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Reproductive ecology of the endemic *Lecocarpus pinnatifidus* (Asteraceae) in an isolated population in the Galápagos Islands

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Lecocarpus pinnatifidus is an endemic member of the Asteraceae occurring on only one island in the Galápagos archipelago. The capitula are large with female ray florets and male disc florets. They are self-compatible but this study suggests fruit set is pollen limited. Visits from *Xylocopa darwini* and other larger insect pollinators are rare, and small insects seem to be the main pollinators. Small insects carry few pollen grains and most likely mediate self-pollinations. Self-compatibility and seed set after selfing are the most common reproductive strategy in the Galápagos Islands and *L. pinnatifidus* seemingly fits well into this group. © 2004 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2004, **146**, 171–180.

ADDITIONAL KEYWORDS: fruit set – phenology – pollen deposition – pollen limitation – pollination – self-compatibility.

INTRODUCTION

Many species are found in naturally isolated populations. This could be due either to fragmentation of former continuous population of the species, or because suitable habitats are found within limited areas only, as on islands. In such isolated populations inbreeding and genetic drift are important evolutionary features influencing the distribution and amount of genetic variation with a speed depending on the size of the populations (Ellstrand & Elam, 1993). Mating systems ensuring outcrossing are the most important factor enabling species to oppose genetic erosion (Hamrick & Godt, 1989). Species in naturally isolated populations of limited size, especially on islands, are often found to be self-compatible with a mixed mating system (Barrett, 1996). The evolutionary stability of mixed mating systems is much debated (Lloyd, 1979; Schoen & Lloyd, 1984; Schemske & Lande, 1985) but largely accepted for islands. This is regarded either as a compromise between the necessity of being able to self during colonization and the necessity, later, of generating individuals with different adaptive properties (Sun & Ganders, 1988), or due to ever changing environmental conditions such as the availability of pollinators (Barrett & Eckert, 1990).

Several species within Asteraceae possess multiallelic sporophytic self-incompatibility systems (Lane, 1996; Messmore & Knox, 1997; Costin, Morgan & Young, 2001; Young *et al*., 2002). In many cases, however, the incompatibility systems are shown to vary between individuals, allowing selfing to happen only in certain individuals (Reinartz & Les, 1994; Lane, 1996; Luijten *et al*., 1996; Cheptou, Lepart & Escarre, 2002; Young *et al*., 2002; Nielsen, Siegismund & Philipp, 2003). Species with such a mating system would be well suited as colonizers and the mating system might afterwards evolve towards more or less outcrossing or selfing according to circumstances.

We examined the pollination and incompatibility system of *Lecocarpus pinnatifidus* Decne, a representative of Asteraceae, which is endemic to one of the Galápagos Islands (Fig. 1) where it occurs in separate populations. We studied pollinator activity, pollen

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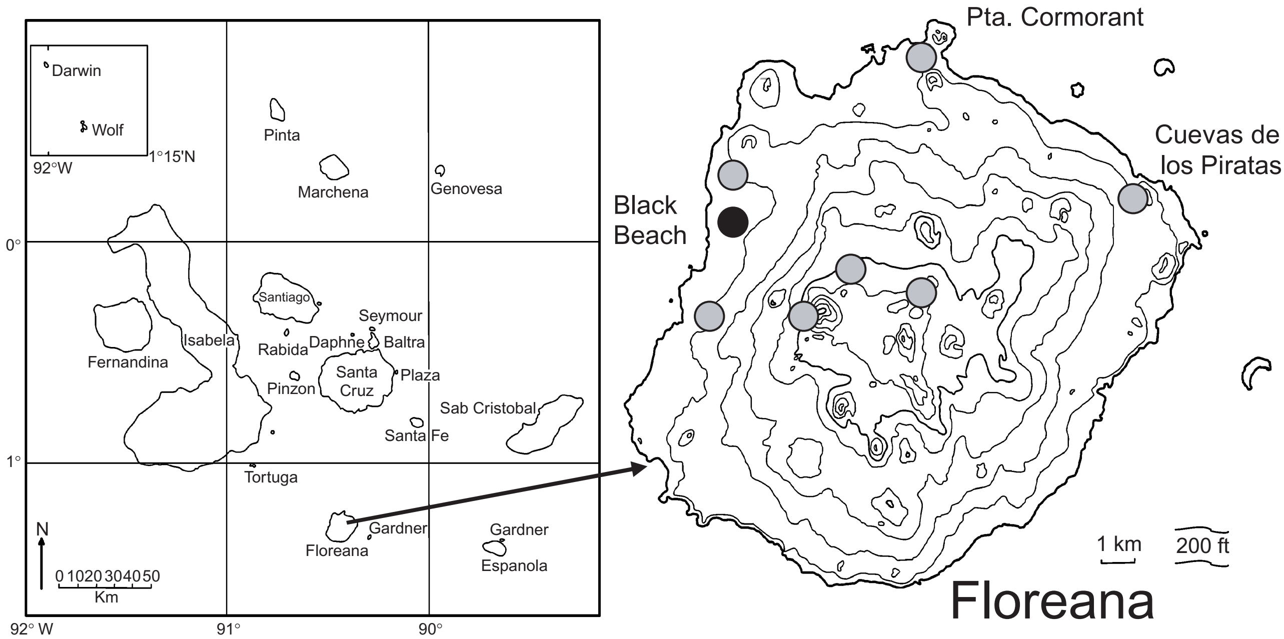

