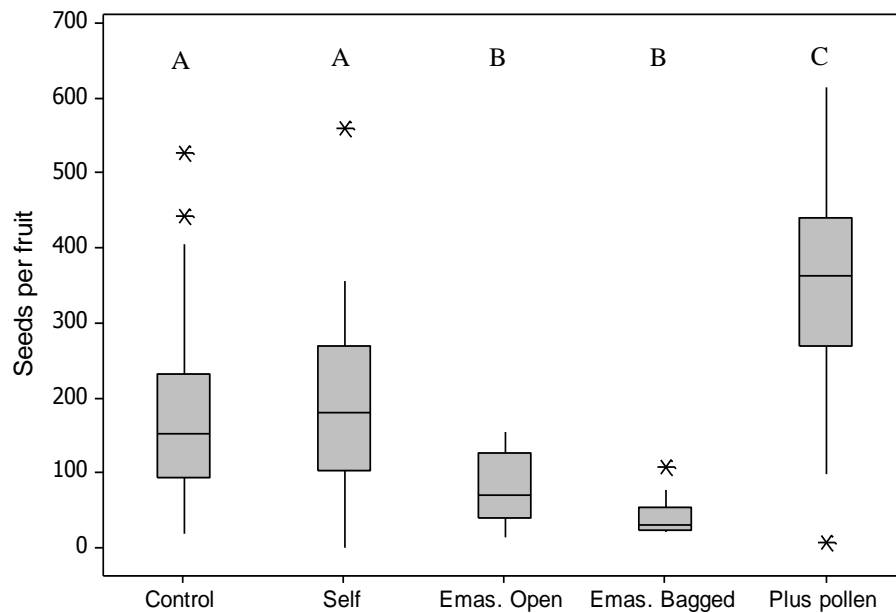


Figure 18. The distribution of *Cl. pyrularia* seed counts resulting from five pollination treatments. Each outlier symbol represents an individual fruit. There were no significant differences in the seed counts between the control and self treatments or between the emasculaton open and emasculaton bagged treatments. However, there were significant differences when comparing the self/control treatments and the two emasculaton treatments ($F = 12.11$, $P = <0.000$). Fruits in the plus pollen treatment produced significantly ($F = 42.21$, $P = <0.000$) more seeds when compared to all other pollination treatments. Letters A-C indicate cases of statistical significance between the treatments.

Frequency of fruit formation

Table 14 summarizes the mean fruit formation frequency for the five pollination treatments in each of the four lobeliad species. For each respective species, there was no significant difference when comparing the control, self and plus pollen treatments or between the emasculaton open and emasculaton bagged treatments. However, there was a significant difference in the frequency of fruit formation between the control, self and plus pollen treatments when compared to both emasculaton treatments in the (Kruskal-Wallis test results: *D. waianaeensis*: $H = 89.59$, 4 d.f., $P = <0.001$; *Cy. superba*: $H = 28.86$ 4 d.f., $P = 0.000$; *Cl. lindseyana*: $H = 88.05$ 4 d.f., $P = <0.001$; *Cl. pyrularia*: $H =$

= 187.34 4 d.f., $P = <0.001$).

Table 14. Proportion of fruit (mean \pm 1 S.D.) that formed in five manipulative pollination treatments in each of the four lobeliad species.

Taxon	Control	Self	Emas. open	Emas. bagged	Plus pollen
<i>D. waianaeensis</i>	0.70 \pm 0.21 n = 95	0.67 \pm 0.19 n = 90	0.45 \pm 0.21 n = 61	0.39 \pm 0.18 n = 53	0.74 \pm 0.15 n = 132
<i>Cy. superba</i>	0.75 \pm 0.19 n = 115	0.75 \pm 0.14 n = 115	0.66 \pm 0.18 n = 101	0.65 \pm 0.11 n = 100	0.79 \pm 0.18 n = 144
<i>Cl. lindseyana</i>	0.69 \pm 0.27 n = 98	0.74 \pm 0.22 n = 72	0.22 \pm 0.26 n = 31	0.24 \pm 0.21 n = 22	0.73 \pm 0.21 n = 37
<i>Cl. pyrularia</i>	0.67 \pm 0.26 n = 121	0.66 \pm 0.29 n = 119	0.20 \pm 0.18 n = 34	0.13 \pm 0.16 n = 23	0.80 \pm 0.17 n = 145

Discussion

The restoration of endangered plant species as a means to promote their recovery has become a widely adopted tool by conservation practitioners. Yet, despite the wide-scale use of restoration, few studies have utilized plant-pollinator interactions as a measure of restoration success (Allen-Wardell 1998, Dixon 2009). Several lines of evidence suggest that neither birds nor insects were wholly effective as pollinators in each of the four lobeliad species studied here. First, birds and insects made infrequent visits to the flowers of the four lobeliad species. Second, the similar mean seed counts in the control and self-pollination treatments in contrast to the few cases of pollination recorded in the emasculation open treatment in each of the lobeliad species suggests that animal-assisted pollination events are rare. The four species appear to have evolved a mixed-mating system whereby autogamy acts to complement animal-assisted pollination. In the absence of pollinator activity, all four species are reproducing primarily by

autogamy and are significantly pollen limited.

Despite native and introduced passerines visiting the restoration plantings of each of the lobeliad species, none appear to be effective pollinators. First, Japanese white-eyes nectar robbed nearly all of the *Cy. superba* and *D. waianaeensis* flowers they visited. The birds tear the corolla tube of *D. waianaeensis* at its upper cleft, causing distinct damage. However, the cleft in the upper surface of *Cy. superba* flowers allowed the birds to access nectar without causing damage to the flowers. Japanese white-eye may nectar rob the flowers of *D. waianaeensis* and *Cy. superba* as both species have relatively long corollas (> 40 mm) that likely exceed the average bill lengths (14.4 ± 0.9 mm, $n = 30$, R. Pender, unpublished data) of these birds. From the results of this and other studies in Hawai‘i, Japanese white-eye appear to potentially act as both pollinators (Cox 1983, Drake and Morden unpublished, Aslan et al. *in press*) and nectar robbers (Drake and Morden unpublished, Elmore 2008) for Hawai‘i’s native bird pollinated flora. The floral morphology of the specific plant species (e.g., inflorescence size, corolla length) determines how Japanese white-eyes approach the flowers (R. Pender, unpublished data).

Despite the fact that ‘i‘iwi visited both *Cl. pyrularia* and *Cl. lindseyana* at Hakalau, their low rates of visitation, and the fact that they nectar-rob a large proportion of the flowers that they visited of both species (32% and 60%, respectively), likely limits their effectiveness as pollinators of either species. Although ‘i‘iwi reach their highest densities in the archipelago at Hakalau, they are still relatively rare at this site (Camp et al. 2010). The birds’ population density, and the rarity of both lobeliad species across the landscape, limits the potential that ‘i‘iwi may interact with either lobeliad species. In addition, the presence of other bird pollinated species (namely; *M. polymorpha*, *Rubus*

hawaiiensis and the invasive *Passiflora tarminiana*), which are common throughout the areas where lobeliad restorations are being undertaken (pers. obs), may effectively support the populations of ‘i‘iwi at the refuge. This may limit the need for ‘i‘iwi to seek out novel food resources. As bird and lobeliad population densities increase at Hakalau, so to may the role that ‘i‘iwi play as pollinators as they adapt to the presence of this presently novel food source.

Hawai‘i ‘amakihi primarily nectar rob the flowers of both *Cl. pyrularia* and *Cl. lindseyana* at Hakalau and appears to be ineffective as a pollinator of either species. The corolla dimensions of both *Cl. pyrularia* and *Cl. lindseyana* are likely to be too large for Hawai‘i ‘amakihi to visit legitimately (i.e., forage for nectar while also contacting the reproductive organs) on all occasions (see chapter two). Although the birds did visit *Cl. pyrularia* flowers legitimately on rare occasions (Figure 15), they often did not make contact with the reproductive organs with their heads (pers. obs.). The birds nectar robbed almost all of the *Cl. lindseyana* flowers that they visited, leaving distinctive holes at the base of the perianth. Similarly, a recent study of *Cl. kohalae*, endemic to Kohala Mountain, has also found that Hawai‘i ‘amakihi in all cases nectar robbed the flowers of this species (R. Pender, unpublished data). Drake and Morden (unpublished) conducted studies that found that the closely related Kauai ‘amakihi nectar robbed the flowers of *Cl. fauriei* and *Trematolobelia kauaiensis* in a manner similar to that observed here, yet they visited the densely-held flowers of *Cy. leptostegia* legitimately. Aslan et al. (2013) found similar results to those of Drake and Morden (unpublished) where Hawai‘i ‘amakihi nectar robbed the flowers of *Cl. montis-loa* and *Cl. hawaiiensis* but legitimately visited the dense inflorescences of *Cy. strictophylla*. Like Japanese white-eye, a clearer picture

of the role that these birds play as pollinators for a broader range of bird pollinated plant species is needed in Hawai‘i.

Insects are not effective substitute pollinators of *Cl. pyrularia* and *Cl. lindseyana*. However, *H. connectens*, and to a lesser extent other insects (e.g., *A. obliqua*), may be responsible for the potential cases of pollination recorded in *Cy. superba* and *D. waianaeensis*. Gardener and Daehler (2006) also recorded *H. connectens* visiting *Cy. superba* flowers. *Hylaeus connectens* and *A. obliqua* appear to visit the flowers primarily to forage on pollen from male phase flowers, but occasionally contact stigmas. The pollination treatments utilized in our study could not distinguish among pollination events carried out by birds or insects. However, given that both lobeliad species were only visited by Japanese white-eye that nectar robbed the flowers in all cases, it seems more likely that these insects are responsible for the rare pollination events recorded in this study.

The production of seeds in the self treatment indicates that all four species are capable of autogamy. Baker's Rule (Baker 1955, 1967) suggests that self-compatible species are better adapted to colonizing remote islands. Other studies of the breeding systems of Hawaiian lobeliads (Cory 1984, Aslan et al. 2013, Pender, unpublished data) found that a further six species are autogamous. It is unknown what role selection has played on the breeding system of these species following the declines of endemic bird pollinators in Hawai‘i. However, it seems likely that selection would favor strongly autogamous individuals. It is possible that this mechanism slowed the decline of the four species in this study, and probably other lobeliads, allowing them to persist (in some cases precariously) in the absence of natural pollinators.

Despite all four species examined here being capable of autogamy, the pollen supplementation treatments indicate that each species was significantly pollen limited. These results highlight two key points. First, they support the visitation data and that of the remaining four pollination treatments that suggest that none of the floral visitors are wholly effective pollinators of each of the lobeliad species. As a consequence, all four species were pollen limited in the second (and likely both) field season(s). Secondly, they suggest that each of the species employs a mixed-mating system, whereby not all of the ovules are fertilized by self pollen that was attached to the stigma as it emerged from the anther. How this mechanism works is not yet known. However, the fact that there was no significant difference in seed set between self and out-cross pollen supplementation in each species suggests that it is unlikely to be due to a late-acting self-incompatibility mechanism. Detailed studies, such as those of Nyman (1992) and Bertin and Sullivan (1988) are needed to better understand the mixed mating systems in Hawaiian lobeliads.

