POLLINATION BIOLOGY OF HAWAIIAN
SCAEVOLA (GOODENIACEAE)

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ABSTRACT

The genus *Scaevola* (Goodeniaceae) occurs as shrubs to small trees in diverse habitats in Hawai'i, from coastal strand to montane rainforests. Extensive variation in floral characteristics suggests pollinators differ among species. Bees (*Hylaeus* spp.) and honeycreepers (Drepanidinae) are the putative native pollinators, but their diversity and abundance have declined over the last century. Due to high rates of extinction among the Hawaiian fauna and the introduction of alien generalists, former roles of some native flower visitors may remain a mystery, and the timeliness of understanding the roles of those still present is underscored. Pollination syndromes may offer clues regarding former pollinators that have become extinct or extirpated, or shifted to new resources. This study quantified flower visitation rates, visitor behavior, nectar volume, sugar concentration and sugar composition along with a series of structural measurements of floral display, attraction, and the mechanics of nectar access for all nine extant species of *Scaevola* in three lineages. Visitation was primarily diurnal, ranging from 0.2 to 3.0 visits $\cdot$ flower^{-1} $\cdot$ hour^{-1} during the day, with 4-15 visitor taxa per species. Non-native visitors, mainly honey bees (*Apis mellifera*) and ants, were the most frequent visitors for most species. *Hylaeus* were infrequent visitors to three species and common only at *S. chamissoniana*. Birds were the main visitors to *S. glabra* and *S. procera*, with the alien *Zosterops japonicus* a primary visitor to both, and the native *Hemignathus kauaiensis* also a primary visitor to *S. glabra*. Visitation was often conducive to pollination for most species. Visitor interactions differed for each species of *Scaevola*, and in several cases may impose limitations on pollination. There were significant differences in nectar volume, concentration and most floral measurements among and within lineages ($P < 0.001$). Flowers ranged from small, pale, and scented ones with small amounts of sucrose-dominant nectar in high concentrations to large, heavy, decurved, and brightly colored ones lacking scent and containing copious amounts of dilute, hexose-dominant nectar. Pollination syndromes corresponded with the putative native pollinators. Nearly all *Scaevola* species exhibit combinations of traits suggesting generalist strategies to allow for visitation by diverse pollinator guilds. Some of these traits may indicate generalist passerine and large moth pollinator guilds that no longer exist among native visitors for some species of *Scaevola*. The prevalence of alien visitors has several implications for both the plants and native flower visitors. Non-native species may be depriving native visitors of floral resources and may limit plant reproduction if alien visitors are less effective pollinators than native species. Alternatively, non-native visitors may pollinate *Scaevola* species whose native pollinators have declined or shifted to new resources for unrelated reasons. The research presented herein provides baseline data on flower biology and flower visitation, from which future pollination investigations may be directed.

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CHAPTER 1. INTRODUCTION AND BACKGROUND ON POLLINATION AND *SCAEVOLA* IN HAWAIʻI

Plant-pollinator interactions may be crucial for the maintenance of ecosystems (Pellmyr, 2002). Such interactions may be disrupted when one member of the association becomes extinct or must compete with introduced species (Traveset and Richardson, 2006). Should native species no longer provide pollination services, visitation by non-native pollinators may be important for plant reproduction (Cox, 1983; Lammers et al., 1987). However, alien flower visitors may be less efficient at pollen transfer, may promote hybridization between different plant species, or may place new selective pressures on floral traits (Traveset and Richardson, 2006).

Few pollination studies have been conducted in Hawaiʻi. Due to high rates of extinction among the Hawaiian fauna, former roles of some native pollinators may remain a mystery, and the timeliness of understanding the roles of those still present is underscored. The extent to which alien species pollinate native plants or compete with native species for floral resources is poorly documented in the Hawaiian Islands.

The genus *Scaevola* (Goodeniaceae) in Hawaiʻi presents an opportunity to examine the pollination biology of a group that has an unusual form of pollen presentation and occurs in diverse lineages and habitats (Table 1.1). The flower visitors of Hawaiian *Scaevola*, both past and present, are not well known. The extensive variation in floral characteristics suggests pollinators differ among species. Not all flower visitation results in pollination, and nectar may be "robbed" (nectar removed by the visitor without coming into contact with reproductive organs). The extent to which

Table 1.1. Summary of Hawaiian *Scaevola* population locations and habitat. Data compiled from Wagner et al. (1999).

Taxa	Common Name	Status	Island	Habitat	Elevation (m)
S. chamissoniana	*Naupaka kuahiwi*	endemic	Moloka'i, Lāna'i, Maui, Hawai'i	wet forest, open areas	350
S. coriacea	Dwarf *naupaka*	endemic, endangered	Maui (Waiehu, Kaupō and Moke‘ehia islet), Moloka'i (Mokuho‘oniki Islet); formerly on all main islands	coastal, sand dune	< 100
S. gaudichaudiana	*Naupaka kuahiwi*	endemic	Kaua'i, O'ahu	wet forest, open areas	170-800
S. gaudichaudii	*Naupaka kuahiwi*	endemic	all main islands except Ni'ihau and Kaho'olawe	dry ridges and flats in open shrubland and forest	75-800
S. glabra	*'Ohe naupaka*	endemic	Kaua'i, O'ahu (Ko'olau range)	wet forest	200-1200
S. hobdyi	N/A	endemic, extinct	West Maui	wet forest	3000
S. kilaueae	*Huahekili uka*	endemic, rare	Hawai'i (Ocean View, Kīlauea)	open ohia forest and scrubland, old lava flows, ash substrate	1000-1460
S. mollis	*Naupaka kuahiwi*	endemic	Kaua'i, O'ahu, Moloka'i (rarely)	wet forest ridges and valleys, boggy areas	600-1400
S. procera	*Naupaka kuahiwi*	endemic	Kaua'i, Moloka'i	wet forest	(150-) 700-1400
S. taccada	*Naupaka kahakai*	indigenous	tropical and subtropical Pacific and Indian Oceans, Hawaiian archipelago except Gardener Pinnacles, Necker and Nīhoa	coastal	< 100

visitors of Hawaiian *Scaevola* rob nectar, and its impact on plant reproduction, is also poorly understood.

The Goodeniaceae and secondary pollen presentation

The genus *Scaevola* is a member of the Goodeniaceae, a family largely centered in Australia and New Guinea, with 11 genera and approximately 400 species (Carolin et al., 1992). Though the majority of *Scaevola* occur in Australia, this is the only genus within the family to have radiated extensively elsewhere (Carolin et al., 1992). Other Goodeniaceae with ranges extending outside Australia include *Goodenia pilosa* (occurring in Indonesia, China and Philippines), and *Selliera radicans* (in New Zealand and Chile). Chromosome numbers in the Goodeniaceae are n = (7) 8 or 9, or multiples of 8 or 9 (Peacock, 1963; Carr, 1978).

The family is generally characterized by herbaceous plants with dry fruits, though species outside (and a small number within) Australia exhibit an evolutionary tendency towards woodiness and fleshy fruits (Carolin et al., 1992). Flowers are protandrous, and have tubular zygomorphic flowers that are split adaxially. Flowers are usually 2-lipped, although most *Scaevola* and a small number of species (the monotypic *Selliera radicans* and a few *Goodenia*) are unilabiate, with the lobes forming a fan-shape (Carolin et al., 1992). Corolla lobes are usually winged with conspicuous thin membranous outgrowths along the lobe margins that are thought to enhance pollinator attraction (Carolin et al., 1992). The Goodeniaceae are generally insect pollinated, with rewards primarily consisting of nectar (Carolin et al., 1992). Many members of the family (including

Scaevola) have nectar guides, which may be visual (with colored lines) and/or tactile (with hairs or hair-like outgrowths on the throat or wing margins)(Carolin et al., 1992).

The Goodeniaceae may be distinguished from other families by a unique means of pollen presentation, as pollen is captured and secondarily presented by a specialized cup-shaped structure (indusium) at the distal end of the style (Leins and Erbar, 1990). A detailed study of this mechanism was described for *Selliera radicans* (Leins and Erbar, 1989). While the flower is in bud, pollen is shed from introrse anthers into the indusium. The pollen grains are coated with pollenkitt (a waxy substance that holds pollen grains together), enabling the pollen to slide into the indusium as the style elongates past the anthers. The indusium then closes and the style continues to grow. As the flower opens, the style tip is bent downwards forming an upper lip, with the fan shaped corolla functioning as a lower lip. Growth of the stigma pushes pollen out of the indusium (male phase), followed by the emergence of the stigma (female phase).

Secondary pollen presentation may have a number of selective advantages. Accuracy of pollen transfer by flower visitors may be facilitated if pollen removal and deposition occur in the same position on different flowers (Carolin, 1960). The concealment of pollen in the indusium may prevent access by unwanted visitors, and/or provide protection against rain or other environmental factors (Carolin, 1960). The portioned release of pollen may also confer an advantage for male fitness, as pollen cannot be completely removed at once by a visitor, and the male phase is prolonged (Leins and Erbar, 1990).

Scaevola

The name *Scaevola* is derived from Latin for "little hand," as the dried fan-shaped flowers appear as a "withered hand" (Carolin et al., 1992), or alternatively from *scaevus*, Latin for "left hand" (Ghisalberti, 2004). The genus includes roughly 130 species, with approximately 40 occurring outside Australia (Howarth et al., 2003). Flower color is variable, including white, blue, mauve, or, less often, yellow (Carolin et al., 1992). Nectaries are located above the ovary in most *Scaevola* (Carolin, 1959). Chromosome numbers are n=8(16) (Peacock, 1963; Carr, 1978).

Molecular data indicate at least six separate dispersal events of *Scaevola* out of Australia (Howarth et al., 2003). Two dispersal events resulted in widespread strand species, *S. taccada* and *S. plumieri*, and subsequent radiations from each. *Scaevola taccada* is widespread in the Pacific and Indian coastal strand, and has radiated in parts of the South Pacific. *Scaevola plumieri* is widespread in the Indian and Atlantic coastal strand, and has radiated in the Hawaiian Islands, Cuba and Socotra. *Scaevola plumieri* and *S. taccada* overlap on the east coast of Africa, Madagascar, the west coast of India, and Sri Lanka. These species also overlap in Florida, where *S. taccada* has become naturalized from ornamental plantings (Thieret and Brandenburg, 1986). At least four additional dispersal events out of Australia are represented by single species lineages, including *S. oppositifolia* (in New Guinea, Indonesia and the Philippines), *S. glabra* (in Hawai'i), *S. beckii* (in New Caledonia), and *S. gracilis* (in Tonga and New Zealand) (Howarth et al., 2003).

Hawaiian *Scaevola*

Ten species of *Scaevola* occur in the Hawaiian Islands, including one indigenous and nine endemic species (Wagner et al., 1999). All are diploid (*n*=8) with the exception of S. *glabra*, which is the only known tetraploid (*n*=16) in the genus (Peacock, 1963; Carr, 1978). Habitats range from coastal strand to high elevation wet forest (Wagner et al., 1999). Historically there have been different views about whether the coastal and inland *Scaevola* were of independent origins (Guppy, 1906) or the result of a single colonization event (Fosberg, 1948). Morphological, chromosomal, chemical and molecular evidence indicate the Hawaiian *Scaevola* resulted from three separate dispersal events represented by 1) *S. taccada,* derived from the Pacific region, 2) *S. glabra,* from Australia, and 3) the diploid endemics, possibly from the Americas (Carlquist, 1969, 1980; Wagner et al., 1990; Patterson, 1984, 1995; Howarth et al., 2003). The relatively high number of colonizations by *Scaevola* in the Hawaiian Islands may have been due to a lack of ecological constraints as *Scaevola* has not colonized any inland continental locations outside of Australia (Howarth et al., 2003).

Scaevola taccada

There has been much debate over the rightful name of the Pacific strand *Scaevola*, currently known as *S. taccada* (Fosberg and Sachet, 1956; St John, 1960; Fosberg, 1961; Fosberg, 1962; Jeffrey, 1980; Green, 1991; Shannon et al., 1997). Research on this species has addressed its physiology, phenology, seed predation and its role in island communities. In one study, *S. taccada* seeds floated in sea water germinated faster than those that were not floated, and seedlings had progressively lowered rates of germination as salinity increased (Lesko and Walker, 1969). Similarly, seedling growth is limited by substrate salinity, and to a lesser extent salt spray (Alpha et al., 1996). On Henderson Island, the phenology of *S. taccada* has no significant seasonality with respect to leaf production/loss; flowering and fruiting occur throughout the year, peaking in the summer months (January and December) (Brooke et al., 1996). Other species examined on Henderson Island have a fairly even distribution of flowering and fruiting throughout year for all species combined, possibly due to a limited number of pollinators and dispersers (Brooke et al., 1996). At Enewetok Atoll in the Marshall Islands, *S. taccada* was the dominant component in rodent stomach material on islands with rodents (Fall et al., 1971), and hermit crabs were presumed to consume the fruits and limit recruitment in inland forests of one islet lacking rodents (Louda and Zedler, 1985). *Scaevola taccada* may play an important role in some communities by protecting inland areas from wind and salt spray. In a study on Cousin Island in the Seychelles, significantly greater reproductive success of the Seychelles Warbler (*Acrocephalus sechellensis*) occurred in sites sheltered by *S. taccada* (Komdeur and Pels, 2005). A negative correlation was found between insect abundance (the food source for the warblers) and wind force. Subsequently, areas not protected by *S. taccada* had four times fewer fledglings and 18 times fewer independent young produced (Komdeur and Pels, 2005).

Few studies have documented flower visitation to *S. taccada*. In the Ogasawara (Bonin) Islands, *S. taccada* are visited by honey bees, carpenter bees (*Xylocopa*), flies, birds, ants and endemic bees (Abe, 2006). An interesting interaction occurs on

Henderson Island (Pitcairn Islands) between *S. taccada* and a lorikeet (Stephen's lorry, *Vini stepheni*), that has a generalist diet of nectar, pollen, fruit and lepidopteran larvae (Trevelyan, 1995). *Scaevola tacccada* is one of the dominant nectar resources for the Lories, that rob and vandalize the flowers. *Scaevola taccada* nectar had variable standing crop volume, ranging from 0-54.1 μL, though most flowers had little to no nectar (mean 6.6 +/- 12.1 μL), and sugar concentrations between 3.7-50.5 % w/w (mean 16.0 +/- 5.5 % w/w)(grams solute per 100 grams solution). Three species of ants were observed feeding on *S. taccada* nectar, and were believed to be responsible for the low nectar supply (Trevelyan, 1995). In conjunction with research on *Sesbania tomentosa* at Ka'ena Point on O'ahu, *S. taccada* was observed to have infrequent visits (< 0.2 visits $\cdot$ flower^{-1} $\cdot$ hour^{-1}) by endemic bees (*Hylaeus*), and was noted as having more frequent visitation by honey bees (*Apis mellifera*) and carpenter bees (*Xylocopa sonorina*)(Hopper, 2002).

Scaevola glabra

Scaevola glabra is unique within the genus in having markedly ornithophilous flowers (Patterson, 1995). The corolla is bright yellow, thick textured and decurved, with a connate tube. Molecular data indicate *S. glabra* is most closely related to the Australian species *S. angulata* and *S. depauperata* (Howarth et al., 2003). Neither of these species resemble *S. glabra*, as they have a more characteristic *Scaevola* flower type, with thin-textured, fan-shaped white or cream to blue flowers (Carolin, 1992). Interestingly, *S. glabra* appears to have convergent characteristics with *S. coccinea* of New Caledonia, which has a strikingly similar long, decurved yellow corolla tube, yet differs in having a more narrow corolla that is split adaxially to the base of the tube and a style that is much longer and densely hairy (Müller, 1990). Though this species was previously suggested as possibly ancestral to *S. glabra* (Carlquist, 1969; Patterson, 1995), molecular evidence indicates they are in separate lineages (Howarth et al., 2003).

Diploid endemic *Scaevola* radiation

A proposed phylogeny for the extant, diploid, endemic Hawaiian *Scaevola* suggests an early divergence from dry to wet habitats, as well as evolution from smaller to larger flowers, and pseudostellate to simple hairs (Carolin, 1970; Howarth, 2002). *Scaevola coriacea* was noted by Carlquist (1969) as morphologically similar to *S. plumieri*, a species that has been found to be most closely related to the diploid endemics (Howarth et al., 2003). Photosynthetic differentiation in *S. coriacea, S. gaudichaudii, S. gaudichaudiana* and *S. mollis* was investigated along a moisture gradient (Robichaux and Pearcy, 1984). Water use efficiency remained constant among species within the lineage, suggesting that characteristics of photosynthesis did not limit their ability to radiate into differing habitats (Robichaux and Pearcy, 1984).

Hybridization has been documented within the endemic radiation of Hawaiian *Scaevola*. Two species (*S. mollis* and *S. gaudichaudiana*) commonly hybridize naturally, forming hybrid swarms (Gillett, 1966; Gillett, 1972). A clinal pattern of flower color, vestiture, fruit diameter, peduncle length and number of leaf hydathodes was documented between these species in 6 sympatric populations on Kaua‘i, O‘ahu and Moloka‘i (Gillett, 1966). Molecular data suggests that hybridization may be recent, possibly resulting from pollination by introduced generalist honey bees (*Apis mellifera*) (Howarth, 2002). Other natural hybrid combinations have been documented between *S. gaudichaudii* and *S. procera* on Moloka'i (Skottsberg, 1927), and between *S. gaudichaudii* and *S. gaudichaudiana* on O'ahu and Kaua'i (Gillett, 1969). Additionally, molecular analyses suggest hybridization has played a role in the radiation of this lineage, as *S. procera* appears to be the result of ancient hybridization between *S. gaudichaudii* and *S. mollis*, and *S. kilaueae* is likely derived relatively recently from hybridization between *S. coriacea* and *S. chamissoniana* (Howarth and Baum, 2005).

A number of studies have been done in association with *S. plumieri*, the closest relative of the diploid endemic radiation of Hawaiian *Scaevola* (Howarth et al., 2003), which are worthy of note. This species is a dune stabilizer that grows primarily as a subterranean "trunkless tree," where sand accumulation results in buried stems with only the branch tips extending above the ground surface, forming dunes as high as 8-10 m (Knevel and Lubke, 2004). In a study of the phenology of this species in South Africa, its reproductive season occurred in mid-winter to fall, lasting 213 +/- 20 days (Knevel and Lubke, 2004). During two study seasons, only a third of the flowers were fertilized, and of those, less than a third produced ripe fruit. Aborted unripe seeds contained larvae of an unidentified fruit fly (Diptera). A third of the ripe fruits were also infected, but the seed apparently hardened prior to infection and remained viable. The pollinators of *S. plumieri* in this region are unknown, but are presumed to be moths or butterflies (Knevel and Lubke, 2004).

Pollination syndromes

Flower color, shape, size, scent, timing of anthesis and types of rewards have traditionally been described as forming "pollination syndromes" in which a suite of traits adapted to a particular type of pollinator are present, and may be used to predict the type of pollinator (Faegri and van der Pijl, 1979). Bird pollinated flowers tend to be brightly colored (especially red), have abundant, dilute (15 to 25% w/w) nectar, lack odor and have a heavy tubular corolla lacking a landing platform (Faegri and van der Pijl, 1979; Proctor et al., 1996). Bee pollinated flowers typically have a landing platform, are scented, have low to moderate amounts of nectar, vivid colors (often yellow or blue) and may have variable shapes (including tubular) (Faegri and van der Pijl, 1979). Honey bees generally forage on nectar with high sugar concentrations, commonly above 50% w/w, but no less than 20% w/w (Proctor et al., 1996). Moth pollinated flowers are characteristically white or dull colored, scented at night, produce larger amounts of nectar than bee pollinated flowers, and have corolla tubes more narrow than those of bird pollinated flowers (Faegri and van der Pijl, 1979). Nectar concentration in moth pollinated flowers may vary widely, from less than 10 % w/w up to 50 % w/w, though generally above 20 % w/w (Stevenson and Thomas, 1977; Howell and Prakrash, 1990; Kato, 1993; Goldblatt et al., 2001; Josens and Farina, 2001; Perret et al., 2001; Kaczorowski et al., 2005).

Flower nectar solutes primarily consist of some combination of sucrose, glucose and/or fructose. Ratios of sucrose to hexose (glucose and fructose) tend to be consistent within species (Baker and Baker, 1983), though intraplant variation (Herrera, 2006) and diurnal fluctuation (Baker and Baker, 1983) have been documented. Sugar ratios tend to correlate with pollinator type, where hummingbirds, moths, butterflies and long-tongued bees tend to visit flowers with sucrose-rich nectar, while generalist passerines, most short-tongued bees, New World bats and flies primarily visit flowers with hexose-rich nectar (Baker and Baker, 1983; Johnson and Nicolson, 2008). The known exceptions to these generalizations may be due to phylogenetic constraints among plants (Baker and Baker, 1983) or shifts in sugar preferences at different concentrations (Lotz and Schondube, 2006).

In recent years the traditional view of pollination syndromes has been criticized as not reflective of generalized pollination systems (diverse groups of pollinators visiting a single species), or situations where plant taxa are phylogenetically constrained such that floral traits commonly associated with a particular pollinator are not present (Johnson and Steiner, 2000). While the predictive power of pollination syndromes may be limited, the concept provides a starting point for developing hypotheses for plant-pollinator interactions (Pellmyr, 2002).

