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(Loganiaceae)**



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ARTIFICIAL HYBRIDIZATION IN THE HAWAIIAN ENDEMIC GENUS *LABORDIA* (LOGANIACEAE)¹

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Cross-pollinations were performed within and among eight species of *Labordia* from Oahu, Molokai, and Hawaii, and one species from Guam of the closely related genus *Geniostoma*. Detailed floral examination confirmed that the species are functionally dioecious, i.e., a given individual lacks either ovules or pollen grains. Female inflorescences bagged to prevent pollination never produced seed, but intraspecific crosses between male and female individuals nearly always yielded fruits with viable seed (>80%). Interspecific crosses between species from different islands and separate taxonomic sections of the genus also yielded good fruit set (>55%). Interspecific F₁ hybrids were vigorous and appeared to be morphologically intermediate to their parents. Intergeneric crosses between *Geniostoma* and *Labordia* failed. Chromosome counts from *Labordia* species were found to be $2n = 80$ or $2n \approx 80$, twice the chromosome complement of two collections examined from its postulated ancestor, *Geniostoma rupestre*. *Labordia* species are distinct morphologically, ecologically, and geographically but apparently lack genetic barriers to interbreeding. This suggests that geographical and ecological isolation, recentness of colonization, and/or rapid speciation have been important factors in the origin of species of *Labordia*.

Key words: cytogenetics; dioecy; *Geniostoma*; Hawaii; hybridization; *Labordia*; Loganiaceae; Pacific.

The indigenous flora of the Hawaiian Islands includes numerous examples of species that have undergone adaptive radiation or speciation subsequent to a single colonization event. Slightly over 10% of the vascular plant genera comprise nearly 48% (~542 species) of the indigenous flora (Wagner, 1991). In some cases, such as with the Hawaiian silversword alliance and lobelioids, several genera have arisen from a single founder event. *Bidens* (Ganders and Nagata, 1984; Helenurm and Ganders, 1985) and the Hawaiian silversword alliance of *Argyroxiphium*, *Dubautia*, and *Wilkesia* (Carr, 1985) of the Asteraceae represent classical examples of adaptive radiation, and much is known about the biology of these groups. However, while the biosystematics of some plant lineages are well known, the biological knowledge of many Hawaiian genera is based solely on unpublished field observations.

It has been postulated that in isolated island systems, rapid adaptive radiation of a single taxon is expected to promote higher interfertility among species because of the lack of reproductive isolating mechanisms (Crawford, Witkus, and Stuessy, 1987). Based on the high fertility of interspecific hybrids, the Hawaiian Asteraceae genera

of *Bidens* (Gillett and Lim, 1970; Ganders and Nagata, 1984), *Tetramolopium* (Lowrey and Crawford, 1985), *Lipochaeta* (Gardner, 1979; Rabakonandrianina, 1980), *Dubautia*, *Argyroxiphium*, and *Wilkesia* (Carr and Kyhos, 1981, 1986; Carr, 1985; Crins, Bohm, and Carr, 1988) support this hypothesis. The same condition has been reported for *Wikstroemia* (Thymelaeaceae; Mayer, 1991) and intrasectional species of *Portulaca* (Portulacaceae; Kim and Carr, 1990). Natural hybridization has only been documented with cytotaxonomic studies of the silversword alliance and *Portulaca* and with randomly amplified polymorphic DNA analysis of the genus *Cyrtandra* (Smith, Burke, and Wagner, 1996), although Gillett (1972), Carlquist (1974), and Wagner, Herbst, and Sohmer (1990) have suggested the occurrence of natural hybrids in many additional Hawaiian genera based upon morphological intermediacy among populations. Although molecular techniques are helping to clarify this situation, the documentation of natural hybrids has been difficult in the Hawaiian flora because the majority of the genera are long-lived, woody perennials that make experimental crossing studies difficult and long term. Moreover, chromosomal markers that might aid in identifying hybrids are relatively infrequent in the Hawaiian flora (Carr, in press).

