Pacific Science (1996), vol. 50, no. 1: 55–65

Evolution of *Rhaphithamnus venustus* (Verbenaceae), A Gynodioecious Hummingbird-Pollinated Endemic of the Juan Fernández Islands, Chile[1]

BYUNG YUN SUN,[2] TOD F. STUESSY,[3] ANA MARÍA HUMAÑA,[4]
MAGALY RIVEROS G.,[4] AND DANIEL J. CRAWFORD[3]

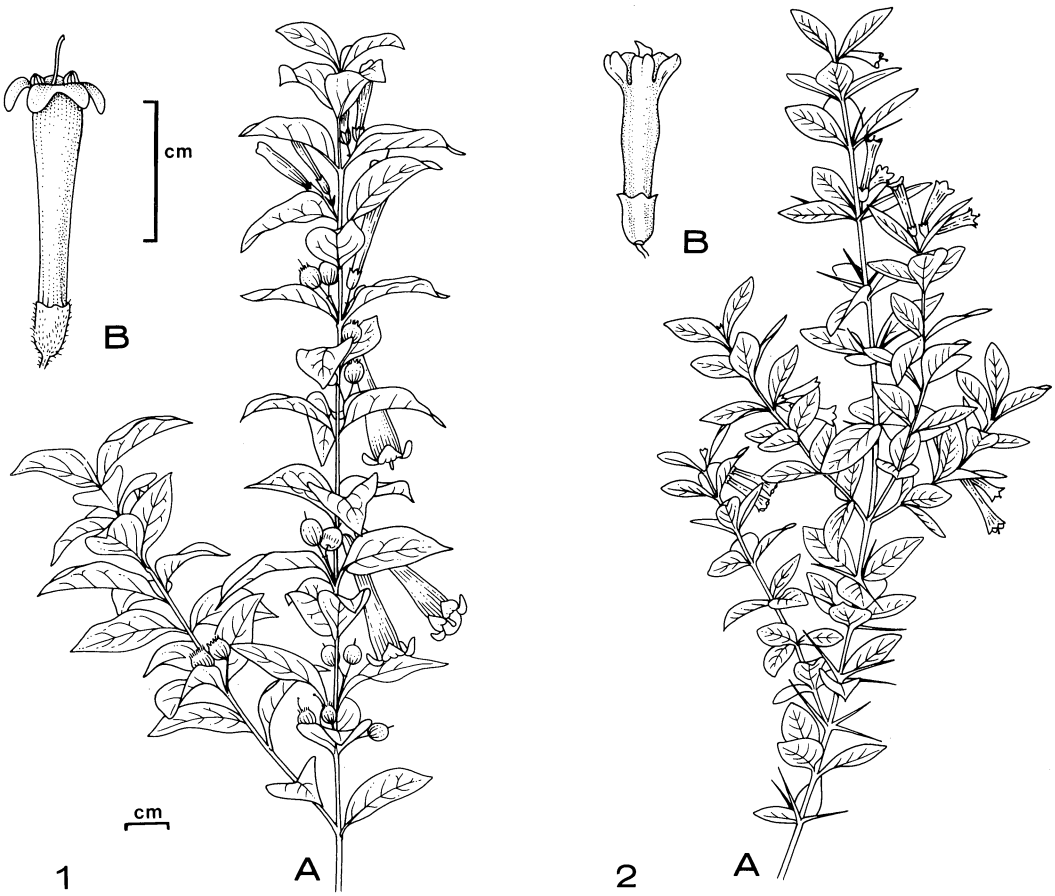
ABSTRACT: *Rhaphithamnus* Miers. (Verbenaceae) consists of two species restricted to southern South America. *Rhaphithamnus spinosus* (A. L. Juss.) Mold. occurs in mainland Chile and adjacent Argentina; *R. venustus* (Philippi) Robinson is endemic to the two islands (Masatierra and Masafuera) of the Juan Fernández Archipelago. Both species are related to *Citharexylum* Miller, a genus distributed from Peru northward along the Andes. Youthful geological ages of the Juan Fernández Islands (1–4 myr) and occurrences of the sister group also on the continent suggest that *R. venustus* evolved from *R. spinosus* rather than the reverse. Morphologically the two species differ primarily in corolla length and color and stem armament, with *R. spinosus* with shorter (ca. 12 mm) and bluish flowers and usually with axillary thorns in contrast to longer (ca. 25 mm) and purple flowers and lack of thorns for *R. venustus*. Studies of pollinators reveal bees, flies, beetles, and infrequently hummingbirds for *R. spinosus* and exclusively hummingbirds for *R. venustus*. *Rhaphithamnus spinosus* is hermaphroditic and partially self-compatible, whereas *R. venustus* is gynodioecious and with an unknown compatibility system. In the latter species female flowers appear to be in an early stage of evolution because anthers are still fully formed, but usually without pollen grains. Embryological studies reveal breakdown of pollen mother cells (and tapetal cells) during meiosis. We hypothesize that evolution of floral features in *R. venustus* is a result of a change from primarily insect to hummingbird pollination; loss of thorns may result from absence of herbivores in the Islands. Gynodioecy in *Rhaphithamnus* may have as its selective basis reduction of inbreeding depression otherwise brought on by geitonogamy in scattered individuals of small populations.