Future directions for research

Restoration is likely to remain a pivotal component of recovery plans for endangered bird pollinated plants in Hawai‘i in the future. The results of this study pose challenging questions for the restoration of endangered ornithophilous plant species in Hawai‘i in the future. First, in the absence of effective pollinators, does pollen limitation lead to seedling limitation and population decline? Field based research similar to Anderson et al. (2011), who compared seedling recruitment of a bird pollinated shrub in New Zealand where birds were present and functionally extinct, would greatly benefit our understanding of these processes. Second, gene flow between the plants is likely to be

low, with each plant genetically isolated from the next. Autogamy may partially compensate although it may also be leading to inbreeding depression. How inbreeding impacts these and other lobeliad species remains to be addressed, but has been shown to affect other bird pollinated island-endemic plant species (Robertson et al. 2011). Future studies that assess out-crossing rates and population level gene flow (e.g., Byrne et al. 2007, Krauss et al. 2009) would provide useful insights regarding the effectiveness of floral visitors as pollinators. Third, substitute pollinators (primarily the Japanese white-eye) were not effective in the species studied, yet have been shown to be effective for other Hawaiian bird pollinated species (Cox 1983, Drake and Morden unpublished, Aslan et al. 2013). Identifying those plant species that are most vulnerable to pollinator loss may be a useful first step for their future restoration. Finally, the effects of global climate change on current bird and plant distributions in Hawai‘i have received some research attention (Benning et al. 2002, Hobbelen et al. 2012, Rock et al. 2012). Understanding how climate change may impact pollination mutualisms in Hawai‘i in the future may allow conservation practitioners to implement effective mitigation measures.

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CHAPTER SIX:
CONCLUSIONS

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The Hawaiian lobeliads are composed of six genera and 140 species (taxonomy follows Lammers 2007 ab, Lammers 2009) of herbs, shrubs, trees and stout caudiciforms (Givnish et al. 2009). *Clermontia*, the second largest genus of Hawaiian lobeliads, is composed of 22 species and nine subspecies of shrubs and small trees found in mesic and wet forests between 150 and 2100 m elevation on the main Hawaiian Islands (Lammers 1991). *Clermontia* species have little interspecific variation in vegetative characters. However, floral characters vary widely among taxa, particularly for corolla size and calyx arrangement that may either be reduced (sepaloid) or petal-like (petaloid; Lammers 1991, 1995). All *Clermontia* taxa produce flowers congruent with a passerine pollination syndrome (Lammers and Freeman 1986), and are believed to have been historically pollinated by now mostly endangered or extinct nectarivorous Hawaiian honeycreepers and Mohoidae (Lammers and Freeman 1986).

The series of studies presented here broadly addressed the floral trait evolution and the pollination ecology of species of *Clermontia*, and to a lesser extent, other Hawaiian lobeliad taxa. The studies were:

1. Chapter two summarized a study of the floral nectar sugar characteristics of 21 *Clermontia* taxa.
2. Chapter three assessed potential interactions between extant nectarivorous passerines and Hawaiian lobeliad species in the genus, *Clermontia*.
3. Chapter four summarized a phylogenetic study of the Hawaiian lobeliad genus, *Clermontia* with special emphasis on floral trait evolution, and
4. Chapter five reported on a pollination ecology study of restoration plantings of four endangered Hawaiian lobeliad species.

Conclusions and future research directions

Chapter two: Floral nectar sugar compositions in the Hawaiian lobeliad genus, *Clermontia* (Campanulaceae)

Hypothesis: The nectar sugar characteristics (sugar composition and concentration) of all 21 *Clermontia* taxa included in the study will be similar to other plant species with a generalist passerine pollination syndrome.

Findings: The hypothesis was supported. All of the 21 *Clermontia* taxa included in the study produced nectar rich in hexose sugars (fructose and glucose) that are dilute. These results are very similar to those of Lammers and Freeman (1986) who included six *Clermontia* species that were also included in the present study. Although these nectar sugar traits have been associated with other animal pollinator guilds (e.g., flies and New World bats; Baker and Baker 1983), the floral morphology of *Clermontia* species (reviewed in Lammers 1991) and recorded observations of nectarivorous Hawaiian Drepanidinae and Mohoidae species visiting the flowers of *Clermontia* taxa (reviewed in Lammers and Freeman 1986), suggests that these passerine species provided the selective pressures for the nectar traits recorded in the present study. However, whether these avian lineages strictly co-evolved with *Clermontia* taxa is now impossible to fully address given the wide-scale passerine extinctions that have occurred since the arrival of humans to Hawai‘i (Banko and Banko 2009).

Future studies: Focusing specifically on *Clermontia*. Individual *Clermontia* flowers remain open for up to one week (R. Pender, unpublished data). Future research could potentially assess nectar production over the life of individual flowers to understand if pollinator selection pressures have influenced nectar production dynamics (e.g., Galetto and Barnardello 2004). In addition, nectar samples could be analyzed for sugar concentration and sugar constituents to assess if these nectar sugar parameters

change during the life of the flower. More broadly, approximately 20% of Hawai‘i’s flora is believed to have evolved for bird pollination (Sakai et al. 2002). High performance liquid chromatography (HPLC) could be usefully employed to confirm if taxa have evolved a generalist passerine pollination syndrome. Such research is needed as part of wider floral biology studies of bird pollinated plant species to inform the conservation and restoration of this unique component of Hawai‘i’s flora.

Chapter three: Predicting potential interactions between extant nectarivorous passerines and Hawaiian lobeliad species in the genus *Clermontia* (Campanulaceae)

Hypothesis: In extant communities, the majority of *Clermontia* species have no avian pollinators present that are capable of effective nectar foraging and pollen movement.

Findings: The hypothesis was supported. This study aimed to predict which *Clermontia* taxa might be visited by six (five endemic, one alien) extant nectarivorous passerine species using existing geographic information system (GIS) distribution layers for the extant nectarivorous bird species and *Clermontia* taxa and morphological comparisons between floral and bill measurements. Of the 25 *Clermontia* taxa included in the study, 12 taxa either have no potential pollinators or the potential pollinators are present over only part of their geographical ranges. Seventeen *Clermontia* taxa appear to be reliant on ‘i‘iwi for pollination as the corolla dimensions of these taxa are too large for the short billed bird species to access the nectar while also contacting the reproductive organs.

Future research: This research provides a framework from which to undertake future studies of the reproductive ecology of *Clermontia*. Field-based pollination studies (e.g., Aslan et al. 2013) should be undertaken to validate the predictions made

in chapter three. Such studies would benefit from molecular analyses to assess rates of out-crossing and gene-flow (e.g., Byrne et al. 2007, Krauss et al. 2009). Species that have lost their potential pollinators could be compared with species where potential pollinators are present to assess if they experience higher levels of inbreeding depression and pollen limitation (e.g. Anderson et. al. 2011, Robertson et al. 2011).

Chapter four: A phylogenetic study of the Hawaiian lobeliad genus, *Clermontia* (Campanulaceae) with special emphasis on floral trait evolution

Hypothesis one: Lammers (1991) subgeneric classification of *Clermontia* does not reflect the evolutionary history of the genus

Hypothesis two: *Clermontia* evolved on Kaua‘i, geologically the oldest of the larger islands of the Hawaiian archipelago and subsequently dispersed eastward to the progressively younger islands of O‘ahu, the Maui-Nui island complex, and the island of Hawai‘i.

Hypothesis three: The petal-like sepal arrangement that occurs in more than half of the *Clermontia* species has evolved multiple times.

Findings: Hypothesis one is supported. The current analysis suggests that the subgeneric classification of *Clermontia* (two sections, each containing three series) utilized by Lammers (1991) does not adequately reflect the evolution of the genus. Lammers (1991) classification was based upon floral characters; principally the arrangement and morphology of the calyx and corolla lobes. However, these floral traits appear to have evolved separately and on multiple occasions. This result was supported by the findings of Givnish et al. (2013) who also concluded that Lammers’ (1991) subgeneric classification did not reflect the evolutionary history of the genus. In addition, the genus, as it is currently circumscribed is not monophyletic. *Clermontia pyrularia* either belongs in *Cyanea* or represents an intergeneric hybrid between *Clermontia* and

Cyanea. Givnish et al. (2013) suggest that *Cl. pyrularia* may represent a hybrid between a member of the purple fruited clade of *Cyanea* and *Cl. clermontioides*. However, more detailed work is needed to resolve the evolutionary history of *Cl. pyrularia*.

Hypothesis two was also supported, although, the study could not specifically identify whether the genus evolved on Kaua‘i or O‘ahu. Generally the biogeographic history of the genus follows the “progression rule” (reviewed in Funk and Wagner 1995). The results of the study suggest that the genus evolved on either Kaua‘i or O‘ahu followed by dispersal events to the younger islands of the Maui-Nui island complex followed by two subsequent dispersal events to Hawai‘i Island. The analysis suggests that there has been at least one back dispersal from either Hawai‘i or Maui-Nui to the older island of O‘ahu (*Cl. kakeana*). These findings are in contrast to Lammers’ (1995) cladistic analysis that suggested that the genus evolved on Hawai‘i Island and dispersed to the older islands. However, they are generally supported by the study of Givnish et al. (2013) and Hofer et al. (2013) who found that the genus evolved either on Kaua‘i or an older island with dispersal events that generally followed the progression rule.

Hypothesis three was not supported. Based on the results of the present study, petaloid sepals appeared to have evolved once on O‘ahu (in *Cl. oblongifolia* subsp. *oblongifolia*) and were largely retained as the genus dispersed down the island chain. Five, possibly six, reversal events (to the sepaloid form) appear to have taken place. By contrast, Givnish et al. (2013) suggest that petaloid sepals arose two times, first in *Cl. persicifolia* from O‘ahu and once in *Cl. grandiflora* (Maui Nui) with three reversal events back to the sepaloid form.

Future research: Taxa that were not included in the present study should be included in future analyses to clarify their relationships within the genus. Recently developed nrDNA primers, designed for DNA bar-coding *Clermontia* taxa (Pillon et al. 2013) should be utilized for future phylogenetic studies of this and other Hawaiian lobeliad genera. Ultimately, the long term goal of phylogeneticists working with Hawaiian lobeliads should be to resolve the biogeographic and evolutionary relationships of all 140 species.

Chapter five: Are restoration plantings of endangered ornithophilous Hawaiian lobeliads (Campanulaceae) being pollinated by nectarivorous passerines in Hawai‘i?

Hypothesis one: The four lobeliad species are not being visited by effective animal pollinators. As a consequence, all four lobeliad species will be pollen limited

Hypothesis two: All four lobeliad species are self-compatible (capable of autogamy). This will be the primary means by which all four lobeliad species sexually reproduce.