Hawaiian *Scaevola* exhibit variation in floral traits among species (Wagner et al., 1999). Flower colors include white, purple, dull yellow, and bright yellow. Some are scented while others lack scent. Shape and size vary, with differences in corolla tube length, curvature, thickness, and presence/absence of petal wings. Ultraviolet light reflectance occurs in *S. gaudichaudiana*, but is lacking in *S. coriacea, S. mollis, S. taccada* and *S. mollis* x *S. gaudichaudiana* (Jones et al., 1999).

Insect pollination of Hawaiian *Scaevola*

Scaevola species are generally considered to be insect pollinated (Carolin, 1992), though there has been little documentation of the types of insects that visit them. Similarly, there is only limited and often anecdotal information available regarding potential pollinators (past and present) of *Scaevola* in the Hawaiian Islands. These include native and non-native bees and birds (Table 1.2).

Perkins (1913) noted that native bees (*Hylaeus*) visit both coastal and inland species of *Scaevola*, but did not identify plants or insects to the species level. Five species of *Scaevola* (*S. chamissoniana, S. coriacea, S. gaudichaudiana, S. procera* and *S. taccada*) are variously listed in the "floral records" of 17 species of *Hylaeus* (Daly and Magnacca, 2003). These records list flowers around which bees have been seen flying, but not necessarily visiting or pollinating. In a study of pollination of *Sesbania tomentosa, Hylaeus* spp. were observed visiting flowers of *Scaevola taccada*, though they were approximately 1.5 times more active at *Sesbania* despite its low abundance relative to *Scaevola* (Hopper, 2002). Stomach contents of female *Hylaeus* spp. collected from both *Sesbania* and *Scaevola* contained 95-100% *Sesbania* pollen in most individuals, though 82% of the pollen from one individual was from *Scaevola*, indicating relative floral constancy and oligolectic to semi-polylectic behavior [female bees collecting pollen from a few allied (oligo-) to many diverse (poly-) plant species] (Hopper, 2002).

Hylaeus bees have radiated extensively in the Hawaiian Islands, including 62 species presumed to have originated from a single ancestor (Magnacca, 2005). *Hylaeus* occur over a wide range of habitats and elevations, from the coast to well above the tree

Table 1.2. Potential pollinators of Hawaiian *Scaevola* observed prior to present study.

Taxa	Invertebrate	References	Vertebrate	References
S. chamissoniana	*Hylaeus coniceps** *H. connectens** *H. haleakalae** *H. unicus** *H.* sp. A*	Daly and Magnacca, 2003		
	Apis mellifera†	pers. obs.		
S. coriacea	*H. longiceps**	Daly and Magnacca, 2003		
S. gaudichaudiana	*H. connectens** *H. mimicus** *H. unicus**	Daly and Magnacca, 2003		
	A. mellifera†	pers. obs.		
S. glabra			*Magumma parva* *Hemignathus kauaiensis* *Vestiaria coccinea*	Conant et al., 1998
			Himatione sanguinea	pers. obs.
S. kilaueae	*A. mellifera*†	pers. obs.		
S. mollis	*A. mellifera*†	Gillett, 1966		
S. procera	*H. connectens** *H. kauaiensis** *H. laetus** *H. specularis**	Daly and Magnacca, 2003	*M. parva*	D. Drake, pers. comm.
			H. sanguinea	D. Drake, pers. comm.
			Zosterops japonicus†	pers. obs.
S. taccada	*H. anthracinus** *H. difficilis** *H. flavifrons** *H. flavipes** *H. hostilis** *H. longiceps** *H. ombrias** *H. solaris**	Daly and Magnacca, 2003		
	Megachile fullawayi †	Swezey, 1954		
	A. mellifera† *Xylocopa sonorina*†	Hopper, 2002		
	Ceratina sp. nr dentipes† *Lasioglossum impavidum* †	Snelling, 2003		
S. gaudichaudiana x *S. mollis*	*A. mellifera*†	Gillett, 1966		
Scaevola spp.	*Hylaeus* spp.	Perkins, 1913 Swezey, 1954		
	A. mellifera†	Swezey, 1954		

*observed visiting plant, not necessarily flowers (Daly and Magnacca, 2003)

† non-native species

line (Perkins, 1913). *Hylaeus* are solitary and nest in the ground in coastal and dry areas, and in dead wood (often in standing trees) in wet areas (Perkins and Forel, 1899). Both nectar and pollen are collected by mouth, and later regurgitated as a food source for larva (Perkins and Forel, 1899). *Hylaeus* visit many different plant species (Perkins, 1913; Hopper, 2002).

Non-native honey bees (*Apis mellifera*) are attracted to *Scaevola* (Swezey, 1954), and visit *S. taccada* (Hopper, 2002), *S. mollis*, *S. mollis* x *gaudichaudiana* (Gillett, 1966), *S. gaudichaudiana*, *S. chamissoniana* and *S. kilaueae* (pers. obs.). In a study at Ka'ena Point, honey bees were the primary visitors of *S. taccada* (Hopper, 2002). *Apis mellifera* are social bees that may be found in many habitats, and may travel several kilometers in search of floral rewards (Schoonhoven et al., 2005). Both pollen and nectar are collected, and pollen is combed from hairs on the body and raked into packets on their hind legs (Schoonhoven et al., 2005). Whether honey bees use *Scaevola* for nectar, pollen, or both resources has not been clearly documented. In one study, 97 pollen types from 29 families were found in pollen packets collected from honey bees returning to their nests at Hakalau, Panaewa and Volcano on Hawai'i Island; however, *Scaevola* pollen was not represented (Arita et al., 1989).

Four other alien bee species have been reported to visit *S. taccada*. Carpenter bees (*Xylocopa sonorina*) were observed preferentially visiting *S. taccada* at Ka'ena Point on O'ahu (Hopper, 2002). *Megachile fullawayi* (Megachilidae), *Ceratina sp. nr dentipes* (Apidae) and *Lasioglossum impavidum* (Halictidae) also feed on flowers of *S. taccada* (Swezey, 1954, Snelling, 2003).

The extent to which insects other than bees pollinate Hawaiian *Scaevola* (presently or in the past) remains unknown. In addition to bees, many other types of insects likely provide pollinator services to the Hawaiian flora, including moths, flies, beetles and wasps (Howarth, 1985). There are only two species of native butterflies in Hawai‘i. The pale-colored, narrow, tubular corollas as well as scent in most Hawaiian *Scaevola* correspond with a moth-pollinated syndrome (Faegri and van der Pijl, 1979). Over 950 species of moths representing 17 families are native to the Hawaiian Islands (Howarth and Mull, 1992). Many of these species are extinct due to the introduction of parasites (Zimmerman, 1958a), and their former roles in ecosystems are poorly known. Moths have not been documented to visit *Scaevola* flowers in Hawai‘i.

The efficacy of pollination by native and non-native visitors to *Scaevola* is unknown. *Hylaeus* lack the profuse feathery hairs common to other bees which facilitate pollen trapping and transfer, and may not be very effective pollinators (Hopper, 2002). Honey bees and carpenter bees, though profusely hairy; rob nectar from some flowers (Barrows, 1980; Hopper, 2002).

Bird pollination of Hawaiian *Scaevola*

Two groups of birds (Meliphagidae and Drepanidinae) in the Hawaiian Islands include species that consume nectar. The Hawaiian Honeycreepers (Drepanidinae) have undergone a spectacular radiation, with over 50 species that evolved from a single ancestor (James and Olson, 1991, 2003, 2005, 2006; James, 2004). The diversity of bill types reflect specialization of feeding behaviors, with the finch-billed type as most ancestral (James, 2004; Pratt, 2005). Nectar consumption is a primary, partial or occasional part of the diet in several of these species (Pratt, 2005). Approximately half the endemic avifauna went extinct during the prehistoric era, though birds with bills specialized for nectar are a minority among the fossil species as compared with those with bills specialized for insects and fruit (James and Olson, 1991; Olson and James, 1991).

While most *Scaevola* are insect pollinated, flower visitation by birds has been reported among Hawaiian *Scaevola*. As noted above, *S. glabra* is unique within the genus in having a corolla highly adapted to bird pollination, with a heavy wide tube, the size and curvature matching the bill of the ‘I‘iwi (*Vestiaria coccinea*, syn. *Drepanis coccinea*) (Pratt, 2005). ‘I‘iwi (Conant et al., 1998), ‘Anianiau (*Magumma parva*, syn. *Hemignathus parva*) (Conant et al., 1998; Pratt, 2005) and ‘Apapane (*Himatione sanguinea*) (personal observation) have been observed visiting flowers of *S. glabra*. Interestingly, each of these have been observed robbing nectar from *S. glabra* (Conant et al., 1998; Pratt, 2005; personal observation), though ‘Anianiau have been reported to also visit the flowers “correctly” in such a way that pollination may occur (Conant et al., 1998). ‘I‘iwi rob nectar by piercing a hole in the base of the corolla tube (Conant et al., 1998), while ‘Apapane have been seen stealing nectar using pre-existing holes (personal observation). It has been suggested that nectar robbing among the honeycreepers is a recently learned behavior, subsequent to the introduction of alien plant species (Conant et al., 1998).

Curiously, the Black Mamo (*Drepanis funerea*, historically extinct) is portrayed with *S. glabra* in paintings by F.W. Frohawk and Rothschild (Wilson, 1890-1899; Berger, 1972). However, *D. funerea* is known only from Moloka'i (historically) and Maui (prehistorically), and *S. glabra* is only known from Kaua'i and O'ahu (James and Olson, 1991; Wagner et al., 1999). It is possible that the artists were using creative license by the inclusion of *S. glabra* in these paintings, although alternatively *S. glabra* may have been formerly present on additional islands and potentially visited by the Black Mamo.

Scaevola procera has a non-ornithophilous floral morphology, and as noted above, is visited by native bees (Daly and Magnacca, 2003). However, birds have also been observed visiting these flowers, including 'Anianiau (D. Drake, pers. comm.; pers. obs.), 'Apapane (D. Drake, pers. comm.) and Japanese White-eye (*Zosterops japonicus*)(pers. obs.).

CHAPTER 2. FLOWER VISITATION AMONG HAWAIIAN *SCAEVOLA*

Abstract

The genus *Scaevola* (Goodeniaceae) occurs as shrubs to small trees in diverse habitats in Hawai'i, from coastal strand to montane rainforests. Extensive variation in floral characteristics suggests pollinators differ among species. Bees (*Hylaeus* spp.) and honeycreepers (Drepanidinae) are the putative native pollinators, but their diversity and abundance have declined over the last century. Although the floral structure of some *Scaevola* suggests large moth pollinators, no native species in this guild are documented as visitors to *Scaevola* flowers. Due to high rates of extinction among the Hawaiian fauna and the introduction of alien generalists, former roles of some native flower visitors may remain a mystery, and the timeliness of understanding the roles of those still present is underscored. Flower visitation rates and visitor behavior were quantified for all nine extant species of *Scaevola* during the day and night (178.5 observation hours). Visitation was primarily diurnal, ranging from 0.2 to 3.0 visits $\cdot$ flower^{-1} $\cdot$ hour^{-1} during the day, with 4-15 visitor taxa per species. Non-native visitors, mainly honey bees (*Apis mellifera*) and ants, were the most frequent visitors for most species. *Hylaeus* were infrequent visitors to three species and common only at *S. chamissoniana*. No native macroleptidopteran visitors were observed. Birds were the main visitors to *S. glabra* and *S. procera*, with the alien *Zosterops japonicus* a primary visitor to both species, and the native *Hemignathus kauaiensis* also a primary visitor to *S. glabra*. Visitation was often conducive to pollination for most species. Visitor interactions differed for each species of *Scaevola*, and in several cases may impose limitations on pollination. The prevalence of alien

visitors has several implications for both the plants and native flower visitors. Non-native species may be depriving native visitors of floral resources and may limit plant reproduction if alien visitors are less effective pollinators than native species. Alternatively, non-native visitors may pollinate *Scaevola* species whose native pollinators have declined or shifted to new resources for unrelated reasons.

Introduction

Plant-pollinator interactions may be crucial for the maintenance of ecosystems (Pellmyr, 2002). Such interactions may be disrupted when one member of the association becomes extinct or must compete with introduced species (Traveset and Richardson, 2006). Should native species no longer provide pollination services, visitation by non-native pollinators may be important for plant reproduction (Cox, 1983; Lammers et al., 1987). In parallel with pollinator extinction worldwide, native insect and bird pollinators have declined in Hawai'i over the last century, and the recent discovery of parasitic mites (*Varroa destructor*) on O'ahu may lead to declines in introduced generalist honey bees (*Apis mellifera*)(Ramadan et al., 2007). Due to high rates of extinction among the Hawaiian fauna, former roles of some native pollinators may remain a mystery, and the timeliness of understanding the roles of those still present is underscored.

The genus *Scaevola* (Goodeniaceae) occurs as shrubs and small trees in Hawai'i, in habitats from coastal strand to montane rainforests. Ten species occur among three lineages represented by 1) *S. taccada,* 2) a radiation of eight species (one extinct), and 3)

S. glabra (Howarth et al., 2003). Variation in flower shape, size, color, scent and nectar properties suggests pollinators differ among species (Chapter 3). At least twenty-seven potential pollinating species have historically been associated with *Scaevola*, including 17 native bees, 4 native birds, 5 non-native bees, and 1 non-native bird (Chapter 1). However, flower visitation has not been quantified for any of the *Scaevola* species. Although the floral structure of some of the *Scaevola* species is suggestive of large moth pollinators, no native species in this guild have been observed visiting flowers.

This study aims to quantify the current flower visitors and visitor behavior among the extant Hawaiian *Scaevola* in order to address the following questions. Are non-native species potentially competing with or replacing native visitors? Are native and alien species visiting flowers in a manner that may result in pollination? It was anticipated that non-native species would be the primary flower visitors, and that visitation behavior of both native and alien species would generally be conducive to pollination.

Methods

Study system. *Scaevola* typically have fan-shaped flowers that are split adaxially to the base of the corolla (Carolin et al., 1992). Corolla lobes are usually winged with conspicuous, thin, membranous outgrowths along the margins (termed "petal wings") that are thought to enhance pollinator attraction (Carolin et al., 1992). Flowers may have nectar guides, which may be visual (with colored lines) and/or tactile (with hairs or hair-like outgrowths on the throat or wing margins)(Carolin et al., 1992). Flowers are protandrous, and pollen is secondarily presented by a specialized cup-shaped structure (indusium) at the distal end of the style (Leins and Erbar, 1990). Growth of the stigma pushes pollen out of the indusium (male phase), followed by the emergence of the stigma (female phase). Ovaries contain two ovules, and nectaries are located above the ovary in most *Scaevola* (Carolin, 1959). Flowers remain open throughout the day and night for approximately five to seven days.

Study sites. Observations of flower visitation for nine *Scaevola* species were conducted at ten locations on four islands between February and October of 2007 (Table 2.1). When possible, study sites were selected that were known to have potential native flower visitors within the community. Two study sites were used for *S. mollis* to increase the sample size, as many plants were inaccessible due to steep terrain at both sites. *Scaevola mollis* and *S. gaudichaudiana* commonly hybridize in areas of sympatry (Gillett, 1966). For this reason, an isolated population of *S. gaudichaudiana* was sampled. Isolated populations are less common among *S. mollis*, and care was taken to avoid sampling intermediate forms.

Field observations. Timed observations were made among randomly chosen individuals for all nine extant species of *Scaevola* with approximately fifteen person-hours during the day and five at night for most species. Observation intervals lasted either fifteen or thirty minutes, wherein between one and twenty-five flowers on an individual plant were continuously watched. Observations at *S. coriacea* were supplemented in part with the aid of video recordings using a MiniDV Handycam®

Table 2.1. Study site locations and habitat description forvisitation observations at nine species of *Scaevola* in Hawai‘i. A single population was used at each location, with the exception of two populations of *S. mollis*.

Species	Common name	Status	Location	Habitat	Elevation (m)
S. chamissoniana Gaud.	*Naupaka kuahiwi*	endemic	Waihe‘e Ridge Trail, West Maui Forest Reserve, Maui	ridge tops and slopes of open mesic forest/shrubland with *Metrosideros, Dicranopteris* and *Machaerina*	670
S. coriacea Nutt.	Dwarf *naupaka*	endemic, endangered	Waiehu Golf Course, Maui	sparsely vegetated fragments in raised inland dry coastal sand dune	20
S. gaudichaudiana Cham.	*Naupaka kuahiwi*	endemic	Mau'umae Ridge, Honolulu Watershed Forest Reserve, O'ahu	ridge tops of mesic forest/shrubland with *Acacia, Dicranopteris, Santalum* and *Metrosideros*	425
S. gaudichaudii Hook. & Arnott	*Naupaka kuahiwi*	endemic	Waimea Canyon State Park, Kaua'i	ridge tops of dry forest/shrubland with *Acacia, Dodonea, Leptecophylla* and *Wilkesia*	720
S. glabra Hook. & Arnott	*'Ohe naupaka*	endemic	Na Pali-Kona Forest Reserves, Pihea and Alaka'i Trails, Kaua'i	raised land in wet forest on margins of bogs with *Metrosideros, Clermontia* and *Melicope*	1200
S. kilaueae Degener	*Huahekili uka*	endemic, rare	Hilina Pali Road, Hawai'i Volcanoes National Park, Hawai'i	lava fields with dry to mesic forest/scrubland with *Metrosideros, Dodonea* and *Leptecophylla*	1000
S. mollis Hook. & Arnott	*Naupaka kuahiwi*	endemic	1) Kōnāhuanui, Honolulu Watershed Forest Reserve, O'ahu; 2) Mt. Ka'ala, Mokulē‘ia Forest Reserve, O'ahu	ridge tops and steep slopes of wet forest/shrubland with *Metrosideros, Dicranopteris* and *Machaerina*	1) 640 2) 1130
S. procera Hillebr.	*Naupaka kuahiwi*	endemic	Kōke'e State Park, Kaua'i	broad ridge top above deeply dissected valleys in wet forest with *Metrosideros, Kadua* and *Coprosma*	1250
S. taccada (Gaertn.) Roxb.	*Naupaka kahakai*	indigenous	Ka'ena Point Natural Area Reserve, O'ahu	dry coastal strand with *Sesbania, Myoporum, Jacquemontia* and *Sida*	5

Camcorder (Model DCR-HC96). All flower visits were documented, even if the visitor did not contact reproductive parts of the flower or did not utilize floral resources, in order to quantify all visitor interactions, and to avoid discounting potential pollen transfer. Each visitor taxon, the length of time (handling time) spent on each flower (measured with a stopwatch to the nearest second) and general notes on visitor behavior were documented. Visitors were determined to family, genus or species level using keys, reference specimens at Bishop Museum and/or expert assistance. The type of floral reward used (nectar and/or pollen) and the potential for pollen transfer (based on contact with pollen or stigma, collectively referred to as the indusium) were documented. Because *Scaevola* flowers are protandrous, the flower stage was also recorded. Flower stages were categorized as early male (flowers not fully open), male (flowers fully open, no stigmatic tissue expanded outside indusium), late male/early female (stigmatic tissue beginning to expand outside indusium, small amounts of pollen may still be present), and female phase (stigma fully developed). Observations were generally made during clear weather, to preclude reduced visitation associated with inclement weather (Tian et al., 2004).

Statistical Analysis. Mean visitation rates were calculated based on the total number of visits per total number of flowers observed per hour for each observation interval. Mean time spent at flowers and relative proportions of visitation by each taxon were calculated based on individual visits independent of observation intervals. Relative proportions of three types of behaviors were calculated for each visitor taxon based on individual visits independent of observation intervals: 1) contact with indusium versus nectar robbing, 2) visits to male versus female phase flowers, and 3) use of nectar versus pollen.

Results

A diverse assemblage of flower visitors was observed, including at least 47 taxa, from two classes and eight orders (Table 2.2-2.3). Total number of visitor taxa ranged from four (*S. glabra*) to fifteen (*S. gaudichaudiana*). Non-native visitors, mainly honey bees and ants (Formicidae) were the most frequent visitors. Native visitors were infrequent with the exception of *Hylaeus connectens* at *S. chamissoniana*. A number of previously documented visitors (Chapter 1) were not observed during the present study, including several species of *Hylaeus* associated with *S. chamissoniana*, *S. coriacea*, *S. gaudichaudiana*, *S.procera* and *S. taccada*, as well as ʻIʻiwi (*Vestiaria coccinea*) associated with *S. glabra* (despite its presence in the vicinity of *S. glabra* during the present study). No native macroleptidoptera visitors were observed. Direct interactions between floral visitors were not evident, barring one instance of a *Hylaeus* apparently being driven off *S. chamissoniana* by honey bees.

Visitation was primarily diurnal, ranging from relatively infrequent (*S. glabra*, 0.20 mean visits $\cdot$ flower^{-1} $\cdot$ hr^{-1}) to recurrent (*S. kilaueae*, 3.03 mean visits $\cdot$ flower^{-1} $\cdot$ hr^{-1}) during the day (Table 2.4). Mean visitation rates at night were < 0.10 visits $\cdot$ flower^{-1} $\cdot$ hr^{-1} for all species. Ants were the most common visitors at night. No visitors were observed at night at *S. glabra*, *S. mollis*, or *S. procera*. Time spent at flowers during a single visit varied greatly, from one second to over 30 minutes (Table 2.5). Ants and

Table 2.2. Percent of flower visits by each visitor taxon at Hawaiian *Scaevola* species based on total number of visits during timed observations.

