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Breeding system of the subantarctic plant species *Pringlea antiscorbutica* R. Br. and search for potential insect pollinators in the Kerguelen Islands

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Abstract The reproductive biology of *Pringlea antiscorbutica* R. Br. (Brassicaceae), an endemic species from the southern Indian Ocean islands, is investigated here. Controlled crosses were performed between plants grown in a common garden on the Kerguelen Islands. Searching for potential insect pollinators, we investigated the flying ability of all insects known from the Kerguelen Islands. We showed evidence for self-compatibility, low pollen limitation in the absence of a biotic pollen vector and slight selfing depression in *P. antiscorbutica*. The insects present on the islands are mostly wingless and are not likely to be efficient pollinators of this species. Since *P. antiscorbutica* shows morphological adaptations to insect pollination despite the absence of pollinators in its present range, we conclude that this species recently evolved from insect pollination to wind and/or autonomous pollination.

Keywords *Pringlea antiscorbutica* · Subantarctic islands · Breeding system · Pollinators

Introduction

In the Southern Indian Ocean province, the islands host a flora that is poor in terms of numbers of species (29 native vascular plants in Kerguelen, Frenot et al. 2001), but rich in terms of endemic plants (Hennion and Walton 1997a; Chown et al. 1998). Among these endemic plants, *Pringlea antiscorbutica* R. Br. (Brassicaceae), the ''Kerguelen cabbage'', is the most famous. It has been described as phylogenetically very isolated, and defined as a monospecific genus and a monotypic tribe. Previous studies have focused on the physiological adaptation of the Kerguelen cabbage to the windy and cold subantarctic climate (e.g. Hennion and Bouchereau 1998; Aubert et al. 1999; Hummel et al. 2004a). Very little is known about its mating system, although this information would be crucial for understanding the current evolution of this species. Indeed, mating systems determine the distribution of genetic diversity within and among populations (Baker 1955; Charlesworth 2003), and influence the sensitivity of species to extinction when the population size is reduced (Husband and Schemske 1996). Populations of *P. antiscorbutica* are under several threats. Hooker (1847) who first described the plant reported it to be a widespread species, but rabbits introduced in 1874 dramatically grazed upon *P. antiscorbutica*, and restricted populations to zones inaccessible to this herbivore. Since the 1960s, the Kerguelen Islands have become even less favourable for *P. antiscorbutica*: the climate has become warmer and drier (Frenot et al. 2001), which represents an important stress factor for this species (Dorne and Bligny 1993; Hennion and Martin-Tanguy 2000; Hummel et al. 2004b). Furthermore, introduced plant species have spread more easily under these new climatic conditions, potentially subjecting *P. antiscorbutica* to increased competition (Frenot et al. 2001). In addition, newly introduced insects could modify the equilibrium of the island insect fauna, which may have important consequences for the mating system of *P. antiscorbutica* if this species can be insect-pollinated.

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The life history of *P. antiscorbutica* is partially known through Werth's early work (1911) and more recent reports by Hennion (1992) and Hennion and Walton (1997a, b). Werth (1911) concluded from his observation of floral morphology that *P. antiscorbutica* is probably a self-pollinating species: indeed, at male maturity, the dehiscent anthers are located just above the receptive stigma. However, Hennion (1992) described protogynous maturation of the flowers, and such a time-lag between female receptivity and male maturity usually promotes outcrossing. It is also unclear whether the species is wind or insect-pollinated: on the one hand, neither the stigmata nor stamens show any morphological adaptation to wind pollination, and the small nectar glands, described by Werth (1911), suggest that the plant may attract biotic pollinators. On the other hand, the species richness of the indigenous insect fauna in this subantarctic archipelago is very low (Chown et al. 1998), the functionality of nectar glands is unknown, and the flowers are often devoid of petals (Hooker 1879b; Hennion 1992). These features argue for a wind or an autonomous mode of pollination.

A shift from a cross-fertilized mating system to a self-fertilized mating system may modify dramatically the fate of a species. The evolution of selfing rate in plants has been the subject of many investigations. Two evolutionary forces could explain evolution of selfing: first, Fisher (1941) demonstrated the 50% automatic advantage of selfing. The maintenance of cross-fertilization despite this automatic advantage of selfing was then explained by the deleterious effects of inbreeding depression, i.e. the relative decrease of fitness of self vs. outcrossed individuals. Genetic models predict a dichotomous outcome of the evolution of selfing in hermaphroditic plants: either complete selfing or complete outcrossing (reviewed in Porcher and Lande 2005). However, natural populations present stable intermediate rates (Barrett 1998). One possible explanation is found when ecological factors, namely pollen limitation, and pollen and seed discounting are taken into account (Porcher and Lande 2005). More studies taking ecology into account to explain mating system evolution are needed (Kalisz and Vogler 2003). Both the distribution of autonomous selfing in nature and the ecological context favouring mixed mating demand more attention.