Findings: Hypothesis one is supported. Pollination ecology studies were undertaken to assess if four endangered ornithophilous Hawaiian lobeliads are pollinated by passerines (native or introduced) and diurnal insects at two separate restoration sites; Hakalau National Wildlife refuge on Hawai‘i and Kahanahāiki and Pahole Gulches on O‘ahu. Two honeycreeper species, ‘i‘iwi (*Vestiaria coccinea*) and Hawai‘i ‘amakihi (*Hemignathus virens*), were infrequent floral visitors to *Cl. lindseyana* and *Cl. pyrularia* at Hakalau National wildlife refuge. However, both ‘i‘iwi and ‘amakihi nectar robbed a significant proportion of the flowers and were not effective pollinators of either lobeliad species. Introduced Japanese white-eye (*Zosterops japonicus*) were not effective pollinators of *Cyanea superba* subsp. *superba* and *Delissea waianaeensis*, nectar robbing all of the flowers they visited of both species. However, on rare occasions, the native

yellow-faced bee, *Hylaeus connectens*, and the introduced hoverfly, *Allograpta obliqua*, may potentially pollinate flowers. Pollination treatments indicated that all four species were significantly pollen limited, suggesting that none of the animals that visited the flowers were effective pollinators.

Hypothesis two is supported. All four lobeliad species are capable of autogamy. Autogamy appears to be the primary means by which all four species are reproducing at each of the restoration sites. However, pollen supplementation treatments suggest that if pollinators were present at the restoration sites of each lobeliad species all four species would likely employ a mixed mating system, whereby both autogamy and animal-assisted pollination would likely occur. Cory (1984), who studied *Cl. kakeana* and *Cy. angustifolia* on O‘ahu and Aslan et al. (2013) who studied *Cl. parviflora*, *Cl. montis-loa* and *Cl. hawaiiensis* on Hawai‘i Island both found that these species were also capable of autogamy. However, only Aslan et al. (2013) undertook pollen supplementation treatments and found that all three lobeliad species also have a mixed mating system similar to the four species included in the present study.

Future research: A fundamental question facing plant conservation practitioners in Hawai‘i is: does the decline or loss of pollinators impact the survival of a plant species? In the absence of effective pollinators, does pollen limitation lead to seedling limitation and population decline? Autogamy may partially compensate although it may also lead to inbreeding depression. How inbreeding impacts these and other plant species has not been widely studied in Hawai‘i (see Culley et al. 1999, Weller et al. 2005 for exceptions). Substitute pollinators were not effective in the present study, yet have been shown to be effective for other species (Cox 1983, Drake and Morden unpublished, Aslan

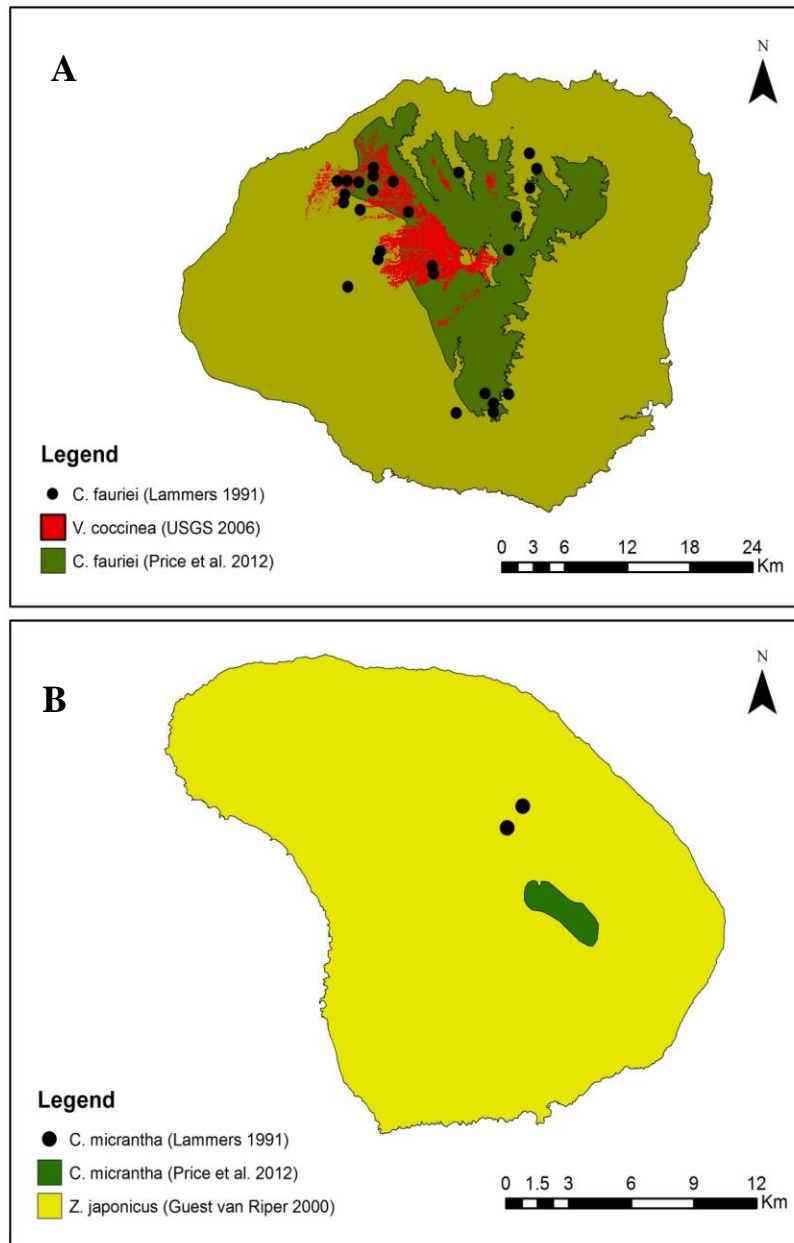
et al. 2013). Finally, how global climate change might impact plant-pollinator mutualisms in the future warrants concerted research attention and effective mitigation strategies.

APPENDIX A. Results of two sample *t*-tests between male and female head and bill dimensions in each of the six nectarivorous passerine species. Statistics (*t* and *p* values) are provided in where cases of statistical significance were recorded.

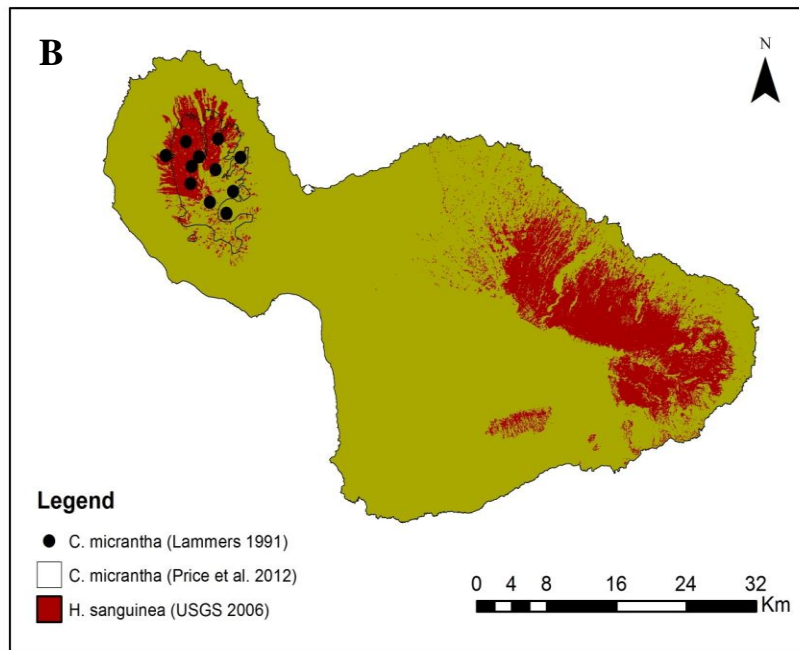
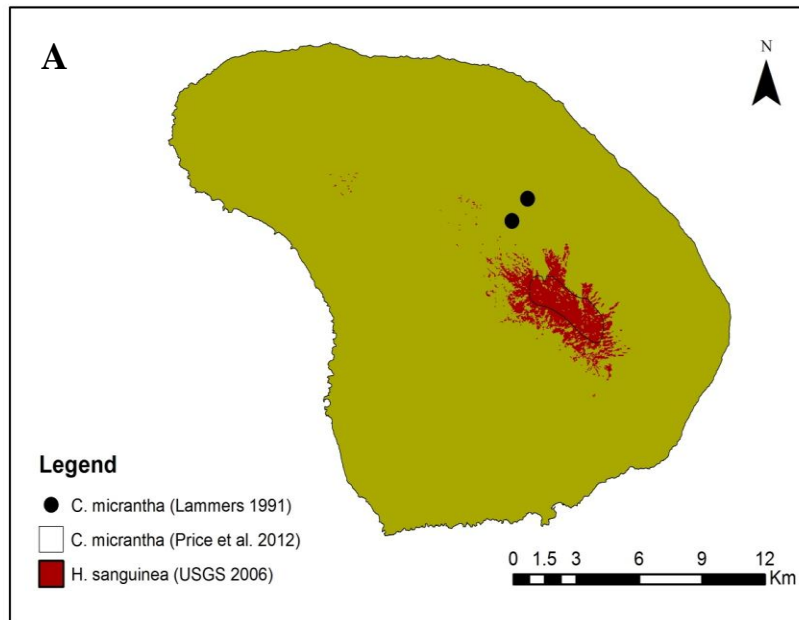
Species	Bill and head dimensions	Exposed culmen	True culmen
‘Iiwi	♂ $\bar{x} = 43.8 \pm 1.3$ (n = 14)	♂ $\bar{x} = 26.4 \pm 1.7$ (n = 14)	♂ $\bar{x} = 30.6 \pm 1.4$ (n = 14)
	♀ $\bar{x} = 42.6 \pm 1.8$ (n = 15)	♀ $\bar{x} = 24.9 \pm 1.4$ (n = 15)	♀ $\bar{x} = 28.1 \pm 1.5$ (n = 15)
	<i>t</i> = 2.07, <i>p</i> = 0.049	<i>t</i> = 2.53, <i>p</i> = 0.018	<i>t</i> = 4.65, <i>p</i> = 0.000
‘Apapane	♂ $\bar{x} = 34.7 \pm 1.2$ (n = 15)	♂ $\bar{x} = 16.4 \pm 1.1$ (n = 15)	♂ $\bar{x} = 19.3 \pm 1.0$ (n = 15)
	♀ $\bar{x} = 34.6 \pm 1.8$ (n = 16)	♀ $\bar{x} = 15.8 \pm 0.7$ (n = 16)	♀ $\bar{x} = 19.3 \pm 1.0$ (n = 16)
	Not significant	<i>t</i> = 2.74, <i>p</i> = 0.013	Not significant
Hawai‘i ‘amakihi	♂ $\bar{x} = 31.9 \pm 1.2$ (n = 15)	♂ $\bar{x} = 13.4 \pm 0.7$ (n = 15)	♂ $\bar{x} = 16.5 \pm 0.8$ (n = 15)
	♀ $\bar{x} = 29.5 \pm 1.2$ (n = 15)	♀ $\bar{x} = 12.2 \pm 0.8$ (n = 15)	♀ $\bar{x} = 16.0 \pm 0.8$ (n = 15)
	<i>t</i> = 5.46, <i>p</i> = 0.000	<i>t</i> = 4.70, <i>p</i> = 0.000	Not significant
O‘ahu ‘amakihi	♂ $\bar{x} = 32.1 \pm 1.8$ (n = 15)	♂ $\bar{x} = 14.6 \pm 0.4$ (n = 15)	♂ $\bar{x} = 16.7 \pm 0.6$ (n = 15)
	♀ $\bar{x} = 30.0 \pm 1.6$ (n = 15)	♀ $\bar{x} = 12.8 \pm 0.6$ (n = 15)	♀ $\bar{x} = 14.7 \pm 0.7$ (n = 15)
	<i>t</i> = 3.26, <i>p</i> = 0.003	<i>t</i> = 9.36, <i>p</i> = 0.000	<i>t</i> = 8.39, <i>p</i> = 0.000

