



Reproductive ecology of *Scalesia cordata* (Asteraceae), an endangered species from the Galápagos Islands

MARIANNE PHILIPP^{1*} and LENE ROSTGAARD NIELSEN²

¹*Department of Biology, University of Copenhagen, Universitetsparken 15, DK-2100 Copenhagen Ø, Denmark*

²*Forest & Landscape Denmark, Faculty of Life Sciences, University of Copenhagen, Hørsholm Kongevej 11, DK-2970 Hørsholm, Denmark*

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The genus *Scalesia* is endemic to the Galápagos Islands. *Scalesia cordata* is a tree occurring only in the southern part of Isabela as small, remnant populations of larger forests. We studied the reproductive ecology of a population protected in an enclosure in order to reveal the extent to which the reproductive system limits the recruitment of young individuals. Pollinator observations revealed that each capitulum received, on average, 2.5 visits per hour and exposed receptive stigmas received many pollen grains. Even so, the seed set and recruitment of trees was poor. Crossing experiments showed that *S. cordata* produced about twice as many fertile seeds after outcrossing than after selfing. In addition, self-pollinated capitula flowered for a longer time than outcrossed capitula (3.8 ± 1.1 days vs. 2.1 ± 0.8 days). These results may suggest that the species is partly self-incompatible, as other species of the genus. The small population size, with its potential derived consequences, is most certainly a prominent threat to *S. cordata*. The pressure from introduced plant species found in the enclosure also contributes to the seemingly poor condition of the population. To recover *S. cordata*, we suggest enlarging the enclosure, removing the introduced plant species and continuing to cultivate plants as a genetic reserve. Introducing new genetic material is likely to increase the seed set of the population. © 2010 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2010, **162**, 496–503.

ADDITIONAL KEYWORDS: pollination experiments – seed set – threatened species.

INTRODUCTION

Small populations are at high risk of extinction as a result of stochastic events of both demographic and genetic nature (Amos & Balmford, 2001; Pullin, 2002). Populations decline in size for several reasons when fragmentation caused by human activities is very common. The genetic diversity of the fragments depends on their size, the amount of gene flow among fragments and the reproductive system of the species. To ensure the best management of small populations, a knowledge of the reproductive system is crucial.

When species become fragmented, selfings or matings among related individuals will increase if the effective number of parents is low (Hamrick, 2004). As

a result, inbreeding depression may occur, especially if outcrossing has been the dominant mating pattern (Barrett & Kohn, 1991; Hedrick & Kalinowski, 2000; Hedrick, 2004). Another related effect of fragmentation in combination with a self-incompatibility system is the possible lack of suitable mates. As most plant species do not disperse their seeds widely, nearby individuals may be close relatives. In recently formed fragments, many individuals may thus be relatives and compatible mates are potentially rare. The effect of this will be a poor and uneven seed set. Such an imbalance in offspring production among individuals is one of several important factors that may reduce the effective size of a population. The effective number of individuals in a given population will be influenced negatively if many members of a population do not contribute to the gamete pool as a result

*Corresponding author. E-mail: Marianp@bio.ku.dk

of compatibility problems or if few members have a high productivity. In disturbed populations, forces such as genetic drift and inbreeding depression may be significantly stronger than first assumed when based on counts of the actual population size. Over time, extinction of the fragmented population is a possibility, enforced by the potentially lowered seed set. Another potential outcome, however, is that inbreeding depression becomes insignificant because of purging of the genetic load (Amos & Balmford, 2001).

The species subject of the present study belongs to the family Asteraceae and is endemic to the Galápagos Islands. Species of Asteraceae are often self-incompatible with a homomorphic sporophytic self-incompatibility system (Young *et al.*, 2002). On islands, however, some degree of self-compatibility is expected as this greatly increases the probability of setting seed during the establishment phase (Baker, 1955). After establishment, populations may increase in size and mechanisms to promote outcrossing may develop.