The present study aimed to investigate the mating system of an endemic plant experiencing extreme ecological conditions. The following questions about the breeding system of *P. antiscorbutica* were asked: (1) is *P. antiscorbutica* self-compatible, and if so, does it suffer from inbreeding depression? (2) is this species able to produce seeds without a biotic pollen vector? and (3) are there insect species likely to be efficient pollinators of *P. antiscorbutica* in the Kerguelen Islands?

We addressed these questions by performing controlled crosses on mature *P. antiscorbutica* plants grown within a common garden close to the scientific base on the Kerguelen Islands. In addition, the flying abilities of all insects observed in the Kerguelen Islands were surveyed. This is the first experimental study of the mating system of a higher plant under the harsh subantarctic climatic conditions.

Description of species

Pringlea antiscorbutica

This species is endemic to the Southern Indian Ocean province, but its origin is unknown. Recent molecular work (Warwick et al. 2002) demonstrated that its closest relatives belong to the genus Sisymbrium (New World Thelypodiae). However, generic reassignments within the New World Thelypodiae are expected to arise from further molecular studies (Al-Shehbaz et al. 2006). Hooker (1847, 1879a) and Werth (1911) first suggested that *P. antiscorbutica* may have been present on the Kerguelen Archipelago since the Tertiary, when a rich temperate flora covered it. Heard, Crozet and Marion Islands, which are more recent, could have been colonized by the plants present on Kerguelen (Moore 1972). *P. antiscorbutica* is a long-lived perennial (usually more than 7 years, Chapuis et al. 2000), and forms ramified prostrate shoots. The shoots end with large rosettes of leaves (up to 80 cm in diameter, from which 3–5 lateral inflorescences arise on plants over 3 or 4 years old (Fig. 1a). A high intra-population variability in plant size and number of flowering stalks is found (Hennion et al. 2006).

The raceme inflorescences are dense and sometimes ramified. Flowers (Fig. 1b) have short peduncles, a calyx of four green, hairy sepals approximately 6 mm long, but the corollas, formed by four small petals, are often incomplete or missing, even in buds (Hooker 1879a). The androecia include six stamens, 1/3 longer than the pistil when mature, with 8 mm long filaments and 2 mm long anthers. The ovaries are oblong and hairy, the styles are short and the stigmata are large and round-shape with many papillae (Hennion 1992). In young flowers, the stamens are shorter than the ovaries, and the stigmata are receptive before the stamens start growing. At male maturity, the anthers are located above the receptive stigmata. Ripe fruits (siliquae) are dry and dehiscent. Seeds have no dormancy and were described by Hennion and Walton (1997a): they are approximately 4 mm long and are covered by specialized cells, which become a mucilaginous sheath in the presence of water, allowing the seeds to float in both fresh and seawater. Successful seed dispersal was observed in freshwater by Hennion (1992) and in seawater by Chapuis et al. (2004).
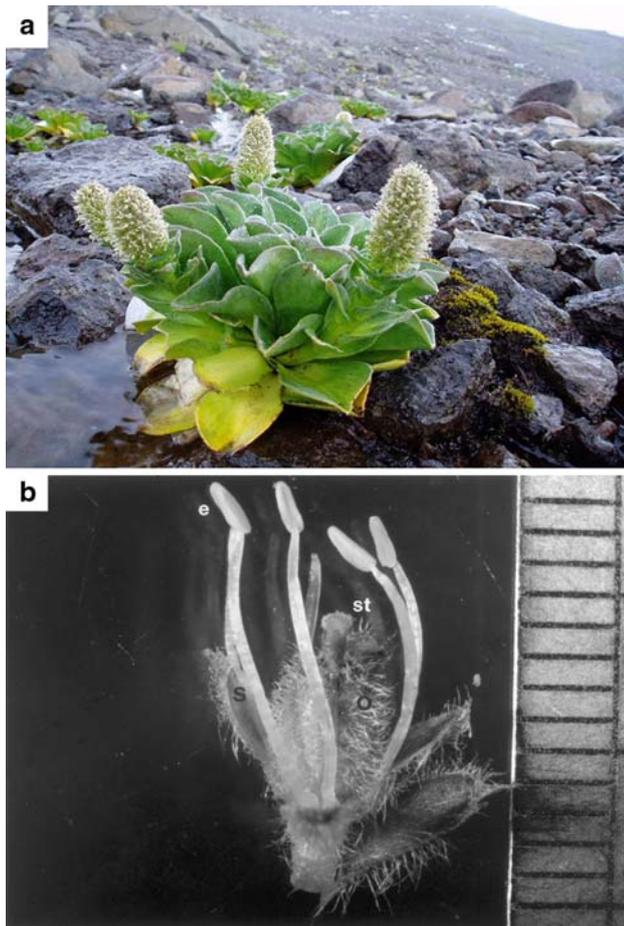
Fig. 1 *Pringlea antiscorbutica* R. Br.: **a** general view of a flowering plant; **b** isolated flower. *s* sepal (4), *e* stamen (6), *o* ovary, *s* stigma. Scale in mm (adapted from Hennion 1992)