Kaua'i 'amakihi	♂ $\bar{x} = 35.4 \pm 1.5$ (n = 9) ♀ $\bar{x} = 34.5 \pm 0.46$ (n = 5) Not significant	♂ $\bar{x} = 17.0 \pm 0.7$ (n = 9) ♀ $\bar{x} = 16.0 \pm 0.81$ (n = 5) $t = 2.45, p = 0.044$	♂ $\bar{x} = 19.0 \pm 0.9$ (n = 9) ♀ $\bar{x} = 17.7 \pm 0.6$ (n = 5) $t = 3.07, p = 0.012$
Japanese white-eye	♂ $\bar{x} = 29.4 \pm 1.5$ (n = 20) ♀ $\bar{x} = 30.5 \pm 1.8$ (n = 10) Not significant	♂ $\bar{x} = 10.5 \pm 0.7$ (n = 20) ♀ $\bar{x} = 11.0 \pm 0.6$ (n = 10) Not significant	♂ $\bar{x} = 14.5 \pm 1.0$ (n = 20) ♀ $\bar{x} = 14.3 \pm 0.7$ (n = 10) Not significant

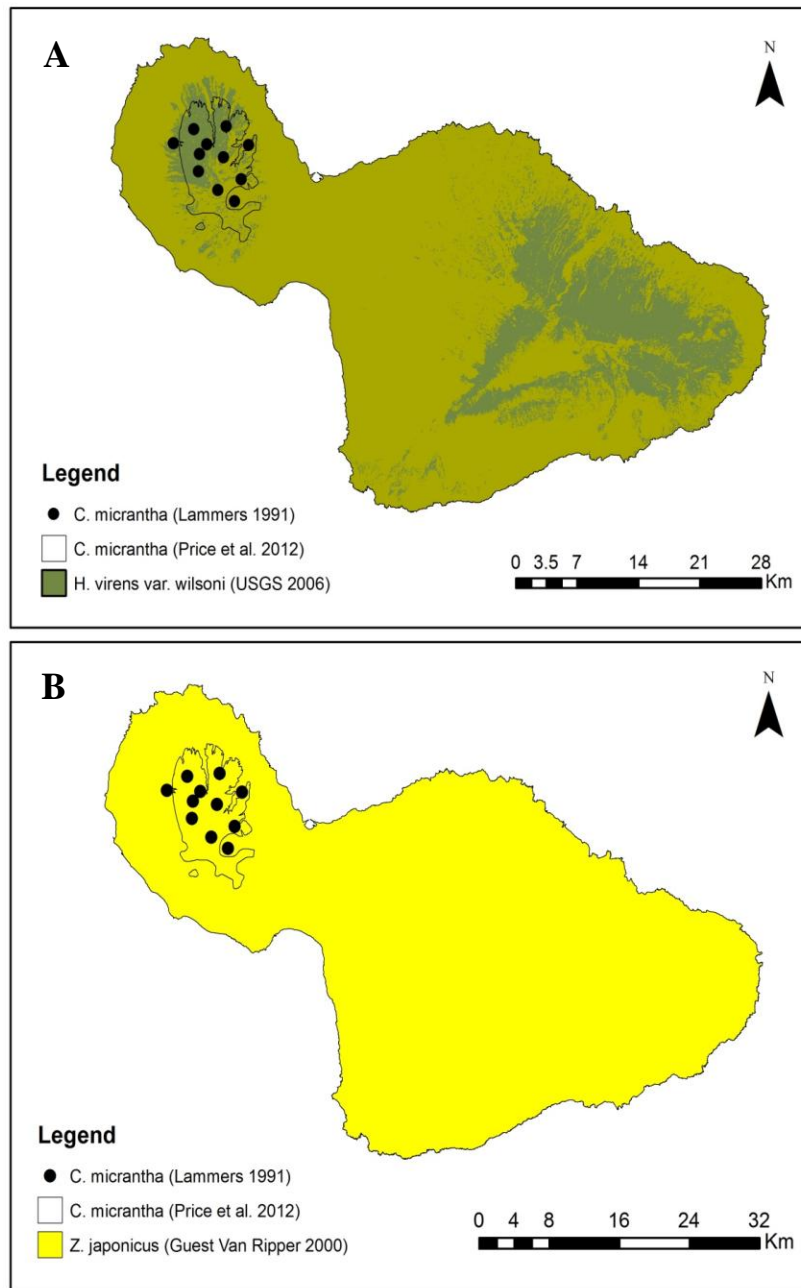
Appendix B: Projected distributions of *Clermontia* and extant nectarivorous passerine species.



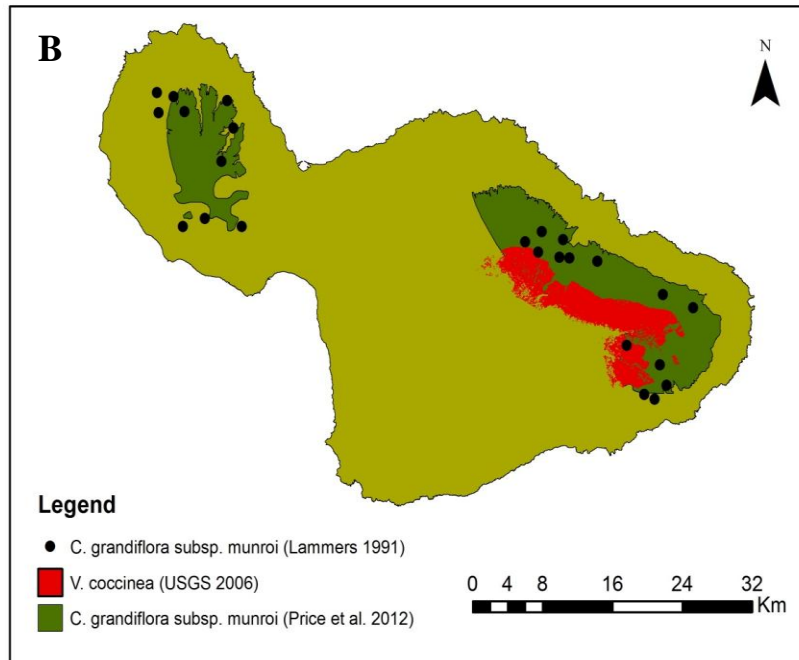
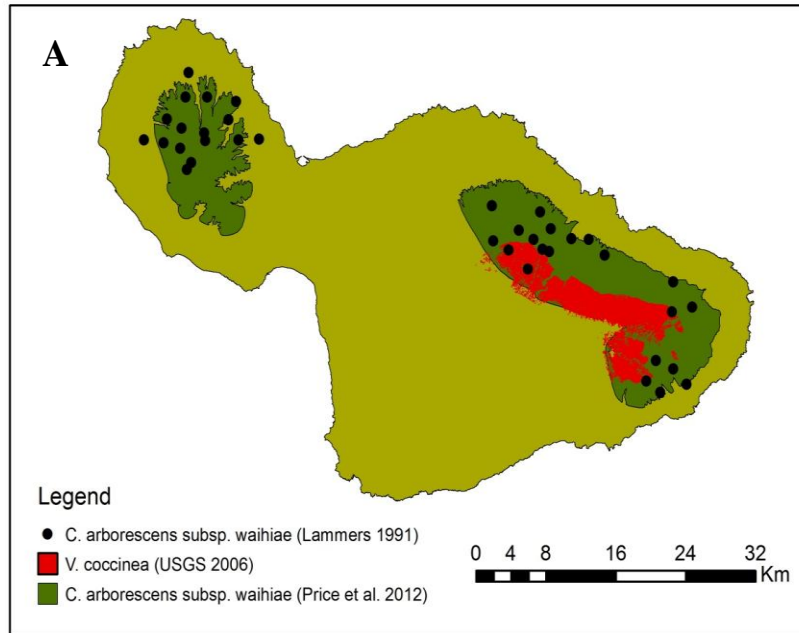
Appendix B: 1(A) The projected distribution of *C. fauriei* and ‘i‘iwi on Kaua‘i. See methods for an explanation of the GIS layers included on the maps. (B) The projected distribution of *C. micrantha* and Japanese white-eye on Lana‘i.



