Dioecy and wind pollination in *Pernettya rigida* (Ericaceae) of the Juan Fernández Islands

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Pernettya rigida is endemic to the Juan Fernández archipelago. Although all flowers are complete, with seemingly fertile stamens and pistils, differences in fruit production and detailed field, anatomical, and morphological studies indicate they are functionally unisexual, and the species is consequently dioecious. A comparison of 15 features demonstrated significant differences between the sexes. The populational sex ratio is 1:1. Nectaries located between the filaments produce small amounts of floral nectar with similar sugar composition in both sexes. There are $\bar{x} = 33,357$ (or $\bar{x} = 133,429$) pollen tetrads (or pollen grains)/male flower and $\bar{x} = 109$ ovules/female flower. No tetrads in either hand self-pollinated or open pollinated male flowers showed any pollen germination. Tetrads on open- and hand-pollinated female flowers germinate. Female flowers do not show autogamy or apomixis. During more than 80 hours of field observation, we recorded only seven floral visitors (representing three insect species). In spite of this, open-pollinated female flowers have

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abundant fruit and seed-set. Thus, we conclude that pollen is transferred abiotically and the ever-present wind over the exposed ridges of the islands is the likely dispersal agent. A number of anemophilous features, such as dry pollen and exposed habitat, support this conclusion. Thus, dioecy and anemophily have evolved independently, *in situ*, in this species in this remote locality. Preservation of habitat and elimination of competitive invasives are the primary conservation challenges.

ADDITIONAL KEY WORDS:—breeding system – conservation – floral morphology – insect pollination – island biology – nectar – reproductive biology – self compatibility.

CONTENTS

INTRODUCTION

Dioecy is found in many families of flowering plants, but at a low frequency and few are entirely dioecious. Phylogenetic analysis of sex forms among angiosperms has determined that dioecy occurs in early angiosperm families as well as in more advanced ones (Yampolsky & Yampolsky, 1922; Richards, 1997). This pattern suggests that it has arisen secondarily from hermaphrodite breeding systems many times. This unusual reproductive system is found in only about 4% of all species (Richards, 1997). Several authors have shown associations between ecological factors and dioecy at various taxonomic levels (summarized in Sakai *et al.*, 1995a), e.g. with fleshy fruits and animal dispersal, entomophily, anemophily, woodiness and longer life spans, various moisture conditions, and tropical and island floras. Thus, it is not surprising that the incidence of this reproductive strategy varies considerably in different regional floras (summarized in Steiner, 1988).

The highest levels of dioecy in regional floras are found in species from oceanic islands of volcanic origin, such as New Zealand and Hawaii (Carlquist, 1974; Godley, 1979; Sakai *et al.*, 1995 a,b). Over 30 years ago, Baker (1967) suggested what has become known as 'Baker's law', that most successful island colonizers will be hermaphrodite and self-compatible. The argument rests on the recognition that

most founders will arrive as single individuals that could not set seed if self-incompatible or unisexual. It is thus remarkable that islands should today be notable for the comparatively high levels of dioecious and gynodioecious species.

The Juan Fernández archipelago (located 667 km W of continental Chile) consists of three islands: Robinson Crusoe (= Masatierra) and Santa Clara to the east, and Alexander Selkirk (= Masafuera) further west. The archipelago is well known for its high level (60%) of endemic vascular plants (Skottsberg, 1956; Stuessy *et al.*, 1997). Around 9% of the native and endemic species are considered to be dioecious (Skottsberg, 1953; Carlquist, 1974; pers. observ.). For the Juan Fernández flora, Carlquist (1974) was more impressed with the groups in which dioecy occurs. Known dioecious species include one monocot (the palm *Juania australis*, Arecaceae) and 13 dicots (in the genera *Robinsonia* [Asteraceae, seven spp.], *Coprosma* [Rubiaceae, two spp.], *Fagara* [Rutaceae, two spp.], and *Cuminia* [Lamiaceae, two spp.]).

The species we report on here is *Pernettya rigida* (Bertero in Colla) DC. (Sect. *Pernettya*), a polyploid shrub (Sanders, Stuessy & Rodríguez, 1983) that is the only member of the Ericaceae in the archipelago and is locally known as 'murtillo.' We studied the reproductive biology of this species because it is a common and striking element of the flora of Robinson Crusoe and Alexander Selkirk Islands, constituting, along with the introduced *Ugni molinae* (Myrtaceae), the most abundant shrub on exposed and barren ridges, often forming low impenetrable thickets. The increase of barren ground due to foraging by feral goats and rabbits in recent decades seems to have contributed to the proliferation of lithophytic and xerophytic plants on the islands, among them *P. rigida* (Sanders, Stuessy & Marticorena, 1982). The presence of subterranean stolons and vegetative growth (pers. observ.) in this particular species, a feature frequent in the genus (Schneidt & Weberling, 1992), may contribute to its success and dominance in these areas. In addition, we studied this species because of an odd distribution of fruit set in the field.

The genus *Pernettya* includes 14 species that occur in Tasmania and New Zealand, South and Central America, and Mexico (Sleumer, 1985; Luteyn, 1995). There are five species in Chile and Argentina and one in Peru (Sleumer, 1952, 1985). This genus is also interesting because, although all flowers have stamens and pistils, they may be functionally unisexual in some species (Sleumer, 1952, 1985; Palser, 1958; Franklin, 1962; Moore, 1983; Cambi & Hermann, 1989; Middleton, 1991), as is the case in a few other ericaceous taxa (Cronquist, 1981). However, as pointed out by Luteyn (1995), there is little in the literature on reproductive biology, pollination, or dispersal in *Pernettya*. One exception is Arroyo & Squeo's report (1987) on anemophily in the Chilean dioecious *P. mucronata*. There are more records on pollination and reproductive biology of the closely related genus *Gaultheria* (Thompson, 1926; Heine, 1937; Hagerup, 1954; Pojar, 1974; Reader, 1975, 1977; Mirick & Quinn, 1981; Primack, 1983; Hermann & Cambi, 1992), with which *Pernettya* is sometimes combined (Middleton & Wilcock, 1990). We follow the opinion of Sleumer (1985) and Luteyn (1995) in treating *Pernettya* as a distinct genus.