Figure 1. Distribution of *Lecocarpus pinnatifidus* in the Galápagos Islands. The population studied is indicated with a black dot, whereas other populations are shown with grey dots.

deposition on stigmas and fruit set after different types of pollinations. The aims were: (i) to see how the capitula in *L. pinnatifidus* functions; (ii) to test whether or not *L. pinnatifidus* is self-compatible like many other species on isolated islands; and (3) to examine the possibility of inbreeding in these isolated populations.

MATERIAL AND METHODS

The species

The genus *Lecocarpus* is one of seven endemic genera on the Galápagos Islands (McMullen, 1990). The genus consists of three species each endemic to one island or a group of neighbouring islets on the south-eastern part of the Galápagos Archipelago (Eliasson, 1971; Adsersen, 1980). The genus belongs to Heliantheae–Melampodiinae (Asteraceae). We worked on *L. pinnatifidus*, which is found in eight populations solely on the island of Floreana (Adsersen, 1980) (Fig. 1).

The plants are single-stemmed, evergreen shrubs *c.* 1 m high (Brok & Adsersen, in press). There is no vegetative propagation, and the individuals possess solitary, yellow capitula in the axils of leaves on most branches (Fig. 2). The capitulum of *Lecocarpus* is heteromorphic, heterogamous, and with ray and disc florets, which is a common arrangement in the Asteraceae (Mani & Saravanan, 1999). The capitula combine, however, female ray florets with tubular

Figure 2. The capitulum of *Lecocarpus pinnatifidus* with female ray florets and male disc florets. Behind the capitulum in flower there is an older capitulum with developed achenes carrying the characteristic wing.

male disc florets (Eliasson, 1971; M. Philipp, pers. observ.). This is a rare monoecious type among capitula in the group with pronounced disc and ray florets, but it is also found in genera such as *Acanthospermum*, *Tussilago* and *Calendula* (Mani & Saravanan, 1999). The capitulum is about 3 cm wide (M. Philipp, pers. observ.) and thus does not belong to the general non-showy flower appearance emphasized by McMullen (1987) to be characteristic of the Galápagos Islands. We did not find any nectar within florets. Achenes (from ray florets) are by maturity equipped with a broad peltate wing developed from the bract enclosing the achene (Adsersen, 1980) (Fig. 2) and probably facilitating dispersal by wind or surface run-off water.

Study population

We selected one main population for our studies (Fig. 1), which were undertaken early in 2001. This population of more than 1000 individuals was located on a barren lava flow on the west coast of Floreana (01°16′14.6″S, 90°29′17.9″W). This 1 km stretch of lava, which emerged from a vent at *c*. 50 m a.s.l., is fairly recent (<2000 years old?) judging from its sparse vegetation cover and low degree of weathering. Although *Lecocarpus pinnatifidus* occurred over the entire flow, we selected the section between 50 m and 200 m from the coast where the substrate is a homogeneous gravelly to stony aa-lava and the terrain is level (~10 m a.s.l.). This allows us to assume that climate and soil conditions are uniform throughout the study area. *Lecocarpus pinnatifidus* was the most prominent plant species but 18 other species were recorded at the locality at very low density. The most prominent of these species were *Walteria ovata* Cav., *Jasminocereus thouarsii* (Weber) Backsb., *Bursera gravolens* (HBK.) Trion. & Planch. and *Opuntia megasperma* Howell. Within this main population, a circular plot with a radius of 24 m enclosing a total of 65 individuals of *L. pinnatifidus* was chosen for detailed analyses of the functioning of the capitula. Mean height and mean diameter of these individuals were 42 and 44 cm, respectively. They had a mean number of flowering capitula per individual of 4.24 (0–23) on 14.i.2001 and 1.73 (0–10) on 4.ii.2001. The mean distance to nearest neighbour was 2.94 m (H. Adsersen, pers. observ.). Individuals used for observations of insect activity and experiments with ray florets were chosen from outside this focal area.