On the Galápagos Islands, most species possess a mixed mating reproductive system (McMullen, 1987, 2009; Philipp *et al.*, 2004). They are able to set seed after selfing, but pollinators are often necessary to transfer pollen from anthers to stigmas. *Scalesia cordata* Stewart is one of 15 species belonging to the genus *Scalesia* Arn. (Asteraceae), which is endemic to the archipelago (Eliasson, 1974; Lawesson, Adersen & Bentley, 1987). We have studied other species of *Scalesia*, and they possess an incomplete self-incompatibility system, allowing some individuals to set seed at diverging numbers after selfing. However, production of viable seed is, in most cases, much lower after selfing than after outcrossing, and the resulting progeny show significant signs of inbreeding depression for fitness-related characters (Nielsen, Siegismund & Hansen, 2007).

Some *Scalesia* spp. are found on several islands, whereas others are found on only one. Several of the species, with rather limited distributions, are categorized as threatened. The threats are a result, in particular, of habitat loss from human activities: the release of goats and pigs on inhabited islands, farming and the introduction of exotic plant species. Fragmentation and the isolation of these fragmented populations have been the fate of some species. Among these is *S. cordata*, which is categorized as endangered (Tye & Loving, 1998). By following permanent plots for 10–12 years on six *S. cordata* sites, it was found that the establishment of new seedlings occurred most frequently after disturbances such as fires or El Niño events (Vries & Tupiza, 1990). It was also found that trees could be 40–60 years old judging from their diameter. Many

trees died within 10 years following germination, often as a result of an El Niño event or severe drought (Vries & Tupiza, 1990). *Scalesia cordata* probably occurred in dense populations in the humid zone of the volcanos Sierra Negra and Cerro Azul on South Isabela, but these areas have been severely transformed by the creation of an agricultural zone, fires and the introduction of animals and invasive plants. One of the largest natural stands of *S. cordata* in the agricultural zone is now in an enclosure (Esperanza) at an altitude of 433 m close to the village of Santo Tomas, 20 km from the coastal town of Villamil on Isabela island. The future of this species is highly dependent on its reproductive success, and the background for this study is to explore the reproductive status of the species.

MATERIAL AND METHODS

THE STUDY SPECIES

Scalesia cordata is a tree which can grow up to 10 m in height. The leaves are crenate, ovate with a cordate base. At the end of the branches, several capitula are found together, each with a long peduncle forming a secondary corymbiform inflorescence (Fig. 1) (Wiggins & Porter, 1971). The capitula are small compared with the capitula of other species of *Scalesia*. Each floret develops into a fruit including one seed (an achene).

THE SITE

The enclosure (Esperanza, 0°51'S, 91°1'W) comprises 168 individuals, all more than 2 m in height (J. Gordillo, Charles Darwin Foundation, Isabela Island pers. com.). We saw a few smaller individuals all in one corner of the enclosure. The adult individuals are the remnants of a much larger *Scalesia* forest. The enclosure holds several introduced species, including *Psidium guajava* L., *Cedrela odorata* L., *Ricinus communis* L., *Passiflora edulis* Sims., *Ipomoea alba* L. and *Delonix regia* (Hook.) Raf. We studied the reproductive system from 29 January to 8 February, 2004.

REPRODUCTIVE SYSTEM ANALYSES

In order to determine the phenology of *S. cordata* and its reproductive success after different crossing treatments, we selected six trees with capitula at a reachable height from a movable platform (height, 2 m). The trees were positioned along different parts of the fence around the enclosure and thus were not neighbouring individuals. For phenological studies, we marked a total of 24 capitula at the bud stage on seven trees. The number of open florets was counted and their sexual status was noted each day. These 24



Figure 1. Branch of *Scalesia cordata* with capitula in anthesis and in bud stage.

capitula were also used as open-pollinated controls. We bagged 90 capitula. Some of these were not treated but left to self-pollinate [passive selfings (19)], whereas others were later either actively self-pollinated (37) or outcrossed (34). As a result of the structure of the florets, emasculation was not possible. We were, however, careful when handling the capitula so as not to transfer pollen between florets within the capitulum. We have experienced from earlier studies of other *Scalesia* spp. that empty achenes often look like achenes containing embryos. The achenes resulting from the different treatments were therefore placed on adhesive transparent plastic and X-rayed with an Xylon, smart 225 apparatus at 35 kW, 2 mA and 4 s exposure time. AGFA Structurise, D4 FW, Industrial X-ray film was used for processing X-ray photographs. From the X-ray photographs, we scored the number of seeds containing embryos.