OCEANIC ISLANDS ARE natural laboratories of plant evolution. Because of their youthful geological age, isolated geography, and rapid ecological change, plant immigrants have become established in oceanic archipelagos and have speciated in response to new selection pressures within short periods of evolutionary time. Because of these circumstances, studies on island taxa in relation to continental ancestors can often reveal much about the processes of plant evolution, sometimes more clearly than among continental taxa.

The Juan Fernández Islands are a good natural laboratory of plant evolution because of their isolation from continental areas (670 km W of mainland Chile at 33°S latitude), the existence of only two major islands of different ages and distances from the mainland (Masatierra, 4 myr old; Masafuera, 1–2 myr old and 180 km W of Masatierra [Stuessy et al. 1984]), and an endemic flora of small size (104 endemic species of angiosperms [Stuessy et al. 1992]).

Rhaphithamnus Miers. (Verbenaceae) is a good genus for revealing evolutionary events

[1] This research was supported by National Science Foundation grant BSR89-06988 to T.F.S. and D.J.C. Manuscript accepted 27 January 1995.
[2] Department of Biology, Chonbuk National University, Chonju 560-756, Korea.
[3] Department of Plant Biology, Ohio State University, Columbus, Ohio 43210.
[4] Instituto de Botánica, Universidad Austral de Chile, Valdivia, Chile.



FIGURES 1–2. Vegetative and floral features of *Rhaphithamnus*. (1) *R. venustus*. (2) *R. spinosus*. A, habit; B, flower. Both drawings same scale.

in the Juan Fernández Islands. It consists of only two species (Figs. 1, 2) worldwide (Moldenke 1937, 1939, 1979): *R. venustus* (Philippi) Robinson, endemic on both islands of the archipelago; and *R. spinosus* (A. L. Juss.) Mold., restricted to southern South America in Chile and Argentina. *Rhaphithamnus* apparently is related to *Citharexylum* Miller, a genus of 130 species distributed in Peru, Bolivia, and Paraguay (Moldenke 1958, 1959, Sanders et al. 1983). It is highly probable, therefore, that *R. venustus* in the islands was derived from *R. spinosus* on the continent. This enables us to examine differences in the biology of these two species and to interpret these differences in a phyletic framework. Preliminary field studies and recent work by Colwell (1989) indicated *R. venustus* to be hummingbird pollinated. *Rhaphithamnus spinosus* of the continent, on the other hand, has been suspected to be pollinated by insects (J. Arriagada, pers. comm.; confirmed herein). These field studies and other reproductive data have facilitated adaptive interpretations of differences in floral morphology between the two species. Further, the sexual configuration of *R. spinosus* is hermaphroditic, whereas that of *R. venustus* is gynodioecious. Another morphological difference between the two species is that *R. spinosus* usually is armed with thorns, whereas *R. venustus* is generally unarmed.

The purposes of this paper, therefore, are

to: (1) document differences in vegetative and floral morphology between *R. spinosus* and *R. venustus*; (2) describe differences in reproductive biology between the two species, focusing on pollination and breeding systems; (3) in the context of phylogeny, speculate on the adaptive value of morphological and reproductive changes with emphasis on floral morphology and the thorn-inflorescence complex; and (4) comment on the origin of gynodioecy in *R. venustus* in relation to changes in the pollination syndrome and its bearing on the evolution of gynodioecy and dioecy on oceanic islands in general.

MATERIALS AND METHODS

New plant specimens were collected during expeditions to the Juan Fernández Islands and central Chile in 1980 (two trips), 1984, 1986, and 1990 by personnel from laboratories of the Department of Plant Biology of Ohio State University and Departamento de Botánica, Universidad de Concepción, Chile. Personnel from the Universidad Austral de Chile also participated in the 1990 expedition. Herbarium specimens from GH, M, MO, and Z were also used in this study. Vegetative and reproductive morphologies were determined and measured from these herbarium materials as well as from FAA (formalin; acetic acid; alcohol) prepared specimens. Quantitative data of floral dimensions were obtained from 30 herbarium specimens of *R. spinosus* and 23 specimens (20 from Masatierra and 3 from Masafuera) of *R. venustus* (Table 1).

For embryological study, buds in various stages of one functionally female plant of *R. venustus* from Masafuera (*Ruiz et al. 8290*) were fixed in FAA and sectioned at 10 μm using the TBA-Paraffin method (Sass 1958). The sections were double-stained in safranin and fast green and mounted in 60% Picolyte.