Morphology of capitula

In order to study the variation in number of ray florets among individuals and its influence on fruit set, we counted well-developed and aborted achenes in up to 20 capitula on each of the 50 flowering plants within the circular area. These counts also gave data on the fruit set variation within the population. Disc florets were counted from each of 29 of the plants in up to five capitula on each.

Phenology

The timing of female and male function of the capitula was followed in 19 capitula on 12 plants. The capitula were marked when they were about to open with all florets still in bud. The status of the rays as well as the number of open disc flowers was noted every day from opening to withering of the florets.

Pollination

Information on pollinator activity was obtained by observing visits to individuals of *L. pinnatifidus* during periods of 15 min per day between 11.00 and 14.00 hours. We observed the same three individuals from a distance of a few metres each day and counted the number of open capitula on the observed plant each time of observation and noted the number of pollinators during each census. As larger pollinators turned out to be rare, we also counted the number of small insects in a number of capitula. These insects could not be observed during the routine 15-min observations as the capitula had to be observed at a much smaller distance. The observation period for small insects was 30 s per capitulum. The influence of the ray florets on attracting pollinators measured by fruit set was investigated by removing the elongated part of the corollas on every other ray floret within a capitulum using scissors, leaving the corolla tubes with the styles intact. This was carried out on a number of pairs of capitula, where the second capitulum in the pair functioned as a control.

Pollen deposition

In order to record the pollen deposition during the course of a day on stigmas in capitula in female stage, we bagged capitula until they were in the female stage. In the morning the bags were removed, and one or two styles were immediately fixed in 50% ethanol. The capitula were left open, and every second hour styles were fixed, and if sufficient styles were present, one was fixed in the afternoon the day after, and also, if possible, the day after that. Later the stigmas were placed in a drop of preheated glycerol gelatine and the number of pollen grains counted using a microscope.

In order to observe the influence of self and foreign pollen on withering of the styles we also used bagged non-flowering capitula. On the first day of anthesis capitula were unbagged and divided into two using string. In six such capitula stigmas were cross-pollinated on one half and not treated on the other half. Three capitula were self-pollinated on one half and not treated on the other. Two were self-pollinated on one side and cross-pollinated on the other.

To quantify the number of pollen grains on stigmas exposed for open pollination, we counted pollen grains on stigmas harvested in capitula exposed for pollination two to three days. On these stigmas we also recorded the presence of pollen from other species.

Controlled pollinations

We studied the self-incompatibility and the capability of spontaneous self-pollination in *L. pinnatifidus* by means of different pollination treatments. Eleven individuals were selected for these experiments and a total of 147 capitula were bagged while all florets were in bud. On each individual four different treatments were performed: (1) spontaneous self-pollination (bagged only); (2) hand self-pollination (bagged and selfed by hand); (3) hand cross-pollination (bagged and outcrossed by hand); and (4) controls (not treated at all). The cross-pollinations were performed in a reciprocal crossing design. Out of the 110 possible crossings, only 40 were realized owing to time restraints and too few available capitula. Pollinations by hand were performed by picking from one bagged capitulum a few male flowers on which plenty of pollen could be seen. The pollen grains were transferred to the stigmas. We checked the success of the pollination using a magnifying glass (×10). The pollination was repeated on successive days, using pollen from the capitulum used for the first pollination, until the styles withered. The capitula (pollinated as well as controls) were kept bagged after anthesis and collected two months later, and the result of the treatments were based on fruit set. Mature and fully developed achenes were easily recognized by a well-developed wing and a hard, brown fruit. We investigated the probability of agamospermy by emasculating and bagging young capitula on cultivated plants grown in a greenhouse in Copenhagen.

RESULTS

Morphology of capitula

Capitula possess 9.2 (SD = 0.67, $N = 736$) ray florets, and 43.4 (SD = 5.88, $N = 130$) disc florets. The range is from 7 to 11 and from 30 to 57 for ray and disc florets, respectively. The 50 individuals for which ray and disc florets were counted, differ significantly in their number of ray and disc florets (Kruskal–Wallis, $P < 0.001$). The number of ray and disc florets were positively correlated (Spearman rank correlation, $r = 0.488$, $P = 0.008$, $N = 29$). No correlation was found between abortion rate and number of either ray or disc florets (Spearman rank correlation $r = -0.1$, $P = 0.61$ and $r = 0.15$, $P = 0.43$, respectively).