Sleumer (1985) concluded that *P. rigida* is "generally dioecious, sometimes gynodioecious" based on examination of a limited herbarium samples. However, there has been very little study of the reproductive biology of this species; even floral sex expression is not well understood. This coupled with our own preliminary field observations on unusual patterns of fruit set in *P. rigida* led us to pursue an intensive and extensive study of its reproductive biology. *Pernettya* was included as part of a broadscale study of the conservation biology of the

Juan Fernández flora (Stuessy *et al.*, 1997). Here, we analyse several aspects of the reproductive biology of this species, based on *in situ* observations, addressing the following questions: (1) What is the functional structure of its flowers? (2) What is the sexuality of the species? (3) Do the flowers offer any reward? and if so, what is its chemical composition? (4) What is its breeding system? (4) How is pollination accomplished? (5) What are the implications of these data relative to the conservation biology of this endemic species?

MATERIAL AND METHODS

Fourteen populations were studied in the field during the course of three field expeditions to Robinson Crusoe Island, Chile (January 1991 and 1996, and December/January 1997). Additional herbarium vouchers from CONN and OS were also examined (Appendix).

Floral anatomy. Flowers were fixed in 70% ethanol, dehydrated in an ethyl alcohol-xylol series, and embedded in Paraplast. Serial cross- and longi-sections were cut at 10 µm, mounted serially, and stained with safranin-fast green-haematoxylin and observed with a compound microscope. To detect stomata in the nectariferous tissue, glands were cleared with standard bleach for 1 min, stained with I/IK solution, and observed with a compound microscope. Photomicrographs were taken with Kodak T-Max film.

Pollen and ovule number and pollen viability. Pollen quantity was estimated using Anderson & Symon's (1989) modification of Lloyd's (1965) technique. All ovules were counted with a dissecting scope. Buds examined for pollen counts were near anthesis, so pollen was mature but anthers had not dehisced. A total of three specimens and a minimum of three buds per specimen were examined.

Pollen viability was estimated by the percent stainability of 100 grains from each of 10 flowers (three specimens) using aniline blue in lactophenol (Hauser & Morrison, 1964).

Ratio of floral morphs. We recorded the ratio of flower types from 14 populations. We walked randomly through each population and scored plant type along various transects, being careful to count only individuals that grew far enough apart that they could not be connected vegetatively.

Sugar nectar chemical composition. Nectar was extracted in the field with glass micro-pipettes. Nectar drops were immediately placed on Whatman #1 chromatography paper and quickly dried. Sugar separation was accomplished by gas chromatography of TMS-derivatives (Sweeley *et al.*, 1963). Nectar was lyophilized and sylilated. The derivatives were then injected into a Konik KNK 3000-HRGS gas chromatograph, equipped with a Spectra-Physics SP 4290 data integrator, a flame ionization detector and a OV 101 column (2-m long), 3% on Chromosorb G/AW-DMCS mesh 100–120. Nitrogen was the carrier gas (30 ml min^{-1}) using the following temperature program: 200°C for 2 min, 1°C × min^{-1} until 215°C was reached, 15°C × min until reaching 310°C for 5 min. Carbohydrate standards (Sigma Chem.) were prepared using the same method. Analyses were repeated for each sample. The

sugar ratio was calculated according to Baker & Baker (1983) as r=sucrose/fructose + glucose. The hexose ratio was estimated as hr=glucose/fructose.

Floral visitors. About 100 plants of both flower types were observed in the field for more than 80 hours, during 1991, 1996, and 1997. Periods of observation ranged from 20 to 40 min, all during daylight hours (from 8:00 to 17:00). All floral visitors were recorded, observed for behaviour, and captured for identification; they were deposited in the insect collections of the Department of Ecology and Evolutionary Biology of the University of Connecticut.

Experimental crosses. Branches with tagged buds were bagged in the field with nylon mesh bags to exclude potential visitors. Hand-pollinations were performed on emasculated flowers by applying pollen from recently opened anthers from large anthered flowers, using the anthers themselves as pollen applicators. About 48 hours after hand pollination, flowers were collected and fixed in 70% ethanol. Autogamy was checked in the flowers with ovules, by bagging young buds and collecting the unmanipulated flowers two days after the flower had opened. To test the possibility of apomixis in the flowers with ovules, we bagged emasculated flowers, which also had the stigmata removed (clipped off).

Pollen tube growth. Gynoecia were softened by soaking in 8N NaOH at 60°C for 1 h, rinsed, and stained in aniline blue-0.1 NK$_3$ PO$_4$ for 2 h (Martin, 1959). The carpels were dissected from the flowers and flattened on a slide in glycerin. Pollen tubes were examined under an epifluorescence microscope.

Seed germination. Seeds were dipped in standard bleach to surface sterilize them, rinsed thoroughly in distilled water, placed in clean petri dishes lined with moistened Whatman #1 filter paper, and kept at 4°C for 14 days (after Arena, Pastur & Vater, 1994). Seeds were checked periodically for mold and retreated if necessary. Seedlings were then transferred to 2 inch pots with Redi-earth (uncovered), and placed under mist.

RESULTS

Floral morphology

Flowers are nodding (Figs 1, 2), actinomorphic, and pentamerous. The pedicels are bracteate at the base with usually 2–6 bracts. Flowers are borne solitary in the axils of the upper leaves, giving the impression of racemes. The calyx is 5-parted, nearly free, glabrous, and has green triangular acute sepals that are persistent in the fruit. The corolla is white and glabrous, the petals fused in a campanulate-urceolate tube with short triangular reflexed lobes (Figs 1, 2).