Labordia has been circumscribed as a Hawaiian endemic genus consisting of 16 species (Table 1) (Wagner, Herbst, and Sohmer, 1990; Motley, 1995). Alternatively, Conn (1980) and Struwe, Albert, and Bremer (1994) considered *Labordia* to be a subgenus of the closely related Pacific genus *Geniostoma*. While it is acknowledged that there is a close relationship between the genera, morphological and cytological differences in the taxa suggest that they are best treated as separate genera (Gray, 1859; Wagner, Herbst, and Sohmer, 1990). Based on limited cytological evidence, Wagner, Herbst, and Sohmer (1990) noted a possible ploidy difference between the genera. Previous studies of two species of *Labordia* yielded counts of $2n \approx 80$ (Carr, 1978), whereas two species of

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TABLE 1. Distribution of species of *Labordia*.

Species	Distribution
Section <i>Darbolia</i>	
<i>L. helleri</i>	Kauai
<i>L. kaalae</i>	Oahu (Waianae Mts.)
<i>L. tinifolia</i>	Kauai, Oahu, Molokai, Lanai, Maui, and Hawaii
<i>L. triflora</i>	Molokai
Section <i>Labordia</i>	
<i>L. cyrtandrae</i>	Oahu
<i>L. degeneri</i>	Kauai
<i>L. fagraeoides</i>	Oahu (Koolau Mts.)
<i>L. hedyosmifolia</i>	Molokai, Lanai, Maui, and Hawaii
<i>L. hirtella</i>	Kauai, Oahu, Molokai, Lanai, Maui, and Hawaii
<i>L. hosakana</i>	Oahu (Koolau Mts.)
<i>L. lydgatei</i>	Kauai
<i>L. pumila</i>	Kauai
<i>L. sessilis</i>	Oahu (Koolau Mts.)
<i>L. venosa</i>	Maui
<i>L. waialealae</i>	Kauai
<i>L. waiolani</i>	Oahu, Molokai, and Lanai

Geniostoma had counts of $2n \approx 38$ [*G. antherotrichum* var. *archboldianum* (= *G. arfakense*), Bismarck Archipelago; Borgmann, 1964] and $2n = 40$ [*G. rupestre* var. *crassum* (= *G. ligustrifolium*) New Zealand; Gadella, 1963; Hair, 1963].

Labordia is a morphologically variable genus, ranging in habit from shrubs to small trees and sometimes scandent lianas, and it occurs in a variety of habitats throughout the wet forests at 300–1500 m on the six largest islands in the Hawaiian Archipelago. The species are scattered in the forest understory and never form large stands. Individual species are highly variable, and this has led in the past to the description of numerous species by various authors (Gaudichaud-Beaupré, 1829; Gray, 1859; Mann, 1866; Wawra, 1872; Baillon, 1880; Forbes, 1916; St. John, 1933, 1936, 1976, 1982, 1984; Skottsberg, 1936; Sherff, 1938, 1939, 1944, 1948, 1951a, b). Prior to the recent revision by Wagner, Herbst, and Sohmer (1990), a total of 28 species and 60 varieties had been recognized for the genus, attesting to the extreme morphological variation exhibited among *Labordia* species. However, Wagner, Herbst, and Sohmer (1990) reduced the genus to 15 species and two varieties, and their treatment, with the addition of the resurrected species *L. triflora* (Motley, 1995) is adopted herein as the taxonomic framework for the present study (Table 1).

The eight major islands of the Hawaiian Archipelago extend 640 km in a linear progression between 18° and 22° north latitude and 154° and 160° west longitude. Isolated from the nearest large land mass by 4000 km, and any other island group by 1600 km, these volcanic islands range in age from ~6 my (million years) (Kauai) to <0.5 my (Hawaii) (MacDonald, Abbott, and Peterson, 1983). The distribution pattern of island endemic and nonendemic *Labordia* species suggests a longer period of occurrence and speciation by the genus on the older islands compared to the younger islands (Fig. 1).