Phenology of capitula

The florets of each capitulum opened centripetally. On the first day of anthesis of the capitula, the ray florets

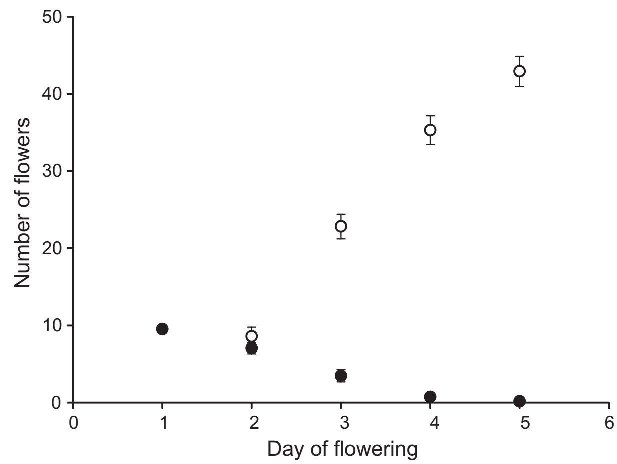

Figure 3. Phenology of 19 capitula in *Lecocarpus pinnatifidus*: ● number of fresh styles in ray florets with SE bars, ○ cumulative number of open disc florets with SE bars.

opened and the stigmas were exposed. The capitulum is in a female stage. By the second day of anthesis some of the styles had withered and the outer ring of male flowers had opened (Figs 2, 3). The third day the second ring of male flowers came out and the fourth or fifth day all male flowers had opened (Fig. 3). On average, the anthesis period of the capitulum was 5.2 days (SD = 1.17). On the sixth day the capitulum looked senescent and the ray florets were withered or fallen off; fruit development could be observed by the seventh day. The average period from exposed stigmas to the observed development of fruits was 7.2 days (SD = 0.67).

Pollination

Capitula were visited by large (> 1 cm) and small insects (< 1 cm). The larger insects were mainly the endemic carpenter bee (*Xylocopa darwini* Cockerell) and syrphid flies (Syrphidae). We observed in total 97 capitula for 240 min and saw five visits from the carpenter bee (all females) and six from syrphid flies. This resulted in a frequency of 0.03 visits per capitula per hour. With a flowering period per capitulum of about five days corresponding to about 60 h of daylight, this corresponded to *c.* 1.8 visits per capitulum during the flowering period. Some of the visits were, however, very short and presumably only marginally effective for pollination. Notwithstanding the few visits noted to focal capitula, carpenter bees were often seen passing our plant population.

Small insects were found in 15% of the capitula (Table 1). In young or female capitula we did not observe any of these small insects, but in capitula with male florets in anthesis 21% of the capitula were vis-

Table 1. Visits by small (< 1 cm) insects in capitula of *Lecocarpus pinnatifidus*. Each capitulum was observed for 30 s

Stage of capitulum	Number of capitula observed	Proportion of capitula visited	Number of capitula visited (average number of insects per capitulum)	
			By flies	By ants
Female	105	0.00	0	0
Hermaphrodite	155	0.25	21 (1.7)	17 (2.3)
Male	150	0.18	14 (1.1)	13 (2.0)
Total	410	0.15	35 (1.5)	30 (2.2)

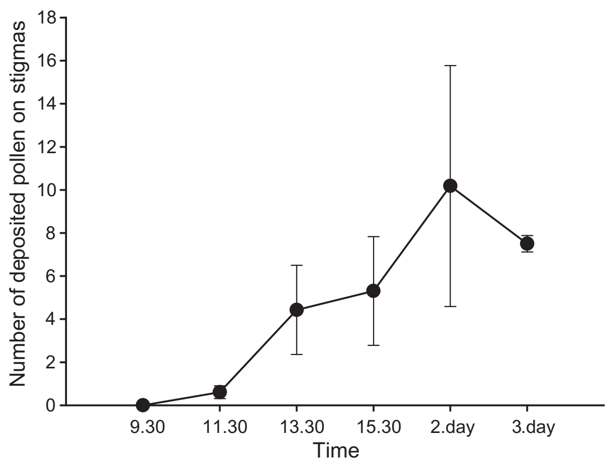

Figure 4. Cumulative deposition of pollen grains per style (± SE) of *Lecocarpus pinnatifidus* during the female stage (1st day) of the capitula (09.30–15.30 hours) and on the 2nd and 3rd day of flowering. The number of stigmas recorded is: day 1: 09.30, 17; 11.30, 28; 13.30, 28; 15.30, 26; day 2, 11; day 3, 2 (sampled from 15 capitula).

ited. The capitula inhabited by either flies or ants were about equal in number, but the amount of ants observed outnumbered the flies as ants tended to be found in higher numbers per capitulum.