	S. chamissoniana	*S. coriacea*	*S. gaudichaudiana*	*S. gaudichaudii*	*S. glabra*	*S. kilaueae*	*S. mollis*	*S. procera*	*S. taccada*
Aves									
Passeriformes									
Drepanidinae: *Magumma parva* ‡								7.9	
Drepanidinae: *Himatione sanguine* ‡					a[a]			1.6	
Drepanidinae: *Hemignathus kauaiensis* ‡					40.0				
Zosteropidae: *Zosterops japonicus* †				a	60.0			47.6	
Hexapoda									
Hymenoptera									
Formicidae: Ant(s) †	0.9	67.2[b]	16.3	64.5[c]		0.4	1.2		22.8
Apidae: *Apis mellifera* †	38.3	a	40.0	18.4		80.2	96.4	23.8	51.5
Apidae: *Xylocopa sonorina* †	a		3.1				a		1.2
Apidae: *Ceratina smaragdula* †									16.4
Apidae: *Ceratina arizonensis* †			8.2						
Megachilidae: *Megachile* †									4.7
Colletidae: *Hylaeus* sp. ‡[d]						2.9			
Colletidae: *Hylaeus* sp. [e]									a
Colletidae: *Hylaeus hostilis* and ? ‡				13.2					
Colletidae: *Hylaeus connectens* ‡	35.7								
Bee 1 ‡ or † [f]									3.5
Bethylidae ‡?						0.4			
Nesodynerus? ‡		10.4							
Wasp 1*		7.5							
Wasp 2*						0.4			
Vespidae: *Vespula pensylvanica* †						6.2			
Hemiptera									
Lygaeidae ‡?	a						a		
Lepidoptera									
Microlepidoptera*		1.5	7.1			0.8			
Nymphalidae †			a						
Pieridae: *Pieris* sp. †									a
Sphingidae †			a						

Table 2.2. (Continued) Percent of flower visits by each visitor taxon at Hawaiian *Scaevola* species based on total number of visits during timed observations.

	S. chamissoniana	*S. coriacea*	*S. gaudichaudiana*	*S. gaudichaudii*	*S. glabra*	*S. kilaueae*	*S. mollis*	*S. procera*	*S. taccada*
Diptera									
Syrphidae †	20.9		2.0	1.3		7.4[g]	1.2	19.0	
Psychotidae*			5.1						
Fly 1*								a	
Coleoptera									
Elateridae*			1.0						
Beetle 1*		7.5							
Beetle 2*			1.0						
Blattaria*	0.9								
Thysanoptera*	3.5		12.2				1.2	a	
Orthoptera									
Tettigoniidae: *Conocephalus saltator* †				1.3					
Orthoptera 1*						1.2			
Larva 1*				1.3					
Unknown 1*		1.5							
Unknown 2 *		3.0							
Unknown 3*		1.5							
Unknown 4*			2.0						
Unknown 5*			1.0						
Unknown 6*			1.0						
Unknown 7*									a
Unknown 8*						a			
Total number of visitor taxa	8	10	15	9	4	11	6	7	8

‡ native; † non-native; *unknown status; a: anecdotal observation

[a] observed twice, both times robbing nectar from pre-existing robbing hole at base of flower

[b] includes *Brachymyrmex obscura* and *Ochetellus glaber*

[c] includes *Ochetellus glaber*, *Pheidole megacephala*, and *Tetramorium* sp.

[d] may include *Hylaeus volcanicus* and/or *H. laetus* (collected near *S. kilaueae*)

[e] may include *Hylaeus athracinus* and/or *H. longiceps* (species documented at Kaena Point (Magnacca, 2007))

[f] may include *Hylaeus anthracinus* and/or *H. longiceps* (species documented at Kaena Point (Magnacca, 2007), *Ceratina arizonensis* or *Lasioglossum impavidum*

[g] includes 2 species

Table 2.3. Percent of plants visited by each visitor taxon at Hawaiian *Scaevola* species during timed observations.

	S. chamissoniana	*S. coriacea*	*S. gaudichaudiana*	*S. gaudichaudii*	*S. glabra*	*S. kilaueae*	*S. mollis*	*S. procera*	*S. taccada*
Aves									
Passeriformes									
Drepanidinae: *Magumma parva* ‡								1.9	
Drepanidinae: *Himatione sanguine* ‡					a[a]			1.9	
Drepanidinae: *Hemignathus kauaiensis* ‡					5.3				
Zosteropidae: *Zosterops japonicus* †				a	7.9			7.4	
Hexapoda									
Hymenoptera									
Formicidae: Ant(s) †	3.7	71[b]	16	41[c]		3.4	2.7		38
Apidae: *Apis mellifera* †	44	a	22	9.4		72	46	7.4	38
Apidae: *Xylocopa sonorina* †	a		2.3				a		6.3
Apidae: *Ceratina smaragdula* †									28
Apidae: *Ceratina arizonensis* †			4.7						
Megachilidae: *Megachile* †									9.4
Colletidae: *Hylaeus* sp. ‡[d]						10			
Colletidae: *Hylaeus* sp. [e]									a
Colletidae: *Hylaeus hostilis* and ? ‡				6.3					
Colletidae: *Hylaeus connectens* ‡	33								
Bee 1 ‡ or † [f]									9.4
Bethylidae ‡?						3.4			
Nesodynerus? ‡		9.5							
Wasp 1*		4.8							
Wasp 2*						3.4			
Vespidae: *Vespula pensylvanica* †						10			
Hemiptera									
Lygaeidae ‡?	a						a		
Lepidoptera									
Microlepidoptera*		4.8	12			6.9			
Nymphalidae †			a						
Pieridae: *Pieris* sp. †									a
Sphingidae †			a						

Table 2.3. (Continued) Percent of plants visited by each visitor taxon at Hawaiian *Scaevola* species during timed observations.

	S. chamissoniana	*S. coriacea*	*S. gaudichaudiana*	*S. gaudichaudii*	*S. glabra*	*S. kilaueae*	*S. mollis*	*S. procera*	*S. taccada*
Diptera									
Syrphidae †	33		4.7	3.1		24[g]	2.7	3.7	
Psychotidae*			4.7						
Fly 1*								a	
Coleoptera									
Elateridae*			2.3						
Beetle 1*		19							
Beetle 2*			2.3						
Blattaria*	3.7								
Thysanoptera*	11		21				2.7	a	
Orthoptera									
Tettigoniidae: *Conocephalus saltator* †				3.1					
Orthoptera 1*						3.4			
Larva 1*				3.1					
Unknown 1*		4.8							
Unknown 2 *		9.5							
Unknown 3*		4.8							
Unknown 4*			4.7						
Unknown 5*			2.3						
Unknown 6*			2.3						
Unknown 7*									a
Unknown 8*						a			

‡ native; † non-native; *unknown status; a: anecdotal observation

[a] observed twice, both times robbing nectar from pre-existing robbing hole at base of flower

[b] includes *Brachymyrmex obscura* and *Ochetellus glaber*

[c] includes *Ochetellus glaber*, *Pheidole megacephala*, and *Tetramorium* sp.

[d] may include *Hylaeus volcanicus* and/or *H. laetus* (collected near *S. kilaueae*)

[e] may include *Hylaeus athracinus* and/or *H. longiceps* (species documented at Kaena Point (Magnacca, 2007))

[f] may include *Hylaeus anthracinus* and/or *H. longiceps* (species documented at Kaena Point (Magnacca, 2007), *Ceratina arizonensis* or *Lasioglossum impavidum*

[g] includes 2 species

Table 2.4. Flower visitation rates from day and night observations of Hawaiian *Scaevola*. Rates represent mean visits $\cdot$ flower^{-1} $\cdot$ hour^{-1} for all flower visits, including those in which the visitor did not contact reproductive parts of the flower or did not utilize floral resources. Mean visitation rates and standard error (se) are based on averages among observation intervals. Observations of *S. coriacea* were not made at night. Sample size is based on number of individual plants observed.

	Day visits $\cdot$ fl^{-1} $\cdot$ hr^{-1}				Night visits $\cdot$ fl^{-1} $\cdot$ hr^{-1}			
	Mean	se	tot hrs*	n	mean	se	tot hrs*	n
S. chamissoniana	1.37	0.28	15.50	26	0.04	0.04	5.00	9
S. coriacea	1.09	0.13	15.00	21			0.00	
S. gaudichaudiana	1.71	0.34	15.00	39	0.88	0.43	5.00	10
S. gaudichaudii	1.07	0.20	15.25	30	0.04	0.04	5.25	9
S. glabra	0.20	0.10	18.75	38	0.00	0.00	1.75	4
S. kilaueae	3.03	0.48	15.00	27	0.15	0.11	5.00	10
S. mollis	0.90	0.25	17.00	32	0.00	0.00	5.00	10
S. procera	0.77	0.33	15.25	45	0.00	0.00	4.75	12
S. taccada	1.53	0.27	15.00	23	0.10	0.10	5.00	10

*total observation person-hours

Table 2.5. Mean time (in seconds) spent at flowers by visitors of Hawaiian *Scaevola* per visit. Diurnal (☼) and/or nocturnal (●) visitation are indicated for each visitor taxon.

	S. chamissoniana	*S. coriacea*	*S. gaudichaudiana*	*S. gaudichaudii*	*S. glabra*	*S. kilaueae*	*S. mollis*	*S. procera*	*S. taccada*
Aves									
Passeriformes									
Drepanidinae: *Magumma parva* ☼								2.0	
Drepanidinae: *Himatione sanguinea* ☼					a			8.0	
Drepanidinae: *Hemignathus kauaiensis* ☼					2.5				
Zosteropidae: *Zosterops japonicus* ☼				a	2.9			1.1	
Hexapoda									
Hymenoptera									
Formicidae: Ant(s) ☼●^{a}	u^{b}	>600	>453	>968^{c}		u	u		>1800
Apidae: *Apis mellifera* ☼	47	a	11	7.6		7.0	7.8	8.3	3.7
Apidae: *Xylocopa sonorina* ☼	a		5.7				a		4.0
Apidae: *Ceratina smaragdula* ☼									36
Apidae: *Ceratina arizonensis* ☼			138						
Megachilidae: *Megachile* ☼									4.1
Colletidae: *Hylaeus* sp. ^{d}☼						9.0			a
Colletidae: *Hylaeus* sp. ^{e}☼									a
Colletidae: *Hylaeus hostilis* and ? ☼				50.3					
Colletidae: *Hylaeus connectens* ☼	22								
Bee 1 ^{f}☼									14
Bethylidae ☼						60			
Nesodynerus? ☼		28							
Wasp 1 ☼		6.0							

Table 2.5. (Continued) Mean time (in seconds) spent at flowers by visitors of Hawaiian *Scaevola* per visit. Diurnal (☼) and/or nocturnal (●) visitation are indicated for each visitor taxon.

	S. chamissoniana	*S. coriacea*	*S. gaudichaudiana*	*S. gaudichaudii*	*S. glabra*	*S. kilaueae*	*S. mollis*	*S. procera*	*S. taccada*
Wasp 2 ☼						2.0			
Vespidae: *Vespula pensylvanica* ☼						8.7			
Hemiptera									
Lygaeidae ☼	a						a		
Lepidoptera									
Microlepidoptera ☼●^{g}		75	26			18			
Nymphalidae ☼			a						
Pieridae: *Pieris* sp. ☼									a
Sphingidae ☼			a						
Diptera									
Syrphidae ☼	5.5		38	20		9.0^h	30	31	
Psychotidae ●			5.6						
Fly 1 ☼								a	
Coleoptera									
Elateridae ☼			14						
Beetle 1 ●		347							
Beetle 2 ●			12						
Blattaria ●	u								
Thysanoptera ☼	>1350		>987				u	a	
Orthoptera									
Tettigoniidae: *Conocephalus saltator* ☼				357					
Orthoptera 1 ☼						50			
Larva 1 ☼				>1800					

Table 2.5. (Continued) Mean time (in seconds) spent at flowers by visitors of Hawaiian *Scaevola* per visit. Diurnal (☼) and/or nocturnal (●) visitation are indicated for each visitor taxon.

	S. chamissoniana	*S. coriacea*	*S. gaudichaudiana*	*S. gaudichaudii*	*S. glabra*	*S. kilaueae*	*S. mollis*	*S. procera*	*S. taccada*
Unknown 1 ☼		6.5							
Unknown 2 ☼		1.0							
Unknown 3 ☼		1.0							
Unknown 4 ☼			2.0						
Unknown 5 ☼			13						
Unknown 6 ☼			45						
Unknown 7 ☼									a
Unknown 8 ☼						a			

u: unknown (not timed), but generally a long handling time

[a] diurnal only at *S. chamissoniana*, *S. coriacea*, *S. mollis*; diurnal and nocturnal at *S. gaudichaudiana*, *S. gaudichaudii*, *S. taccada*; nocurnal only at *S. kilaueae*

[b] includes *Brachymermex obscura* and *Ochetellus glaber*

[c] includes *Ochetellus glaber*, *Pheidole megacephala*, and *Tetramorium* sp.

[d] may include *Hylaeus volcanicus* (collected near S. kilaueae) at *S. kilaueae*; may include *Hylaeus anthracinus* and/or *H. longiceps* (species documented at Kae‘na Point (Magnacca, 2007) visiting for nectar and pollen at *S. taccada*

[e] may include *Hylaeus athracinus* and/or *H. longiceps* (species documented at Kaena Point (Magnacca, 2007))

[f] may include *Hylaeus anthracinus* and/or *H. longiceps* (species documented at Kaena Point (Magnacca, 2007), *Ceratina arizonensis* or *Lasioglossum impavidum*

[g] diurnal only at *S. coriacea*, diurnal and nocturnal at *S. gaudichaudiana*, nocturnal only at *S. kilaueae*

[h] includes 2 species

thrips (Thysanpotera) were often present on flowers during the entire observation interval. Visits by birds were generally brief (< 10 seconds), while handling time by insects (other than ants and thrips) were variable.

Flowers were visited for nectar and/or pollen, though in some instances no floral resource was used (i.e., the visitor merely rested or crawled on the flower) or the visit was too brief to determine the intended resource (Figure 2.1). Both nectar and pollen were harvested from all species except *S. glabra*, which was visited only for nectar. Nectar was the primary resource used among most *Scaevola* species, with the exception of *S. chamissoniana*, at which visitors mainly harvested pollen. Pollen was obtained most frequently by honey bees, *Hylaeus*, and syrphid flies (Syrphidae).

Visitors approached flowers both in a manner conducive to pollination (indusium contacted inadvertently or in search of pollen) and non-conducive to pollination (nectar was robbed or visitors merely rested on the flowers)(Figure 2.2). Some visits included behaviors both conducive and non-conducive to pollination, particularly among honey bees, which often contacted the indusium and robbed nectar from the base of the corolla in a single visit. Nectar robbing occurred at all *Scaevola*, most commonly by ants and honey bees. Kaua'i 'Amakihi (*Hemignathus kauaiensis*) were also observed piercing the base of *S. glabra* flowers; the resulting holes are common (Appendix A). Kaua'i 'Amakihi, Japanese White-Eyes (*Zosterops japonicus*), and 'Apapane (*Himatione sanguinea*) used pre-existing holes to rob nectar at *S. glabra*. The indusium was contacted in at least half of the visits at all species except for *S. gaudichaudiana* and

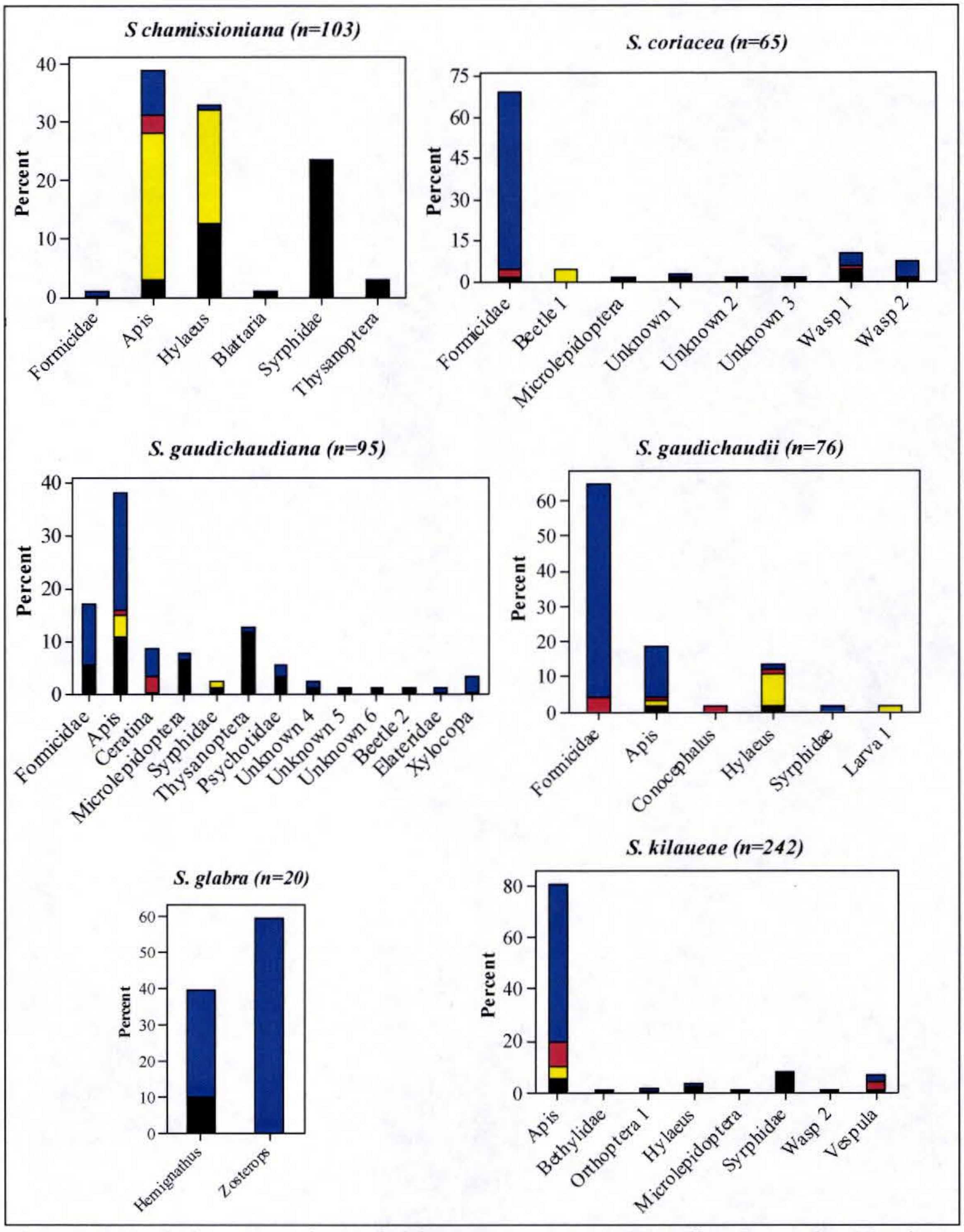


Figure 2.1. Relative proportions of visitor resource use at Hawaiian *Scaevola*. Floral resources are nectar (🟦), nectar and pollen (🟥), pollen (🟨) and unknown/none (⬛). Frequency based on individual visits rather than mean frequencies per observation interval. Full taxonomic names are listed in Table 2.2.