The genus *Labordia* is divided into two taxonomic sections (Table 1). Section *Darbolia*, with branches terete, leaves widely spaced, stipules free from the petiole, flow-

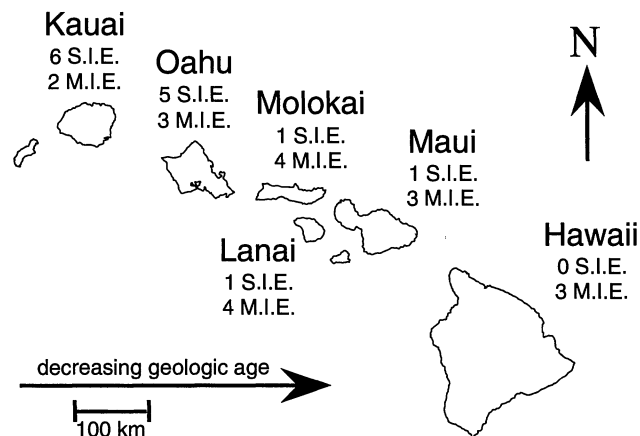


Fig. 1. Distribution of *Labordia* species in Hawaii. S.I.E. = number of single-island endemic taxa, M.I.E. = number of multiple-island endemic taxa.

ers small, fragrant, in open, paniculate cymes, corollas green and urceolate (Wagner, Herbst, and Sohmer, 1990), and pollen grains with a smooth exine and prominent pores (Selling, 1947) appears to be entomophilous. In contrast, section *Labordia*, with branches angled, leaves closely spaced, stipules adnate to the petiole base, flowers larger, nonfragrant, in dense, subumbellate, corymbiform to paniculate inflorescences, corollas yellow and salverform-funnelform (Wagner, Herbst, and Sohmer, 1990), and pollen grains with an irregular, coarse exine and shallow pores (Selling, 1947) appears to be ornithophilous.

The sexual system in *Labordia* was first described by Baillon (1880) as “polygamous-dioecious.” Solereder (1892–1895) and Rock (1913) simply stated that the flowers were “hermaphroditic or unisexual.” Mann (1866), in his description of *L. membranacea* also remarked that the flowers were hermaphroditic. Skottsberg (1936) looked at the floral morphology of five *Labordia* species in detail and concluded that the flowers were unisexual and “probably dioecious.” This observation was confirmed by Sherff (1939) in his work, but he cited a specimen of *L. hedyosmifolia* (Cranwell, Selling & Skottsberg 3166b GB), which had mostly bisexual flowers and one staminate flower. Wagner, Herbst, and Sohmer (1990) described the flowers as “unisexual (and plants dioecious), rarely perfect(?)”.

There have been no published studies of the occurrence of natural hybrids between species of *Labordia* other than the observations of Wagner, Herbst, and Sohmer (1990) in their discussion of species complexes and morphological intergradation between species. In order to better understand the reproductive biology and evolution of *Labordia* and its species lineages in island and isolated ecosystems, the following field and laboratory tests were conducted: (1) flowers were sampled in individual populations over a 3-yr period to determine whether *Labordia* species are, indeed, dioecious; (2) artificial crosses were performed within and among eight species of *Labordia* and one species of the closely related genus *Geniostoma* to test the crossability and interfertility among species and between genera; (3) additional chromosome counts of species of *Labordia* and *Geniostoma* were com-

TABLE 2. Floral examination of *Labordia*, sample sizes (*N*), and collection data for populations studied.