We removed the elongated part of the corollas on half of the ray florets in 36 capitula and had 41 as controls. The treatment did not, however, influence the fruit set in ray florets (Mann–Whitney, $P = 0.547$).

Pollen deposition

Stigmas received an increasing amount of pollen during the first day of anthesis of the capitulum (Fig. 4). On the second day, the amount of deposited pollen increased further but on the third day the number of pollen grains per stigma decreased. On this day only two stigmas were harvested and this could be the reason for the unexpectedly low value. The number of stigmas without any pollen grains, however, was high

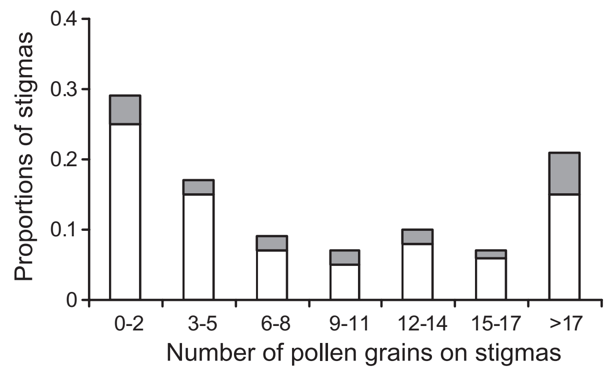

Figure 5. Number of pollen grains deposited on stigmas harvested from well-exposed (2–3-day-old) capitula in *Lecocarpus pinnatifidus*. The grey parts of the columns indicate the proportions of stigmas also carrying pollen from other plant species. $N = 100$.

at the end of days one (73%) and two (27%). On the third day, pollen grains were found on both the stigmas. The distribution of pollen grains on stigmas was very uneven. On day one most stigmas received no pollen grains, whereas a few received a lot with a maximum of 61 pollen grains.

The number of pollen grains counted on stigmas exposed to natural pollination for two to three days showed great variation, ranging from 0 to 116 pollen grains per stigma, average 12.7 (SD = 18.1). Sixteen percent of these stigmas carried no pollen grains (Fig. 5). A further 19% of the stigmas carried pollen from other plant species, and these were distributed nearly evenly among stigmas with few to many conspecific pollen grains.

Controlled pollination

Emasculated capitula on individuals in cultivation did not set any achenes (seven capitula) indicating that agamospermy had not occurred in *L. pinnatifidus*. The fractions of fruit set after the four different pollination treatments (in the field) were high. The open-pollinated group (controls) had the lowest fruit set rate of 0.56 (SD = 0.30, $N = 22$). The fruit set rate in hand cross-pollinated capitula was 0.74 (SD = 0.24, $N = 47$), in hand self-pollinated capitula 0.68 (SD = 0.23, $N = 24$), and in spontaneously self-pollinated capitula 0.61 (SD = 0.25, $N = 76$). The treatment classes are significantly different (Kruskal–Wallis, $P = 0.012$). The fruit set after controlled cross-pollinations was significantly different from the open-pollinated and the spontaneously selfed capitula (multiple comparison procedure by Dunn's method, $P < 0.05$).

The counts of mature fruits in up to 20 capitula per individual within our study area showed that fruit set varied significantly among individuals (Kruskal–Wallis, $P < 0.001$). The average fruit set rate was 0.66 (SD = 0.17) in those capitula which flowered before our study period. This value was not significantly different from the fruit set values in the above-mentioned controlled pollination experiments (Kruskal–Wallis, $p = 0.011$, multiple comparison procedure by Dunn's method, $P < 0.05$, the above-mentioned significant differences among some treatments were still valid).

The ability to self-pollinate was also demonstrated by the experiments where we pollinated the two halves of the ray florets differently. When we self-pollinated half a capitulum and cross-pollinated the other half, the result was that all the pollinated styles were withered by the day after. If one half were either self- or cross-pollinated, and the other half were non-pollinated, the non-pollinated styles were unaffected while the pollinated ones were withered by the next day.

DISCUSSION

The present study has revealed that the capitula of *L. pinnatifidus* were in flower on average for five days; only female florets were open on the first day, and both male and female florets on the remaining days. The capitula were mainly pollinated by insects smaller than 1 cm during our period of study, even though *Xylocopa darwini* was frequently seen in the area. *Lecocarpus pinnatifidus* turned out to be self-compatible and seeds set after self- and cross-pollinations were of equal size but greater than after open pollination. In the following we will discus the reproductive ecology of the *L. pinnatifidus* capitulum in relation to what is known about other species of Asteraceae and, furthermore, discuss the possibility of inbreeding in the studied population.