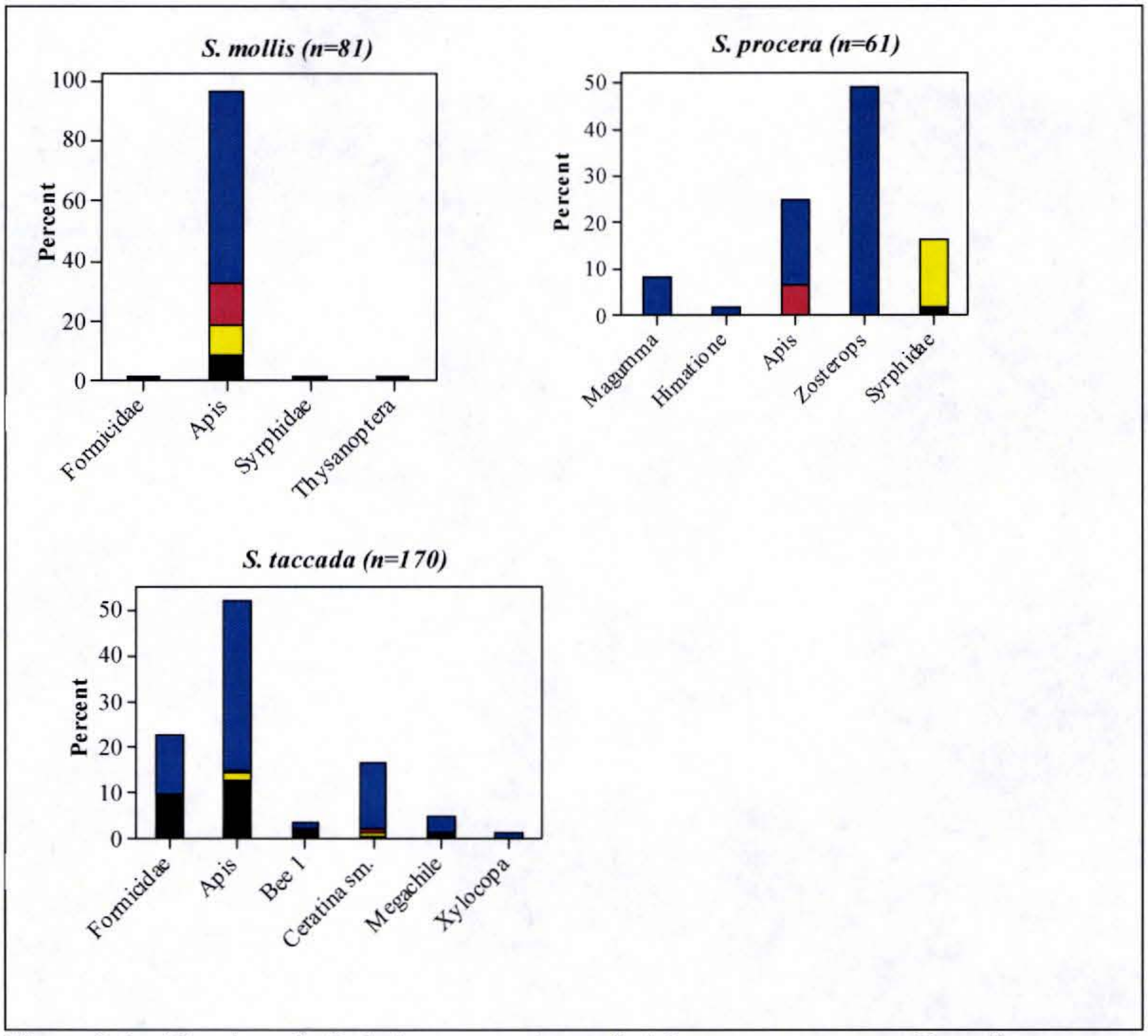


Figure 2.1. (Continued) Relative proportions of visitor resource use at Hawaiian *Scaevola*. Floral resources are nectar (🟦), nectar and pollen (🟥), pollen (🟨) and unknown/none (⬛). Frequency based on individual visits rather than mean frequencies per observation interval. Full taxonomic names are listed in Table 2.2.

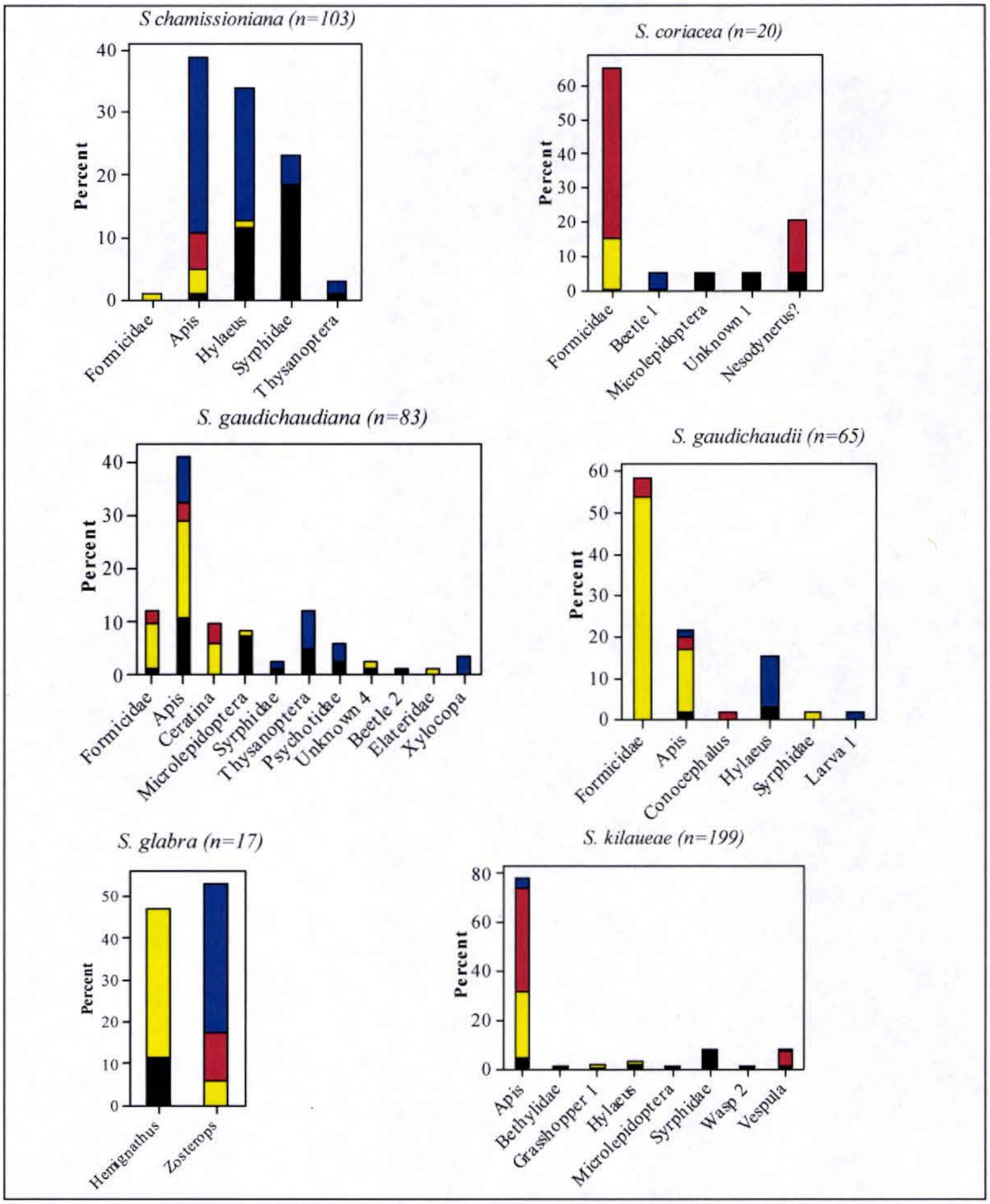


Figure 2.2. Visitor behavior, showing frequency of visitation with relative proportion of types of behavior during visit. Includes contact with indusium and no nectar robbing (🟦), contact with indusium and nectar robbing (🟥), no contact with indusium and nectar robbing (🟨), no contact with indusium and no nectar robbing (⬛). Frequency based on individual visits rather than mean frequencies per observation interval. Full taxonomic names are listed in Table 2.2.

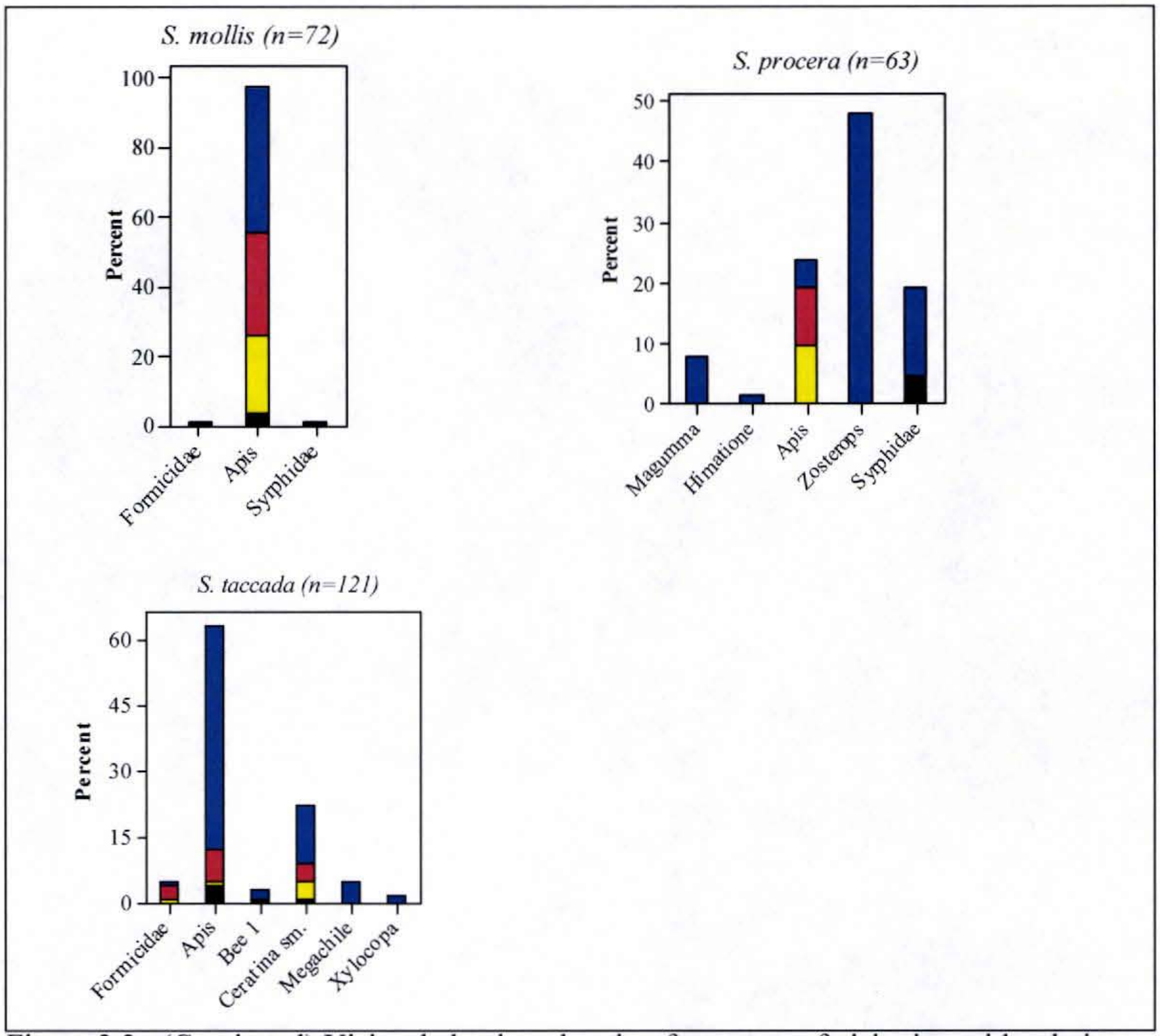


Figure 2.2. (Continued) Visitor behavior, showing frequency of visitation with relative proportion of types of behavior during visit. Includes contact with indusium and no nectar robbing (🟦), contact with indusium and nectar robbing (🟥), no contact with indusium and nectar robbing (🟨), no contact with indusium and no nectar robbing (⬛). Frequency based on individual visits rather than mean frequencies per observation interval. Full taxonomic names are listed in Table 2.2.

S. gaudichaudii, where contact with the indusium occurred only a third and a quarter of the time, respectively.

Because *Scaevola* is protandrous, in order for pollination to occur, visitors must not only contact the indusium, but they must also visit both male and female phase flowers. Among instances in which the indusium was contacted, the most common visitors utilized flowers of both male and female stages, with noted exceptions at *S. coriacea*, *S. glabra*, and S. *procera* (Figure 2.3). [The indusium was contacted only at male phase flowers among all visitors to *S. coriacea*; only at female phase flowers among all visitors to *S. glabra*; and only at male phase flowers by syphids at *S. procera*.] Additionally, contact with indusia of (early to late) male phase flowers was in roughly equal proportion with contact at female phase flowers among the most common visitors, with the above-noted exceptions as well as *S. chamissoniana*. Only 10% of the visits at *S. chamissoniana* were to female phase flowers, as pollen was the primary resource. Some of the less common visitors that contacted the indusium only visited male or female phase flowers, though sample sizes were small. However, situations in which visits were limited to a single flower phase may be an artifact of limited observation time and infrequent visitation involving contact with reproductive parts of flowers.

Discussion

Non-native species were the main flower visitors for all *Scaevola* species, except for *S. chamissoniana* and *S. glabra*, where the primary visitors included both alien and native species. The majority of visits were conducive to pollination at all species with the

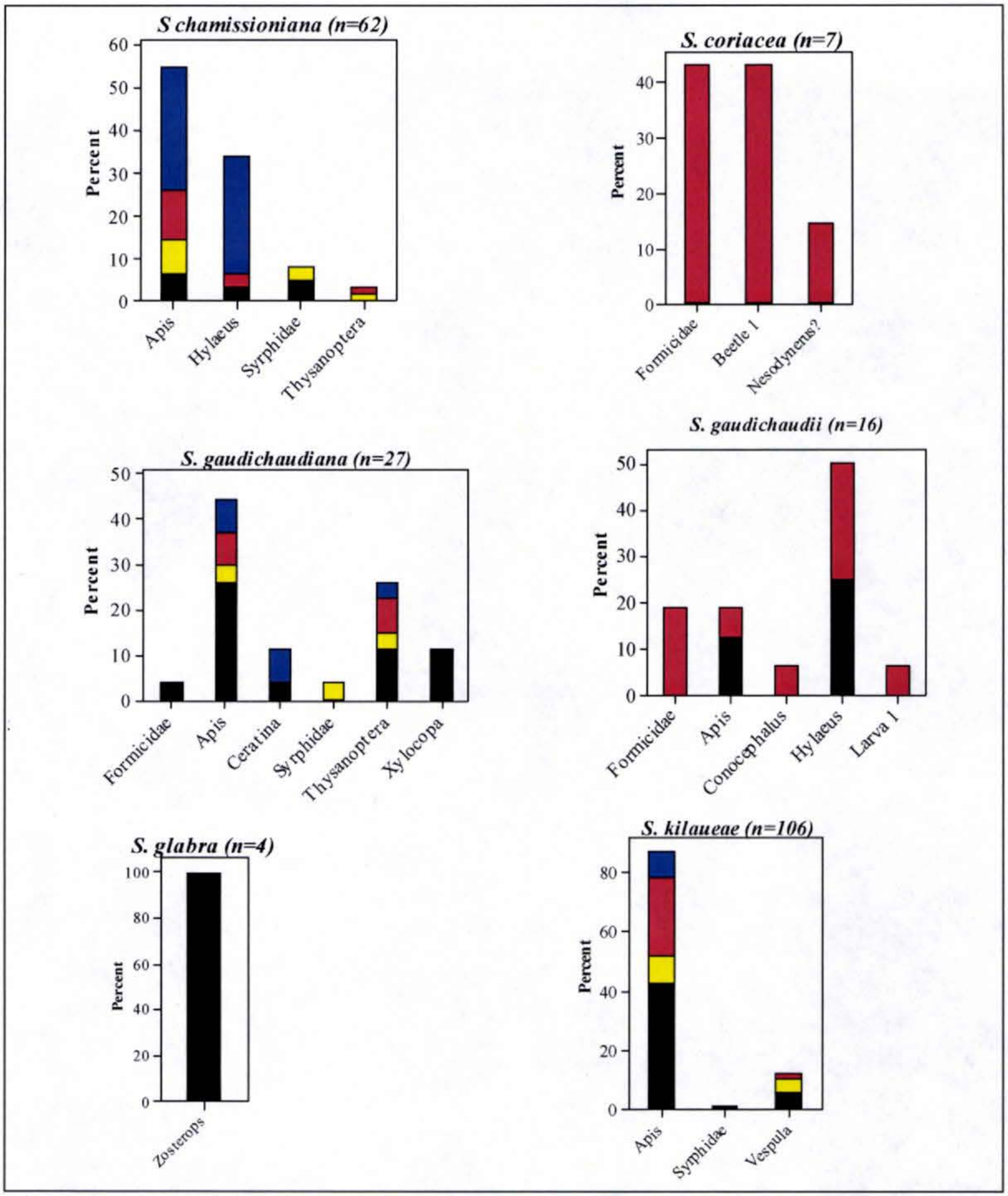


Figure 2.3. Potential pollen transfer to female phase flowers, showing frequency of flower visits in which indusium was contacted, with relative proportion of flower phase indicated. Flower phases are early male (🟦), male (🟥), late male/early female (🟨) and female (⬛). Frequency based on individual visits rather than mean frequencies per observation interval. Full taxonomic names are listed in Table 2.2.

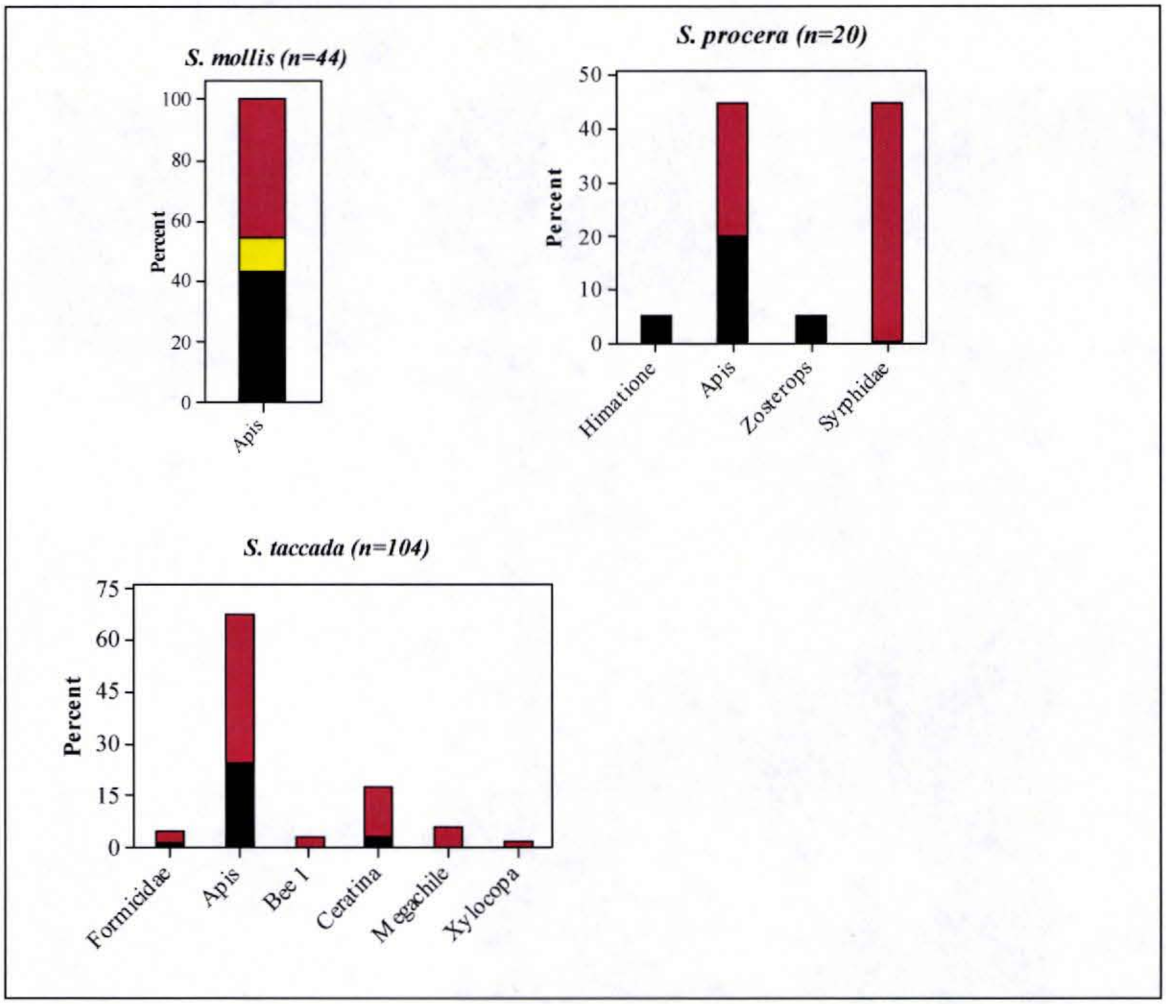


Figure 2.3. (Continued) Potential pollen transfer to female phase flowers, showing frequency of flower visits in which indusium was contacted, with relative proportion of flower phase indicated. Flower phases are early male (🟦), male (🟥), late male/early female (🟨) and female (⬛). Frequency based on individual visits rather than mean frequencies per observation interval. Full taxonomic names are listed in Table 2.2.

exception of *S. gaudichaudiana* and *S. gaudichaudii*. Visitor interactions differed for each species of *Scaevola*, and in several cases may pose limitations on pollination (Table 2.6). Pollination limitations may occur due to infrequent visitation (*S. glabra*, *S. mollis*, *S. procera*), infrequent contact with indusia (*S. gaudichaudiana* and *S. gaudichaudii*), or infrequent-to-no contact with female phase indusia (*S. chamissoniana* and *S. coriacea*). Only two species (*S. kilaueae* and *S. taccada*) received relatively frequent visitation in a manner conducive to pollination.

Despite potential pollination limitations, *Scaevola* species that have low visitation rates or infrequent visits conducive to pollination may be less at risk for limits on reproduction than genera with high numbers of ovules or short-lived flowers. *Scaevola* flowers only have two ovules, and the flowers are open for about five days. *Scaevola glabra* had a visitation rate of only 0.2 visits $\cdot$ flower^{-1} $\cdot$ hour^{-1} and the indusium contacted in only half the visits, but, over the life of a flower, this may translate to approximately five visits conducive to pollination, and has the potential to result in fertilization of both ovules. The lack of visits to male phase flowers at *S. glabra* may represent an artifact of the small number of visits, rather than visitor preference for female phase flowers.