Pollen-ovule ratios (Cruden 1977) were determined by counting the number of fertile pollen grains in four microsporangia (two each from one small and one large anther) in a flower, multiplying that number by four (a total of four anthers and 16 microsporangia in each flower), and dividing by the number of ovules in the ovary (always 4). Pollen-ovule ratios of *R. venustus* were determined from 19 populations from both islands, Masatierra and Masafuera. Those of *R. spinosus* were determined from eight populations from var-

TABLE 1

SPECIMENS EXAMINED AND MEASURED FOR FLORAL CHARACTERISTICS IN *Rhaphithamnus* (SEE TABLE 2)

SPECIES	MATERIAL EXAMINED
R. spinosus	Prov. Arauco: *Kunkel M11* (M), *M199* (M), *M212* (M); *M et al. 1614* (OS). Prov. Aysén: *S et al. 7375* (OS). Prov. Bío-Bío: *M et al. 942* (OS). Prov. Chiloé: *M 1743* (OS); *M et al. 165* (OS); *Pennell 12499* (GH); *S et al. 7050* (OS); *Werdermann 55* (Z). Prov. Concepción: *Neger 3968* (M); *S et al. 6699* (OS). Prov. Coquimbo: *Muñoz B-147* (GH); *Ricardi 2111* (OS). Prov. Llanquihue: *Cantino 36* (OS); *Ricardi 5282* (OS). Prov. Malleco: *Greer 797-R* (OS), *981* (OS). Prov. Maule: *Weldt & Rodríguez 1197/492* (OS). Prov. Osorno: *Grau s.n.* (M); *Zollitsch 47* (M), *64* (M), *124b* (M). Prov. Palena: *S et al. 7197* (OS). Prov. Valdivia: *Aravena 18030* (GH); *Athanasius et al. 152* (M); *Buchtien s.n.* (GH). Prov. Valparaíso: *Bertero 1298* (GH); *Schlegel 5930* (OS).
R. venustus	Masafuera: Q. del Tongo, *Landero & Gaete 8492* (OS); Q. Larga, *Ruíz et al. 8290* (OS), *8307* (OS). Masatierra: N slope of Damajuana, *Kubitzki 188* (M); Puerto Inglés, *M & Ugarte 9062* (OS), between Plazoleta del Yunque and El Camote, *9073* (OS); near Mirador de Selkirk, *M et al. 9017* (OS), Cordón Salsipuedes, *9106* (OS), Cerro Pascua, *9156* (OS); Valle Villagra, *Ruíz & Pacheco 6603* (OS); Damajuana, *Solbrig et al. 3788* (GH), N of El Yunque, *3903* (GH); La Vaquería, *S & Crawford 6453* (OS), Valle Inglés, *6581* (OS), La Pascua, *6654* (OS); below Mirador de Selkirk, *S & Sanders 5029* (OS), Cerro Pascua, *5122* (OS), Q. Villagra, *5198* (OS); La Vaquería, *S et al. 5477* (OS), Mirador Selkirk, *5520* (OS), Valle Villagra, *6226* (OS), up toward Damajuana from CONAF, *6243* (OS); Valle Inglés, *Valdebenito & Landero 6589* (OS).

M, Marticorena; Q., Quebrada (ravine); S, Stuessy.

ious localities in Chile. Although pollen-ovule ratios can sometimes be misleading (Cruden and Miller-Ward 1981), they can at least give some clues to possible breeding systems (see pertinent discussions in Preston [1986], Mione and Anderson [1992]).

Breeding system studies in *Rhaphithamnus* on the continent as well as in the Islands were completed by bagging individual flowers and checking for seed set. In automatic self-pollination, flowers were bagged and left alone to set seed. In manual self-pollination, flowers were bagged and manually pollinated 2 days later, coincident with onset of anthesis and opening of the corolla. In natural (open) pollination, the level of seed set in unbagged flowers without any experimental manipulation was recorded. Manual cross-pollinations were also accomplished in the same fashion between flowers of different individuals.

Data on natural pollinators were obtained by observing, collecting, and recording the visitors to flowers of both species of *Rhaphithamnus*. Four 6-hr observation periods were devoted to four different plants for *R. venustus* in the Islands. On the continent, 39 10-min observation periods were completed for *R. spinosus*. Insect visitors were identified and deposited in the entomological collections of Universidad Austral.

RESULTS

Morphology

The most obvious distinguishing characteristic between the two species of *Rhaphithamnus* is that *R. spinosus* usually is armed with thorns in the axils of the leaves, whereas *R. venustus* usually is not. Very infrequently, *R. venustus* also has thorns in the axils of the leaves (e.g., *Marticorena et al. 9106*, *Stuessy et al. 11147*), but the degree of armature is much less than that of *R. spinosus*. Likewise, sometimes unarmed individuals occur in *R. spinosus*. For example, Kunkel (1968) described a new variety, *R. spinosus* var. *inermis*, based on the absence of thorns. The type specimen of this variety (*Kunkel M212*; isotype, M!) shows no tendency toward *R. venustus* except for the absence of thorns. The leaves of *R. venustus* are usually larger and more coriaceous than those of *R. spinosus*.

Either thorns, inflorescences, or thorn-inflorescence combinations (e.g., sometimes in *R. spinosus* flowers are borne on thorns [*Stuessy et al. 7197*, OS]) are located in axils of leaves in the two species of *Rhaphithamnus*. The typical inflorescence is a two-flowered dichasium usually ending in a sharp-pointed thorn in *R. spinosus* or a very short threadlike thorn in *R. venustus*. Infrequently, 3- to 5-flowered racemes with or without basal bracts are developed in both species. Sometimes the thorn in *R. spinosus* bears a lateral flower bud and bract, and sometimes it bears only bracts or even small bracteate lateral thorns. In *R. spinosus*, some branches have only inflorescences in axils of the leaves while others have opposite or ternate thorns. The case is similar in *R. venustus*: some branches have only inflorescences in axils of the leaves (called a "vegetative-floral shoot" by Skottsberg [1953]), whereas other branches lack inflorescences (and thorns called a "vegetative-prolongation shoot" by Skottsberg [1953]).