Species	Flowers (<i>N</i>)	Plants (<i>N</i>)	Populations (<i>N</i>)	Collection data
Section <i>Darbolia</i>				
<i>L. helleri</i>	40	4	2	Awaawapuhi trail (K): 1264.
<i>L. kaalae</i>	150	26	2	Pahole gulch (O): Pali Kea trail (O) ^{a,b} : 1199.
<i>L. tinifolia</i>	750	50	2	Tantalus (O) ^{a,b} : 1005; Hawaii Loa ridge (O): 1216.
<i>L. triflora</i>	80	8	1	Kua gulch (Mo): 1256.
Section <i>Labordia</i>				
<i>L. cyrtandrae</i>	20	5	1	Scholfield Barracks (O) ^b : 1131.
<i>L. degeneri</i>	20	4	1	Alakai swamp trail (K): 1411.
<i>L. fagraeoides</i>	43	10	1	Poamoho ridge (O) ^b : 1094.
<i>L. hedyosmifolia</i>	500	50	8	Kamakou (Mo): 1145; Puu Kukui (M): 1162; Hanaula (M): 1336; Waikamoi (M): 1329; Lanai Hale (L): 1286; Volcano dump (H): 1030; Kulani prison (H) ^{a,b} : 1117; Puu Makaala (H): 1119.
<i>L. hirtella</i>	25	6	4	Wahiawa stream (K): 1445; Kamakou (Mo) ^b : 1253; Hanaula (M): 1340; Waikamoi (M): 1318.
<i>L. hosakana</i>	100	25	1	Aiea ridge (O) ^{a,b} : 1070.
<i>L. venosa</i>	30	12	2	Hanaula (M): 1330; Waikamoi (M): 1326.
<i>L. waialealae</i>	100	25	2	Hanakoa (K): 1087; Pihea trail (K): 1074.
<i>L. waiolani</i>	500	50	3	Mt. Kaala (O) ^{a,b} : 1034; Kamakou (Mo): 1062; Lanai Hale (L): 1292.

^a Populations sampled for 4-yr period and used as female parents in the hybridization study.

^b Populations used as male parents in the hybridization study.

pleted; and (4) pollinator visitations to flowers in field populations of *Labordia* were recorded.

MATERIALS AND METHODS

Flowers collected from individual plants were dissected in the laboratory to determine the sexual expression of individuals. Thirteen of the 16 species in 24 populations were examined from six of the major islands (Kauai, Oahu, Molokai, Lanai, Maui, and Hawaii) (Table 1). The five populations used in the hybridization portion of this study were monitored for 4 yr. Flowers were considered hermaphroditic if they contained ovules and viable pollen grains, staminate if they lacked ovules and contained viable pollen, and pistillate if they contained ovules but lacked viable pollen. Pollen grains were tested for stainability in cotton blue-lactophenol stain (Radford et al., 1974) as an estimate of pollen viability. The pollen was stained for 24 h on a microscope slide and 100 grains were counted per sample to determine stainability. Pollen grains that stained blue were considered viable.

Cross-pollinations were performed in the field at five sites on two islands. These crossing attempts involved three species from section *Labordia* (*L. hedyosmifolia*, *L. hosakana*, and *L. waiolani*) and two species from section *Darbolia* (*L. kaalae* and *L. tinifolia*). Initially, crosses were attempted intraspecifically to test the percentage of successful fertilizations between individuals, the functionality of flowers manipulated during hand-pollination, and the ability of the nylon mesh bag to exclude pollinators without inhibiting fruit development. Staminate flowers were removed and taken to plants producing suitable pistillate flowers. Pistillate flowers on inflorescences that had undergone anthesis were removed since it could not be determined whether they had already been pollinated by natural means. Flower buds that appeared immature and thus presumably unreceptive were also removed. Buds near anthesis were considered suitable and their corollas were removed to expose the receptive stigma. Because the androecium in *Labordia* is adnate to the corolla, this procedure also removed the non-functional stamens. The crosses were then made by transferring the pollen from male plants to the exposed stigmas of female plants with a fine camel-hair paintbrush. The entire inflorescence was then bagged and marked. Staminate and pistillate inflorescences and pistillate buds that were manipulated as above for pollination but left unpollinated were also bagged and marked. After ~4 mo the sites were revisited, the mature fruits collected, and the seeds were tested for viability by germination.

For interspecific crosses, the same five populations that were used for intraspecific crosses (Table 2) provided individuals that served as potential female parents. The pollen donors included individuals from these populations as well as from populations of four additional species (*L. cyrtandrae*, *L. fagraeoides*, *L. hirtella*, and *G. rupestre*). Because of time and distance factors in some of these cases, branch cuttings of male plants rather than individual flowers were transported between populations. The branches were usually put in florist's cutflower tubes with water and stored under cool conditions until used.