Honey bees, in particular, appear to be important visitors to *Scaevola*, as they were among the most common visitors to nearly all species, and at least sometimes visited flowers in a manner conducive to pollination among species with moderate-to-high visitation rates (for species with low visitation rates, the majority of visits were conducive to pollination). Though honey bees (both worldwide and in Hawai'i) are known for generalist behavior and visit many different species, they tend to limit their

Table 2.6. Summary of key points of the most frequent flower visitors' interactions with each *Scaevola* species.

	Most frequent visitors (relative handling time in parentheses[a])	Relative frequency of visitation[b]	Primary resource used	Primary behavior of visitors at flower (robbing nectar and/or contact with indusium)	Potential pollination limitations
S. chamissoniana	honey bees (long), *Hylaeus* (long), Syrphids (moderate)	moderate	pollen	contact without robbing (honey bees, *Hylaeus*), no contact or robbing (Syrphids)	infrequent contact with indusia of female phase flowers
S. coriacea	ants (very long)	moderate	nectar	robbing with contact	contact with indusia only at male phase flowers
S. gaudichaudiana	honey bees (moderate), ants (very long), thrips (very long)	moderate	nectar	robbing (ants, honey bees), contact (thrips)	infrequent contact with indusia
S. gaudichaudii	ants (very long), honey bees (moderate)	moderate	nectar	robbing without contact	infrequent contact with indusia
S. glabra	Japanese White-Eye (brief), Kaua'i 'Amakihi (brief)	very low	nectar	contact (Japanese White-Eye), robbing (Kaua'i 'Amakihi)	very infrequent visitation
S. kilaueae	honey bees (moderate)	high	nectar	contact and/or robbing	none apparent
S. mollis	honey bees (moderate)	low	nectar	contact	infrequent visitation
S. procera	Japanese White-Eye (brief), honey bees (moderate), Syrphids (long)	low	nectar	contact	infrequent visitation
S. taccada	honey bees (brief), ants (very long), *Ceratina* (moderate)	moderate	nectar	contact	none apparent

[a] flower handling time (mean seconds $\cdot$ flower^{-1}) brief: < 5; moderate: 5-15; long: 16-60; very long: >60

[b] visitation rate (mean visits $\cdot$ flower^{-1} $\cdot$ hour^{-1}) very low: < 0.1; low: < 1; moderate: 1-2; high: > 2

visits to a single species on individual foraging bouts, thereby exhibiting a behavior beneficial for plant reproduction (Arita et al., 1989; Schoonhoven et al., 2005). While honey bees may be pollinating many *Scaevola* species, they may also compete with native species for floral resources. Nectar feeding by honey bees limits use of nectar by *Hylaeus* at *Sesbania tomentosa* at Ka'ena Point on O'ahu (Hopper, 2002).

Ants were common visitors to four species. Nectar was robbed during most visits, and contact with indusia occurred either infrequently (*S. gaudichaudiana* and *S. gaudichaudii*) or frequently (*S. coriacea* and *S. taccada*). Ants are often viewed as inefficient pollinators (Faegri and van der Pijl, 1979). In Hawai'i, ants may disrupt plant-pollinator interactions by reducing nectar availability, aggressively guarding floral resources, and preying on larvae of native pollinators or excluding them from nest sites (Cole et al., 1992; Hopper, 2002, Lach, 2005).

Evidence that a particular visitor is a pollinator should include presence of pollen on the visitors' body, verification of conspecific pollen deposition on stigmas, seed set resulting from visitor access, and lack of or lessened seed set upon visitor exclusion (Pellmyr, 2002). Though effectiveness of pollen transfer factors into which visitors are most important for plant reproduction, recent meta-analyses suggest visitation frequency generally is a better predictor of pollinator importance than effectiveness (Vazquez et al., 2005; Sahli and Conner, 2006). The importance of rare visitors for *Scaevola* reproduction remains uncertain.

Prior to the introduction of alien species, *Scaevola* were presumably visited and pollinated by native species. In this study, native visitors were among the more common visitors at only two species. *Hylaeus connectens* harvested pollen frequently at newly opened flowers of *S. chamissoniana*. Though visitation was infrequent, roughly half the visits to *S. glabra* were by Kaua'i 'Amakihi, which only robbed nectar. Other species of *Hylaeus* and honeycreepers along with native Lygaeids (Hemiptera: Lygaeidae) and wasps (Hymenoptera: Bethylidae and possibly *Nesodynerus*) were only rare visitors to *Scaevola*, suggesting they (and possibly other species) are being replaced by non-native flower visitors.

The prevalence of non-native flower visitors to *Scaevola* species in Hawai'i has conservation implications for both the plants and native flower visitors (Table 2.7). Non-native flower visitors may be disrupting plant-native pollinator interactions. This may be of particular concern if native visitors do not have alternate resources, or if non-native flower visitors are less effective pollinators than native species. Alternatively, non-native flower visitors may provide important pollination services for *Scaevola* species whose native pollinators (potentially e.g., 'I'iwi or large moths) have declined or shifted to new resources for unrelated reasons. Native macrolepidoptera have declined over the last century in Hawai'i as a result of species introduced for biocontrol of agricultural pests (Zimmerman, 1958a). 'I'iwi have declined or become extirpated in some locations, and may presently feed more frequently than in the past on *Metrosideros* flowers subsequent to the extinction of the behaviorally dominant 'ō'ō (Meliphagidae: *Moho nobilis*), which sometimes excluded 'I'iwi from *Metrosideros* (Smith et al., 1995).

Future research must be done to address conservation implications of alien visitor interactions with *Scaevola* species in Hawai'i. Observations at multiple populations on

Table 2.7. Conservation implications concerning plants and/or pollinators.

S. chamissoniana	**Plants:** primary visitors may not be effective pollinators due to infrequent visits to female phase flowers; **Pollinators:** pollen of *S. chamissoniana* may be an important resource for native bees; pollination syndrome suggestive of macrolepidopteran pollinator (Chapter 3), though none observed visiting plants
S. coriacea	**Plants:** primary visitor (ants) may not be effective pollinator; **Pollinators:** no native visitor observed; ants may hinder visitation by native species
S. gaudichaudiana	**Plants:** primary visitors mostly rob nectar (ants, honey bees) or may not be effective pollinators (ants, thrips); **Pollinators:** pollination syndrome suggestive of large moth pollinator (Chapter 3), though no native species observed visiting plant
S. gaudichaudii	**Plants:** primary visitors mostly rob nectar (ants, honey bees); **Pollinators:** 2 species of *Hylaeus* frequent visitors at only 2 out of 32 observed plants, may be limited by abundance of ants
S. glabra	**Plants:** reproduction may be limited by infrequent visitation; **Pollinators:** loss of and/or behavioral shift in native pollinators may have occured, as the anticipated pollinator ('I'iwi) did not make any visits despite its presence in the community, and the 2 observed native species were primarily robbing nectar; a non-native bird species may be replacing the role of native bird pollinators
S. kilaueae	**Pollinators:** visits by native species (*Hylaeus*) rare, despite abundance in community (frequent visitors of *Dodonea* and *Leptecophylla*), may indicate loss of native pollinator other than *Hylaeus,* or that high rate of visitation by honey bees may hinder *Hylaeus* visitation
S. mollis	**Pollinators:** visits by native species (Lygaeids) rare, and did not contact indusial
S. procera	**Pollinators:** visits by native species ('Anianiau, 'Apapane) infrequent
S. taccada	**Pollinators:** visits by native species (*Hylaeus*) infrequent, may be hindered by primary visitors (honey bees, ants, *Ceratina*)

multiple islands and across several field seasons will provide a more thorough understanding of the nature of visitation to *Scaevola*. Different visitors may occur in different areas, may shift resources during the year, and may fluctuate in abundance from one year to the next. Prior to the decline of *Hylaeus*, when their presence was largely ubiquitous across the landscape, Perkins (1913) noted that bee numbers were variable from year to year. Controlled experiments must also be done to investigate the efficacy of flower visitors as well as interactions between native and non-native flower visitors.

CHAPTER 3. FLOWER BIOLOGY OF HAWAIIAN *SCAEVOLA*

Abstract

Hawaiian *Scaevola* have floral traits that suggest pollinators differ among species, yet knowledge of native pollinators that may have selected for these traits is limited. Because the diversity and abundance of native pollinators have declined over the last century in Hawai'i, pollination syndromes may offer clues regarding former pollinators that have become extinct or extirpated, or shifted to new resources. Nectar volume, sugar concentration and sugar composition along with a series of structural measurements of floral display, attraction, and the mechanics of nectar access were quantified among all nine extant Hawaiian *Scavola* species in three lineages. These were examined in the context of pollination syndromes and compared with visitors associated with each species. There were significant differences in nectar volume, concentration and most floral measurements among and within lineages ($P < 0.001$). Flowers ranged from small, pale and scented ones with small amounts of sucrose-dominant nectar in high concentrations to large, heavy, decurved, and brightly colored ones lacking scent and containing copious amounts of dilute, hexose-dominant nectar. Pollination syndromes corresponded with the putative native pollinators: endemic bees (*Hylaeus*) and honeycreepers (Drepanidinae). Nearly all *Scaevola* species exhibit combinations of traits suggesting generalist strategies to allow for visitation by diverse pollinator guilds. Some of these traits may indicate generalist passerine and large moth pollinator guilds that no longer exist among native visitors for some species of *Scaevola*.

Introduction

Flower color, shape, size, scent, timing of anthesis and types of rewards have traditionally been described as forming "pollination syndromes" in which a suite of traits adapted to a particular type of pollinator are present, and may be used to predict pollinator guilds (Faegri and van der Pijl, 1979). For example, bird pollinated flowers tend to be brightly colored (especially red), have abundant, dilute (15 to 25% w/w)(grams solute per 100 grams solution) nectar, lack odor and have a heavy tubular corolla lacking a landing platform (Faegri and van der Pijl, 1979; Proctor et al., 1996). Bee pollinated flowers typically have a landing platform, are scented, have low to moderate amounts of nectar, are yellow or blue, and may have variable shapes (including tubular) (Faegri and van der Pijl, 1979). Honey bees generally forage on nectar with high sugar concentrations, commonly above 50% w/w (Proctor et al., 1996). Moth pollinated flowers are characteristically white or dull colored, scented at night, produce larger amounts of nectar than bee pollinated flowers, and have narrow corolla tubes (Faegri and van der Pijl, 1979). Nectar concentration in moth pollinated flowers may vary widely, from less than 10 % w/w up to 50 % w/w, though generally above 20% w/w (Stevenson and Thomas, 1977; Howell and Prakrash, 1990; Kato, 1993; Goldblatt et al., 2001; Josens and Farina, 2001; Perret et al., 2001; Kaczorowski et al., 2005).

Flower nectar solutes primarily consist of some combination of sucrose, glucose and/or fructose. Ratios of sucrose to hexose (glucose and fructose) tend to be consistent within species, though intraplant variation and diurnal fluctuation have been documented (Baker and Baker, 1983; Herrera, 2006). Sugar ratios tend to correlate with pollinator type; hummingbirds, sunbirds, moths, butterflies and long-tongued bees tend to visit flowers with sucrose-rich nectar, while generalist passerines, most short-tongued bees, New World bats and flies primarily visit flowers with hexose-rich nectar (Baker and Baker, 1983; Johnson and Nicolson, 2008). The known exceptions to these generalizations may be due to phylogenetic constraints among plants (Baker and Baker, 1983) or shifts in sugar preferences at different concentrations (Lotz and Schondube, 2006).

In recent years the traditional view of pollination syndromes has been criticized as not reflective of generalized pollination systems (diverse groups of pollinators visiting a single species), or situations where plant taxa are phylogenetically constrained such that floral traits commonly associated with a particular pollinator are not present (Johnson and Steiner, 2000). While the predictive power of pollination syndromes may be limited, the concept provides a starting point for developing hypotheses for plant-pollinator interactions (Pellmyr, 2002).

The genus *Scaevola* (Goodeniaceae) occurs as shrubs and small trees in Hawai‘i in habitats from coastal strand to montane rainforests (Wagner et al., 1999). Ten species occur among three lineages represented by 1) *S. taccada,* 2) a radiation of eight species (one extinct), and 3) *S. glabra* (Howarth et al., 2003). Floral characteristics vary among Hawaiian *Scaevola* species (Wagner et al., 1999). Flower colors include white, purple, dull yellow, and bright yellow. Some are scented while others lack scent. Shape and size vary, with differences in corolla tube length, curvature, and width. Ultraviolet light reflectance has been documented in *S. gaudichaudiana*, but is lacking in *S. coriacea, S.*

mollis, S. taccada and *S. mollis* x *S. gaudichaudiana* (Jones et al., 1999). Variation in floral traits suggests native pollinators differ among species. Most *Scaevola* worldwide are visited by insects (Carolin et al., 1992), though one species in Hawai'i, *S. glabra*, has striking ornithophilous adaptations (Pratt, 2005). Shifts in pollinators may have occurred either as a cause or consequence of evolution in new environments (Price and Wagner, 2004). The diversity of floral characteristics may be due to selective pressure by different pollinators, though genetic drift or founder effects may also have been a factor.

Current flower visitors of Hawaiian *Scaevola* include endemic short-tongued bees (*Hylaeus* spp.) and honeycreepers (Drepanidinae), along with a diverse assemblage of alien taxa - primarily honey bees (*Apis mellifera*), ants (Formicidae), and Japanese White-Eye (*Zosterops japonicus*)(Chapter 2). Prior to the introduction of alien species, additional native species may have visited flowers of the Hawaiian *Scaevola*. Pollination syndromes of *Scaevola* species may offer clues as to types of native pollinators that may be extinct, extirpated or in decline. Because current animal community compositions and interactions do not necessarily reflect those prior to the arrival of humans in the Hawaiian Islands (Olson and James, 1982; Burney et al., 2001), an examination of floral rewards and cues may provide indirect evidence for plant-animal interactions that may have occurred previously (Lammers and Freeman, 1986). Flower rewards may also provide insight into resource use by flower visitors in Hawai'i.

This study aims to explore flower characteristics of Hawaiian *Scaevola* associated with floral display/attraction, the mechanics of nectar access, and nectar properties including volume, sugar concentration and sugar composition. These traits may then be examined in the context of known molecular-based phylogenetics (Howarth et al. 2003; Howarth and Baum, 2005). From this, it is anticipated that some, but not all, species will have a pollination syndrome that corresponds with known flower visitors, and that closely related species will have more similar floral traits than those in different lineages.

Methods

Study system. *Scaevola* typically have fan-shaped flowers that are split adaxially to the base of the corolla (Carolin et al., 1992). Corolla lobes are usually winged with conspicuous, thin, membranous outgrowths along the margins (termed "petal wings") that are thought to enhance pollinator attraction (Carolin et al., 1992). Flowers may have nectar guides, which may be visual (with colored lines) and/or tactile (with hairs or hair-like outgrowths on the throat or wing margins)(Carolin et al., 1992). Flowers are protandrous, and pollen is secondarily presented by a specialized cup-shaped structure (indusium) at the distal end of the style (Leins and Erbar, 1990). Growth of the stigma pushes pollen out of the indusium (male phase), followed by the emergence of the stigma (female phase). Ovaries contain two ovules, and nectaries are located above the ovary in most *Scaevola* (Carolin, 1959). Flowers remain open throughout the day and night for approximately five to seven days.

Study sites. Nectar properties and flower measurements for nine *Scaevola* species were conducted at ten locations on four islands between February and October of 2007 (Table 3.1). Two study sites were used for *S. mollis* to increase the sample size, as many plants were inaccessible due to steep terrain at both sites. *Scaevola mollis* and

Table 3.1. Study site locations and habitat description for nectar analysis and flower measurements at nine species of *Scaevola* in Hawai'i. A single population was used at each location, with the exception of two populations of *S. mollis*.

Species	Common name	Status	Location	Habitat	Elevation (m)
S. chamissoniana Gaud.	*Naupaka kuahiwi*	endemic	Waihe'e Ridge Trail, West Maui Forest Reserve, Maui	ridge tops and slopes of open mesic forest/shrubland with *Metrosideros, Dicranopteris* and *Machaerina*	670
S. coriacea Nutt.	Dwarf *naupaka*	endemic, endangered	Waiehu Golf Course, Maui	sparsely vegetated fragments in raised inland dry coastal sand dune	20
S. gaudichaudiana Cham.	*Naupaka kuahiwi*	endemic	Mau'umae Ridge, Honolulu Watershed Forest Reserve, O'ahu	ridge tops of mesic forest/shrubland with *Acacia, Dicranopteris, Santalum* and *Metrosideros*	425
S. gaudichaudii Hook. & Arnott	*Naupaka kuahiwi*	endemic	Waimea Canyon State Park, Kaua'i	ridge tops of dry forest/shrubland with *Acacia, Dodonea, Leptecophylla* and *Wilkesia*	720
S. glabra Hook. & Arnott	*'Ohe naupaka*	endemic	Na Pali-Kona Forest Reserves, Pihea and Alaka'i Trails, Kaua'i	raised land in wet forest on margins of bogs with *Metrosideros, Clermontia* and *Melicope*	1200
S. kilaueae Degener	*Huahekili uka*	endemic, rare	Hilina Pali Road, Hawai'i Volcanoes National Park, Hawai'i	lava fields with dry to mesic forest/scrubland with *Metrosideros, Dodonea* and *Leptecophylla*	1000
S. mollis Hook. & Arnott	*Naupaka kuahiwi*	endemic	1) Kōnāhuanui, Honolulu Watershed Forest Reserve, O'ahu; 2) Mt. Ka'ala, Mokulē'ia Forest Reserve, O'ahu	ridge tops and steep slopes of wet forest/shrubland with *Metrosideros, Dicranopteris* and *Machaerina*	1) 640 2) 1130
S. procera Hillebr.	*Naupaka kuahiwi*	endemic	Kōke'e State Park, Kaua'i	broad ridge top above deeply dissected valleys in wet forest with *Metrosideros, Kadua* and *Coprosma*	1250
S. taccada (Gaertn.) Roxb.	*Naupaka kahakai*	indigenous	Ka'ena Point Natural Area Reserve, O'ahu	dry coastal strand with *Sesbania, Myoporum, Jacquemontia* and *Sida*	5

S. gaudichaudiana commonly hybridize in areas of sympatry. For this reason, an isolated population of *S. gaudichaudiana* was sampled. Isolated populations are less common among *S. mollis*, and care was taken to avoid sampling intermediate forms.

Nectar analysis. Standard techniques were used to measure nectar volume, sugar concentration and sugar composition at randomly chosen plants (Kearns and Inouye, 1993; Corbet, 2003; Dafni et al., 2005). Standing crop nectar (nectar available at any given time, where visitors have not been excluded from flowers) was sampled at varying times of day and night, and was removed from flowers by capillary action using microcapillary pipettes (Drummond Scientific Company Microcaps®, with pipette sizes of 1, 2, 5, 10 and 20 µL). Sampling was conducted during clear weather to preclude rainwater dilution of nectar. Nectar volume was estimated by measuring the length of nectar within pipettes of known volume. Nectar concentration (grams solute per 100 g solution, or %w/w) was measured in sucrose equivalents with portable refractometers capable of measuring lower (0-50% w/w) and higher (45-80% w/w) sugar concentrations (Bellingham & Stanley No.:45-81 and No.:45-82). A small amount of nectar (0.04 to 20.0 µL) was collected and air dried in glass shell vials (8 x 35 mm) to examine sugar composition. For species containing minute amounts of nectar, flowers were bagged using drawstring bags made from fine mesh cloth to exclude visitors and allow nectar to accumulate, and Vaseline was applied to stems below flowering branch tips to prevent nectar removal by ants. In the laboratory, dried nectar samples were eluted with 200 µL of deionized water. Sucrose, glucose and fructose were separated and quantified by isocratic high-performance liquid chromatography (HPLC) using a Shimadzu

Prominence liquid chromatograph (Model LC-20AT) 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 x 7.8 mm with a precolumn, Bio-Rad Laboratories, Hercules, CA) was run at 1 mL min^{-1} and 80°C with degassed deionized water. The ELSD was run at 40°C. Retention times of the sugars were compared with that of pure standards. Peak area of the individual sugar peaks was calculated by the HPLC software. Classification of sucrose/hexose ratios is based on Baker and Baker (1983): hexose-dominant (< 0.1), hexose-rich (0.1-0.499), sucrose-rich (0.5-0.99) and sucrose-dominant (>0.999).

Flower measurements. Flowers on randomly chosen plants were photographed in the field with a reference scale, including front, side and top views of each flower. Only female phase flowers were used in the analysis in order to consistently represent fully mature flowers within the same developmental stage. Measurements were obtained using image analysis software (ImageJ Version 1.38). Measurements included width of corolla "fan," maximum petal lobe width (inclusive of petal wings), maximum petal wing width, corolla tube length (from base of tube to base of petal lobes), corolla tube width (measured from midpoint of tube), style length and corolla tube connation length (Figure 3.1). Presence or absence of scent (as recognized according to M. Elmore's perceptions) was noted at varying times of day.