A superficial examination of the flowers suggests they are hermaphroditic because all possess an androecium and gynoecium (Figs 1, 2). The androecium is bicyclic and composed of 10 included equally sized stamens, with dorsifixed anthers and filaments free from the corolla tube. The filaments have a peculiar shape: linear and flattened at the base, they enlarge strikingly into two showy protuberances of around 0.7 mm wide, then become cylindrical (Fig. 3). The epidermis of the basal third of the filament is papillate (Figs 6, 7). The anthers are typically tetrasporangiate
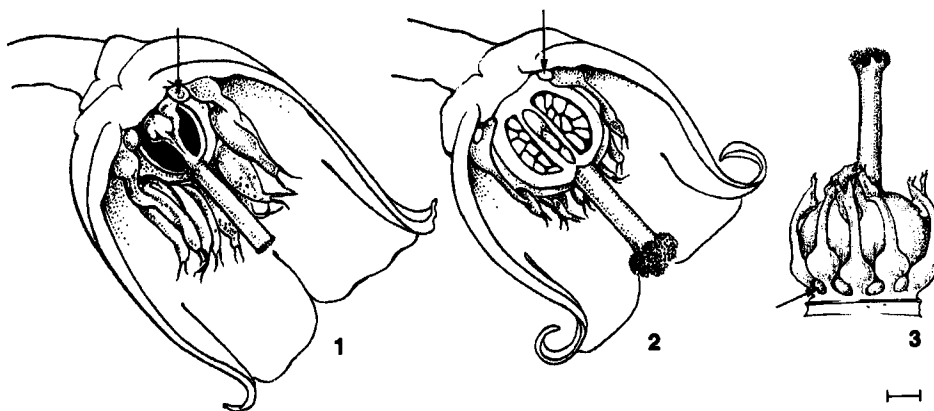
Figures 1–3. Semi-schematic diagrams of *Pernettya rigida* flowers. Fig. 1. Large anthered flower, long-section. Fig. 2. Small anthered flower, longi-section. Fig. 3. Detail of androecium and gynoecium from small anthered flower. Arrows point to nectaries. All diagrams are at the same scale. Scale bar = 1 mm

and dithecal, releasing the pollen by apical pores. The anther apex is drawn out into a pair of slender tubules with two thin appendages, like small horns (Figs 1, 2). The gynoecium has a superior, plurilocular ovary composed of five united carpels with the ovules inserted in axile placentation (Figs 2, 7). The style is gynobasic (Figs 1, 2, 4), slender, and has a stylar canal. The latter is angled, with the angles corresponding in position to the locules, and the ridges of the stylar tissue between the angles corresponding to the septa.

Differences in fruit production among individuals were detected in the field. In effect, certain plants had many fruits (with 'small anthers'), while others were completely fruitless (with 'long anthers') even though the two types produced about the same quantity of flowers. This, and major differences in anther size between them, suggested that they might be functionally unisexual. Descriptions of the differences between the two flower types follow.

Flowers with long anthers. The ratio of filament: anther is about 1:1. The anthers are brown to yellowish brown, and dehisce roughly simultaneously. The mature anther has a papillate epidermis, two middle layers, and a tapetum (Fig. 5). Pollen grains are shed in tetrahedral tetrads and are highly stainable ($\bar{x}$ = 94% ± 2.3). The average number of tetrads per flower is $\bar{x}$ = 33 357 ± 9 007, for an average of $\bar{x}$ = 133, 429 ± 36 029 pollen grains (n = 14 flowers, 10 specimens). Several characteristics of the gynoecium of these long anthered flowers indicate that is non-functional. The ovary is comparatively smaller than the one of the small anthered flowers (see below) and has locules that contain either no ovules (Figs 1, 6), small rudimentary ovules