The two species also show differences in the size of flower and floral tube, calyx, and length of peduncle and pedicel (Table 2). *Rhaphithamnus venustus* has much larger flowers and more deeply purple-colored corollas. The length of the floral tube and entire flower of *R. venustus* is twice that of *R. spinosus*. The length of peduncle and pedicel of *R. venustus* is more than twice that of *R. spinosus*. In *Rhaphithamnus* the flowers are more pendulous than in the related genus *Citharexylum*, which has a spike or very short-pediceled raceme. In addition to presence and absence of thorns, size of leaves in the two species of *Rhaphithamnus* helps differentiate them (Figs. 1, 2); they also differ by quantitative nonoverlapping floral characters (Table 2). Moldenke (1937, 1979) described the differences between these two species in detail.

Pollen-Ovule Ratio (P/O) and Breeding System

As in related genera such as *Citharexylum* and *Duranta* L., each flower of *Rhaphithamnus* has two carpels each containing two ovules (four ovules total). Each flower has

TABLE 2

COMPARISON OF FLORAL CHARACTERISTICS (IN MM; SHOWING MEANS AND STANDARD DEVIATIONS) IN SPECIES OF *Rhaphithamnus*

	FLOWER			FLORAL TUBE		CALYX		PEDUNCLE	PEDICEL
SPECIES	LENGTH	DIAMETER	COLOR	LENGTH	DIAMETER	LENGTH	DIAMETER	LENGTH	LENGTH
R. spinosus	11.9 ± 1.9	3.5 ± 0.5	Blue	6.0 ± 1.3	2.1 ± 0.4	2.7 ± 0.5	2.0 ± 0.2	1.2 ± 0.6	1.7 ± 0.5
R. venustus	25.6 ± 3.8	5.3 ± 0.9	Purple	14.4 ± 2.4	2.8 ± 0.5	4.2 ± 0.7	2.3 ± 1.3	2.3 ± 1.3	3.3 ± 0.8

TABLE 3

POLLEN-OVULE RATIOS OF POPULATIONS OF *R. spinosus* AND *R. venustus* IN CONTINENTAL CHILE AND THE JUAN FERNÁNDEZ ISLANDS (MEANS ± STANDARD DEVIATIONS)

TAXON	POLLEN-OVULE RATIO	NO. OF ABORTED POLLEN GRAINS	VOUCHER
R. spinosus	1,847	211	*Munoz B-147* (GH)
	1,962	118	*Aravena 18030* (GH)
	1,964	89	*Bertero 1298* (GH)
	1,975	48	*Buchtien s.n.* (GH)
	2,107	62	*Schlegel 5930* (OS)
	2,187	132	*Gunckel 1778* (GN)
	2,321	67	*Körner 1375* (M)
	2,504	92	Collector unknown "*750*" (M)
Mean	2,108 ± 205	102 ± 49	
R. venustus (hermaphrodite)	2,508	214	*Bock 51* (GH)
	2,605	214	*Solbrig et al. 3802* (GH)
	2,634	184	*Stuessy & Sanders 5122* (OS)
	2,643	418	*Stuessy et al. 6243* (OS)
	2,818	242	*Solbrig et al. 3788* (GH)
	2,863	104	*Stuessy et al. 5520* (OS)
	3,059	168	*Chapin 1083* (GH)
	3,507	147	*Marticorena et al. 9017* (sheet 1; OS)
Mean	2,803 ± 304	217 ± 82	
R. venustus (female)	0	0	*Marticorena et al. 9017* (sheet 2; OS)
	0	0	*Marticorena et al. 9156* (OS)
	0	0	*Morrison 17334* (MO)
	0	0	*Ruiz et al. 8290* (OS)
	0	0	*Stuessy & Crawford 6654* (OS)
	0	0	*Stuessy & Sanders 5029* (OS)
	0	0	*Stuessy & Sanders 5198* (OS)
	0	0	*Stuessy et al. 5477* (OS)
	0	342	*Downton s.n.* (GH)
	7	32	*Stuessy & Crawford 6581* (OS)
	273	9,464	*Valdebenito & Landero 6589* (OS)
Mean	26	894	

four anthers, two larger anterior ones and two smaller posterior ones, each with four microsporangia. The average P/O of *R. spinosus* obtained from the anthers dissected from preanthesis flowers of eight populations was 2108 with small intraspecific variation (Table 3). However, dramatic intraspecific variation occurred in *R. venustus*. The average P/O of *R. venustus* from eight hermaphroditic populations was 2803, similar to that of *R. spinosus*. Eleven other populations examined were female. Eight of them showed a

TABLE 4

RESULTS OF PRELIMINARY BREEDING SYSTEM STUDIES IN *R. spinosus* IN CONTINENTAL CHILE

TEST	NO. OF FLOWERS	FRUITS OBTAINED	
		NO.	%
Automatic self-pollination	267	10	3.75
Manual self-pollination	42	6	14.29
Natural (open) pollination	821	261	31.79
Manual cross pollination	35	24	68.57

P/O value of 0 in which no pollen grains were produced. Another population (*Valdebenito & Landero 6589*) showed a very low value of P/O but produced a high number of aborted pollen grains (Table 3). In spite of intraspecific variation in amount of pollen produced, the morphology of anthers was the same. The data indicate, therefore, that *R. venustus* is gynodioecious.