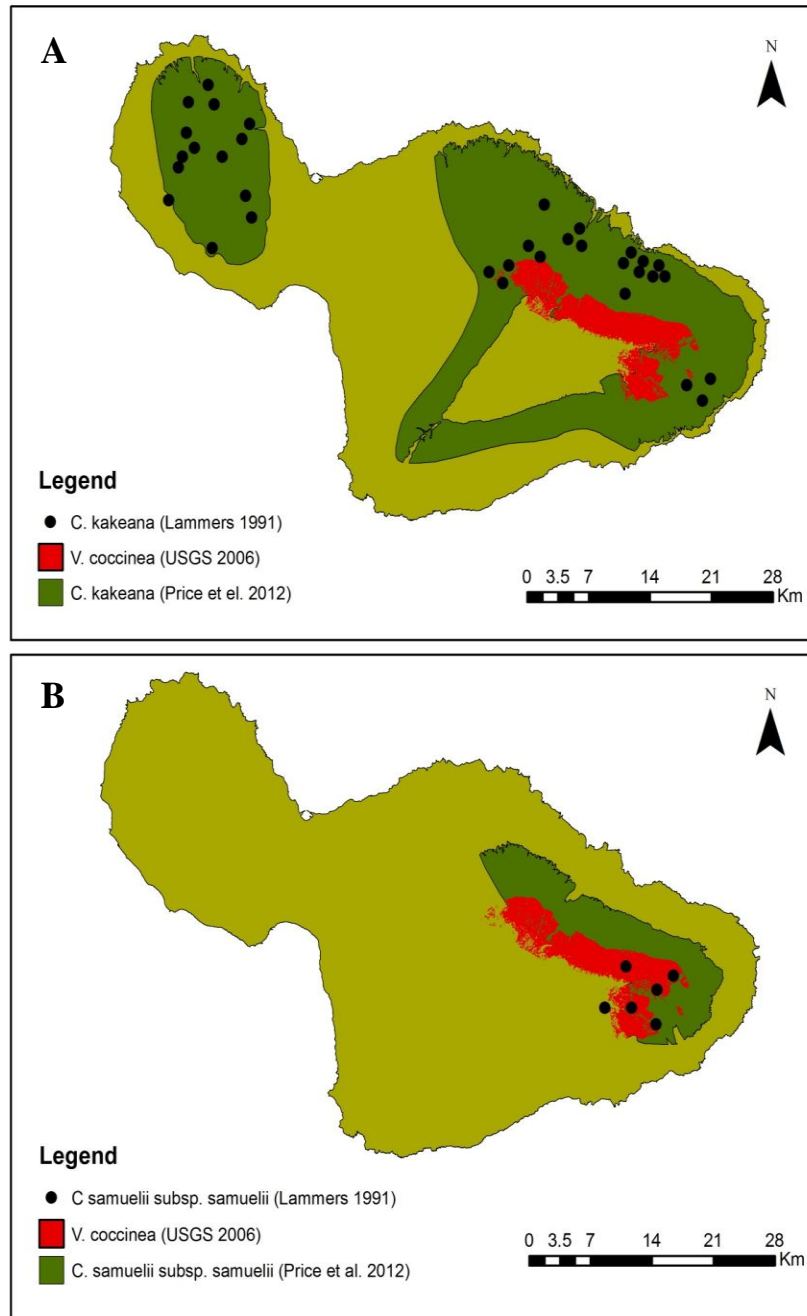
Appendix B: 2 (A) The projected distribution of *C. micrantha* and 'apapane on Lana'i. (B) The projected distribution of *C. micrantha* and 'apapane on Maui.



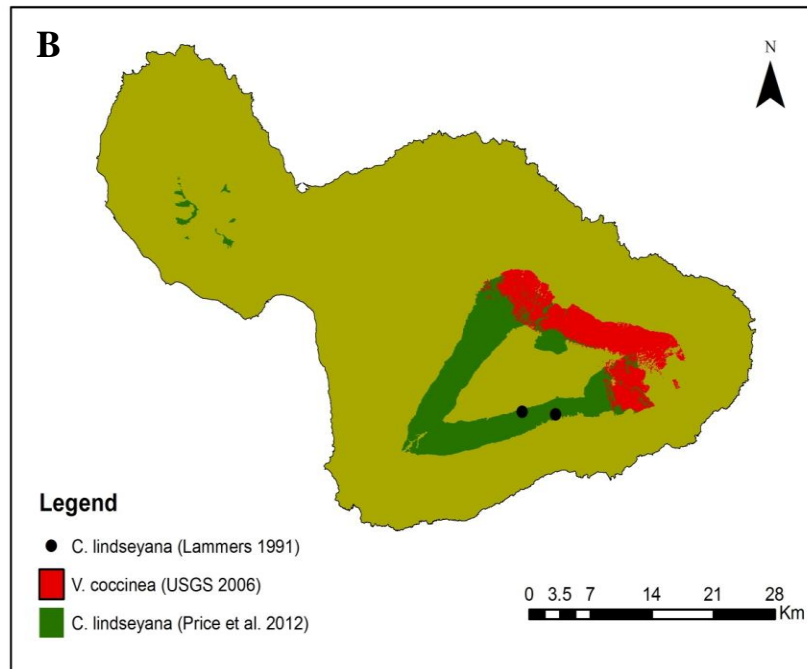
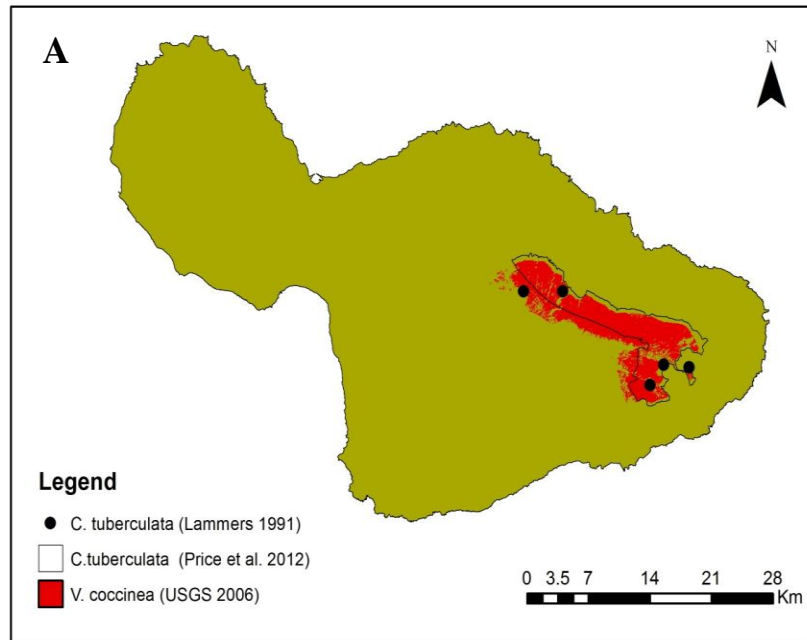
Appendix B: 3 (A) The projected distribution of *C. micrantha* and Hawai‘i amakihi on Maui. (B) The projected distribution of *C. micrantha* and Japanese white-eye on Maui.



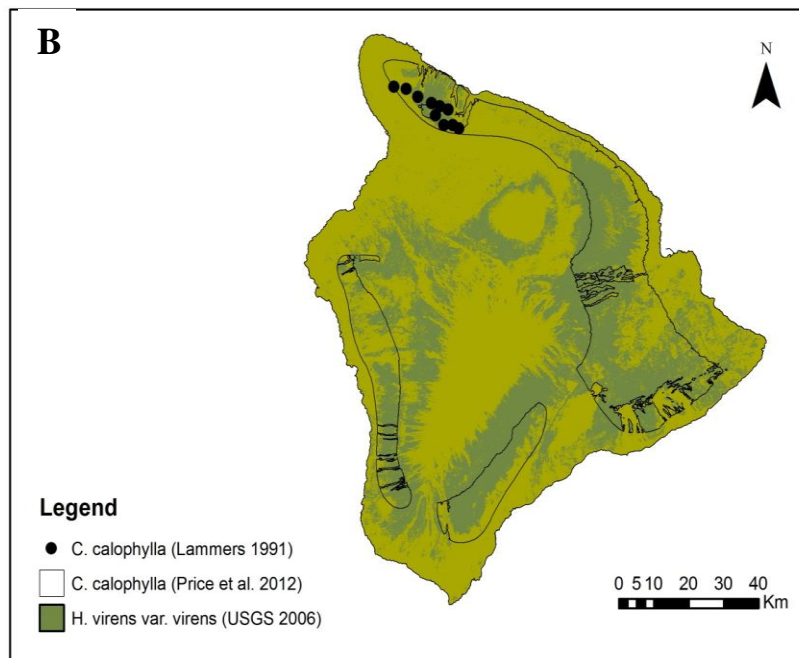
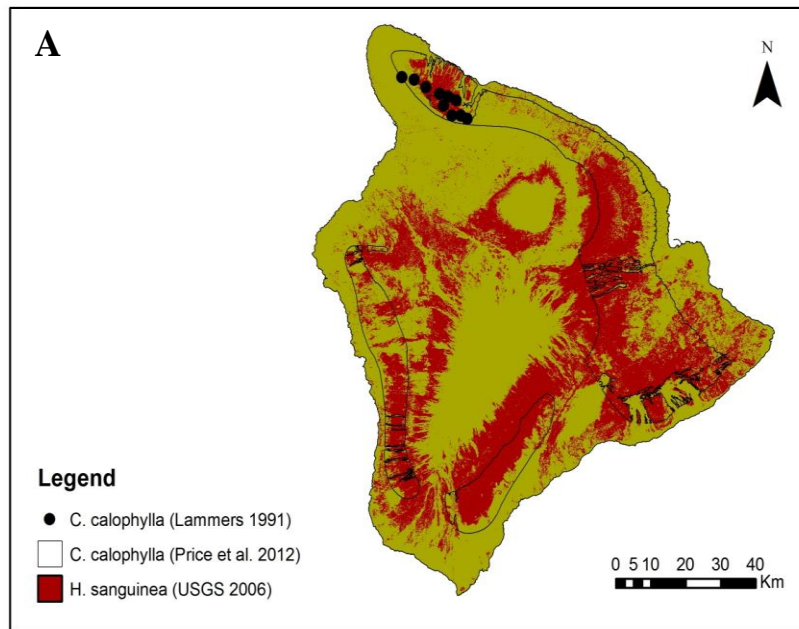
Appendix B: 4 (A) The projected distribution of *C. arborescens* subsp. *waihiaae* and ‘i‘iwi on Maui. (B) The projected distribution of *C. grandiflora* subsp. *munroi* and ‘i‘iwi on Maui.



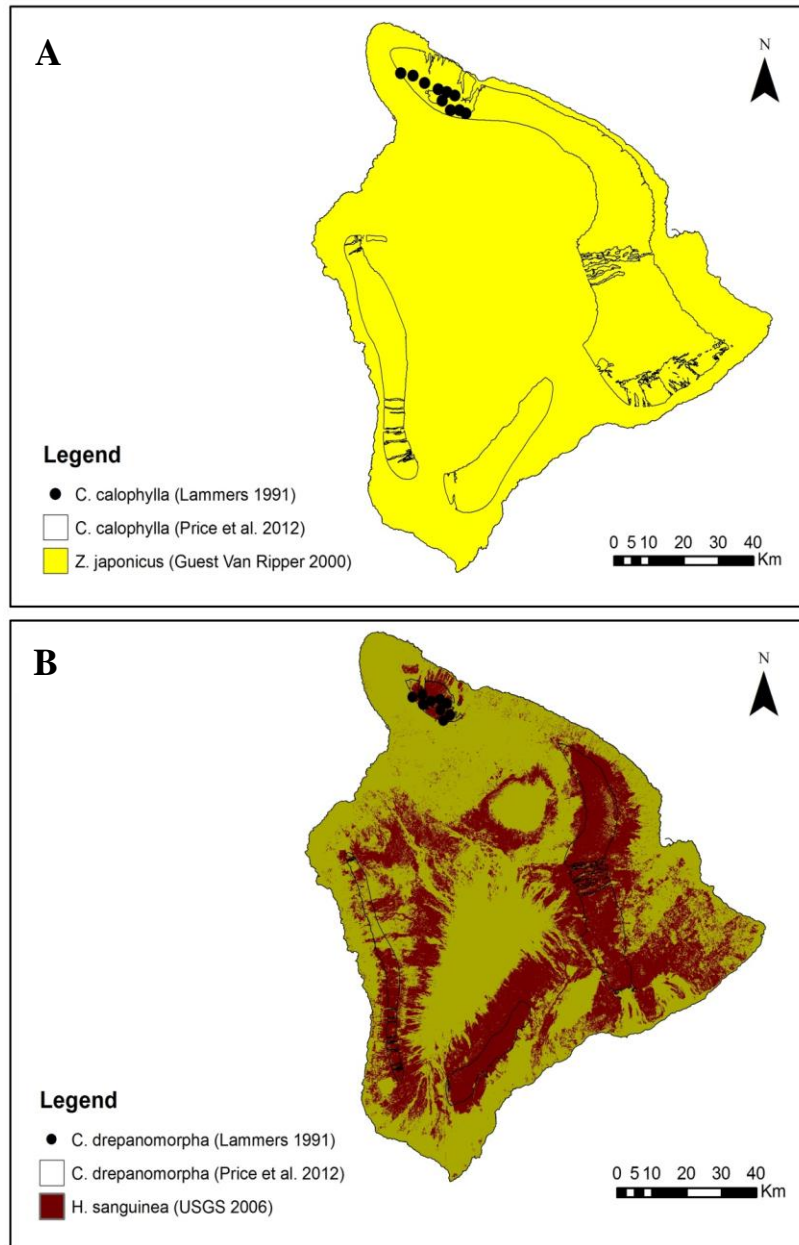
Appendix B: 5 (A) The projected distribution of *C. kakeana* and 'i'iwi on Maui. (B) The projected distribution of *C. samuelii* subsp. *samuelii* and 'i'iwi on Maui.