Figures 4–9. Anatomical floral features in *Pernettya rigida*. See scale bar on Figure 9. Fig 4–6. Large anthered flower: Fig. 4. Flower long-section. Scale bar = 750 μm. Fig. 5. Thecae trans-section showing two microsporangia with tetrads. Scale bar = 50 μm. Fig 6. Detail of longi-section showing one nectary and one locule with no ovules. Scale bar = 215 μm. Figs 7–9. Small anthered flower. Fig. 7. Detail of a longitudinal section showing a nectary and locule with ovules. Scale bar = 250 μm. Fig. 8. Detail of a cross-section showing nectaries. Scale bar = 75 μm. Fig. 9. Anther cross-section showing four empty microsporangia. Scale bar = 300 μm. Abbreviations: c, corolla; f, filament; n, nectary; l, locule.
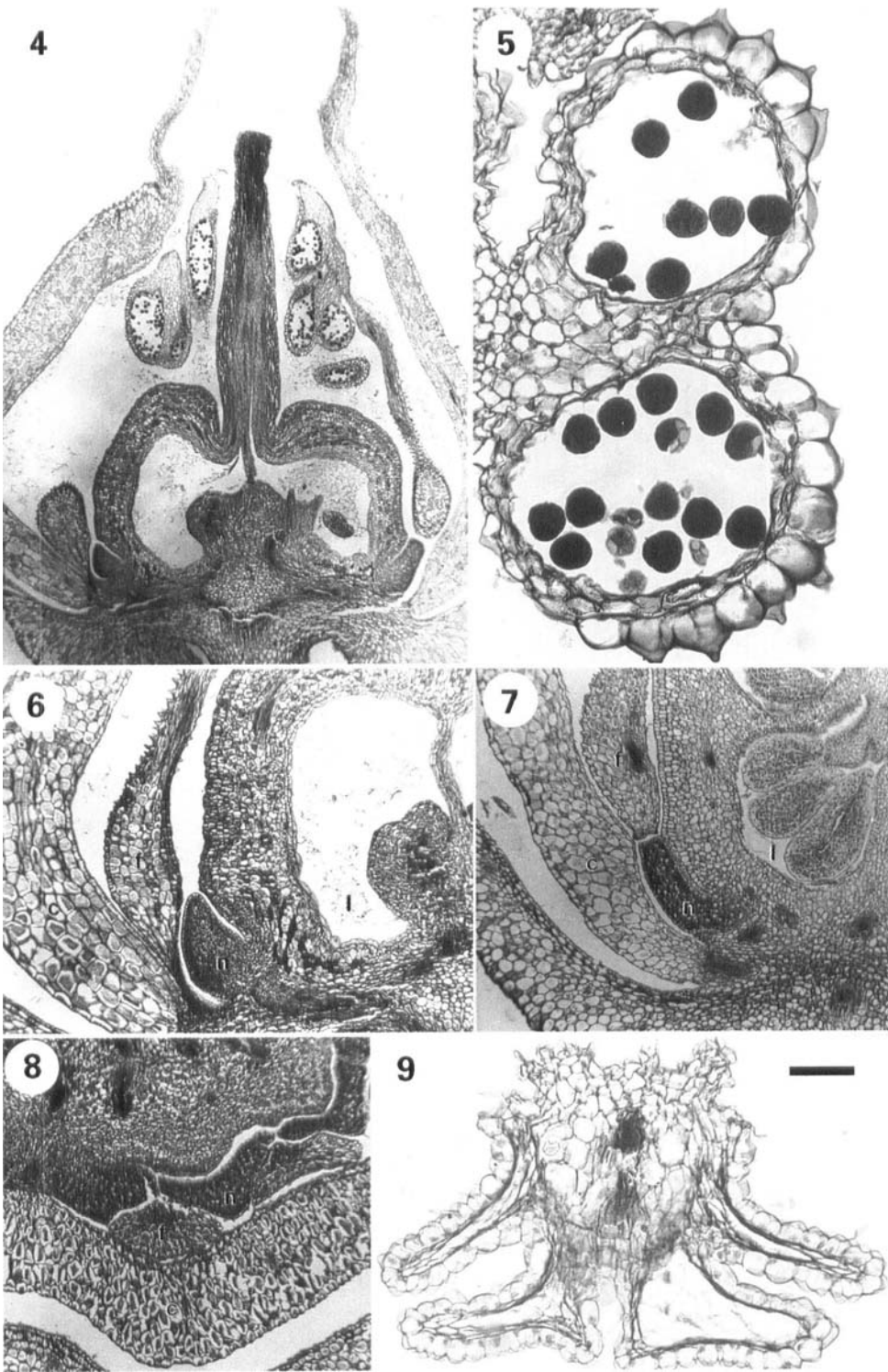
(Fig. 4), or larger ones that do not fill the locules, and subsequently do not develop into viable seeds. The few flowers that bear ovules had an average of $\bar{x} = 69.4 \pm 15.8$ ($n = 5$, one specimen). The stylar canal is narrow. The stigma is dry, pinkish or green, blunt or slightly capitate, and not expanded, i.e. almost not differentiable from the style (Figs 1, 4). The stigmatic papillae are small ($\bar{x} = 24$ μm ± 6.8, range 15–25 μm; $n = 5$, one specimen), mostly isodiametrical, and clustered together. The height of the style is the same as or slightly above that of the anthers.

These flowers did not set fruit. Nonetheless, a few such flowers did bear enlarging berries. Detailed examination and comparison of these ovaries showed them to have a lighter reddish color and be smaller than those produced by flowers with small anthers (see below). Upon dissection, no seeds or a few small aborted seeds with no embryos were found. In several such enlarged ovaries, insect larvae were discovered (i.e. ovary development was presumably stimulated by oviposition by an insect).

Flowers with small anthers (Figs 2, 3). The stamens, although present, appear to be non-functional, i.e. staminodes. They barely reach the base of the style (Fig. 2) and bear small indehiscent anthers. The ratio of filament: anther length is about 1:0.4. The anther sacs are either collapsed or filled with a viscous material and have no pollen (Fig. 9). The anther wall is reduced to a non-papillous epidermis and 2–3 collapsed layers (Fig. 9). A few rare specimens had flowers with comparatively longer anthers and several inviable pollen grains in them. Upon anatomical examination, one such flower exhibited completely collapsed vascular bundles of the filaments and collapsed layers of the anther wall.

In comparison, the ovary always has many ovules that completely fill the locules (Figs 2, 7), and produce seeds with embryos. Ovule counts ($n = 10$, 10 specimens) indicate an average of $\bar{x} = 109 \pm 11.2$. The stigma (Figs 2, 3) is wet, conspicuous, capitate, and red to purplish in color. It has five shallowly expanded lobes with fairly long stigmatic papillae ($\bar{x} = 59 \pm 16$ μm, range 37–80 μm, $n = 5$, one specimen) that are unicellular and narrowly elliptical. These flowers develop into dark purple berries with viable seeds that have a typically reticulated brown coat and endosperm. The average number of seeds is $\bar{x} = 111 \pm 20$ ($n = 10$ fruits from three specimens). Seed germination took about 4 weeks and was about 60%.

Comparisons between flower types

The differences in primary reproductive characteristics described above are complemented by a number of secondary features. Some of the differences were: larger vs, smaller flowers, campanulate-urceolate vs urceolate corollas, and mostly curved vs. straight pedicels, for large anthered and small anthered flowers respectively (Figs 1, 2). In order to detect significant differences in both the primary and secondary characters between the sexes, we recorded 15 quantitative morphological measurements from individuals on both islands (Table 1). Preserved material was studied exclusively from Robinson Crusoe Island, and dried material was studied from both Robinson Crusoe and Alexander Selkirk Islands (Appendix).