Preliminary studies conducted on the continent on *R. spinosus* reveal this species to be partially self-compatible (Table 4). Emasculation and bagging of flowers to test for agamospermy gave negative results. Preliminary studies on Masatierra with *R. venustus* were not conclusive, but of 52 manually self-pollinated flowers in four different plants, 20 initiated fruits, suggesting that this species may be partially self-compatible. It is also likely that both species are outcrossing, as suggested by the high P/O above 2000 (Cruden 1977) and the spatial separation of stigma above the anthers. The occurrence of fruits on isolated individuals of *R. venustus* on Masatierra (e.g., *Stuessy et al. 11147*) suggests that the species is also geitonogamous.

Pollinators

The pollinators of *R. spinosus* are primarily 20 species of insects that belong to three orders and seven families: Coleoptera (Chrysomelidae, Nitidulidae); Diptera (Muscidae, Syrphidae); Hymenoptera (Apidae [including *Apis mellifera* L.], Halictidae, Colletidae). Among them the Hymenoptera, family Apidae, are the most frequent visitors to this species. One less frequently observed visitor is an unidentified hummingbird. *Rhaphithamnus venustus* is hummingbird pollinated; no insect pollinators have been observed (Skottsberg 1928, field data of T.F.S. from 1986, Brooke 1987, Colwell 1989). Colwell (1989) reported that two hummingbird species inhabit the Islands, one endemic to the archipelago (*Sephanoides fernandensis*) and the other found both on the mainland and in the Islands (*S. sephanoides*). In spite of remarkable differences in body size, the bill lengths of both species and both sexes (15–16 mm) are nearly identical and closely match the flower tube length of the several species of endemic plants in the Islands, including *R. venustus*. Both bird species, therefore, are likely pollinators of *R. venustus*.

Microsporogenesis

To understand absence of pollen in female plants of *R. venustus* from a developmental standpoint, a study of microsporogenesis was undertaken. The sequence of events is as follows. The archesporial cells begin to divide periclinally into a primary parietal layer and primary sporogenous tissue when the bud is ca. 2–3 mm long. Easily discernible at that stage are seven or eight archesporial cells, each with a large, conspicuous nucleus, arranged in one row just beneath the epidermis. The primary sporogenous tissue appears to function directly as pollen mother cells (PMCs) because there is no evidence that this layer undergoes mitosis. The parietal layer normally divides mitotically into endothecium, middle, and tapetal layers. At this stage, most of the tapetal cells have only one nucleus, and the microspore mother cells have thin, primary walls. Cell size at this stage for the tapetal cells is 10–12 by 13–14 μm and for PMCs is 10–12 by 15–20 μm. The tapetal cells undergo endomitotic divisions, resulting in a multinucleate condition and increasing their size to 25–30 by 17–21 μm. At this stage, the PMCs are enclosed in callose walls. However, the protoplast (11–12 by 13–17 μm) is smaller than the

callose wall (17–31 μm), and therefore a cavity exists between the callose wall and protoplast. Throughout the remaining stages, neither meiotic divisions of PMCs nor tetrads or microspores have been observed. The tapetal cell layer now detaches from the middle layer, and the callose wall of the PMCs appears to dissolve. The shapes of the tapetal cells in this stage are very irregular. Finally, the callose wall of the PMC completely disappears, leaving the PMC protoplast alone in the locule of the anthers. At that point the walls of the tapetal cells break down and the protoplasts move into the anther cavity. Ultimately, the middle layers and endothecia also dissolve, and the whole locule fills with these dissolved materials, which stain dark with safranin. By contrast, microsporogenesis in hermaphroditic plants of *R. venustus* (*Stuessy et al. 6243*) forms all normal tetrads with thick callose walls; the tapetal layer eventually also disappears.

DISCUSSION

Phylogeny of Rhaphithamnus

The genus most closely related to *Rhaphithamnus* is *Citharexylum*, which is distributed in Peru, Bolivia, and Paraguay (Moldenke 1937, 1958, 1959). The close distributional and morphological affinities of the two genera suggest that at one time they might have formed a single generic complex throughout southern South America. It is suspected that the drying that developed as a result of the southern Andean orogeny and subsequent Pleistocene glaciation and that gave rise to the vast, arid Atacama Desert in northern Chile (Ochsenius 1982) may have isolated the populational systems and led to generic differentiation. From an ancestral population in Chile and Argentina of *R. spinosus* (or its immediate precursor), therefore, came a dispersal event leading to the establishment of the genus in Masatierra sometime during the past 4 myr. Isolation resulted in phyletic speciation with the development of *R. venustus*. This occurred with no change in chromosome number; both species are $n = 18$ (Sanders et al. 1983, Spooner et al. 1988). Subsequent dispersal to Masafuera may have occurred recently as suggested by its scarcity on the younger island and lack of morphological differentiation. Although direct observations are lacking, the dark blue-black, berrylike, fleshy fruits up to 1 cm diam. of both species may be bird dispersed, having thus traveled to the archipelago from the continent and between islands. Bird traffic on these proposed routes, although not extensive, has been documented (see Valdebenito et al. [1990] for discussion).