Chromosome numbers were determined in *Labordia* and *Geniostoma* species to examine the ploidy levels within the genera. Meiotic chromosomes were examined in the microsporocytes of floral buds fixed in Carnoy's solution (6 parts chloroform:3 parts absolute ethanol:1 part one glacial acetic acid) for 4–6 d and stored at –10°C. Anthers were excised from preserved floral buds, squashed in acetocarmine, and mounted in Hoyer's solution for microscopic examination using phase contrast optics. Mitotic chromosomes were examined from root tips in one species of *Labordia*, since floral material was unavailable. Root tips were treated in saturated PDB (paradichlorobenzene) solution at 4°C for 4–6 h and fixed in Carnoy's Solution (Beeks, 1955). The root tips were then hydrolyzed in HCl, squashed in acetocarmine, and mounted in Hoyer's solution for microscopic examination using phase contrast optics.

Pollinator visitations were observed and recorded for 30- and 60-min periods in five *Labordia* populations over a 4-yr period (Table 1). Observations were taken in each population (5–40) on 5–10 individual plants, over several days (3–5), during the flowering season. In three of the species (*L. hedyosmifolia*, *L. tinifolia*, and *L. waiolani*) observations were also taken at dawn, dusk, and in the evening. While it was possible to observe the movements of pollinators on flowers of individual plants, it was not feasible to follow the pollinators to other individuals due to the forest terrain and density. All voucher specimens were deposited at (BISH) unless otherwise noted.

RESULTS

Labordia flowers were collected and examined to determine the sexual expression of each plant in 30 separate populations representing 13 of the 16 species in the genus. More than 2300 flowers were examined from 275 individuals. Individuals in five of the populations were

TABLE 3. The percentage fruit set for intra- and interspecific crosses made within the genus *Labordia* and between *Labordia* and *Geniostoma*. The island locality for each population follows the name in parentheses, O = Oahu, Mo = Molokai, H = Hawaii, and G = Guam. The percentages of fruit set for crosses are listed, followed by the sample size (*N*) and an * if the cross was intersectional within the genus *Labordia*. Male parents are on the *x*-axis, female are on the *y*-axis.

Species	<i>kaalae</i> (O)	<i>tinifolia</i> (O)	<i>waiolani</i> (O)	<i>hosakana</i> (O)	<i>hedyosmifolia</i> (H)	<i>hirtella</i> (Mo)	<i>fagraeoides</i> (O)	<i>cyrtandrae</i> (O)	<i>Geniostoma</i> (G)
<i>kaalae</i> (O)	88 (80)	50 (30)		50 (16)*	33 (12)*	67 (18)*			
<i>tinifolia</i> (O)	64 (14)	100 (80)		0 (9)*	33 (27)*			73 (15)*	0 (6)
<i>waiolani</i> (O)			92 (50)		43 (14)		60 (15)		
<i>hosakana</i> (O)		60 (20)*		80 (50)					
<i>hedyosmifolia</i> (H)	100 (10)*	100 (10)*			100 (50)				

re-examined over a 4-yr period (Table 2). Individual plants in all cases were found to be either entirely pistillate (flowers with ovules and no pollen grains in the anthers, the latter small and necrotic) or entirely staminate (pollen grains present, no ovules in the ovary locules). Pollen grains in species examined were found to have a stainability and presumed viability of >98%. The study thus confirms the occurrence of strict functional dioecism in all species of *Labordia* investigated. The flowers have both "male" and "female" parts present but only one sex is functional in each plant. Over the 4-yr study period, the sexual expression of individual plants remained constant.

Crosses performed between individuals within populations had a fruit-set success rate ranging from 80 to 100%, with a mean success rate of 92% for all populations (Table 3). However, nonpollinated, bagged inflorescences never set any fruit. Pistillate plants under natural conditions in the populations produced much fruit, except in the case of *L. waiolani*. These results indicate that pistillate flowers require pollination by a vector and do not set fruit by selfing or apomixis (at least in the absence of pollination) and that the nylon bags effectively excluded pollinators.