We first compared the statistical differences within each type between the islands. The vegetative features recorded were not significantly different between the islands, except for leaf width (Table 1). Thus, we pooled the data for each type from both islands and made overall comparisons.

TABLE 1. Morphological features of dried specimens of *Pernettya rigida*. Measurements are averages in mm unless otherwise noted. L=large anthered flowers, S=small anthered flowers, RC=Robinson Crusoe Island, AS=Alexander Selkirk Island. See text for detailed description of characters measured. The *P* values are from *t*-tests that compared large anthered flowers and small anthered flowers. *n* represents the number of specimens examined

Flower type/ Island	n	Pollen count	Pollen size (μm)	Anther length	Filament length	Stigma width	Stigma-throat distance	Style length	Ovary width	Ovary length	Ovule size	Pedicel length	Corolla width	Corolla length	Leaf length	Leaf width
L/RC	9	26402	29.26	1.55	1.79	0.43	1.55	2.02	1.45	1.33	0.20	9.39	4.54	5.79	11.62	5.70
L/AS	3	21944	29.44	1.56	1.75	0.42	1.69	1.92	1.55	1.44	0.31	8.22	3.60	4.89	10.89	5.67
Mean		25288	29.31	1.55	1.78	0.43	1.59	1.99	1.48	1.35	0.23	9.10	4.31	5.57	11.43	5.69
SD		8004	1.50	0.16	0.20	0.06	0.54	0.27	0.20	0.14	0.07	1.55	0.97	0.81	1.52	0.71
S/RC	9	—	—	0.55	1.33	0.73	0.30	1.68	1.55	1.60	0.33	6.38	3.97	5.52	12.47	5.92
S/AS	3	—	—	0.51	1.25	0.83	0.62	1.55	1.49	1.49	0.33	6.67	3.81	4.69	10.33	7.00*
Mean		—	—	0.54	1.31	0.75	0.38	1.65	1.54	1.58	0.33	6.45	3.93	5.32	11.93	6.19
SD		—	—	0.09	0.20	0.13	0.33	0.21	0.16	0.21	0.09	1.37	0.58	0.57	2.13	1.19
t-test		10.44**	67.75**	18.90**	5.70**	7.60**	6.67**	3.46*	NS	3.01*[1]	2.74*	4.43***	NS	NS	NS	NS

[1]This is the only significant difference in any feature compared between the islands; *P<0.01, **P<0.001, NS=non-significant

TABLE 2. Morphological features of pickled specimens of *Pernettya rigida* from Robinson Crusoe Island. Island. Measurements are means in mm. n = number of flowers measured. L = large anthered flowers, S = small anthered flowers. The *P* values are from *t*-tests that compared large anthered and small anthered flowers

Flower type	n	Anther length	Filament length	Stigma width	Stigma-throat distance	Style length	Ovary width	Ovary length	Ovule size	Corolla width	Corolla length
L	10	2.18	2.30	0.49	2.14	2.58	2.30	2.18	0.18	6.43	7.30
SD		0.25	0.32	0.09	0.51	0.75	0.72	0.66	0.02	1.07	0.54
S	10	0.76	2.02	1.00	0.38	2.21	2.16	1.98	0.39	4.99	5.47
SD		0.07	0.11	0.07	0.41	0.22	0.26	0.23	0.04	0.38	0.43
t-test		12.73**	NS	11.78*	5.60*	NS	NS	NS	19.20**	2.74*	7.51**

* P<0.01, ** P<0.001, NS = non-significant

Almost all the reproductive characters studied from dried material showed significant differences (using *t*-tests) between the flower types, with the exceptions of ovary width and corolla size (Table 1). In addition to the differences in the androecium (pollen presence, filament and anther length) and gynoecium (ovule size, ovary and style length, stigma width), the pedicels of large anthered flowers are longer, and the distance from stigma to corolla throat is less in the flowers with small anthers, i.e. stigmata are more exposed in this type.

We also compared preserved material from Robinson Crusoe Island to assess possible variations deriving from different preservation of the material (Table 2). We assayed ten reproductive features that were also measured in dried material (Table 1). The pattern of variation for these features was the same between the two data sets (i.e. always larger in one flower type) regardless of the preservation of the material used. However, the differences were not always statistically significant (as they were in the dried samples). The preserved material did allow thin sections of stigmata to be prepared. The sections showed that papillae are significantly larger in small anthered flowers (two specimens, one of each type, small anthered: $\bar{x}$ = 16.07, large anthered: $\bar{x}$ = 6.75; t = 4.490, P = 0.002).

In the field, the flower types appeared to differ in floral density, with the inflorescences of small anthered flowers appearing to be more clustered at the ends of branches. We attempted to quantify this variation in five specimens of each flower type from Robinson Crusoe island, counting the number of flowers present in three successive 3 cm segments starting from the tip of a branch. The comparative proportions of flowers in each segment were not significantly different between the types (total combined mean [large anthered and small anthered flowers] for each of the segments from tip to 9 cm: $\bar{x}$ = 40% ± 0.005, $\bar{x}$ = 31% ± 0.043, $\bar{x}$ = 27% ± 0.048).

Ratio of the flower types

In the field, each plant had only one flower type. The numbers of plants of each type were counted in a total of 14 populations (n = 816 individuals) from Robinson Crusoe Island (Table 3). A statistical analysis of the pooled samples showed that the ratio of the two types were not significantly different from a 1:1 ratio (χ^2 = 0.15).