Statistical Analysis. Mean values for nectar volume and concentration were calculated for 1-28 flowers per plant, including male and female phase flowers. Statistical analyses of nectar properties and flower dimensions were conducted using a

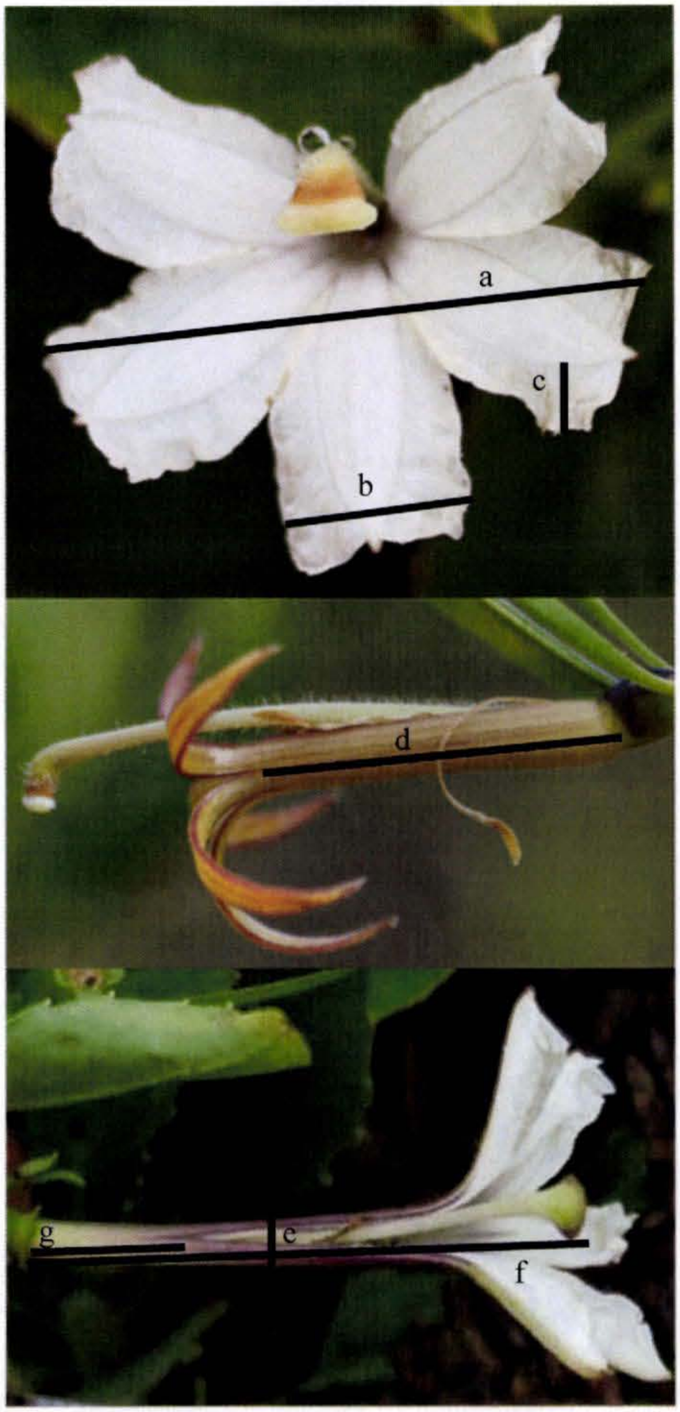


Figure 3.1. Diagram of types of flower measurements obtained for nine species of *Scaevola* using image analysis software. Measurements include (a) corolla "fan" width, (b) maximum petal width, (c) maximum petal "wing" width, (d) corolla tube length, (e) corolla tube width, (f) style length, and (g) corolla connation length.

non-parametric Kruskal-Wallis test for variance between lineages and within the radiation. An ANOVA test for variance could not be used due to unequal variance among data distributions. Regression analyses were performed for both nectar volume and concentration as compared with time of day. Multivariate analyses were made using principal components analysis (PCA) to examine relationships among species using all nectar and flower measurements as well as mean annual rainfall (Giambelluca et al., 1986) and elevation (determined with a Garmin Rino 120 GPS). A correlation matrix was used with components having eigenvalues of >1. Categorical data (scent and color) were layered atop the score plot to examine potential relationships with quantitative data. Minitab 14 was used for all statistical analyses.

Results

Nectar properties. There were significant differences in nectar volume, sugar concentration, and relative sugar composition among and within *Scaevola* lineages ($P < 0.001$)(Table 3.2, Figures 3.2-3.3). Mean (± standard error) nectar volume differed among species by three orders of magnitude, from minute (0.05 ± 0.02 µL in *S. coriacea*) to relatively copious (56.1 ± 11.2 µL in *S. glabra*) amounts. There was an eight-fold difference in mean sugar concentration among species, from relatively dilute (6.3 ± 0.4 %w/w in *S. glabra*) to concentrated (51.8 ± 1.1 %w/w in *S. taccada*). There were no significant relationships between nectar volume or concentration and time of day, with the exception of *S. coriacea* and *S. gaudichaudiana*, wherein nectar volume decreased during the day (Table 3.3 and Figure 3.4). All species had a combination of sucrose,

Table 3.2. Summary statistics for nectar volume, sugar concentration and relative sugar composition among *Scaevola* species in Hawai'i. Mean and standard error (se) values are based on averages among plants with 1-30 flowers subsampled per plant, except for relative sugar composition for *S. chamissoniana*, *S. coriacea*, *S. glabra* and *S. procera*, where a single flower was sampled per plant. Volume represents standing crop per individual flowers. Sugar concentration is in sucrose equivalents. Nectar composition is based on relative proportions of sucrose, glucose and fructose.

								Composition								
		Volume (µL)			Concentration (%w/w)			% sucrose		% glucose		% fructose		sucrose/hexose ratio		
Lineage	Species	mean	se	n	mean	se	n	mean	se	mean	se	mean	se	mean	se	n
1	*S. taccada*	0.07	0.02	32	51.8	1.1	30	62.53	5.98	23.2	3.7	14.3	2.5	10.5	4.1	24
2	*S. chamissoniana*	0.90	0.16	25	17.1	0.5	25	0.50	0.09	51.8	0.3	47.7	0.3	0.005	0.001	19
	S. coriacea	0.05	0.02	12	41.6	2.9	9	0.00	0.00	49.4	0.9	50.6	0.9	0.000	0.000	10
	S. gaudichaudiana	0.14	0.02	29	29.7	1.6	26	0.77	0.07	51.0	0.3	48.2	0.2	0.008	0.001	22
	S. gaudichaudii	1.14	0.15	29	27.2	1.8	24	0.00	0.00	50.3	0.3	49.7	0.3	0.000	0.000	21
	S. kilaueae	0.16	0.04	27	35.9	2.5	26	34.25	4.55	32.3	2.0	33.4	2.7	0.674	0.122	20
	S. mollis	2.78	0.45	35	22.8	0.7	35	1.55	0.59	52.5	0.5	45.9	0.6	0.017	0.007	20
	S. procera	2.53	0.43	22	15.9	0.7	20	0.10	0.04	54.2	0.4	45.7	0.3	0.001	0.000	21
3	*S. glabra*	56.10	11.20	20	6.3	0.4	35	0.07	0.03	53.8	1.1	46.1	1.1	0.001	0.000	8
Significance among lineages		H = 77.30 DF = 2 P < 0.001			H = 136.14 DF = 2 P < 0.001			H = 57.46 DF = 2 P < 0.001*								
Significance within lineage 2		H = 100.90 DF = 6 P < 0.001			H = 92.41 DF = 6 P < 0.001			H = 91.80 DF = 6 P < 0.001*								

*Significance test for sucrose/hexose ratio.

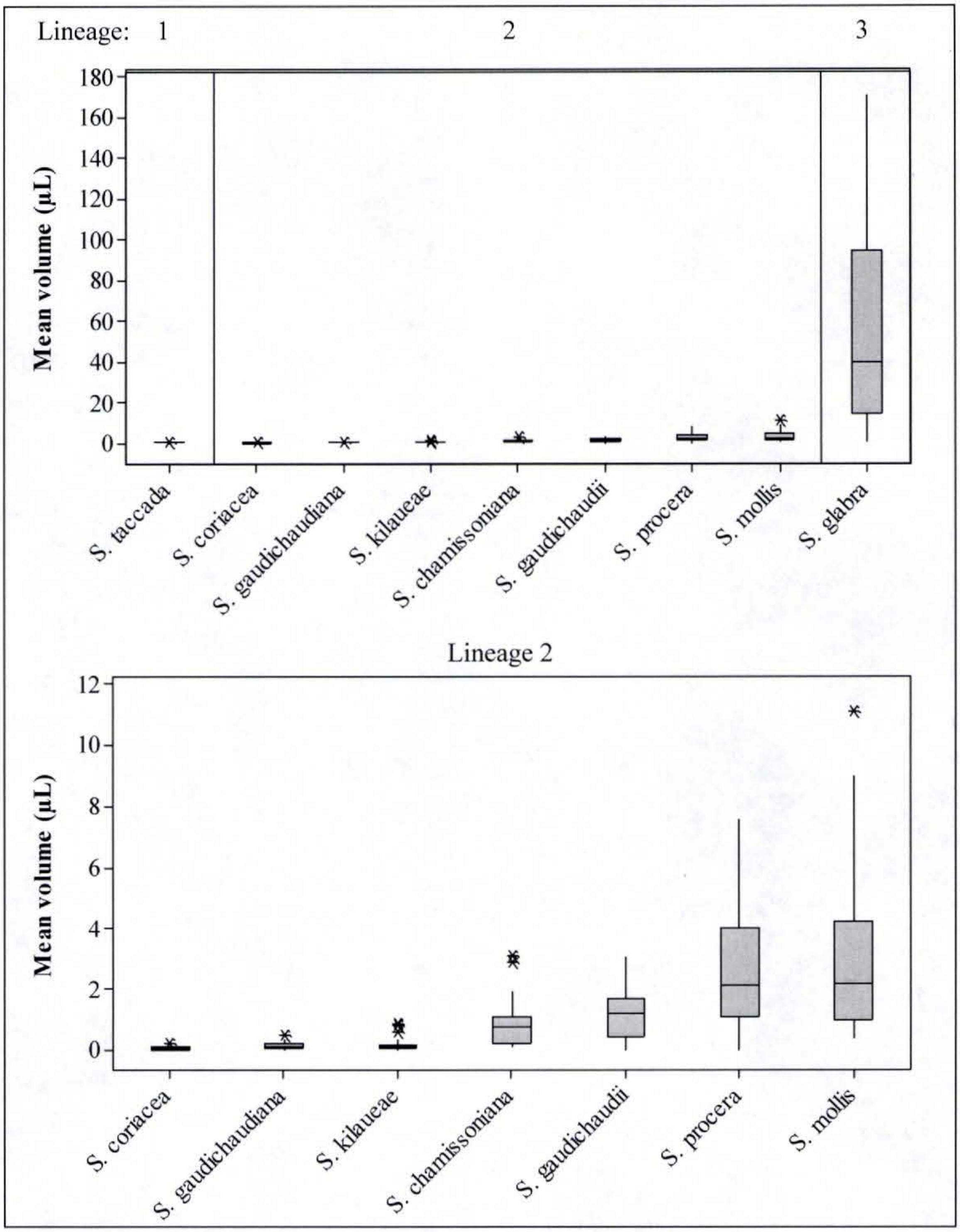


Figure 3.2. Boxplot distributions of nectar volume between and within Hawaiian *Scaevola* lineages. Volume represents standing crop per individual flowers measured at various times of day and night.

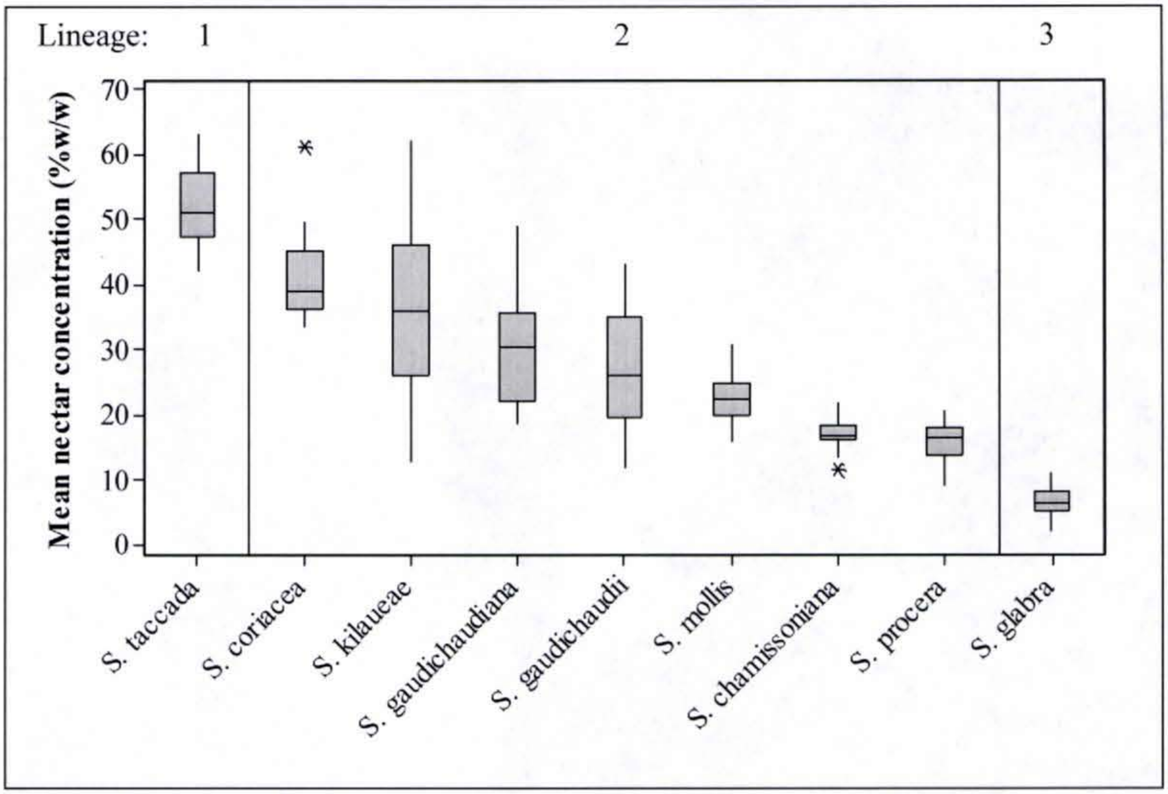


Figure 3.3. Boxplot distributions of nectar concentration between and within Hawaiian *Scaevola* lineages. Sugar concentration is in sucrose equivalents.

Table 3.3. Linear regression of Hawaiian *Scaevola* nectar volume and concentration versus time during the day. Volume represents standing crop per individual flowers. Sugar concentration is in sucrose equivalents. Significant relationships are in boldface.

	volume (µL)		concentration (%w/w)	
	R^2(%)	p	R^2(%)	p
S. chamissoniana	0.0	0.623	10.4	0.064
S. coriacea	**29.0**	**0.041**	0.0	0.362
S. gaudichaudiana	**22.3**	**0.006**	1.8	0.239
S. gaudichaudii	0.0	0.558	0.0	0.491
S. glabra	0.0	0.355	2.2	0.193
S. kilaueae	0.0	0.697	0.9	0.288
S. mollis	0.0	0.469	0.0	0.442
S. procera	3.9	0.189	8.7	0.111
S. taccada	0.0	0.833	0.0	0.795

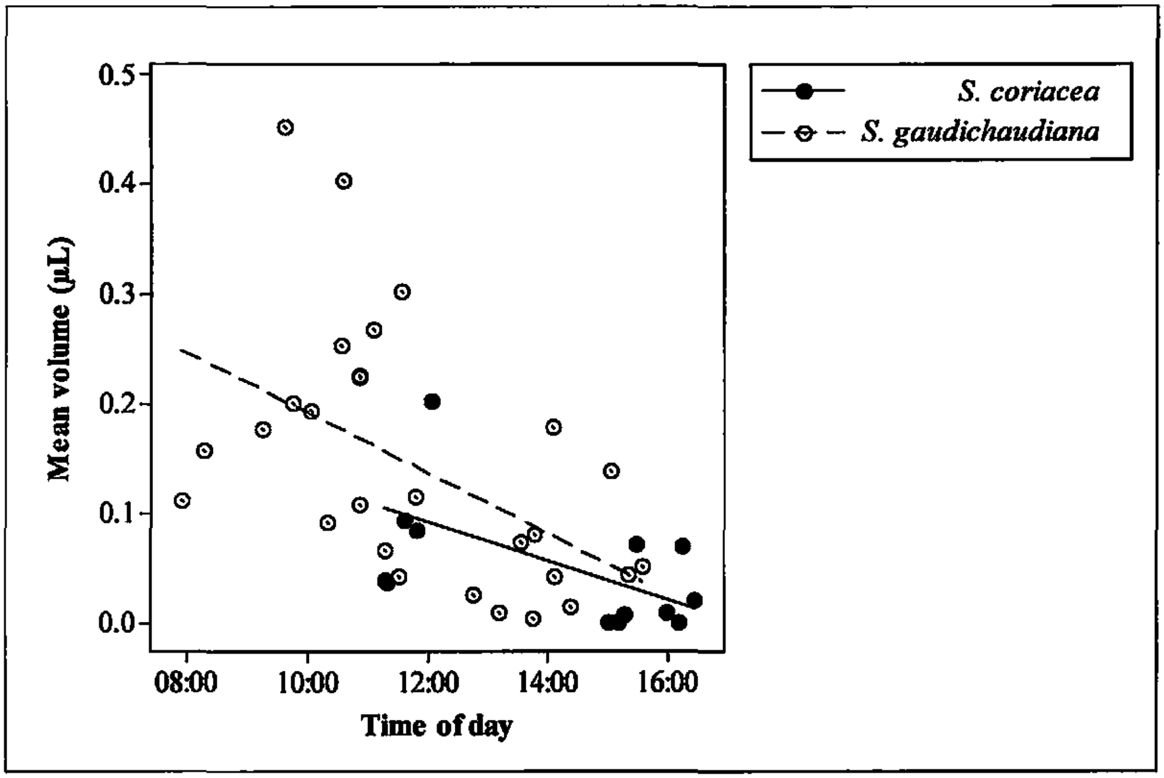


Figure 3.4. Scatterplot of nectar volume by time of day for *S. coriacea* and *S. gaudichaudiana*. Volume represents standing crop per individual flowers.

glucose and fructose, except for *S. coriacea* and *S. gaudichaudii*, which lacked sucrose entirely. Glucose and fructose were present in similar proportions to one another among all species. All species had hexose-dominant sugar (sucrose absent or in small amounts) with consistent ratios among samples, except for *S. kilaueae* and *S. taccada*. Mean sugar composition for *S. kilaueae* was hexose-rich (sucrose, glucose and fructose in nearly equal proportions), though sucrose/hexose ratios were quite variable with samples ranging from hexose-dominant to sucrose-dominant. Mean sugar composition for *S. taccada* was sucrose-dominant (hexose in small amounts), and also had variable sugar proportions wherein a third of the samples were either sucrose-rich or hexose-rich.

Flower measurements. There were significant differences in all flower measurements among and within *Scaevola* lineages ($P < 0.001$), with the exception of wing width among lineages ($P = 0.072$)(Tables 3.4-3.5 and Figure 3.5). Mean (± standard error) widths of the corolla "fan," petal lobe and petal wing varied among species, with *S. chamissoniana* having the largest of all flowers (27.9 ± 0.8 mm "fan," 7.4 ± 0.2 mm petal lobe, 2.1 ± 0.1 mm petal wing), twice the width of the smallest corolla (*S. coriacea*, 12.4 ± 0.8 mm), four times that of the smallest petal lobes (*S. gaudichaudii*, 1.8 ± 0.03 mm), and double that of the narrowest petal wings (*S. procera*, 0.9 ± 0.1 mm). Petal wings were lacking in *S. gaudichaudii*. There were approximately four-fold differences in mean corolla tube length and width, with *S. glabra* having the longest and widest tubes (35.8 ± 0.8 mm long, 7.9 ± 0.15 mm wide), *S. taccada* with the shortest (9.2 ± 0.4 mm), and *S. gaudichaudiana* having the narrowest tube (1.8 ± 0.04 mm). Style lengths were between 6 and 77% longer than that of the tube length, with the exception of

Table 3.4. Measurements of Hawaiian *Scaevola* flower visual cues and attractants. All data represent straight line measures (mm), and do not account for flower curvature. All measures based on female phase flowers. se = standard error. Significance based on Kruskal-Wallis test for differences among groups.