Reproductive ecology

The function of any plant mating system is influenced by a number of features among which we studied pollinator activity, pollen deposition on stigmas and fruit set after different types of pollinations.

Pollinator activity

McMullen (1989) states that the endemic carpenter bee (*Xylocopa darwini*), which is the only bee occurring in the archipelago, is responsible for the majority of insect pollinations of the Galápagos flowers. Visits from *X. darwini* as well as from syrphid species was, however, rarely seen in *L. pinnatifidus* during the study period. These insect species preferred other species at the locality such as *Waltheria ovata* even though *L. pinnatifidus* was the dominant species covering the study area. This observation was confirmed by our finding that 19% of the recorded stigmas exposed for pollination two to three days were carrying pollen from other species. On average, a capitulum got 1.8 visits from these insects during the entire 5-day flowering period of the capitulum. We observed the pollinator activity around noon, but from Figure 4 it can be seen that the optimal time for deposition was after noon. Our estimated pollinator activity could therefore be lower than in reality. That the frequency of visits by the carpenter bees to capitula of *L. pinnatifidus* was generally low is supported by the fact that *L. pinnatifidus* is not mentioned in the otherwise comprehensive list of plant species observed being visited by this species in Linsley, Rick & Stephens (1966). The low visitation rate of *L. pinnatifidus* by the carpenter bee was in contrast to the much higher number of visits in the two endemic Galapágos species of *Scalesia*. *Scalesia divisa* received 41 and *S. affinis* 84 visits per capitulum from *X. darwini* during their flowering period (Nielsen *et al.*, 2000 and Nielsen, Philipp & Siegismund, 2002, respectively).

Small flies and ants were much more frequently seen in the capitula (Table 1). These small insects were not found in young capitula at the purely female stage. This could be because it takes a while until the small insects find the newly opened capitula or because these visitors were interested in pollen. As the small flies move almost constantly, we suggest that the pollen reward was the decisive factor for the distribution of small flies and ants. Furthermore, small insects mostly move short distances compared with, for instance, *X. darwini*. The small insects seemed to be the main pollinators during our study period for *L. pinnatifidus*, and their contribution to the pollination of the female florets would probably predominantly be self-pollination, whereas *X. darwini* and the syrphid flies provide the possibility of cross-pollination.

The function of ray florets is to attract pollinators (Mani & Saravanan, 1999). Capitula of *L. pinnatifidus* have on average 9.2 ray florets (the modal value is 9) and 43.4 disc florets (modal value 48), which in total is 52.6 florets. Neither of these figures matched the expected Fibonacci numbers of 8 ray florets and 55 florets in total (Battjes & Bachmann, 1996). The significant differences found in number of ray florets among individual plants at the studied locality was probably the result of genetic variation and not to environmental differences because the studied plants were found in a rather homogeneous environment over an area of 1810 m^2. The limited influence of environment on the variation in number of ray florets was determined in *Senecio vulgaris* (Abbott & Schmitt, 1985). In the present study the experimental variation of the number of ray florets did not influence the resulting number of mature achenes in the capitula. In a study on the self-incompatible species *Centaurea nigra*, capitula where the outer larger florets were totally removed received significantly fewer visits than intact capitula (Lack, 1982). This was also the case in a study on the self-incompatible *Helianthus grosseserratus*, where rayless capitula set about 20% fewer fruit than the rayed capitula when rayed and unrayed capitula were mixed in the population (Stuessy, Spooner & Evans, 1986). In *Scalesia affinis* (with ray florets) and *S. pedunculata* (without ray florets) experiments in which rays were removed and fake rays added to capitula showed that rays florets were important for pollinator attraction and pollen deposition in areas with low pollinator density (Nielsen *et al*., 2002). In *L. pinnatifidus* we do not know how pollen grains on stigmas were distributed between self pollen and pollen from another individual; hence, the importance of the ray florets for cross-pollination remains unknown.

Pollen deposition

In *L. pinnatifidus* the only open ray florets during the first day of anthesis of a capitulum are female owing to the centripetal development of the capitula, as in most Asteraceae (Heywood, 1978). Thus, on their first day, as the only pollen available to them is from outside, there is a possibility of outcrossing for this self-compatible species (Abbott & Schmitt, 1985). We found no small insects in young, first-day capitula. This means that syrphid flies and carpenter bees were responsible for any pollen deposition on the first day of opening. The counts of pollen grains on the stigmas of bagged and afterwards naturally pollinated flowers on the first day of anthesis of the capitulum (Fig. 4) gave a rather uneven distribution of pollen on stigmas, with an average of 15 pollen grains per stigma (range 0–61). Furthermore, these counts showed that only 27% of the stigmas received pollen grains by the end of this day. Similarly, the counts of pollen after 2–3 days of exposed stigmas also revealed a large variation in the number of deposited pollen among the stigmas (Fig. 5) and that 16% of the stigmas were without pollen grains. These results correspond nicely with our observations that the large insect pollinators did not stay in the capitula for long or visit the florets thoroughly; they probably contact only a few stigmas before leaving. That only 19% of the stigmas possessed pollen from other species also emphasizes the irregular deposition of pollen grains. Later during anthesis of the capitulum, small insects might, likewise, touch only a few stigmas. The behaviour of the insects contributed to an unequal distribution of pollen among the stigmas in the capitulum.