TABLE 3. Number of plants with large anthered (L) and small anthered (S) flowers in 14 population samples of *Pernettya rigida* at various locations on Robinson Crusoe Island

Date	Location	L	S
20.i.91	Cordón Central	64	56
10.i.96	Mirador A. Selkirk	10	10
10.i.96	Top of Salsipuedes	46	18
10.i.96	Top of Salsipuedes	32	20
14.i.96	Sendero and Mirador A. Selkirk	49	26
14.i.96	Villagra	1	4
18.i.96	Top of Dama Juana	19	11
18.i.96	Dama Juana	15	5
18.i.96	Top of Corrales de Molina	21	11
20.i.96	Lower Cordón Central	34	40
21.i.96–23.i.96	Lower Salsipuedes	32	28
24.i.96	Cordón Central	42	48
26.i.96	Centinela/Pangal	35	34
3.i.97	Ridge between Pirámide and Corrales de Molina	51	60
	Total	451	365

TABLE 4. Percent concentration of sugar nectars of large anthered (L) and small anthered (S) flowers of *Pernettya rigida*. Data are percent means ± SD

| Flower type | Sugar type | | |
	Sucrose	Fructose	Glucose
L	0.18 ± 0.11	43.75 ± 2.91	56.05 ± 2.98
S	0.43 ± 0.04	44.64 ± 1.97	54.91 ± 1.91
Total mean	0.28 ± 0.15	43.82 ± 2.12	55.82 ± 2.61

Nectaries and nectar

Both flower types produce small but similar amounts of floral nectar (1 µl or less). The exudate accumulates as droplets at the base of the corolla tube. Through a macroscopical analysis, we detected ten lunate, subtriangular to elliptic protuberances of about 0.3 mm long × 0.8 mm wide. These protuberances are located between the ovary and the corolla and alternate with the stamens, filling the spaces between the filament bases (Figs 1–3). Each receptacular nectary ("type 3d", Fahn, 1979) is laterally surrounded by the filament bases and protected from above by the basal expansion of the filament.

Microscopical cross-sections showed that these are nectarial glands (Figs 6, 7). The nectariferous tissue is composed of isodiametrical cells, intensely stained, with dense cytoplasm and small vacuoles. The nectaries are not supplied with special vascular bundles; thus, the vascular bundles of the androecium and gynoecium must fulfil that function. As the epidermis has no stomata, the nectar has to be exuded through the rugose cuticle that covers it.

The nectar has the three most common sugars: sucrose, fructose, and glucose. The proportions found in the different flower types are comparable, as shown in Table 4. The overall sugar ratio obtained for the species with the mean values of both flower types is $r = 0.002$, i.e. a hexose-dominant nectar. It has closely balanced proportions of fructose and glucose (hr = 1.27).

appear to be more dispersed over branch ends, this is not the situation. The looser appearance perhaps may be a manifestation of the longer pedicels of the male flowers.

Nectaries and nectar

The floral nectaries found in *P. rigida* are similar to those observed in other Ericaceae (Palser, 1958). The minute amount of nectar produced is characteristic of other ericaceous species as well (e.g. Reader, 1977; Real & Ratchcke 1991; Aparicio & García-Martín, 1996). Both sexes have similar sugar nectar composition; thus, nectar chemistry seems to be characteristic of the species, not the sexes. Reports on nectar sugar composition in the Ericaceae, mainly for *Rhododendron* and *Erica* species, indicate that most have sucrose rich or dominant nectars, followed by hexose rich or dominant and balanced nectars (Percival, 1961; Harborne, 1993; Stiles & Freeman, 1993; Barnes, Nicolson & van Wyk, 1995). *Pernettya rigida*, however, is hexose rich with balanced proportions of fructose and glucose, a combination similar to that in an unidentified species of *Pernettya* studied by Percival (1961). According to Baker & Baker (1983), hexose rich or dominant nectars are typical of flowers visited by short-tongued bees or flies, this perhaps being a good indication of the ancestral pollination system of *P. rigida*.

Breeding system

Species in the Ericaceae are characterized by self-compatibility and insect-pollination (Fryxell, 1957; Jain, 1976; Reader, 1977; Mirick & Quinn, 1981; Aparicio & García-Martín, 1996), or less commonly by gametophytic self-incompatibility (GSI) (Fryxell, 1957; Pandey, 1960; de Nettancourt, 1977; Charlesworth, 1985; Mau *et al.*, 1991). Reproductive characteristics of the genus *Pernettya* are unknown. We observed a few tetrads on the diminutive male stigmata, most probably from their own anthers, but none showed any germination. This might reflect incompatibility, but more likely in this instance the non-functionality of the male stigmata. Residual incompatibility is apparently expressed in the dioecious *P. insana* from southern Chile and Argentina, where the stigma of the male flower is expanded and apparently functional (deposited pollen germinates, but the tubes grow abnormally, see Cambi & Hermann, 1989).

The confirmation of cryptic dioecy in *P. rigida* increases the possibility that more ericaceous taxa possess this breeding system. The various degrees of androecium and gynoecium sterilization observed in *P. rigida* have been recorded for female and male flowers from several other dioecious ericaceous species as well (Gray, 1876; Wilson, 1893; Stevens, 1911; Aalders & Hall, 1963; Cambi & Hermann, 1989). This circumstance suggests a widespread genetic potential for expression of floral unisexuality and perhaps an evolutionary trend towards dioecy in the family.

As is the situation for other *Pernettya* species, the founders of *P. rigida* may have expressed sexual variation when they first arrived on the island. The existence of a closely related species in southern South America (*P. mucronata*, Sleumer, 1935) with a cryptically dioecious system (Arroyo & Squeo, 1987) and parallel floral structure (Palser, 1958), might be taken as an indication that variation in sexual expression

may have characterized the ancestors of *P. rigida* before the arrival of the first colonizers. Thus, the acquisition of dioecy in *P. rigida* on the Robinson Crusoe Island may have resulted simply from the selection among variants in the colonizers.