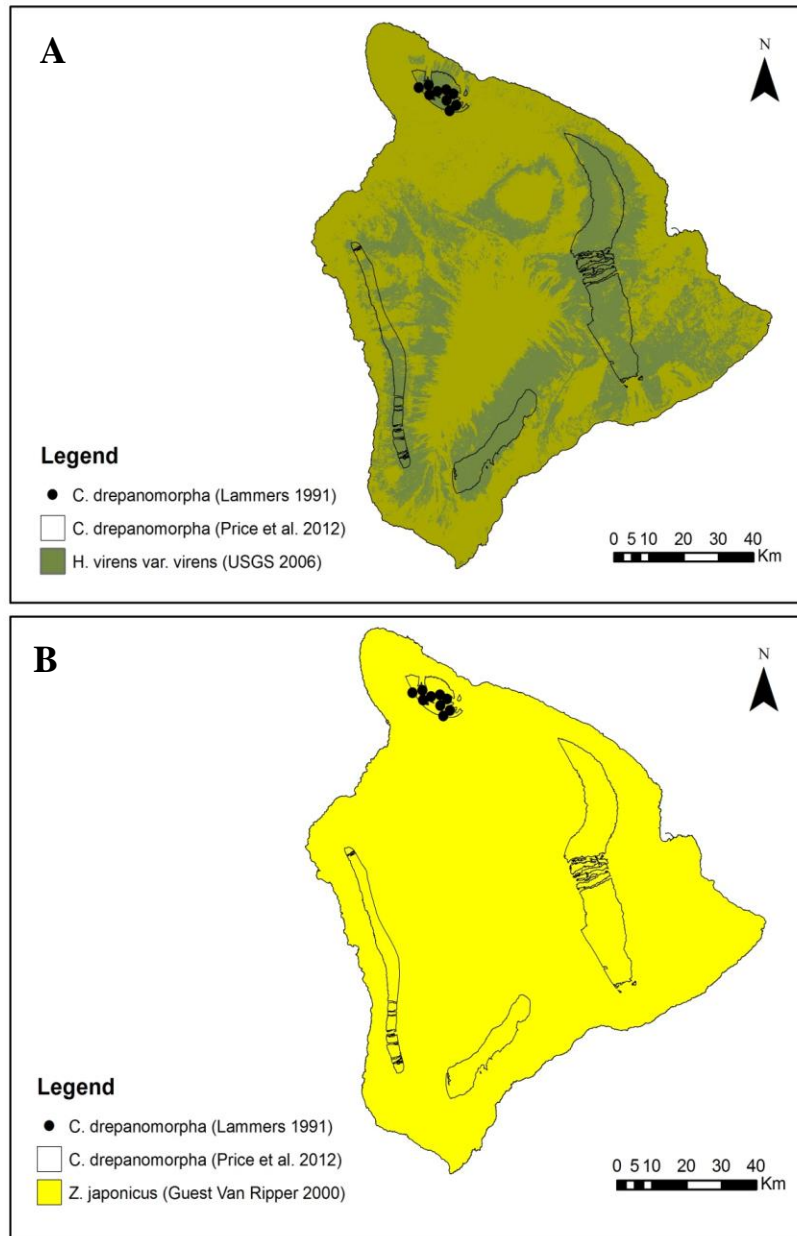
Appendix B: 6 (A) The projected distribution of *C. tuberculata* and ‘i‘iwi on Maui. (B) The projected distribution of *C. lindseyana* and ‘i‘iwi on Maui.



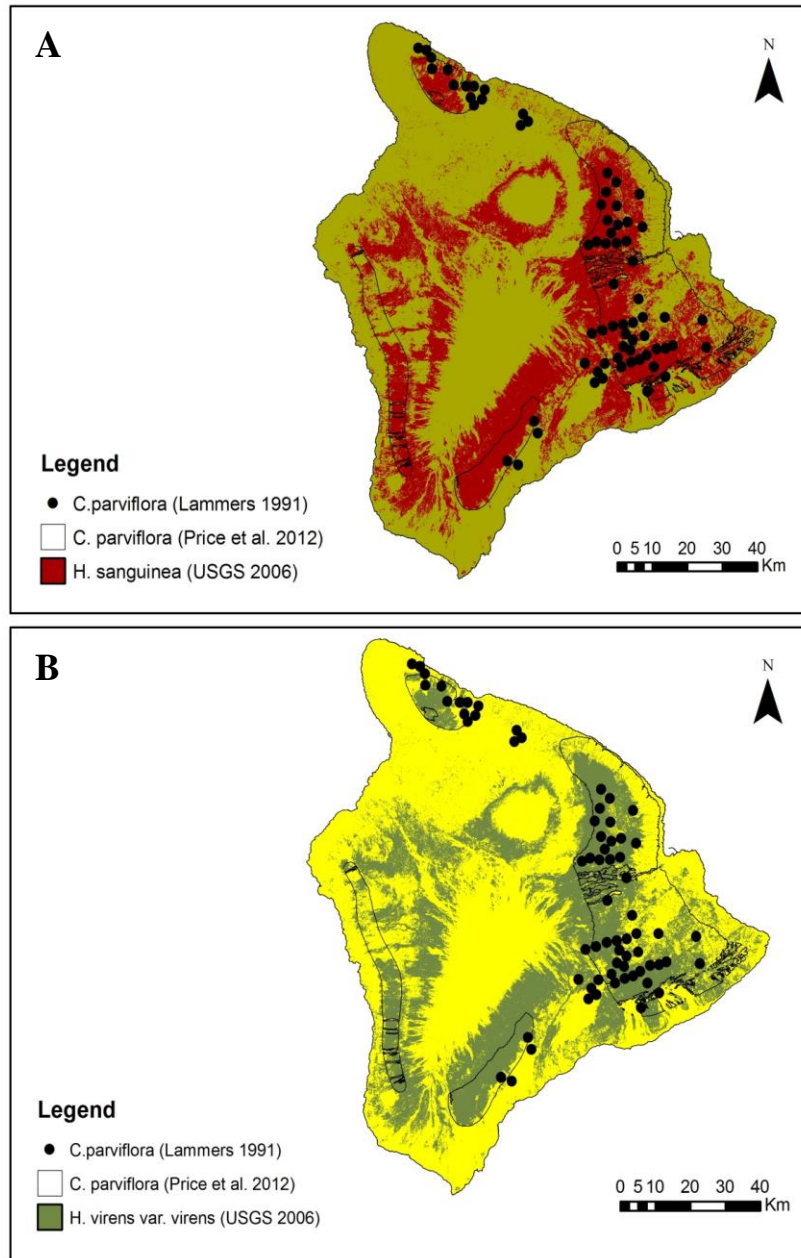
Appendix B: 7 (A) The projected distribution of *C. calophylla* and ‘apapane on Maui. (B) The projected distribution of *C. calophylla* and Hawai‘i amakihi on Maui.



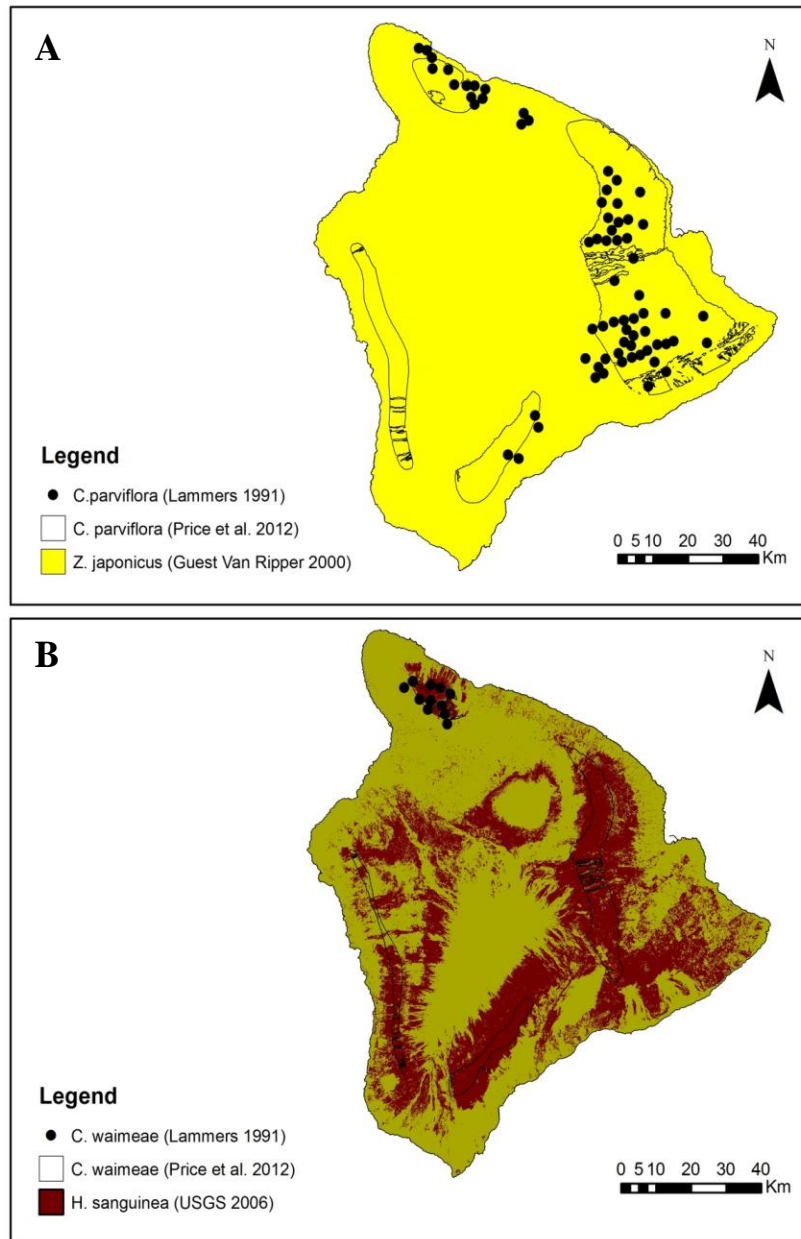
Appendix B: 8 (A) The projected distribution of *C. calophylla* and Japanese white-eye on Hawai‘i. (B) The projected distribution of *C. drepanomorpha* and ‘apapane on Hawai‘i.