FRUIT SET AFTER DIFFERENT TYPES OF POLLINATION

We concluded from our greenhouse experiments that *L. pinnatifidus* is not agamospermous. We could not, however, exclude the possibility of pseudogamous agamospermy.

Self-incompatibility is common in Asteraceae (Richards, 1986) and several species show an incomplete self-incompatibility system (Reinartz & Les, 1994; Lane, 1996; Luijten *et al*., 1996; Cheptou *et al*., 2002; Young *et al*., 2002; Nielsen *et al*., 2003). *Lecocarpus pinnatifidus* produced seeds from all capitula after all types of pollinations and no signs of an incompatibility system were revealed. A low level of self-compatibility was found in another endemic genus *Scalesia* within Asteraceae (Nielsen *et al*., 2000, 2002). Self-compatibility and seed set after self-pollination is generally found on islands and is in particular found in most (76%) of the species investigated in the Galápagos Islands (McMullen, 1987). We can thus add *L. pinnatifidus* to the list of species having the common reproductive strategy at the Galápagos Islands.

The low visitation rate of larger insects (1.8 visits per capitulum during its flowering time) combined with a significantly lower fruit set in open-pollinated capitula compared to artificially outcrossed capitula could suggest pollen limitation on fruit set during this part of the flowering period of *L. pinnatifidus*. On the other hand, counts of 20 capitula on each of 50 individuals indicated equal success in fruit set of capitula that had been open-pollinated and artificially outcrossed capitula in a previous period. The association between pollen limitation on fruit set and other life history and ecological traits were studied in 224 flowering plants (Larson & Barrett, 2000). Pollen limitation was found to be associated with, among other traits, self-incompatible, woody, nectarless plants in tropical areas. The most important of these traits was self-incompatibility. *Lecocarpus pinnatifidus* is, however, self-compatible and the two above-mentioned somehow contradictory results could, perhaps, reflect different levels of pollen deposition due to different pollinator behaviour in different times of the year.

The average abortion rate in open-pollinated (control) capitula of *L. pinnatifidus* during our study period was 0.44 and Figure 5 shows that 44% of the stigmas received five or fewer pollen grains. An abortion rate of 34% in the capitula flowering earlier corresponds similarly to three or fewer pollen grains per stigma. Lane (1996) mentions that multiple pollen grains are necessary for fertilization in some Asteraceae. This suggests that even though there is only one ovule per floret, more than two to five grains are needed to ensure fertilization. The necessity of multiple pollen grains for fertilization could be the consequence of selection against self pollen or that several pollen grains per stigma are needed for a pollen–pistil interaction to be established (Stephenson *et al.*, 1995).

A morphological investigation of *L. pinnatifidus* revealed little differentiation among populations on Floreana (Brok & Adsersen, in press). The reason for this limited diversification could be that the populations are not completely isolated from each other owing to gene flow among populations. The heavy achenes possess adaptations for wind dispersal, but most often piles of achenes were found below individuals. This means that dispersal by achenes in most cases is close to the parent plant (Eliasson, 1971) and probably does not contribute to the gene flow amongst populations. Pollen dispersal by *X. darwini* may be considerable as these bees fly long distances (H. R. Siegismund, pers. observ.). Even though the carpenter bee visited the capitula of *L. pinnatifidus* rarely during our study period, its role in the genetic communication among populations on Floreana could be considered an important reason for the low level of differentiation within the species.

Variation in fruit set among individuals

The large and significant variation in average fruit set among the individuals could be the consequence of differences in resources, gender or because of inbreeding.

Concerning differences in resources, we suggest this to be unlikely because the individuals were growing quite close to each other and the substrate and climatic conditions probably were the same throughout the locality. A difference in seed set owing to resource availability therefore appears unconvincing.