Pollination

In spite of a virtual absence of biotic vectors, open pollinated female flowers set abundant fruits and seeds. The dioecious condition means that pollen must be transported between plants, and the lack of pollinators means it must be transported abiotically. The strong, and ever-present wind is the obvious pollen dispersal agent. Wind is considered a significant pollination agent for remote island floras and early successional systems (Carlquist, 1966; Whitehead, 1969, 1983; Regal, 1982; Barrett, 1995; Bernardello *et al.*, 1999), characterizing significant percentages of species in Hawaii, Juan Fernández, and New Zealand floras (Carlquist, 1974). An apparent exception to this general rule seems to be the flora of the Galápagos Islands (McMullen & Close, 1993), although additional studies are needed.

The clustering of flowers at the end of branches, the nodding flower position (see Figs 1, 2), the poricidally dehiscent anthers, the small dry pollen grains with smooth exine, and dioecy itself are features facilitating pollen shedding when wind gusts shake the branches of these shrubs. When recently opened male flowers are even slightly shaken, small clouds of pollen billow from the anthers. The longer and more curved pedicels of the male flowers would seem to enhance the possible agitation of the flowers and the consequent distribution of pollen into the air. In turn, female flowers clearly have features (straighter pedicels, more exposed larger stigmata with fairly long papillae) that facilitate collection of air-borne pollen (an ability demonstrated by the pollen capture in open pollination tests). Furthermore, the sex ratio, and the fact that the individuals also reproduce vegetatively and grow in almost pure stands (usually only with *Ugni molinae*), facilitates pollen distribution by reducing the dispersal distance, and eliminating obstacles that disrupt wind patterns. The steep open habitats and rocky ridges where the plants grow are also conducive to pollen dispersal by wind.

The P/O ratio in *P. rigida* is low for an anemophilous species. It was calculated by considering the average ovule number of female flowers and the average pollen number of male flowers, the sex ratio of 1:1, and the assumption (based on the data showing a lack of difference in inflorescence clustering) of an equal number of flowers per plant. Given this, the two possible P/O calculations are: 305 tetrads/ovule or 1220 pollen grains/ovule, i.e. the facultatively autogamous and facultative xenogamous categories of Cruden (1977).

In the Ericaceae, three main pollination mechanisms are known: autogamy in self-compatible species (e.g. Hagerup & Hagerup, 1953; Hagerup, 1954; Mirick & Quinn, 1981), entomophily (e.g. Müller, 1883; Merrill, 1936; Hagerup & Hagerup, 1953; Wood, 1968; Reader, 1977; Rebelo, Siegfried & Oliver, 1985; Aparicio & García-Martín, 1996), and anemophily (Arroyo & Squeo, 1987). As indicated above, *P. rigida* has a number of features typical of anemophily, but several others imply an entomophilous history (Faegri & van der Pijl, 1979; Whitehead, 1983; Proctor, Yeo & Lack, 1996). In this instance, where there are no obvious dependable pollinators, the entomophilous features would seem important primarily to provide insight into past ancestry. In more than 80 hours, we observed only seven insect

visits to thousands of flowers in field studies. None of the insects went from flower to flower, and those examined bore no pollen on their bodies. For pollination to be effective in a dioecious species like this, insects must be abundant and regularly fly from flowers of males to flowers of females. With virtually no floral visits that is unlikely.

In the related dioecious *P. mucronata* from the mountains of southeastern Chile, no floral visitors were recorded. Thus, this species was considered exclusively anemophilous, even though other plant species in that habitat were entomophilous (Arroyo & Squeo, 1987). Like *P. rigida*, the anemophilous *P. mucronata* has a number of entomophilous features as well. The lack of insect pollinators to *P. rigida* may be a reflection of a very depauperate pollinator fauna on the islands in general (Skottsberg, 1928; pers. observ.). However, the data on *P. mucronata* imply that even with locally available potential floral visitors, *Pernettya* flowers may not be visited, and anemophily may be the primary pollen distribution system.

Skottsberg (1928) listed *P. rigida* as entomophilous, perhaps because he observed nectar, but he did not record any insect visitor to the flowers. It may be that, in general, features persist longer—essentially vestigial features of a former entomophilous system in this case—in island habitats where there may not be as much competition and thus selection (largely because of the relatively depauperate flora resulting from insufficient time for recruitment) for new adaptive features. Or, it may be that anemophily is related more to the increasing severity of the abiotic conditions encountered by the early colonizers. Ancestral populations of *P. rigida* may have become established because they were pre-adapted: i.e. they were proto-dioecious and proto-anemophilous, perhaps deriving from mountainous continental habitats. Thus, it is possible that dioecy and anemophily did not arise *in situ*, but become established *in situ*.

Shifts to wind pollination and unisexual flowers are correlated in some species, but the order of these evolutionary events is not clear in the Ericaceae (as in many groups; Charlesworth, 1993; Weller *et al.*, 1998). The shift from hermaphroditism to dioecy in populations where gynodioecy occurs may be influenced by the lack of pollinators in windswept habitats (Weller & Sakai, 1990). Self-pollination in hermaphrodites may lead to inbreeding depression and allow establishment of females within the populations. Their continued survival would mandate a shift to an effective xenogamous pollination system, and without appropriate biotic pollinators, to anemophily. This situation may well have played out in the case of *P. rigida*.

Finally, seed dispersal appears to be endozoic because the fruits are fleshy and edible and there are some red-fruit eating birds on the island (Brooke, 1987). Bird dispersal also characterizes the mainland *P. mucronata* (Arroyo & Squeo, 1987). Association among dioecy, anemophily, and fleshy bird dispersed fruits has been noted independently by Bawa (1980) and Givnish (1980), traits that are also combined in several angiosperms in southern Chile (Arroyo & Squeo, 1987).