POLLINATOR OBSERVATIONS

Insects visiting the capitula were observed twice a day during the study period. We used observation periods of 20 min and observed an average of 7.6 capitula (range, 2–12) during each observation period.

POLLEN DEPOSITION ON STIGMAS

To determine whether exposed stigmas received conspecific pollen, we bagged 39 capitula until a fraction of the florets were in the female stage. The bag was removed and one or two stigmas per capitulum were fixed in 50% ethanol hourly, starting at 09.00 h, until all florets in the female stage were collected. The number of pollen grains deposited on the stigmas was then counted under a microscope. The stigmas were placed in a drop of 50% ethanol under a cover glass and pollen attached to the edge of the stigmatic profile was counted. Thus, the resulting figure does

Table 1. Results of the pollination experiments in *Scalesia cordata*

Treatment	Florets per capitulum*	Embryo per floret	Number of capitula
Passive selfing	17.7	0.06 ± 0.09	5
Active selfing	15.9	0.04 ± 0.08	17
Active outcrossing	16.3	0.12 ± 0.13	13
Control	16.6	0.10 ± 0.15	13

*Standard deviations on 'florets per capitulum' are not given as some replicates per treatment group were pooled for practical reasons.

not give the total amount of deposited pollen grains, but rather a measure of the pollen deposition rate over time.

CAPITULA PRODUCTION

On 10 February 2004, we estimated the height and measured the diameter at breast height of 91 *Scalesia* trees in one part of the enclosure. On these trees, we estimated the number of capitula by scoring each tree in predefined classes: Class 0, no capitula; Class 1, 1–99 capitula; Class 2, 100–999 capitula; Class 3, 1000–3000 capitula.

RESULTS

NUMBER OF FLOWERS PER CAPITULUM

We recorded the number of florets in 67 capitula; they contained a mean (\pm SD) of 18.2 ± 3.6 florets. The six individuals enclosed in our pollination experiment had significantly different numbers of florets per capitulum (Kruskal–Wallis one-way ANOVA on ranks, $P = 0.038$) (Table 1).

PHENOLOGY OF CAPITULA

Each capitulum opens from the periphery and towards the centre. On the first day of flowering, the open florets were in the male stage, although rare occurrences of florets in the female stage were observed. As shown in Figure 2, the male stage of each floret lasts one day. On the next day, the former male floret will be in the female stage. Most capitula flower within 4–5 days.

POLLINATION EXPERIMENTS

In our pollination experiment, one-half of the treated capitula were lost before harvest as a result of accidental breakage of branches, eaten capitula or missing bags. The results of the X-ray treatment revealed that only a fraction of the achenes contained embryos. Forty-seven capitula were harvested and, of these, seeds containing embryos were found in 20

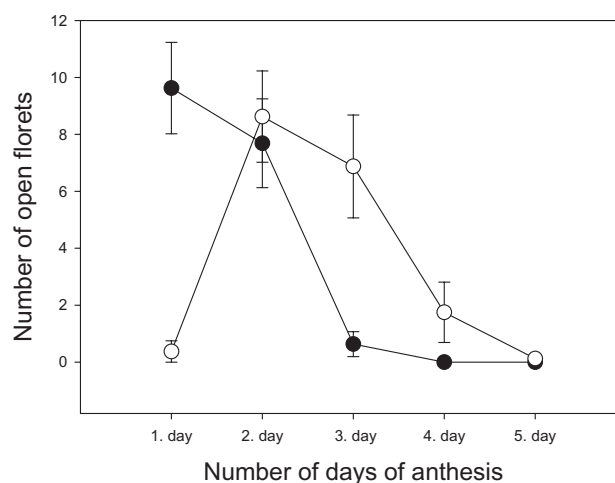


Figure 2. Flowering pattern of an average capitulum in *Scalesia cordata*. Mean number of florets open on days 1–5 of anthesis of the capitula with error bars. Filled circles, florets in male stage; open circles, florets in female stage.

capitula. Our results show that *S. cordata* is able to set on average 1.1 viable seeds per capitulum after passive and 0.6 viable seeds after active selfing (e.g. passive selfing: $17.7 \text{ florets/capitulum} \times 0.06 \text{ embryos/floret} = 1.1 \text{ embryos/capitulum}$). The seed set increases, however, after visits from pollinators, to 1.7 per capitulum in the control group and 2.0 per capitulum in the actively outcrossed group (Table 1). The differences found in the number of embryos per floret are not significant because of the small sample size and large variation. The production of twice as many seeds after outcrossing compared with after active selfing also means that an occasional contamination of otherwise outcrossed florets with self-pollen during handling of capitula would not influence our results significantly.