The sexual reproduction in most flowering plants is functionally hermaphroditic, but there are often variations among individuals in their relative contribution in male and female function (Lloyd, 1980). The high variation in fruit set in *L. pinnatifidus* in open-pollinated capitula could, perhaps, be influenced by differences in functional gender among the studied individuals. We performed the present crossing experiments such that seven individuals participated both as males and females. The resultant fruit set can be used to evaluate the ability of these individuals as female as well as male in respect to fecundity (Table 2 and Fig. 6). We found no trade-off between the female and male function. However, within these seven indi-

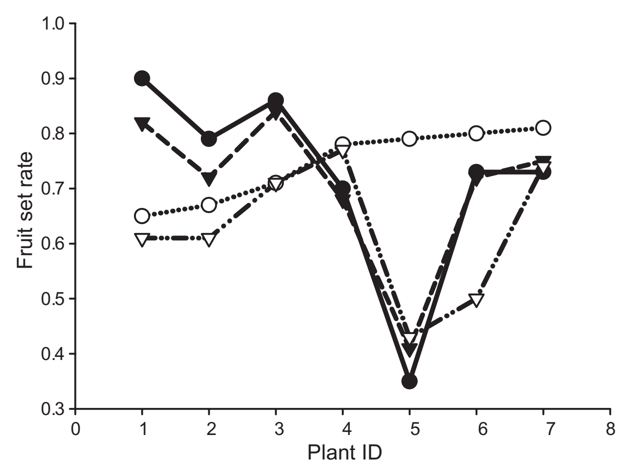

Figure 6. Fruit set in seven individuals of *Lecocarpus pinnatifidus* either as females after cross-pollination by hand (●), as females after open pollination (▼), as females after selfing (▽) or when used as males (○).

Table 2. Fruit set from the pollination experiment in *Lecocarpus pinnatifidus*. Average fruit set when a plant was functioning as female after different treatments or as male. Numbers in parentheses, SE; numbers in italics, number of capitula. Hand- and spontaneous-selfed capitula are pooled in the self-category

Plant ID	Female (cross-pollinated[1])	Female (open-pollinated)	Female (self-pollinated)	Male
1	0.90 (0.03) *4*	0.82 (0.04) *20*	0.61 (0.16) *3*	0.65 (0.10) 6
2	0.79 (0.06) *4*	0.72 (0.05) *20*	0.61 (0.14) *3*	0.67 (0.13) *6*
3	0.86 (0.05) *8*	0.84 (0.06) *20*	0.71 (0.14) *4*	0.71 (0.15) *4*
4	0.35 (0.10) *5*	0.41 (0.04) *20*	0.44 (0.07) *9*	0.77 (0.02) *3*
5	0.70 (0.16) *2*	0.68 (0.09) *9*	0.77 (–) *1*	0.78 (0.07) *4*
6	0.73 (0.05) *4*	0.72 (0.04) *20*	0.50 (0.07) *4*	0.80 (–) *1*
7	0.73 (0.11) *2*	0.75 (0.06) *20*	0.74 (0.08) *6*	0.81 (0.13) *5*

[1]Performed experimentally by hand.

viduals the fruit set varied twice as much when calculated for females than for males.

In the present study we found that average fruit set after selfing is as high as after outcrossing. During our study period, small insects probably were the main pollinators and mostly caused inbreeding through small-scale movements. The fruit set of the seven individuals after selfing (Fig. 6) showed that some of the individuals possessed the same seed set after self-pollination as after cross-pollination and open-pollination, whereas others had a lower fruit set after self-pollination. The high fruit set found after self-pollination could be interpreted as the consequence of inbreeding in the population (either through self-pollination or biparental inbreeding due to the putative short seed dispersal distance) over a long period of time, purging the deleterious alleles. The low fruit set cases might show that the period had not been long enough for a total purging of deleterious alleles to be fulfilled (Schemske & Lande, 1985). The variation in fruit set among individuals may thus not be because of differences in resources or gender, but rather an expression of inbreeding depression in some individuals.

Individuals could thus have different fecundity as a result of inbreeding. Recent investigations have inferred that offspring from inbreeding events primarily show their lower fitness through their female function rather than male function (L. B. Hansen, pers. comm.), which is in agreement with the above-mentioned larger variation in fruit set when the plants were functioning as females than when functioning as males and similar to the findings of Schlichting & Devlin, 1989.

The present study provides an example of the consequences of being an isolated population. The type of capitulum of *L. pinnatifidus* seems rather specialized, as male disc florets are rare in Asteraceae as a whole, but the capitula are visited by generalist pollinators. The fruit set is, however, during some periods limited by the low frequency of pollinations. The population possesses the prerequisites for having a mixed mating system and from the scarcity of pollinators and the size of the fruit set it can be suggested that self-pollinations can play an important role.

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