Adaptive Evolution of Morphological and Reproductive Features

The principal suites of features that distinguish the two species of *Rhaphithamnus* are the leaves, the thorn-inflorescence complex, and the flowers (Figs. 1 and 2). The leaves are larger in *R. venustus* and more coriaceous. Although both species are small trees that occupy forest habitats, *R. spinosus* occurs in drier and warmer zones than does *R. venustus*. The increased size of leaves in the latter species may relate to the darker forest conditions in which the species grows.

The thorn-inflorescence complex differs between *R. spinosus* and *R. venustus*. In the extreme, *R. spinosus* has long thorns up to 3 cm long that occur on branches that do not possess flower buds. Flowers often occur along with the thorns and even as part of the thorn itself. The thorns of *Rhaphithamnus* are judged to be homologous with those in some species of the related genus *Citharexylum*, such as seen in the Peruvian *C. andinum* Mold., *C. flexuosum* D. Don, and *C. weberbaueri* Hayek in Engl. In this latter genus the inflorescences are usually racemes in contrast to the dichasia of *Rhaphithamnus*, but the position of the thorns relative to the inflorescence and leaves is the same. The loss of thorns in *R. venustus* in the island habitat may relate to absence of herbivores that are found normally on the continent. Direct evidence of thorns as herbivore deterrents in any plant group, however, has yet to be obtained (Myers and Bazely 1991). It is worth noting, however, that the unarmed variety of *R. spinosa*, var.

inermis, described by Kunkel (1968) occurs on Mocha Island (Province Arauco) 30 km west of the continent.

The flowers of *R. venustus* and *R. spinosus* differ in size, color, and phenology. In the former the corollas are deep blue-purple, whereas in the latter they are light blue to blue-pink. The orientation of mature flowers also differs, with *R. spinosus* more erect and *R. venustus* more pendant. *Rhaphithamnus spinosus* flowers Sept.–Nov. and *R. venustus* Dec.–Mar. Furthermore, the size of the corollas varies conspicuously, with those of *R. venustus* twice the size of those of *R. spinosus*. We believe that these differences relate to changes in pollinators between the continent and the Islands. Preliminary observations by G. Anderson in 1991 on Masatierra suggested that insect pollinators are not abundant in the Islands; our own field observations during five expeditions have suggested this. Because *R. spinosus* is occasionally visited by hummingbirds on the continent, it may have been preadapted (a "latent potential" [Stebbins 1989]) for a shift to obligate hummingbird pollination as speciation occurred on Masatierra, the older of the two islands.

Origin of Gynodioecy in R. venustus

The data show that *R. spinosus* from the continent is hermaphroditic whereas *R. venustus* from the Islands is gynodioecious (i.e., with both hermaphrodite and female plants). Gynodioecy in *R. venustus*, however, has gone undetected in previous floristic and revisionary studies (e.g., Skottsberg 1922, Moldenke 1937, 1939, 1979), because the anthers appear normal and functioning in female plants. This would be another case of "cryptic dioecy" as defined by Mayer and Charlesworth (1991). In fact, we would not have detected the absence of pollen if it were not for completing pollen-ovule studies to gain insights on breeding systems. Despite failure of pollen to develop in anthers of female plants, the external normal appearance of the anthers is maintained apparently by filling of the anther cavity by protoplasm of degenerating cells of the PMCs and tapetum. In other reported cases of gynodioecy, anthers of female plants are clearly abortive, often shriveled and shrunken (e.g., Stevens and Richards 1985).

The cryptic nature of nonfunctional anthers suggests that gynodioecy in this species in the Juan Fernández Islands is in initial stages of development. For this reason it is profitable to consider hypotheses for its initiation that may bear on the origin of this condition more generally in flowering plants. Because gynodioecy has been considered one of the possible steps to the evolution of dioecy (Richards 1986) and because interest has also centered on the presumptively high levels of dioecism in oceanic islands (e.g., Carlquist 1974, Baker and Cox 1984), it is of further interest to consider the mode of origin of gynodioecy in *R. venustus*.