Insect fauna

The species list for indigenous and introduced insects (excluding Collembola) was compiled from unpublished records (Marchand 1995) and published reviews or taxonomic notes (Eaton 1875, 1879; Verrall 1879; Waterhouse 1879; Jeannel 1940; Séguy 1940; Duckhouse 1970; Gressitt 1970; Harrison 1970; zur Strassen 1982; Chown 1994; Chown et al. 1998; Dahl 1970; Hullé et al. 2003; Frenot et al. 2005). Insect fauna on *P. antiscorbutica* has been observed during growth and flowering monitoring in several natural populations from Kerguelen and in the experimental garden since several decades (Chapuis et al. 2000; F. Hennion unpublished data).

Materials and methods

Controlled pollinations were carried out between plants grown in a common garden close to the scientific base at Port-aux-Français (Kerguelen Island, 49°20′S, 70°15′E), in order to limit the need for favourable weather conditions for trips to the natural stands, and thus allow for more manipulations. The scientific base is located near a natural harbour, which has a climate typical of coastal locations in Kerguelen. *P. antiscorbutica* plants were grown from seeds originating from one population in Mayes Island (Southeastern Kerguelen Archipelago) seven years before the present experiment. Biotic and abiotic conditions were similar in Mayes Island and in Port-aux-Français, and the seed yield obtained in this garden is within the same range as the seed yield in other natural populations from the Kerguelen Archipelago (F. Hennion, unpublished data). *P. antiscorbutica* used to occur naturally around Port-aux-Français in stands similar to that of the garden at the base (*Acaena* herbfield, mesic conditions) but it severely decreased from the main island during the 20th century after rabbit expansion. Nowadays, the closest natural populations occur at around 20 km distances from the base, in stands physically out of reach of the herbivorous species, such as on rocks or cliffs.

In preliminary experiments, anther removal led to flower abortion. We therefore designed a protocol with complete hermaphroditic flowers. Four different treatments were applied:

– Obligate Self pollination (S) was achieved by bagging.
– Hand Self pollination (HS) was achieved by bagging and assisted pollination. Assisted self pollination was obtained by rubbing the mature stigmas with a stamen taken from the same plant; this process was repeated several times for each flower. Each inflorescence was visited for several days in order to adjust the treatment to the maturity of the flowers.
– Hand Cross-pollination (HC) was achieved by bagging and assisted pollination. Assisted cross-pollination was obtained by rubbing the mature stigmas with a paint brush carrying a mixture of pollen from different plants. This process was repeated several times for each flower on different days. Plants were therefore mainly cross-pollinated, although self-pollination cannot be excluded because of the presence of anthers.
– Open pollination (O) without bagging or assisted pollination.

In order to allow for pairwise comparisons, two different treatments were applied per plant, each one on two randomly chosen inflorescences (Fig. 2).

– Experiment I: treatments O and S, to estimate the success of obligate selfing.
– Experiment II: treatments HS and S, to estimate the self pollen limitation (the more usual comparisons between
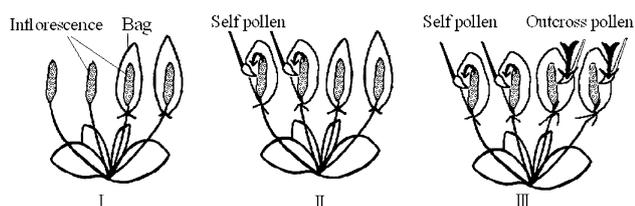
Fig. 2 Controlled pollination experiments in *Pringlea antiscorbutica* R. Br. Five plants with four inflorescences were used for each experiment. Experiment I: open pollination vs. autonomous self pollination. Experiment II: hand-pollination (HS) vs. autonomous self pollination. Experiment III: hand self pollination vs. hand cross-pollination

open pollination (O) and hand cross-pollination (HC) would have led to a mixing of the effects of inbreeding depression and pollen limitation).