Interspecific crosses revealed no genetic barriers to hybridization between *Labordia* species. Fifty-five percent of the crosses produced fruits containing seed. Moreover, there was no significant statistical difference between fruit set for intrasectional (55%) and intersectional crosses (53%). Nor was there any significant statistical difference between interisland vs. intrainland crosses, the for-

mer having a hybridization success rate of 56% and the latter 54%. Seed resulting from successful hybridization were tested for germination in the laboratory, and in all cases nearly every seed germinated (97%). However, because *Labordia* plants are extremely slow-growing none of the F_1 's have yet flowered, and thus no fertility data are available. The young plants do seem to display morphological characters intermediate to those of the parent species, and were as vigorous as the parent species in cultivation. The attempt to produce an intergeneric hybrid between *Labordia tinifolia* and *Geniostoma rupestre* from Guam was unsuccessful.

New observations of meiotic chromosomes in eight species, including both sections of the genus, failed to substantiate any variation in chromosome numbers among the populations of *Labordia* examined (Table 4). However, several of the preparations yielded only approximate determinations because of the small size and poor staining of the chromosomes. Therefore, a deviation of up to two or three pairs of chromosomes from the reported ~40 pairs is possible, at least in some cases. Nevertheless, the ploidy level of *Labordia* determined here is constant and essentially or exactly twice that found in populations from Guam and Tonga of *G. rupestre* with $2n = 20_{II}$.

Under natural conditions all *Labordia* populations studied, except the Mt. Kaala population of *L. waiolani*, had high seed set. Unfortunately, no native and few non-native pollinators were observed visiting any of the populations during numerous hour-long daytime and evening observation periods. Two nonindigenous insects were observed visiting flowers in the Tantalus population of *L. tinifolia*. On two separate days of observation, one in 1994, the other in 1995, European honey bees (*Apis mellifera*) were seen visiting both male and female individuals, and, on the same occasion in 1995, flower flies (*Allograpta exotica*) were observed moving among the flowers on a male individual. While it has not been established whether these visitors were carrying out pollination, they were the only visitors to the flowers observed.

DISCUSSION

Breeding system—It has been hypothesized that the endemic genus *Labordia* resulted from one (Wagner, Herbst, and Sohmer, 1990; Wagner, 1991) or two (Fosberg, 1948; Carlquist, 1974) original introduction(s) to the Hawaiian Islands from a *Geniostoma*-type ancestor that was transported to the archipelago internally by a

TABLE 4. Chromosome numbers ($2n$) in *Labordia* and *Geniostoma*.

Species	Chromosome no.	Voucher or reference
Section <i>Darbolia</i>		
<i>L. helleri</i>	~80	Motley 1264
<i>L. kaalae</i>	~80	Motley 1199
<i>L. tinifolia</i>	~80	Motley 1005
Section <i>Labordia</i>		
<i>L. fagraeoides</i>	~80	Motley 1246
<i>L. hedyosmifolia</i>	80	Motley 1030
<i>L. hirtella</i>	~80	Motley 1340 and (Carr, 1978)
<i>L. hosakana</i>	~80	Motley 1070
<i>L. waialealae</i>	~80	Motley 1275
<i>L. waiolani</i>	~80	(Carr, 1978)
<i>G. rupestre</i>	40	Motley 1196; Guam.
<i>G. rupestre</i>	~40	Drake et al. 280 (WELTU); Vava'u Is., Tonga.

bird (Fosberg, 1948; Carlquist, 1974; Wagner, 1991). Plants subsequently underwent speciation and evolutionary diversification, involving geographical isolation and ecological divergence.

Observations reported here indicate that *Labordia* is strictly dioecious. However, there is little evidence to exclude the possibility that the ancestor was a self-compatible, monoecious, or hermaphroditic individual. To the contrary, a survey of *Geniostoma* in Tonga and Samoa revealed only hermaphroditic individuals (T. Motley, unpublished data).

Although the presence of monomorphic hermaphroditic flowers is the most common condition in the genus *Geniostoma* (Conn, 1980), Valetton (1902) and Smith and Stone (1962) reported heterostyly in a species from Java, and Rattenbury (1980) reported gynodioecious plants in New Zealand. However, in a detailed investigation, Smith and Stone (1962) could find no definite example of dioecy within *Geniostoma* in the Pacific, with the possible exception of one variety of *G. rupestre* from Fiji. Nevertheless, observations such as these led Sakai et al. (1995a) to conclude that the colonizing ancestor of *Labordia* was gynodioecious.