Because of the differences in pollinators of *R. spinosus* on the continent and of *R. venustus* in the Islands, one might hypothesize that directional selection has occurred in the Juan Fernández Islands that has resulted in floral adaptions to hummingbird pollination. The changes are in purple and longer corollas and more pendant flowers. Because the corollas of *R. venustus* in the Islands are substantially larger than those on the continent, and if we assume that all other resources are equal between the two species, then resources would need to be reallocated to corolla tissue from other organs in *R. venustus*. The concept of ecological resources allocation in an evolutionary context still is regarded as valid (Doust 1989). One source of energy gain would be the thorns, which are usually absent in *R. venustus*. This would involve, however, a somewhat complex developmental reallocation from vegetative to reproductive tissue. Another possibility might be reduction in numbers of pollen grains in some plants, which would yield functionally female individuals. The loss of one-half of male gametes (or male fitness) would be reproductively negative, except that in this particular case the shift to hummingbird pollination may provide a more precise and efficient system in which less pollen would be needed to maintain the same level of seed set. Because fe-

male plants make up nearly one-half of the plants surveyed, it is believed that gynodioecy in *R. venustus* is genetically controlled. The exact genetic mechanism, however, such as possibly a two-gene plus cytoplasmic factor system that has been reported in other gynodioecious species (Richards 1986), has not been determined. In any event, the gynodioecious condition does appear to be stable.

Hypotheses for the origin of dioecy have suggested several avenues (see review by Thomson and Brunet [1990]). The first deals with increased size of fleshy fruits for avian dispersal in groups that have limited wind dispersal capability (Muenchow 1987). This will lead to an accelerating female gain curve (Charnov 1982). The second deals with the difficulty of maintaining synchronous dichogamy in woody, perennial species with greater reproductive flexibility. This could lead to geitonogamy (crossing between flowers of the same plant) and then to inbreeding depression (Thomson and Barrett 1981). The third avenue involves plants with small unspecialized pollinators. If pollinators stay on the plants longer, surely geitonogamy will increase and so also will inbreeding depression (Bawa 1980). Small pollinators may select for smaller flowers (Thomson et al. 1989) or they may be more selective in seeking food rewards (Bawa 1980), with the end result being an accelerating male gain curve.

A combination of factors is believed to have been involved with the origin of gynodioecy in *R. venustus*. The initial selection brought about by a change in pollinators may have caused deepening of color and increase in size of the corollas. Because the size of the populations of *R. venustus* in the Islands is small, at least as seen now on both islands (it is rare to find more than three plants at any one locality), increased geitonogamy resulting from more efficient and persistent working of all receptive flowers by the hummingbirds might have led to inbreeding depression. That such reduction in fitness can and does occur has been shown in *Schiedea* Cham. & Schlechtend. (Caryophyllaceae) of the Hawaiian Islands (Sakai et al. 1989). Resource allocation from anther to corolla tissue may have been eventually genetically fixed by increased fitness of outcrossed progeny between hermaphroditic and female plants.

The most important point here is that the initiation of gynodioecy in *R. venustus* appears to be correlated with a change from insect to hummingbird pollination. Only two other bird-pollinated gynodioecious angiosperm species are known, and they are in *Fuchsia paniculata* Lindley (Onagraceae [Breedlove et al. 1982]) and possibly also in *F. hartwegii* (Berry 1982). In those species the origin of the gynodioecious condition does not correlate with changes in pollinators because most of the genus, including relatives, are also known to be bird pollinated (Berry 1982).

ACKNOWLEDGMENTS

It is a pleasure to acknowledge the National Science Foundation for support; the Korean Science and Engineering Foundation (KOSEF) for a postdoctoral fellowship to B.Y.S. for a research visit to Ohio State University; CONAF of Chile for permission to work and collect in the Juan Fernández Islands including Ivan Laeva, Chief of the Robinson Crusoe Islands National Park, for his help and support during fieldwork in the archipelago; the Memorandum of Understanding between Ohio State University and the Universidad de Concepción that facilitated access to literature and Chilean plant specimens; Tracy Archangelsky for drawing Figures 1 and 2; and the curators of GH, M, MO, and Z for loans of specimens.

LITERATURE CITED

BAKER, H. G., and P. A. COX. 1984. Further thoughts on dioecism and islands. Ann. Mo. Bot. Gard. 71:244–253.

BAWA, K. S. 1980. Evolution of dioecy in flowering plants. Annu. Rev. Ecol. Syst. 11:15–39.

BERRY, P. E. 1982. The systematics and evo-

lution of *Fuchsia* Sect. *Fuchsia* (Onagraceae). Ann. Mo. Bot. Gard. 69:1–198.

BREEDLOVE, D. E., P. E. BERRY, and P. H. RAVEN. 1982. The Mexican and Central American species of *Fuchsia* (Onagraceae) except for Sect. *Encliandra*. Ann. Mo. Bot. Gard. 69:209–233.

BROOKE, M. DE L. 1987. The birds of the Juan Fernandez Islands, Chile. Study Report No. 16, International Council for Bird Preservation, Cambridge, England.

CARLQUIST, S. 1974. Island biology. Columbia University Press, New York.

CHARNOV, E. 1982. The theory of sex allocation. Princeton University Press, Princeton, New Jersey.

COLWELL, R. K. 1989. Hummingbirds of the Juan Fernandez Islands: Natural history, evolution and population status. Ibis 131: 548–566.

CRUDEN, R. W. 1977. Pollen-ovule ratios: A conservative indicator of breeding systems in flowering plants. Evolution 31:32–46.

CRUDEN, R. W., and S. MILLER-WARD. 1981. Pollen-ovule ratio, pollen size, and the ratio of stigmatic area to the pollen-bearing area of the pollinator: An hypothesis. Evolution 35:964–974.