The flowering duration of the capitula used for the pollination experiments was recorded, and actively selfed capitula flowered for significantly longer times (mean \pm SD): 3.8 ± 1.1 days for actively selfed capitula and 2.1 ± 0.8 days for actively outcrossed capitula (t -test, $P = 0.0006$).

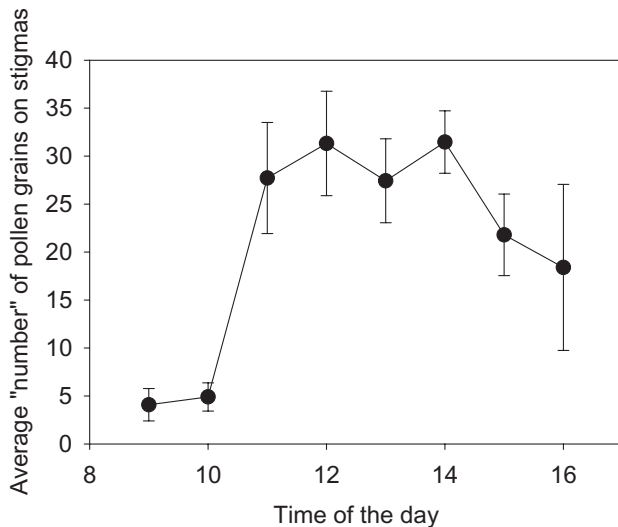


Figure 3. Pollen deposition on stigmas of *Scalesia cordata* at different times (09.00–16.00 h) of the day. The number of pollen grains is not the absolute number deposited but a standardized counting of grains along a specific part of the stigmas. Bars are standard errors.

POLLINATOR OBSERVATIONS

We observed 212 capitula for 9 h and 20 min in periods of 20 min from 09.00 h to 13.00 h. Predominantly females of the endemic carpenter bee, *Xylocopa darwini*, visited the capitula of *S. cordata* 171 times. Different butterfly species visited 19 capitula and syrphids visited four capitula during these observation periods. This gives a total of 194 visits to 212 capitula. Overall, in the 28 periods of 20 min, each of the observed capitula received a mean (\pm SD) of 0.86 ± 0.73 visits, translating to 2.52 visits per hour. *Xylocopa darwini* was responsible for 2.22 visits per hour, or 88% of all visits.

POLLEN DEPOSITION ON STIGMAS

We followed the pollen deposition on stigmas in the capitula from four individuals. In total, the pollen deposition rate increased between 10.00 h and 12.00 h. After 12.00 h, the amount of pollen on stigmas was quite constant (Fig. 3). Late in the day, fewer pollen grains were found on stigmas, a result most probably primarily caused by random variations in the amount of pollen found on stigmas combined with the small number of stigmas recorded.

CAPITULA PRODUCTION

As illustrated in Figure 4, only a small proportion of the population consisted of small trees. Only plants more than 2 m in height flowered. In each size class, some trees did not flower and, for individuals above

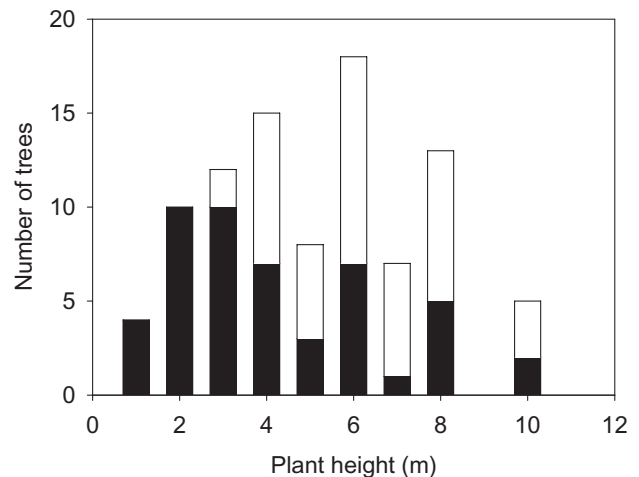


Figure 4. Demographic population structure of *Scalesia cordata*. Black parts of the columns are the nonflowering individuals, white parts are the flowering individuals.