Moreover, Baker's Rule (Baker, 1955, 1967) states that self-compatible individuals are better suited to establishment following long-distance dispersal and that successful colonists are likely to develop mechanisms that promote obligate outcrossing and increase genetic variation. On the other hand, if *Labordia* is the descendent of a dioecious colonizing ancestor, it would be yet another exception to Baker's Rule.

The question of whether dioecy originates before or subsequent to introduction and establishment of many island plants has been the subject of much debate (Carlquist, 1966; Baker, 1967; Gilmartin, 1968; Bawa, 1980, 1982; Thompson and Barrett, 1981; Baker and Cox, 1984; Carr, Powell, and Kyhos, 1986; Mayer and Charlesworth, 1992; Sakai et al., 1995a, b). Outcrossing conditions, such as monoecy and gynodioecy, have been postulated to be evolutionary pathways to dioecy or subdioecy (Bawa, 1980, 1982; Ross, 1982). Further investigations of *Labordia*, which so far have revealed only dioecious populations, and the species of *Geniostoma* throughout the Pacific may provide answers to some of these questions.

Artificial crosses—Intraspecific, interspecific, and inter-sectional hybrids including those involving populations from the same or different islands were easily produced within the genus *Labordia*. Only one interspecific hybridization attempt was unsuccessful, and may have been due to factors other than genetic incompatibility. The unsuccessful attempt involved *L. hosakana* and *L. tinifolia* at the end of the flowering season for *L. tinifolia*. The *L. tinifolia* female plant on which the cross was attempted was already heavily laden with fruit and perhaps resources were unavailable to this individual for the production of more fruit late in the season. A rainstorm with high winds that occurred a few minutes after the pollination may have further contributed to the failure of this attempt. Variable conditions encountered in the field may also account for some of the variation in fruit set observed among the cross-pollinations attempted.

The unsuccessful intergeneric crosses between *L. tinifolia* and *G. rupestre* suggest that genetic barriers may be preventing hybridization between these genera. Alternatively, other species of *Geniostoma* in the Pacific Basin may be more closely related to *Labordia* and may be more likely to yield successful intergeneric crosses. In any case, additional efforts to produce hybrids between the two genera need to be attempted before firm conclusions can be drawn regarding their crossability.

Labordia species, at the level examined (F_1), are similar to many other Hawaiian plant lineages (Rabakanandrianina, 1980; Ganders and Nagata, 1984; Lowrey and Crawford, 1985; Carr and Kyhos, 1986; Kim and Carr, 1990; Mayer, 1991) in that they appear to lack genetic barriers that prevent hybridization. Many plant lineages previously investigated included fast-growing species (many from a single family, Asteraceae) that were cultivated under greenhouse conditions. *Labordia* differs from these plant lineages in that the genus contains woody, slow-growing tree species that require field investigation and observations in order to characterize reproductive modes. Although these restrictions and logistics limited sample size, number of replications, and further F_1 progeny analysis, the information obtained in this study broadens the taxonomic scope of knowledge related to reproduction in Hawaiian plants, and thus enhances a broader perspective on the genetics, evolution, and conservation of island systems.

Carr (1995) suggested that in insular floras such as that of the Hawaiian Islands, where species have experienced founder events, genetic bottlenecks, diverse ecological opportunities, and population fragmentation, hybridization may play an important evolutionary role in speciation and the enhancement of gene pools of species with limited genetic variation. As documented here, *Labordia* species are like many others in the Hawaiian flora in their lack of genetic barriers that prevent hybridization. However, while it is possible for natural hybridization to occur, and while it may have been important in the past, it is apparently a rare event among *Labordia* species at present. Flowering periods of species, and in some cases, populations, are not synchronous, and the dimorphic flower types found within sections of the genus are indicative of pollinator differentiation. Furthermore, *Labordia* species are often separated by large distances, and many are endemic to a single island or mountain range. Thus, geographical, phenological, and morphological barriers may act to reduce the frequency of hybridization among species of *Labordia*. Although some plants have been suspected to be hybrids (Wagner, Herbst, and Sommer, 1990), none have yet been verified.