DOUST, J. L. 1989. Plant reproductive strategies and resource allocation. Trends Ecol. Evol. 4:230–234.

KUNKEL, G. 1968. Die Phanerogamen der Insel La Mocha (Chile). Willdenowia 4: 329–352.

MAYER, S. S., and D. CHARLESWORTH. 1991. Cryptic dioecy in flowering plants. Trends Ecol. Evol. 6:320–325.

MIONE, T., and G. J. ANDERSON. 1992. Pollen-ovule ratios and breeding system evolution in *Solanum* section *Basarthrum* (Solanaceae). Am. J. Bot. 79:279–287.

MOLDENKE, H. N. 1937. A monograph of the genus *Rhaphithamnus*. Feddes Repert. Specierum Nov. Regni Veg. 42:62–82.

———. 1939. Additional notes on the genera *Chascanum*, *Recordia*, *Rehdera* and *Rhaphithamnus*, together with some miscellaneous taxonomic notes. Rev. Sudam. Bot. 6:15–30.

———. 1958. Materials toward a monograph of the genus *Citharexylum*. I. Phytologia 6:242–256.

———. 1959. Materials toward a monograph of the genus *Citharexylum*. VI and VII. Phytologia 7:7–48, 49–77.

———. 1979. Additional notes on the genus *Rhaphithamnus*. I. Phytologia 43:307–323.

MUENCHOW, G. E. 1987. Is dioecy associated with fleshy fruit? Am. J. Bot. 74:287–293.

MYERS, J. H., and D. BAZELY. 1991. Thorns, spines, prickles, and hairs: Are they stimulated by herbivory and do they deter herbivores. Pages 325–344 *in* D. W. Tallamy and M. J. Raupp, eds. Phytochemical induction by herbivores. John Wiley & Sons, New York.

OCHSENIUS, C. 1982. Atacama: The hologenesis of the Pacific coastal desert in the context of the tropical South American Quaternary. Striae 17:112–131.

PRESTON, R. E. 1986. Pollen-ovule ratios in the Cruciferae. Am. J. Bot. 73:1732–1740.

RICHARDS, A. J. 1986. Plant breeding systems. George Allen & Unwin, London.

SAKAI, A. K., K. KAROLY, and S. G. WELLER. 1989. Inbreeding depression in *Schiedea globosa* and *S. salicaria* (Caryophyllaceae), subdioecious and gynodioecious Hawaiian species. Am. J. Bot. 76: 437–444.

SANDERS, R. S., T. F. STUESSY, and R. RODRIGUEZ. 1983. Chromosome counts from the flora of the Juan Fernandez Islands, Chile. Am. J. Bot. 70:799–810.

SASS, J. E. 1958. Botanical microtechnique, 3rd ed. Iowa State College Press, Ames.

SKOTTSBERG, C. 1922. The phanerogams of the Juan Fernandez Islands. Nat. Hist. Juan Fernandez & Easter Is. 2:95–240.

———. 1928. Pollinationsbiologie und Samenverbreitung auf den Juan Fernandez-Inseln. Nat. Hist. Juan Fernandez & Easter Is. 2:503–547.

———. 1953. The vegetation of the Juan Fernandez Islands. Nat. Hist. Juan Fernandez & Easter Is. 2:793–960.

SPOONER, D., T. F. STUESSY, D. J. CRAWFORD, and M. SILVA. 1988. Chromosome

numbers from the flora of the Juan Fernandez Islands. II. Rhodora 89 : 351–356.

STEBBINS, G. L. 1989. Adaptive shifts toward hummingbird pollination. Pages 39–60 *in* J. H. Bock and Y. B. Linhart, eds. The evolutionary ecology of plants. Westview Press, Boulder, Colorado.

STEVENS, D. P., and A. J. RICHARDS. 1985. Gynodioecy in *Saxifraga granulata* L. (Saxifragaceae). Plant Syst. Evol. 151 : 43–54.

STUESSY, T. F., K. A. FOLAND, J. F. SUTTER, R. W. SANDERS, and M. SILVA O. 1984. Botanical and geological significance of potassium-argon dates from the Juan Fernandez Islands. Science (Washington, D.C.) 225 : 49–51.

STUESSY, T. F., C. MARTICORENA, R. RODRIGUEZ, D. J. CRAWFORD, and M. SILVA O. 1992. Endemism in the vascular flora of the Juan Fernandez Islands. Aliso 13 : 297–307.

THOMSON, J. D., and S. C. H. BARRETT. 1981. Selection for outcrossing, sexual selection and the evolution of dioecy in plants. Am. Nat. 118 : 443–449.

THOMSON, J. D., and J. BRUNET. 1990. Hypotheses for the evolution of dioecy in seed plants. Trends Ecol. Evol. 5 : 11–16.

THOMSON, J. D., M. A. MCKENNA, and M. B. CRUZAN. 1989. Temporal patterns of nectar and pollen production in *Aralia hispida*: Implications for reproductive success. Ecology 70 : 1061–1068.

VALDEBENITO, H. A., T. F. STUESSY, and D. J. CRAWFORD. 1990. A new biogeographic connection between islands in the Atlantic and Pacific Oceans. Nature (Lond.) 347 : 549–550.