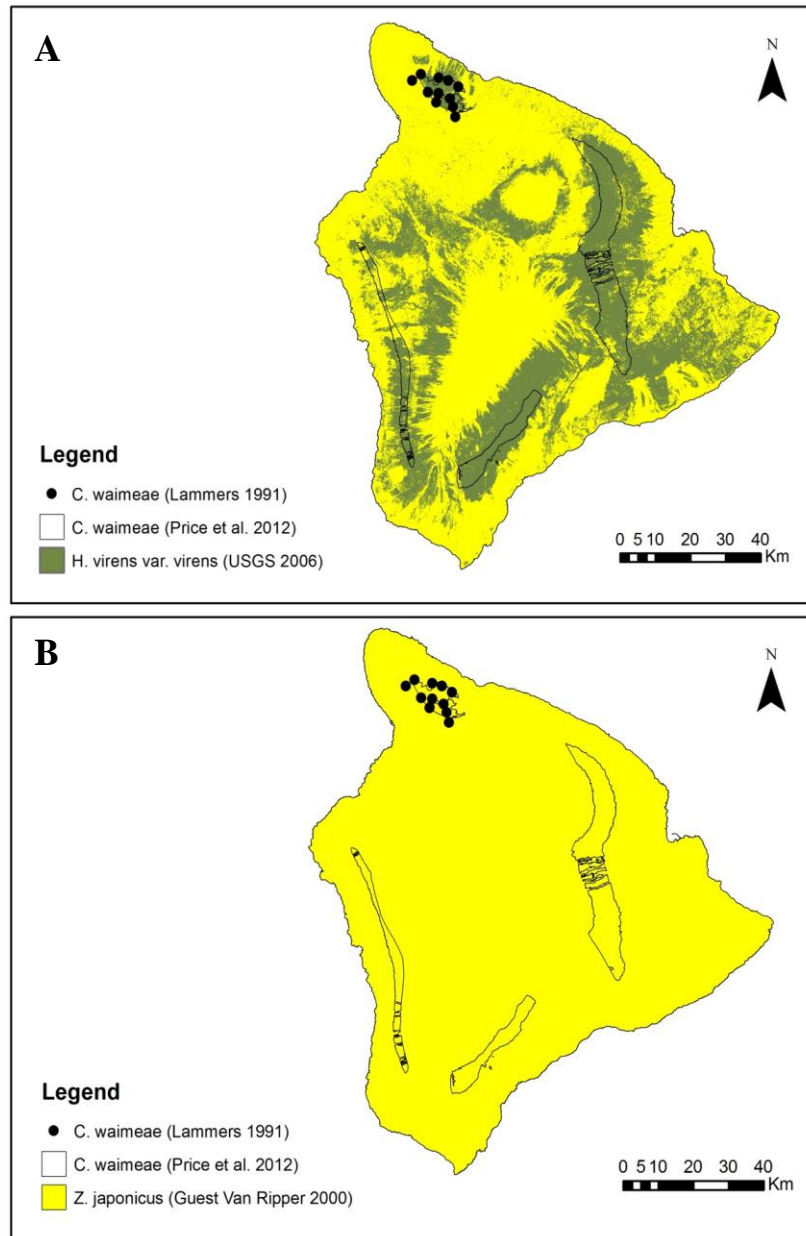
Appendix B: 9 (A) The projected distribution of *C. drepanomorpha* and Hawai'i amakihi on Hawai'i. (B) The projected distribution of *C. drepanomorpha* and Japanese white eye on Hawai'i.



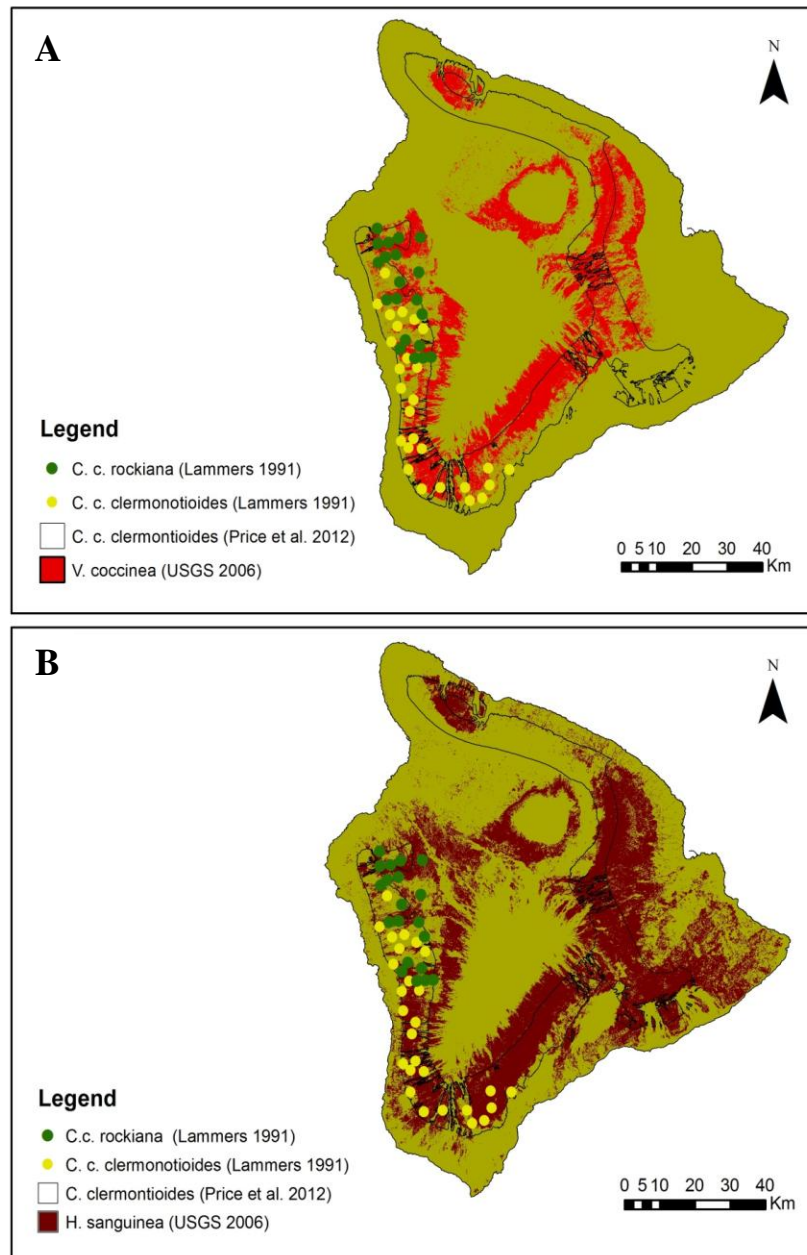
Appendix B: 10 (A) The projected distribution of *C. parviflora* and ‘apapane on Hawai‘i. (B) The projected distribution of *C. parviflora* and Hawai‘i amakihi on Hawai‘i.



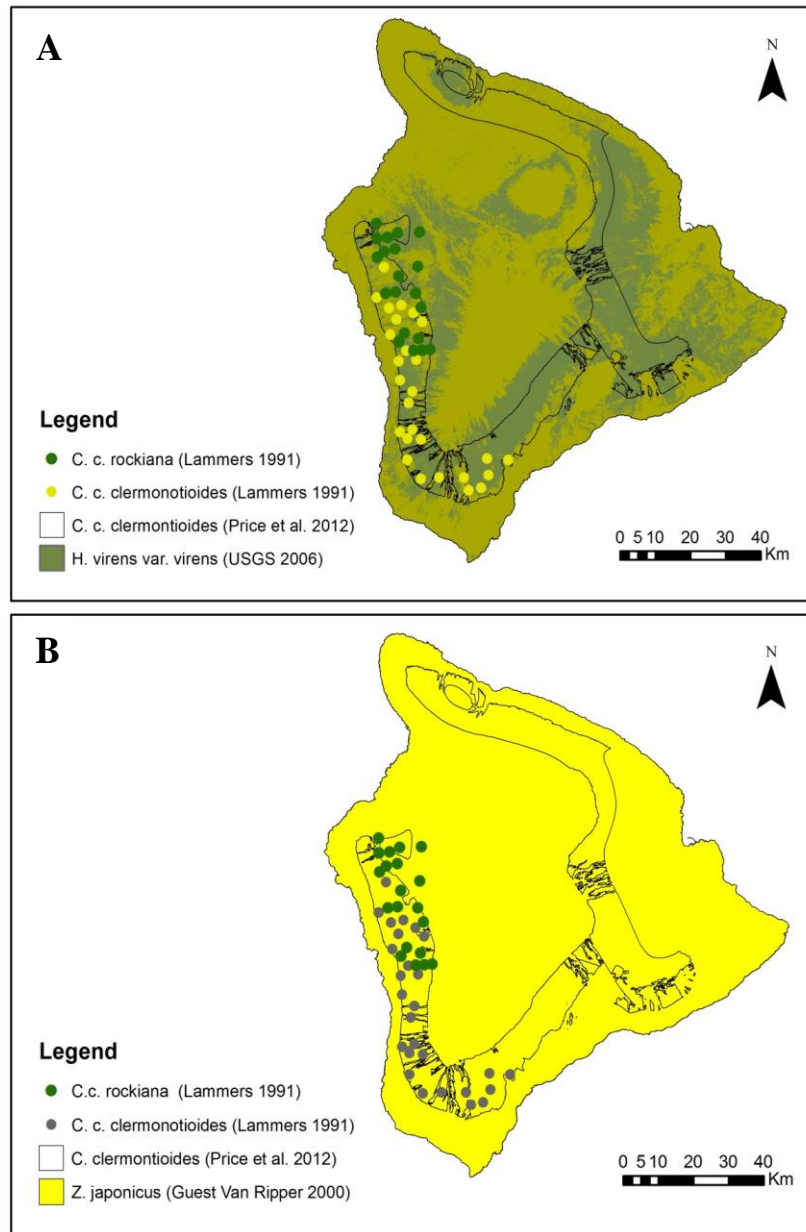
Appendix B: 11 (A) The projected distribution of *C. parviflora* and Japanese white eye on Hawai‘i. (B) The projected distribution of *C. waimeae* and ‘apapane on Hawai‘i.