Cytotaxonomy—The Hawaiian flora apparently has the highest incidence of polyploidy known (Grant, 1963; Goldblatt, 1980; Sanders, Stuessy, and Rodriguez, 1983; Carr, in press). Most polyploid Hawaiian species are paleopolyploid, having evolved polyploidy prior to dispersal of their ancestors to Hawaii (Carr, in press). The situation in *Labordia* is ambiguous. Most available data indicate that *Labordia* has twice the number of chromosomes as its presumed ancestor, *Geniostoma* ($2n = 80$ vs. $2n = 40$). However, two unpublished counts by M. Kiehn (University of Vienna) suggest the occurrence of chro-

mosome numbers in the range of $2n = 40-60$ in *Labordia*. More chromosome determinations in *Labordia* and *Geniostoma* are required to effectively address the issue of autochthonous origin of polyploidy in the endemic Hawaiian genus *Labordia*.

Pollination—The Hawaiian Islands comprise just 0.2% of the total land mass of the United States, yet is home to over one-third of the plant and animal species already listed or awaiting listing as rare or endangered organisms by the federal government. Presently, over half of the native Hawaiian bird species are extinct and 29 of the remaining 44 species are endangered (Murphy, 1996). These figures do not include the insect fauna, for which little information exists regarding the number of species extinctions or population levels. Since most Hawaiian ecosystems are no longer intact, studies on co-evolution and pollination ecology are increasingly difficult to conduct because of the loss of organisms in the ecosystems. This is supported by the situation observed in the Mt. Kaala population of *L. waiolani*, in that no fruit set is seen over the study period in the population under natural conditions, but when pollen is artificially transferred, plants set fruit in 92% of the cases. In some cases the pollinators may have become extinct and subsequently may have been replaced by generalist pollinators. Under these conditions data collected and conclusions reached are not applicable to intact ecosystems. In the case of *Labordia*, introduced bees seem to be acting as effective pollinators contributing to the success of the plant species, but perhaps at a high cost to the native pollinators that are outcompeted by these more aggressive alien species.

In general the biosystematics and reproductive biology of plant species in temperate, continental floras are better known than in tropical floras. In Hawaii, a great deal is known about a few genera, but little is known about the biology of most plant groups. The present research on *Labordia* adds to this knowledge and broadens the taxonomic base of information pertinent to the understanding of evolution in insular ecosystems. The Hawaiian flora has the highest percentage of dioecious species of any recorded flora (Sakai et al., 1995a). This research revealed that *Labordia* species are functionally dioecious. Evidence pertaining to the breeding systems found in *Geniostoma* (Conn, 1980), the likely ancestor of *Labordia*, suggests that dioecy may have evolved autochthonously within the latter genus. If future studies support this evidence, *Labordia* would raise the number of independent lineages considered to have undergone autochthonous evolution of dioecy in Hawaii to 13 (Sakai et al., 1995a).

It was stated above that *Labordia* species are interfertile at the levels investigated in this study. Thus, *Labordia* is like many other Hawaiian plant lineages (Rabakondrianina, 1980; Ganders and Nagata, 1984; Lowrey and Crawford, 1985; Carr and Kyhos, 1986; Kim and Carr, 1990; Mayer, 1991) in apparently lacking genetic barriers to hybridization. While hybridization may have played an important evolutionary role in speciation and enhancing gene pools of species of *Labordia*, it has not been documented during the course of this field-oriented study.

There is an urgency for detailed evolutionary studies, because species and ecosystems are disappearing at

alarming rates in Hawaii and throughout the world. Three *Labordia* species have already been federally listed as endangered and two other taxa are candidate species for federal listing. It became evident during this research that the more common *Labordia* species are also no longer as widespread or as abundant as in the recent past. Furthermore, no native Hawaiian pollinators were seen visiting the remaining individuals in the existing populations. Therefore, in order to get a better understanding of the reproductive biology, biosystematics, and species interactions among Hawaiian organisms, it is necessary to act quickly and initiate research before valuable information is lost forever.

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