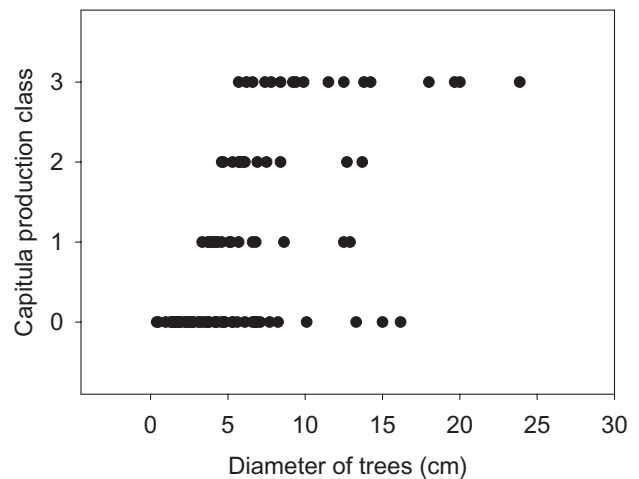


Figure 5. Relationship between diameter of the trunk at breast height and estimated capitulum production of 91 *Scalesia cordata* trees. Production scores: Class 0, no capitula; Class 1, 1–99 capitula; Class 2, 100–999 capitula; Class 3, 1000–3000 capitula.

3 m in height, the proportion of flowering individuals was quite constant, with a mean (\pm SD) of 0.64 ± 0.11 . When investigating the number of flowers produced in relation to the diameter of the trees, it was found that all trees with a large diameter (≥ 18 cm) produced many capitula (Fig. 5).

DISCUSSION

Studies of the reproductive systems of most species of flowering plants on the Galápagos Islands have revealed a mixed mating system: individuals are able to set seed after selfing but, in several cases, insect

visits are necessary for seed set or increase the seed set considerably (Rick, 1966; McMullen, 1987; Philipp *et al.*, 2006). Similar patterns are seen on other islands (e.g. Hawaii, New Zealand; Carr, Powell & Kyhos, 1986; Webb & Kelly, 1993). This apparent lack of self-incompatibility systems in the majority of the island species indicates that the chance of establishment on these remote islands is greatly increased by a capacity to set seed after selfing. Within the endemic genus *Scalesia*, we have studied previously the reproductive systems of *S. divisa* Andersson and *S. affinis* Hook.f. Genetic analysis of the mating system in *S. divisa* revealed that offspring from open-pollinated capitula were fully outcrossed even though the main pollinator (*Xylocopa darwini*) visited many capitula of the same individual (Nielsen *et al.*, 2000). Pollination experiments in *S. affinis*, similar to those in the present study, revealed a partial self-incompatibility system in which some individuals were able to set viable seeds after selfing, whereas others were not. Furthermore, access to pollinators increased the production of viable seeds considerably (Nielsen *et al.*, 2003). In the present study, we found that the seed set was much lower than in the two other *Scalesia* spp. studied, even in the outcrossed groups (open-pollinated and controlled crossings). In selfed capitula, 72% (13 of 18) had no seeds containing embryos, whereas this percentage in the open-pollinated controls plus the controlled outcrossed capitula was 30% (seven of 23). Similar to our findings in *S. affinis* (Nielsen *et al.*, 2003), the longer flowering period of selfed relative to outcrossed capitula indicates that fertilization by self-pollen is less efficient than fertilization by pollen from other individuals. Finally, the seed set is twice as high when pollen grains from other individuals are transferred to the capitula. The three *Scalesia* spp. studied by us (*S. affinis*, *S. cordata*, *S. divisa*) are all highly outcrossing, but able to self at a low rate, probably only when self-pollen alone is present. The mechanism responsible may reflect an incomplete self-incompatibility system. In addition to this, autogamy may be prevented by a combination of protandry and the centripetal maturation of the florets in the capitulum.