		Fan width[a]			Petal width[b]			Wing width[c]				
Lineage	Species	mean	se	n	mean	se	n	mean	se	n	Color	Scent[d]
1	*S. taccada*	15.6	0.6	28	3.6	0.1	28	1.2	0.1	28	white, sometimes with purple throat	subtle
2	*S. chamissoniana*	27.9	0.8	24	7.4	0.2	24	2.1	0.1	24	white	sweet
	S. coriacea	12.4	0.8	11	3.3	0.2	11	1.2	0.1	11	white	subtle, clove
	S. gaudichaudiana	19.1	0.5	21	4.8	0.1	21	1.7	0.1	21	white	sweet
	S. gaudichaudii	16.2	0.5	21	1.8	0.0	21	0.0	0.0	21	dull yellow	absent
	S. kilaueae	17.3	0.4	26	4.2	0.2	26	1.5	0.1	26	white	sweet
	S. mollis	25.1	0.6	20	5.8	0.1	20	1.8	0.1	20	purple to white	subtle, sweet
	S. procera	21.0	0.8	20	3.0	0.1	20	0.9	0.1	20	white, often with purple streaks	absent
3	*S. glabra*	22.8	0.7	31	6.0	0.2	31	1.5	0.1	31	bright yellow	absent
Significance among lineages		H = 35.88 DF = 2 P < 0.001			H = 30.46 DF = 2 P < 0.001			H = 5.27 DF = 2 P = 0.072				
Significance within lineage 2		H = 105.26 DF = 6 P < 0.001			H = 124.32 DF = 6 P < 0.001			H = 101.63 DF = 6 P < 0.001				

[a] maximum span of corolla

[b] petal width is inclusive of wing width

[c] membranous extension of tissue along petal margins

[d] scent does not differ during night versus day, though *S. coriacea* scent at night is unknown

Table 3.5. Flower measurements of Hawaiian *Scaevola* associated with nectar access and pollen transfer. All data represent straight line measures (mm), and does not account for curvature. All measures based on female phase flowers. se = standard error. Significance based on Kruskal-Wallis test for differences among groups.

		Corolla tube length			Corolla tube width			Style length			Corolla tube connation length		
Lineage	Species	mean	se	n	mean	se	n	mean	se	n	mean	se	n
1	*S. taccada*	9.2	0.4	27	2.7	0.07	28	9.8	0.2	28	0.0	0.0	28
2	*S. chamissoniana*	28.6	0.7	23	2.5	0.06	24	35.2	0.6	23	10.3	0.9	21
	S. coriacea	10.1	0.4	11	2.9	0.09	11	8.1	0.4	11	0.0	0.0	11
	S. gaudichaudiana	20.2	0.4	21	1.8	0.04	21	26.2	0.4	21	0.0	0.0	21
	S. gaudichaudii	11.7	0.3	21	2.4	0.05	20	20.6	0.5	21	0.0	0.0	21
	S. kilaueae	19.9	0.5	26	2.3	0.06	26	22.9	0.6	26	0.0	0.0	26
	S. mollis	16.7	0.4	20	3.7	0.07	20	23.4	0.3	20	0.0	0.0	20
	S. procera	12.9	0.2	18	3.3	0.06	24	18.8	0.5	24	0.0	0.0	24
3	*S. glabra*	35.8	0.8	32	7.9	0.15	23	46.7	1.2	23	29.3	1.1	22
Significance among lineages		H = 115.33 DF = 2 P < 0.001			H = 61.22 DF = 2 P < 0.001			H = 100.53 DF = 2 P < 0.001			H = 126.59 DF = 2 P < 0.001		
Significance within lineage 2		H = 123.67 DF = 6 P < 0.001			H = 118.63 DF = 6 P < 0.001			H = 117.16 DF = 6 P < 0.001			H = 144.71 DF = 6 P < 0.001		

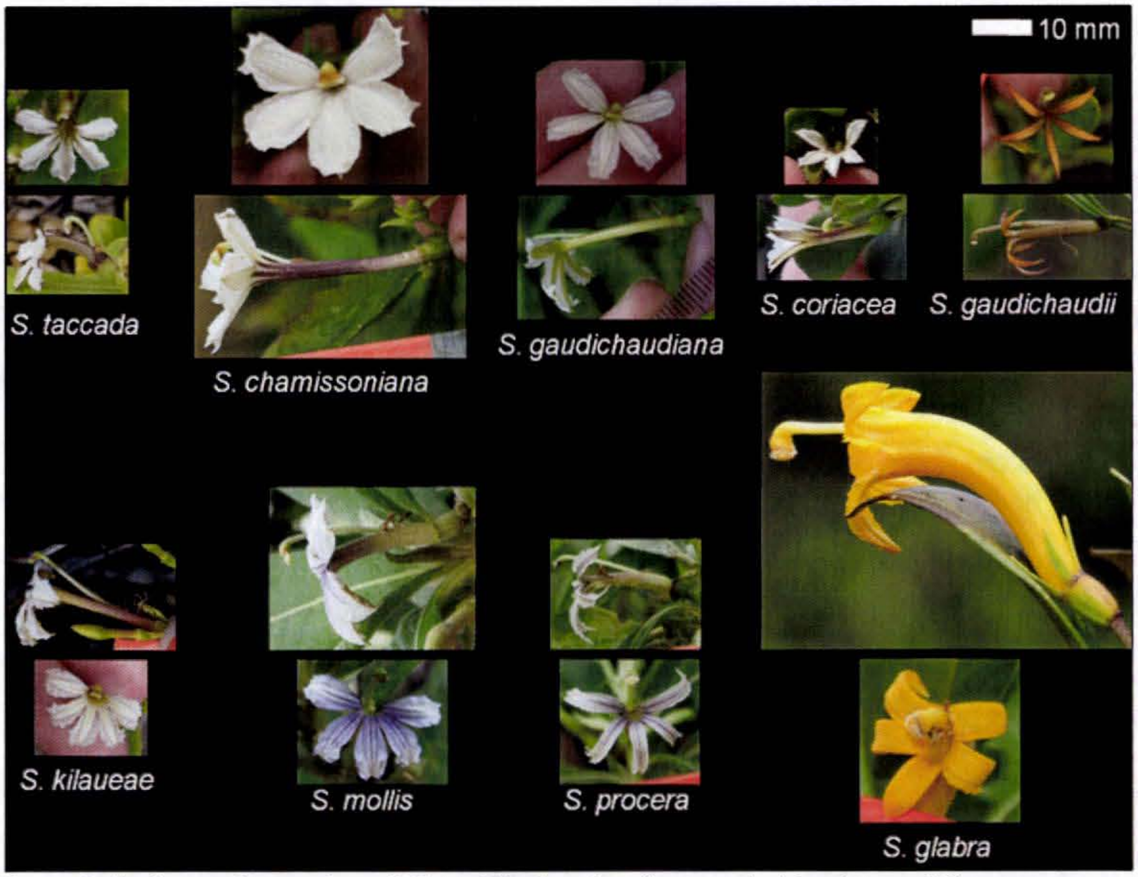


Figure 3.5. *Scaevola* species of Hawai'i, showing front and side views of flowers to scale. All photos taken with scale bar.

S. coriacea, having a style 20% shorter than the tube length. Corolla tube connation occurred in two species, with *S. chamissoniana* connate for approximately one-third the length of the tube, and *S. glabra* connate for over 80% of the tube.

PCA. PCA was used to reduce 12 variables (nectar traits, flower measurements, mean annul rainfall and elevation) to a smaller number of components for comparison. The first three components explained 88% of the variance within the data (Table 3.6). No single trait had a high loading value; instead, all had similarly low values. Nectar concentration and measurements relating to the mechanics of nectar access contributed the most to PC1, while measurements associated with pollinator cues were the primary contributors to PC2. PC3 was mainly determined by elevation and sugar composition. *Scaevola glabra* falls at a considerable distance from all other species on the score plot (Figure 3.6). Two species of ancient homoploid hybrid origins (*S. procera* and *S. kilaueae*, from *S. gaudichaudii* x *S. mollis* and *S. coriacea* x *S. chamissoniana*, respectively)(Howarth and Baum, 2005) plot intermediately between parental species. Species with scented flowers are clustered separately from those without scent (Figure 3.7), and yellow flowers cluster apart from white to purple flowers (Figure 3.8).

Discussion

Scaevola nectar properties, pollinator cues and flower structure vary considerably (Table 3.7). The greatest contrast in floral traits is between the two-single species lineages, wherein *S. taccada* is characterized by small, pale, scented flowers with small amounts of sucrose-dominant nectar in high concentrations, while *S. glabra* has large,

Table 3.6. Principal components of Hawaiian *Scaevola* floral and environmental characteristics. PCA based on correlation matrix with components having eigenvalues >1. Higher loadings for each component are highlighted.

	PC1	PC2	PC3
Eigenvalue	7.0086	1.9083	1.6356
Proportion	0.584	0.159	0.136
Nectar volume	0.292	-0.337	-0.323
Nectar concentration	**-0.334**	0.125	-0.277
Fan width	0.295	0.324	0.200
Petal width	0.273	**0.475**	-0.149
Wing width	0.173	**0.590**	-0.138
Tube length	**0.346**	0.101	-0.174
Tube width	0.286	-0.342	-0.269
Style length	**0.355**	0.013	-0.059
Connation length	**0.319**	-0.169	-0.355
Mean annual rainfall	0.285	-0.069	0.271
Elevation	0.265	-0.167	**0.435**
Sucrose/hexose ratio	-0.175	0.060	**-0.492**

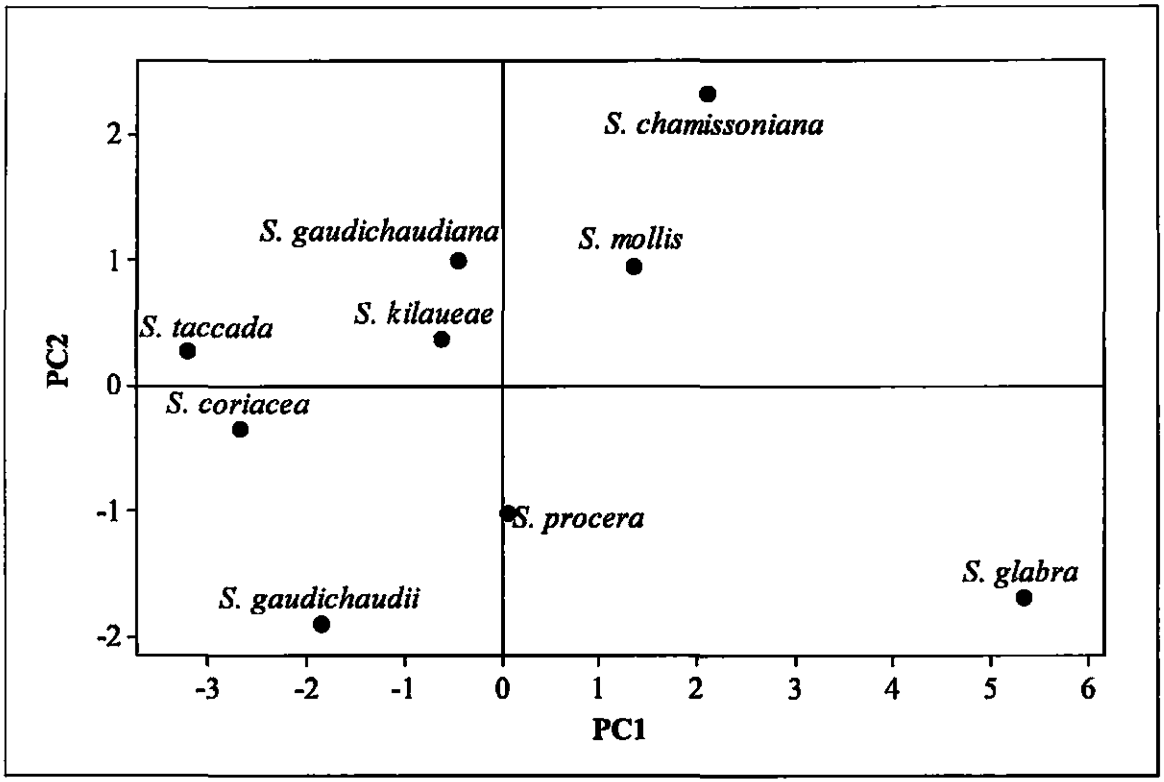


Figure 3.6. Score plot of principal components of flower and environmental measures for Hawaiian *Scaevola* species.

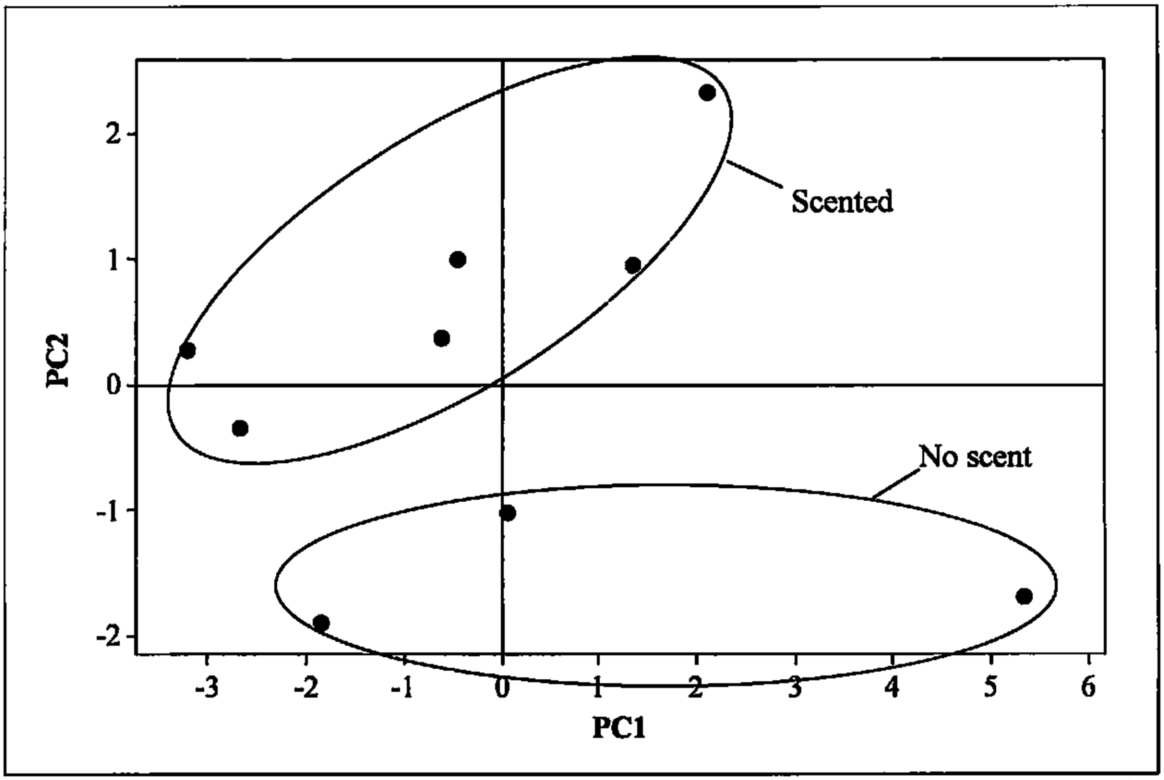


Figure 3.7. Score plot of principal components of flower and environmental measures for Hawaiian *Scaevola* species coded for scent.

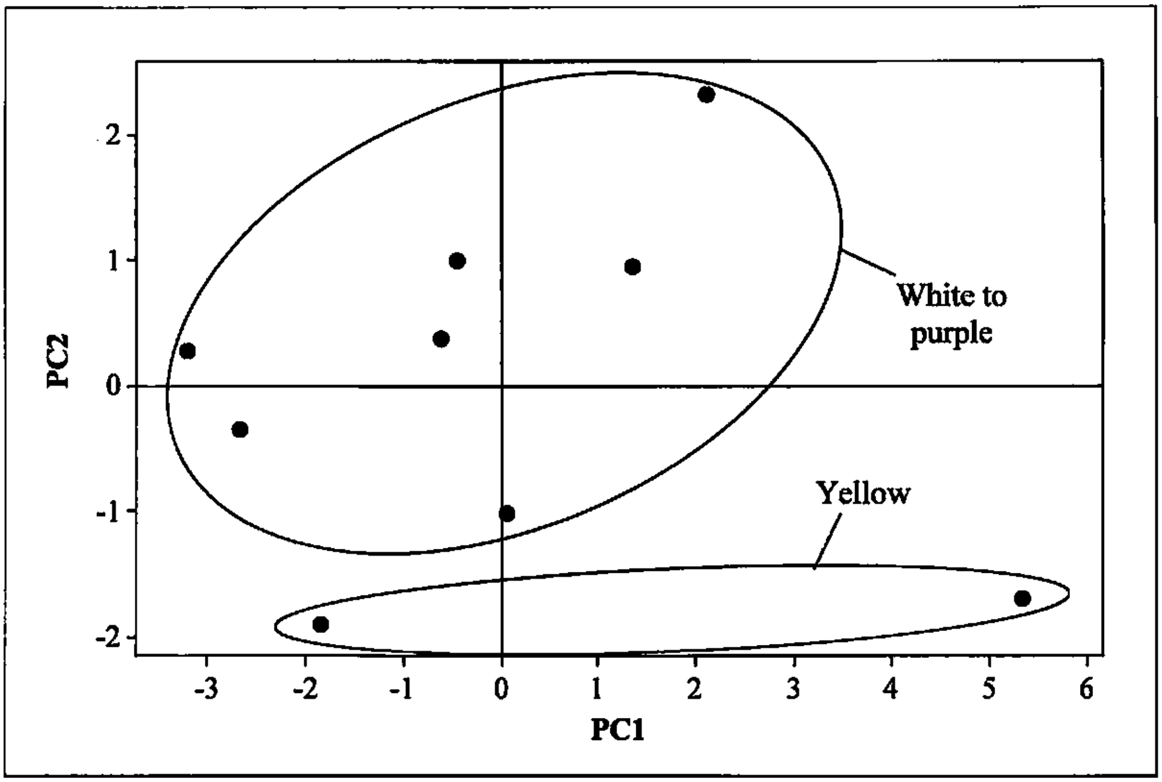
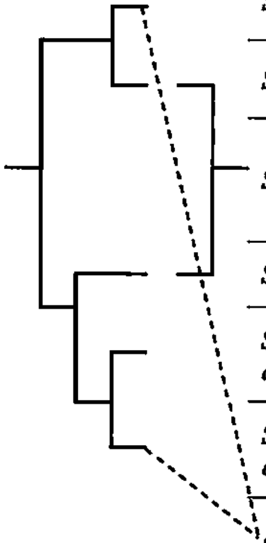


Figure 3.8. Score plot of principal components of flower and environmental measures for Hawaiian *Scaevola* species coded for flower color.

Table 3.7. Summary of Hawaiian *Scaevola* floral traits and flower visitors by phylogeny. Phylogeny based on Howarth et al. (2003) and Howarth and Baum (2005).

		Nectar properties			Pollinator cues and flower structure				Flower visitors	
Lineage		volume	concentration	sugar ratio	floral display[a]	tube size	scent	color	primary[b]	native[c]
1	*S. taccada*	very low	very high	sucrose-dominant	small	short	+	white/ purple	short-tongued bees, ants	short-tongued bees
2 	*S. coriacea*	very low	high	hexose-dominant	small	short	+	white	ants	short-tongued bees
	S. gaudichaudii	low	medium	hexose-dominant	medium-small	medium-short	-	yellow	ants, short-tongued bees	short-tongued bees
	*S. procera**	medium	low to medium	hexose-dominant	medium	medium-short	-	white/ purple	generalist passerines, short-tongued bees, flies	generalist passerines, short-tongued bees
	S. mollis	medium	low to medium	hexose-dominant	large	medium	+	purple/ white	short-tongued bees	Hemiptera
	S. gaudichaudiana	very low	medium	hexose-dominant	medium	medium-long	+	white	short-tongued bees, ants, thrips	short-tongued bees
	S. chamissoniana	low	low to medium	hexose-dominant	large	long	+	white	short-tongued bees, flies	short-tongued bees, Hemiptera
	*S. kilaueae**	very low	medium to high	hexose-rich	medium	medium-long	+	white	short-tongued bees	short-tongued bees, small wasps(?)
3	*S. glabra*	very high	low	hexose-dominant	large	very long	-	yellow	generalist passerines	generalist passerines

[a] accounts for relative fan, petal and wing widths

[b] current main visitors (not necessarily pollinators) based on timed observations (Chapter 2)

[c] all native visitors past and present (not necessarily pollinators), from Perkins, 1913; Swezey, 1954; Gillett, 1966; Conant et al., 1998; Hopper, 2002; Daly and Magnacca, 2003; Snelling, 2003; Pratt, 2005; Magnacca, 2007; D. Drake, pers. comm; and Chapter 2

*Homoploid hybrid

brightly colored flowers lacking scent and containing copious amounts of dilute hexose-dominant nectar. When compared with the known phylogeny based on molecular evidence (Howarth and Baum, 2005), there have been noteworthy divergences in floral traits within the endemic radiation. A basal split within the radiation resulted in one clade (*S. coriacea* and *S. gaudichaudii*) characterized by smaller flowers, subtle or no scent, and nectar that lacks sucrose. The second clade (*S. mollis*, *S. gaudichaudiana* and *S. chamissoniana*) has larger flowers, stronger scent and nectar containing small amounts of sucrose. Two homoploid hybrids (*S. procera* and *S. kilaueae*) are derived from these two separate clades within the radiation, and both have intermediate traits for nectar properties, pollinator cues and flower structure.

Known flower visitors of *Scaevola* generally fall within expectations based on floral characteristics. However, some floral traits indicate pollinator guilds not observed among native visitors. Most species have combinations of traits that allow for visitation by generalist taxa. The extent to which genetic drift or founder effects have influenced floral traits remains unknown.