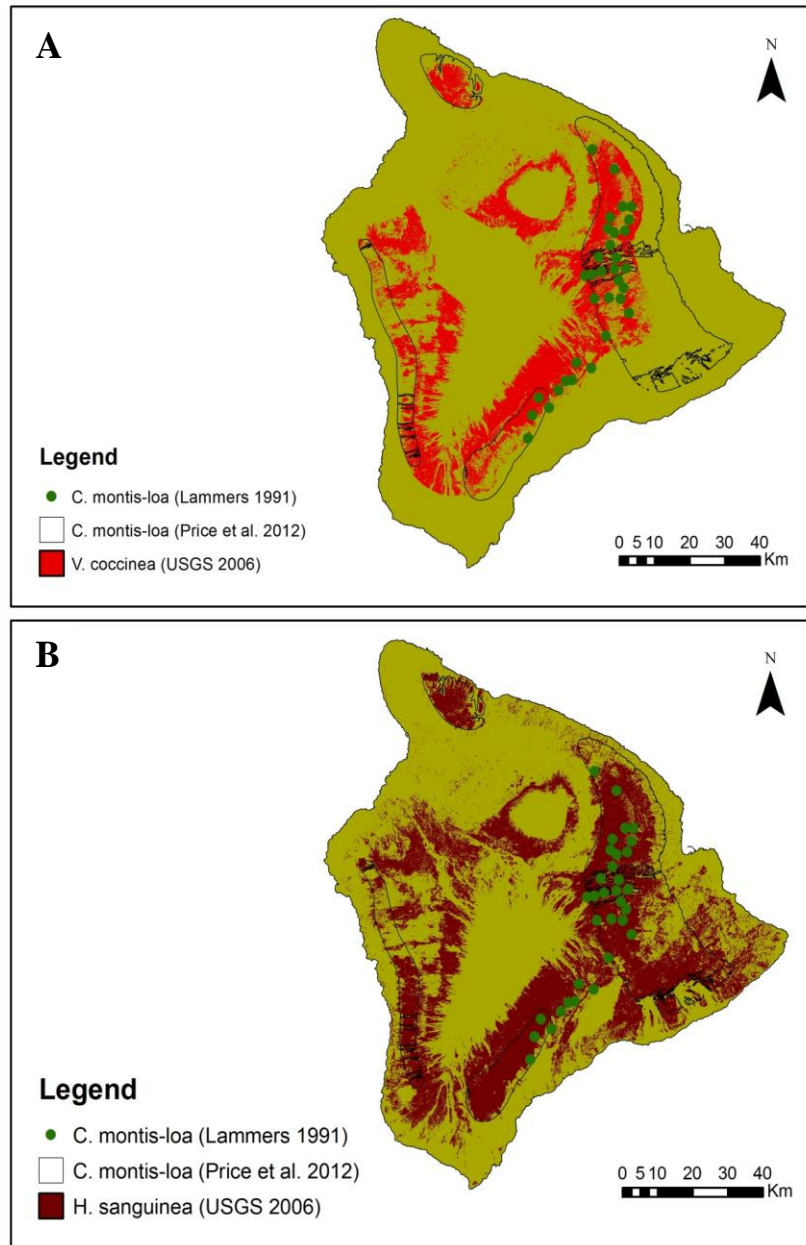
Appendix B: 12 (A) The projected distribution of *C. waimeae* and Hawai'i amakihi on Hawai'i. (B) The projected distribution of *C. waimeae* and Japanese white eye on Hawai'i.



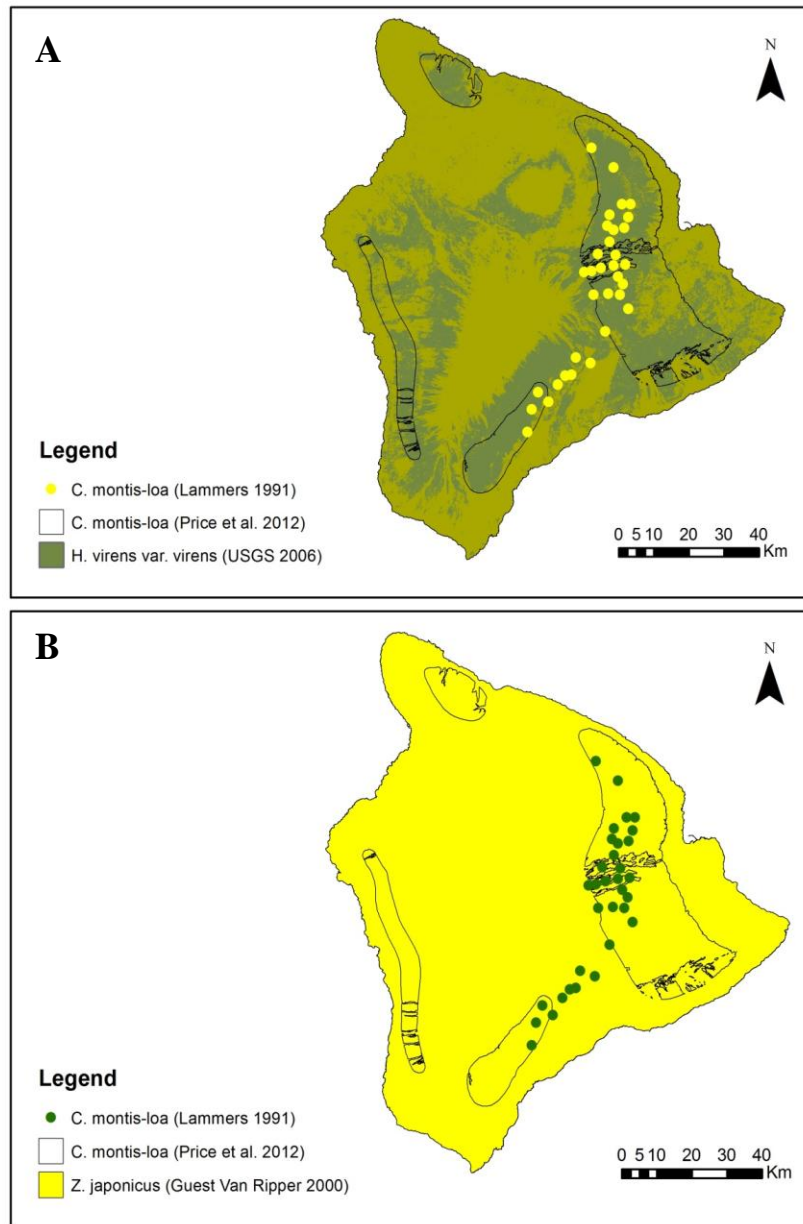
Appendix B: 13 (A) The projected distribution of *C. clermonotioides* subsp. *clermonotioides* and *C.c. subsp. rockiana* and ‘i‘iwi on Hawai‘i. (B) The projected distribution of *C. clermonotioides* subsp. *clermonotioides* and *C.c. subsp. rockiana* and ‘apapane on Hawai‘i.



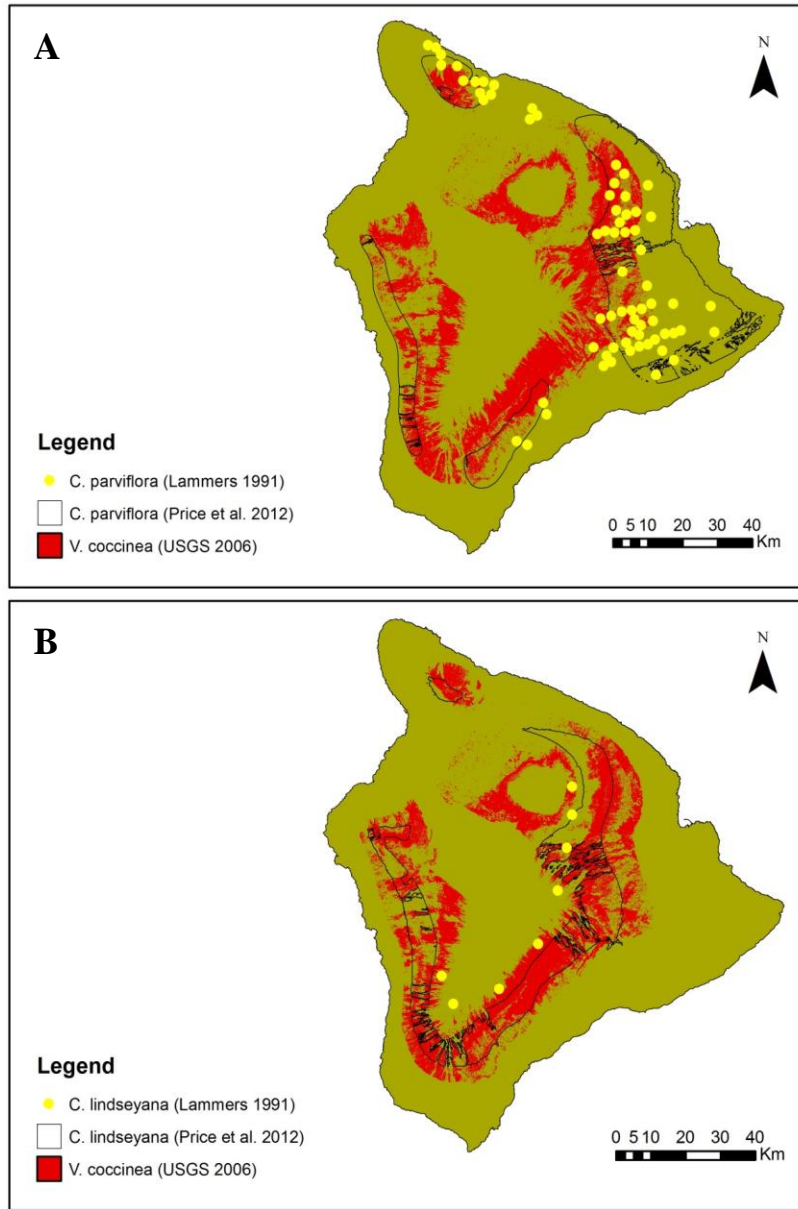
Appendix B: 14 (A) The projected distribution of *C. clermontoioides subsp. clermontoioides* and *C.c. subsp. rockiana* and Hawai'i amakihi on Hawai'i. (B) The projected distribution of *C. clermontoioides subsp. clermontoioides* and *C.c. subsp. rockiana* and Japanese white eye on Hawai'i.



Appendix B: 15 (A) The projected distribution of *C. montis-loa* and ‘i‘iwi on Hawai‘i. (B) The projected distribution of *C. montis-loa* and ‘apapane on Hawai‘i.



Appendix B: 16 (A) The projected distribution of *C. montis-loa* and Hawai'i amakihi on Hawai'i. (B) The projected distribution of *C. montis-loa* and Japanese white eye on Hawai'i.



Appendix B: 17 (A) The projected distribution of *C. parviflora* and ‘i‘iwi on Hawai‘i. (B) The projected distribution of *C. lindseyana* and ‘i‘iwi on Hawai‘i.

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