Conservation

The vegetative reproduction through stolons, the abundant sexual seed production mediated by wind, and the bird dispersal of berries appear to be combined in *P. rigida* to promote colonizing of new habitats. The same traits may also be relevant

to explain the presence of this species on both main islands of the archipelago (only 13% of the flora occurs on both islands). In the first palaeoecological record for the archipelago that includes data on pre-occupation vegetation, Haberle (1997) recorded *P. rigida* pollen in the upland vegetation of Alexander Selkirk island indicating that this shrub (together with *Empetrum* and *Coprosma*) was part of a subalpine heathland.

In plants that possess both vegetative and sexual reproductive mechanisms, density might affect the relative amount of energy allocated to the alternate methods of reproduction (Abrahamson, 1975; Williams, 1975). At low densities, it has been hypothesized that vegetative propagation should be favoured, while at high densities, relatively more energy should be put into seed production for dispersal (Abrahamson, 1975; Williams, 1975). The density of *P. rigida* populations in Robinson Crusoe Island is intermediate, and the plants show a balance between both reproductive methods.

The leaves as well as the nectar (in honey) of some *Pernettya* species are thought to be poisonous (White & Riethof, 1959; Lampe & McCann, 1985). Although we have not yet tested this for *P. rigida* (study of leaves in progress), this could be the reason why the rabbits and feral goats do not feed on them, another attribute promoting the persistence, and conservation, of this species.

As this study shows, and in contrast to most other species on the islands (e.g. *Lactoris*, Bernardello *et al.*, 1999), the endemic *P. rigida* thrives in the Juan Fernández and it seems not to be endangered from a reproductive point of view. Nevertheless, the depauperate soil it grows in is mostly eroded, exposing bedrock to degradation, and retaining less of the available rain water. Erosion is, in fact, a considerable problem in these islands, particularly on the steep volcanic terrain (Sanders *et al.*, 1982). The preservation of habitat seems to be the central challenge to indirectly protect this species. Even in the exposed habitats that *P. rigida* occupies successfully, the highly invasive *Rubus ulmifolius* (Rosaceae) and *Aristotelia chilensis* (Elaeocarpaceae) are aggressive colonizers. Thus a further clear challenge is the control of these highly noxious invasive exotics (Stuessy *et al.*, 1997).

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APPENDIX

Pernettya rigida specimens studied. All were collected on Archipelago Juan Fernández, Chile. Unless otherwise noted after collector/#, specimens are deposited at CONN. Elev. = metres above sea level. Study references are as follows: M = morphology (external), F = fluorescence microscopy for pollen tube growth, P/O = pollen/ovule count, A = anatomy, N = nectar, P = pollen viability.

Collection Site	Collector/#	Sex	Date	Elev.	Study
ROBINSON CRUSOE ISLAND					
Salsipuedes	Anderson 2000	♀	10.i.96	490	M
Sendero and Mirador trail	Anderson 2022	♀, ♂	14.i.96	350	P/O, P
Cordón Central	Anderson 2058	♂	20.i.96	190–250	M, A
Cordón Central	Anderson 2059	♀	20.i.96	190–250	M, A
Salsipuedes	Anderson 2067	♂	21.i.96	200–250	M
Salsipuedes	Anderson 2068	♀	21.i.96	200–250	M
Cordón Central	Anderson 3021	♂	24.i.96	370	M, N
Cordón Central	Anderson 3022	♀	24.i.96	370	M
Pangal trail	Anderson 3039	♂	26.i.96	100	M
Pangal trail	Anderson 3040	♀	26.i.96	100	M, N
CONAF	Anderson 3051	♂	25.i.96	60	F
Mirador trail	Anderson 4038	♂	5.i.97	160–270	M, F
Mirador trail	Anderson 4039	♀	5.i.97	160–270	M, F, P/O
Mirador trail	Anderson 4040	♂	5.i.97	160–270	P/O, P
Mirador trail	Anderson 4042–46	♂	5.i.97	160–270	P/O, P
Mirador trail	Anderson 4047–51	♀	5.i.97	160–270	P/O
Piramide to Cordón					
Falda Larga	Anderson 4061–71	♂	7.i.97	360–550	M
Mirador Sendero	Anderson 4164	♀	16.i.97	165	M, F
Puerto Francés	Ruiz 6673 (OS)	♀	8.ii.84	400	M
Mirador Selkirk	Stuessy 5018 (OS)	♂	28.i.80	575	M
Ridge from San Juan					
Bautista to Cordón Central	Stuessy 5160 (OS)	♂	13.ii.80	500	M
Centinela Ridge above Pangal	Stuessy 5372 (OS)	♂	22.xi.80	470	M
Valle Inglés	Stuessy 6546 (OS)	♀	4.ii.84	100	M
El Camote	Stuessy 11176 (OS)	♂	24.i.90	580	M
Ridge above Corrales de Molina	Stuessy 11311 (OS)	♂	31.i.90	580	M
Salsipuedes	Stuessy 11749 (OS)	♀	15.i.90	620	M
Ridge west of Cerro Alto	Stuessy 11837 (OS)	♀	19.i.91	520	M
Corrales de Molina	Stuessy 12034 (OS)	♀	25.i.91	450	M
ALEXANDER SELKIRK ISLAND					
Cuchillo del Imán	Landero 9152 (OS)	♀	20.i.86	1100	M
Quebrada Pasto	Landero 9384 (OS)	♂	28.i.86	940	M
Quebrada Pasto	Stuessy 9018 (OS)	♂	16.i.86	600–800	M
Quebrada Pasto	Stuessy 9116 (OS)	♂	20.i.86	865	M
Cordón La Cuchara	Valdebenito 9029 (OS)	♀	16.i.86	880	M