As the genus *Scaevola* worldwide is generally associated with insect pollinators, it is noteworthy that two separate lineages of Hawaiian *Scaevola* have adaptations to bird pollinators. All floral traits of *S. glabra* are typical of generalist passerine-pollinated flowers. Indeed, this species is known only to be visited by birds. The flowers are similar in shape and size to the bills of ʻIʻiwi (*Vestiaria coccinea*), which are among known flower visitors (Conant et al., 1998; Pratt, 2005). Both bird and insect visitors are associated with *S. procera*. This species has traits characteristic of bird pollination with respect to nectar properties, the absence of scent, and corolla tube size, but otherwise has traits similar to other *Scaevola* species that are visited only by insects. *Scaevola gaudichaudii* and *S. mollis* have similar tube configurations, nectar properties and lack of scent (or subtle, in the case of *S. mollis*) as *S. procera*. However, bird visitation (Japanese White-Eye) was observed once at *S. gaudichaudii* in this study (Chapter 2). It is conceivable that in addition to insects, native birds may have been important visitors to *S. gaudichaudii* and *S. mollis* in the past. If this were the case, the hybrid evolution of *S. procera* could have resulted from cross pollination by birds of *S. mollis* and *S. gaudichaudii*, which occur in disjunct habitats that may be too far for most insects to travel.

Scaevola glabra is the only known tetraploid species in the entire genus (all others are diploid), and has flowers that look nothing like a typical member of the genus. It is notable that *S. procera* did not evolve highly ornithophilous traits in parallel with *S. glabra*, given that both species are associated with nectarivorous birds. It is possible that the higher level of ploidy in *S. glabra* conferred greater evolutionary lability, and/or that a generalist strategy for pollination by both birds and insects at *S. procera* precluded the evolution of a more distinctive ornithophilous syndrome.

Hylaeus are the primary native insects associated with Hawaiian *Scaevola*. Short-tongued bees typically, though not always, visit flowers with hexose-dominant (< 0.1) to hexose-rich (0.1-0.499) nectar. *Hylaeus* visit flowers for nectar at *Scaevola* species that have sugar compositions with mean (± standard error) sucrose/hexose ratios ranging from 0.0 ± 0.0 (*S. gaudichaudii*) to 10.5 ± 4.1 (*S. taccada*). This suggests that *Hylaeus* do not discriminate between these different sugar ratios. Tube size and style length are such that *Hylaeus* access to nectar would be conducive to pollination only for *S. taccada* and *S. coriacea*. Nectar properties and the mechanics of nectar access are irrelevant to visitors seeking only pollen. Though *Hylaeus* feed on nectar, female bees must also harvest pollen to provision their nests. Because pollen is easily accessed on all *Scaevola*, *Hylaeus* (or other pollen feeders, including flies, beetles and thrips) may visit flowers for pollen at any species, regardless of the pollination syndrome. The corolla fan forms a landing platform on all species allowing for diverse guilds of flower visitors.

The combination of long narrow tubes, strong scent and pale coloration of *S. gaudichaudiana*, *S. chamissoniana* and *S. kilaueae* suggest large, long-tongued moth visitation, though visits by members of this guild (alien Sphingidae) have only rarely been observed at *S. gaudichaudiana*. Tube connation is an unusual feature within the genus, and this trait in *S. chamissoniana* along with higher proportions of sucrose (and possibly nectar with higher volume and lower sugar concentration at night; see Appendix B) in *S. kilaueae*, also suggest selection by large moths. Native Lepidoptera have declined in diversity and abundance over the last century in Hawai'i as a result of species introduced for biocontrol of agricultural pests (Zimmerman, 1958a). Large native moths include Sphingidae (six taxa) as well as some Noctuidae and Oecophoridae (Howarth and Mull, 1992; Nishida, 2002). It is possible that members of this guild were once important pollinators of these species, but are presently either extinct or extirpated.

Additional investigations must be done to have a better understanding of the flower biology of Hawaiian *Scaevola*. Future research of nectar properties may include timing and rates of nectar production, as well as environmental effects on nectar volume and concentration. Nectar may contain solutes other than the three sugars investigated in this study. Analyses of other nectar properties, including amino acids, alkaloids, phenolics, lipids, etc., may reveal additional nutritive (or deterrent) aspects of nectar, particularly for *S. mollis*, in which an unusual spicy/acidic flavor was noted during the course of this study. Sugar composition is generally thought to be constant within species (Baker and Baker, 1983), with exceptions rarely investigated (Herrera et al., 2006). Variation in sugar composition in *S. taccada* and *S. kilaueae* may either suggest a generalist strategy to attract different pollinators, or that some type of contamination is occurring. Damage to floral tissue or the presence of floral yeasts may result in hydrolysis of sucrose, and subsequently higher proportions of glucose and fructose (Kearns and Inouye, 1993). Future research may reveal how and why nectar composition is variable in these species. *Scaevola* pollen is frequently harvested by visitors, and the nutritional content of pollen may provide additional insight in resource requirements of flower visitors, particularly *Hylaeus*. Lastly, non-native visitors appear to be replacing native visitors (Chapter 2) and may be placing new selective pressures on *Scaevola* flowers, and the resulting new floral traits may be disadvantageous to native visitors.

CHAPTER 4. CONCLUSIONS AND ADDITIONAL SUGGESTIONS FOR FUTURE RESEARCH

I have long wished some one to observe the fertilization of Scaevola... Now I hope you will get two plants of Scaevola, and protect one from insects, leaving the other uncovered, and observe the result both in the number of capsules produced, and in the average number of seed in each. It would be well to fertilize half a dozen flowers under the net, to probe that the cover is not injurious to fertility.

- excerpt from a letter by Charles Darwin to Fritz Müller April (9 and) 15, 1866

Hawaiian *Scaevola* have interspecific differences in flower structure and nectar traits that provide different cues, nectar accessibility, and nectar rewards for pollinators. Trait dissimilarities suggest selection by different pollinators, though genetic drift and founder effects may also have been a factor. The current prevalence of a few primary alien flower visitors to most species indicates visitor make-up is different from what it must once have been prior to the introduction of non-native species. Infrequent flower visitation, the loss of native pollinators, and alien visitation behaviors non-conducive to pollination may impose limitations on reproduction for some *Scaevola* species. Such limitations may be assuaged, should the capacity for selfing exist. However, selfing may pose hidden consequences wherein potential deleterious effects of inbreeding are not immediately apparent. The sharing of generalist flower visitors may also allow gene flow between closely related sympatric species that may previously have been reproductively isolated by having different pollinators, and could result in the formation of new hybrid species or loss of species via introgression. The research presented herein provides baseline data on flower biology and flower visitation, from which future pollination investigations may be directed. A number of specific research needs have been discussed in Chapters 2 and 3 with respect to flower visitation and flower biology of Hawaiian *Scaevola*. In light of the current findings, additional research is warranted to examine breeding and mating systems as well as the promotion of introgressive hybridization of *Scaevola* by alien species.

Breeding systems (anatomical, morphological and physiological characteristics of reproduction) and mating systems (outcrossing rates) among the Goodeniaceae have received little attention. Flowers in the family are protandrous, but whether male and female phases overlap enough within a flower to allow for self-fertilization, or if they are capable of selfing from pollen on a separate flower on the same plant, remains unknown. While protandry likely promotes outcrossing (Silvertown and Charlesworth, 2001), the mechanism of pollen presentation atop the developing stigma in most Goodeniaceae suggests a potential for selfing (Carolin, 1960). Charles Darwin was intrigued by secondary pollen presentation in this family, and investigated *Lechenaultia formosa* (Goodeniaceae), finding it to be sterile without the aid of an insect vector (Darwin, 1900). *Lechenaultia*, however, is an anomalous genus within the family, as the stigma is located outside of the indusium lip rather than at the base of the cup (Darwin, 1860a; Carolin, 1960). Darwin also observed and described the mechanism of secondary pollen presentation in *Scaevola microcarpa* (syn. *albida*), but could not be certain if it was capable of self fertilization (Darwin, 1860b). Upon Darwin's request (quoted above), Fritz Müller intended to examine the fertilization of *Scaevola*, but his plants died before he was able to complete the experiment (Müller, 1867).

A better understanding of reproductive biology in the Hawaiian *Scaevola* may indicate the extent to which they are dependent upon biotic pollen vectors for reproduction. This is an important conservation consideration in light of the vanishing native fauna as well as the potential deleterious effects of selfing (Silvertown and Charlesworth, 2001). Future research on *Scaevola* breeding systems is needed to determine if selfing can occur on an individual flower by an overlap in male and female flower phases (with or without a pollen vector) or from separate flowers on the same plant via a pollen vector. Mating systems should also be examined to assess the extent of outcrossing and to determine if plants are pollen limited.

While most species of *Scaevola* occur either in different habitats or on separate islands, *S. gaudichaudiana* and *S. mollis* are sympatric in some locations. It has been suggested that non-native honey bees (*Apis mellifera*) are crossing former ecological barriers to hybridization between these species, which may have been reproductively isolated via different pollinators (Howarth and Baum, 2005). Research is needed to determine if honey bees (or any other visitors) transfer pollen between *S. gaudichaudiana*, *S. mollis* and hybrids of these species in areas of sympatry.

APPENDIX A. FLOWER DAMAGE AT *SCAEVOLA GLABRA*

Nectar robbing occurs worldwide among diverse plant taxa, often in the form of corolla-piercing (Faegri and van der Pijl, 1079). This form of flower damage has been observed in both non-native and native plants in Hawai'i, including *Scaevola glabra* (Conant et al., Pratt 2005)(Figure A.1). *Scaevola glabra* flowers have long, decurved, connate tubes with copious, dilute nectar at the base of the tube (Chapters 2 and 3). 'I'iwi (*Vestiaria coccinea*) (Conant et al 1998) and Kaua'i 'Amakihi (*Hemignathus kauaiensis*)(Chapter 2) have been observed making holes in the base of the corolla to rob nectar, while 'Apapane (*Himatione sanguinea*) and Japanese White-Eye (*Zosterops japonicus*) have been seen utilizing pre-existing holes to access nectar (Chapter 2). Among the observed flower visitors, only 'I'iwi have a bill of similar shape and size to that of *S. glabra* flowers (Pratt, 2005).

A brief survey was conducted to assess the extent of damage among *S. glabra* flowers along the Pihea Trail in the Na Pali-Kona Forest Reserve on Kaua'i in April of 2007. The total number of damaged and intact flowers per plant was counted, and the size of the robbing hole was measured. At the time of the survey, it was noted that the distal end of the corolla tube was often ripped on the upper side of the flower, and this form of damage was similarly documented.

Overall, 19% of *S. glabra* individuals had flowers with distally ripped corollas, and 56% had flowers with between 1 and 3 holes pierced in the base of the corolla (n=32). Among plants containing ripped corollas, a mean of 57% of the flowers contained rips (n=6). Among plants containing flowers with holes, a mean of 65% of the

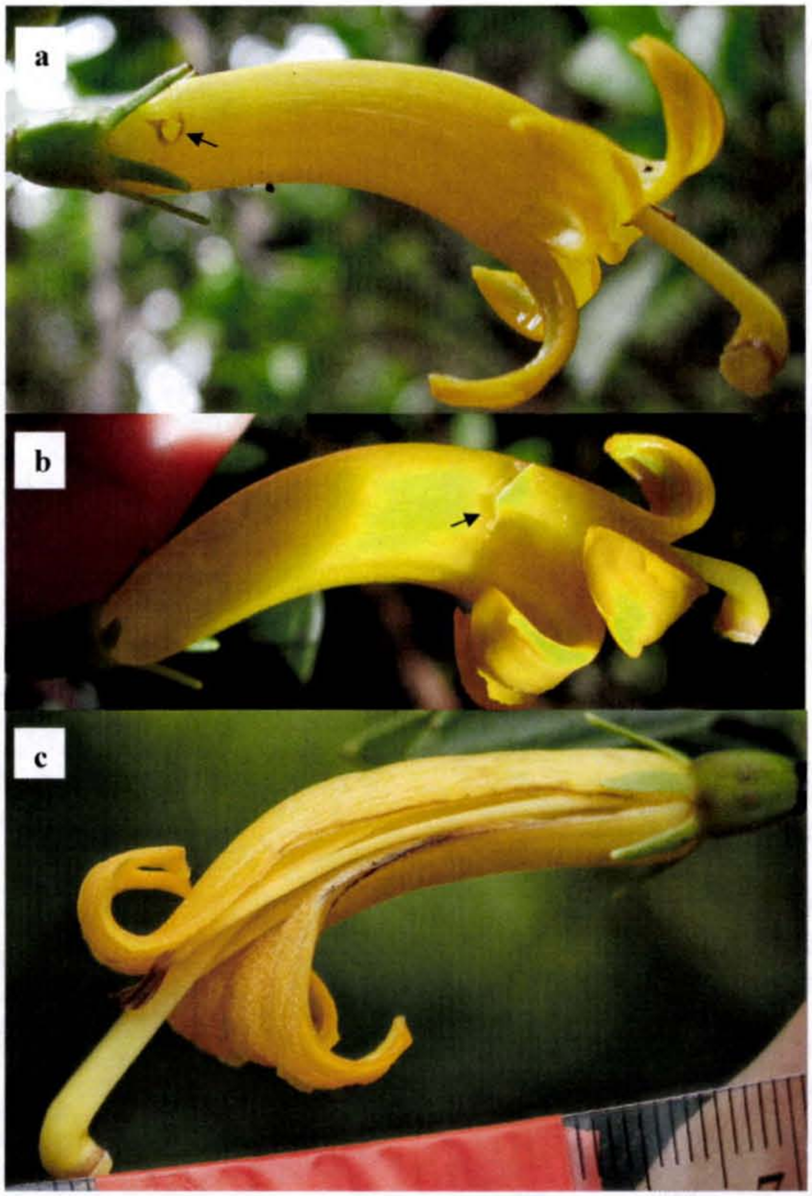


Figure A.1. Flower damage at *Scaevola glabra*: (a) robbing hole, (b-c) ripped corolla.

flowers contained holes (n=18). Rip lengths ranged from 4.8 mm to the entire length of the corolla (n=12), and hole size ranged from 0.5 to 19.2 mm (mean 4.8 mm; se 0.48 mm; n=78).

While access to nectar via corolla-piercing negates the possibility of pollination by the nectar thief, distally ripped corollas may indicate pollination is potentially occurring. Tears may result when a visitor with a bill shorter than the length of the flower tube forcefully inserts its head into the corolla, and in the process contacts the stigma or pollen. However, rips may also result from visitors (with a bill of any size) ripping the tube from atop the flower. Additional observations are needed to observe visitor behavior.

APPENDIX B. *SCAEVOLA* NECTAR PROPERTIES DURING THE DAY VERSUS NIGHT

Comparisons were made between nectar volume and sugar concentration during the day and night among five species where nectar was sampled during the night. Mood's Median test was used to maximize the power of statistical analysis due to the small nighttime sample size, the presence of outliers, and differences in shape and spread of sample distributions. Mean nectar volume (standing crop) per flower was greater during the night versus day among all species, though differences were significant for only two species (*S. kilaueae*, $p < 0.01$; *S. taccada*, $p < 0.05$), likely due to small sample sizes (Table B.1 and Figure B.1). Sugar concentrations were the same or lower at night than during the day, with a significant difference for one species (*S. kilaueae*, $p < 0.01$). Higher standing crop volume at night may be due to decreased visitation rates, environmental influences (decreased temperature and wind speed at night resulting in less evaporation) or may be genetically driven if selection for different pollinator guilds during the night versus day has occurred. Lower concentrations during the night may be due to environmental influences or genetics. The general trend among *Scaevola* species having higher volume yet similar concentration (except for *S. kilaueae*) at night versus the day suggests the differences are not likely due to environmental factors, wherein lower concentrations would be expected among all species. Visitation rates were lower at night than during the day for all species (Chapter 2), and may be the primary influence on differences in nectar properties at night versus day. *Scaevola kilaueae* may also be influenced by genetically driven differences in nectar concentration, wherein selection

Table B.1. Results of Mood's Median test to compare nectar volume and sugar concentration of Hawaiian *Scaevola* during the day versus night. Volume represents standing crop per individual flowers. Sugar concentration is in sucrose equivalents. Significant results are in boldface.

	volume (µL)							concentration (%w/w)						
	day			night				day			night			
	mean	se	n	mean	se	n	p	mean	se	n	mean	se	n	p
S. chamissoniana	0.89	0.16	25	1.42	0.47	5	0.142	16.89	0.53	25	17.21	0.77	5	0.624
S. gaudichaudiana	0.14	0.02	29	0.32	0.19	3	0.544	31.34	1.76	26	23.76	4.58	3	0.584
S. kilaueae	0.08	0.02	23	0.53	0.12	6	**0.004**	39.53	2.26	22	18.66	2.00	6	**0.006**
S. mollis	1.11	0.17	20	4.90	0.39	2	0.138	25.09	0.70	20	20.77	2.44	2	0.138
S. taccada	0.05	0.01	29	0.22	0.06	5	**0.015**	52.32	1.22	27	50.22	3.75	5	0.626

se: standard error

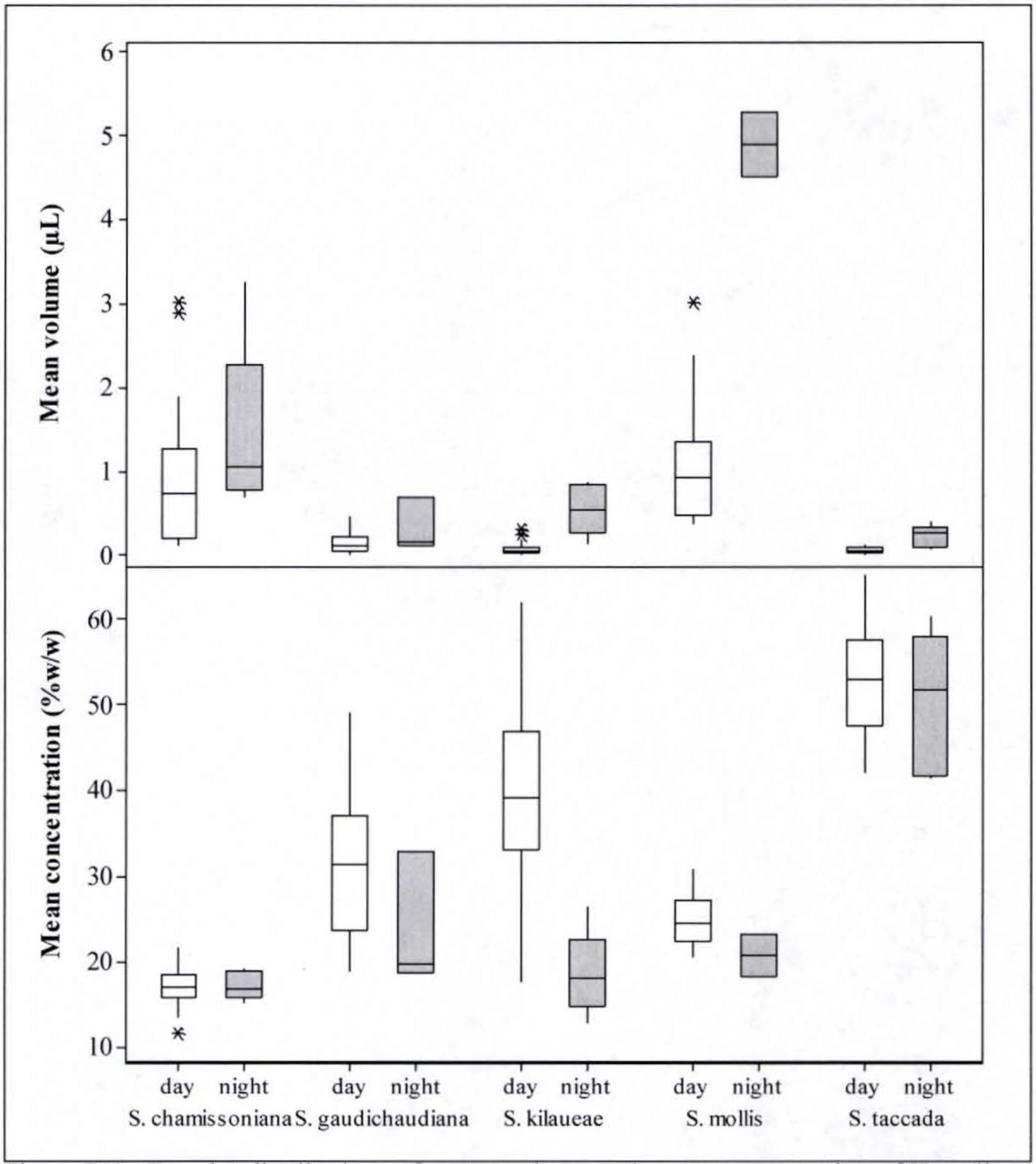


Figure B.1. Boxplot distributions of nectar volume and sugar concentration of Hawaiian *Scaevola* during the day versus night. Volume represents standing crop per individual flowers.

has occurred by differing pollinator guilds at night and during the day. Larger sample sizes and controlled bagging experiments may further clarify distinctions among nectar properties during the day versus night in future investigations.

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