The frequency of insect visits to several plant species, including the two other *Scalesia* spp. mentioned above, was observed in earlier studies (Nielsen *et al.*, 2000, 2003; Philipp *et al.*, 2004, 2006). In most cases, including the present study, *Xylocopa darwini*, the only bee in the archipelago, was the dominant visitor, as also recorded by McMullen (1987). Our observations of pollinator visitations and pollen deposition rates in *S. cordata* showed that florets are well visited and, furthermore, that most stigmas hold several pollen grains. Theoretically, one successful

pollen grain is sufficient to fertilize the one ovary (containing one ovule) per floret in the capitula of *S. cordata*. However, despite the abundant pollen on the observed stigmas, the overall seed set was low in all four treatments. One explanation is that pollen grains deposited on open-pollinated stigmas are the result of geitonogamy, as flowering individuals often carry many capitula at the same stage of maturity and insect visitors were observed visiting many capitula on each tree. Alternatively, they could come from related nearby trees, as seed dispersal distances are most probably short because the achenes lack efficient dispersal equipment. In both cases, the low seed set could be the outcome of self-incompatibility and inbreeding depression. Another explanation for the low seed set is that most plants in the enclosure are related, as controlled crossings between distant individuals also gave a low seed set. This result supports the assumption of relatedness among the plants in the enclosure. Given that the species has a suggested partial self-incompatibility system, each individual may have a limited number of compatible possibilities within the pollen dispersal range in the population (Allee effect; Courchamp, Berec & Gascoigne, 2008). This could be even more pronounced, as the number of incompatibility alleles may decrease as a result of genetic drift in such a small population (Young *et al.*, 2000b; Glémin *et al.*, 2008). Being a tetraploid species, *S. cordata* may be particularly vulnerable to size reduction. Young *et al.* (2000a) found that tetraploid populations of *Rutidosia leptorhynchoidea* F.Muell. (Asteraceae) showed a 20–25% decrease in compatibility when compared with diploid populations for a given level of relatedness. When the population size is small, some tetraploid species may suffer more than diploid species from mate incompatibility. This can be a result of the potential increased probability of tetraploids sharing alleles at the *S* locus, or greater masking of alleles through dominance (Young *et al.*, 2000a; Pickup & Young, 2008).

The results of our study of the population of *S. cordata* in the enclosure show that it is, indeed, threatened. Firstly, the population is highly isolated and has been reduced markedly in size by human-induced disturbances. This reduction in size may lead to: (1) substantial genetic drift, resulting in low genetic variation; (2) low mate availability and thus decreased seed set; and/or (3) decreased fitness among offspring as a result of exposure to inbreeding depression (e.g. Amos & Balmford, 2001; Nielsen, Siegismund & Hansen, 2007). A positive relationship between population size, genetic variation and fitness was demonstrated in a meta-analysis of several plant populations. The population size was suggested to be the most important variable responsible for lower

genetic variation and lower fitness of the species (Leimu *et al.*, 2006). In the present case, we do not know the genetic status of the population in the enclosure, but genetic erosion over time is to be expected. Secondly, we detected a low production of seeds with embryos per capitulum, which is in accordance with the fact that young individuals recruited from seeds were rarely seen in the enclosure. Recruitment is probably also influenced negatively by interference with dense low vegetation in the enclosure, including many introduced species. A visit to the enclosure 3 years later clearly showed that the balance between native and introduced species had progressed in the direction of the introduced species, and the *S. cordata* individuals appeared to be less vigorous than when we collected data for the present study.

We can conclude that a small population size, the low production of viable seeds and the low frequency of young trees, combined with the possibility of low genetic variation as a result of genetic drift, make *S. cordata* extremely threatened. This is probably intensified by the interference of introduced species in the enclosure. The enclosure should, if possible, be enlarged and introduced species should be removed. Cultivation of the species (Gordillo, 2007) will provide a valuable genetic reserve, provided that the seeds are representatively collected. Introducing new genetic material from small *S. cordata* populations from other parts of Isabela island into the enclosure may increase the viability of the population by increasing the diversity of *S* alleles and, potentially, via heterosis.

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