– Experiment III: treatments HS and HC, to estimate the relative decrease of seed set of self-pollinated flowers, compared to that of mixed-pollinated flowers. This decrease of seed set could be due to partial self-incompatibility (less seeds are obtained because self pollen is less efficient at siring seeds on the same plant), or to inbreeding depression (less seeds are obtained because selfed progeny are less viable than outcrossed progeny). The combined outcome of these two effects is called ''selfing depression.''

In August 2003, plants with four inflorescences were selected. The 15 available plants were randomly divided into three groups of five plants, one group for each experiment. Bagging was performed before flower opening. Bags of non-woven polypropylene fabric (30 cm × 50 cm, two layers used to prevent the accidental entrance of free pollen) were fixed to the base of each inflorescence and adjusted periodically during inflorescence elongation. The experiments began at the onset of flowering (mid-September).

Five plants and 20 inflorescences were available per experiment. The relatively small number of plants reduced the power of the statistical analyses, but neither their robustness nor their reliability. As a consequence, only large treatment effects can be detected, but we believe that those which provide significant results despite this lack of power are reliable. Furthermore, this sample size is sufficient to ascertain qualitative aspects of the reproductive system of *P. antiscorbutica*, such as its self-compatibility or its capacity to set seeds in the absence of a pollen vector.

Number of flowers

The density of flowers within the inflorescence is so high that an accurate count of their number requires the use of

a destructive method, consisting in removing all of them. An indirect estimate of the number of flowers was therefore used, by measuring height and perimeter of the study inflorescences when maximum elongation was reached. To establish the relationship between these measurements and the number of flowers, a calibration experiment was carried out. Twenty plants were randomly chosen among those not used for the controlled crossing experiments. On each plant, the height and maximum perimeter of one randomly chosen inflorescence were measured at maximum elongation. Then, all flowers were removed and counted.

Fruit and seed set

Fruit and seed maturation took from 2 to 3 months. First flowering was observed in mid-September, and the first ripe fruits were observed in mid-November 2003. All fruits from each inflorescence were collected together with the inflorescence stalk when mature, but prior to dehiscence, between January 15 and January 30, 2003. Ripe inflorescences were stored in individual paper bags and shipped at 4°C to France. For each mature inflorescence, the number of fruits and the number of seeds per fruit were counted in 20 randomly chosen fruits. Flat seeds were not taken into account. Fifty seeds (when available) were kept at 4°C for germination trials.

Seeds were germinated in May and June 2004. First, they were decontaminated in 10% sodium hypochlorite solution for 15 min, and then rinsed twice in distilled water. Each batch of seeds was then spread on a sterile filter paper in a Petri dish and soaked with distilled water. Dishes were randomly placed on a table at room temperature. Moisture was regularly adjusted during the 6 weeks of the experiment. Germinated seeds were counted twice a week. After six weeks, no new germinations appeared, the experiment was stopped and the germination rates were calculated.

Data analysis

Three variables were analysed: (1) the mean number of mature fruits per flower, obtained for each inflorescence by dividing the number of mature fruits by the estimated number of flowers (one measurement per inflorescence); (2) the number of mature seeds per fruit, obtained by counting the seeds of 20 randomly selected fruits in each inflorescence (20 measurements per inflorescence); (3) the proportion of germinated seeds (one measurement per inflorescence).

The numbers of ripe seeds per fruit were analysed with a nested mixed-model ANOVA:

$$Y = \mu + \text{plant} + \text{treatment} + \text{plant} \times \text{treatment}$$
$$+ \text{inflorescence (plant} \times \text{treatment)} + \varepsilon$$

The mean numbers of ripe fruits per flower, and proportions of germinated seeds were compared using a fixed effect model ANOVA:

$$Y = \mu + \text{plant} + \text{treatment} + \text{plant} \times \text{treatment} + \varepsilon$$

The proportion of germinated seeds was analysed following arcsine square root transformation. All analyses were performed using freeware R (R Development Core Team 2004).

Results

Estimation of the number of flowers

The inflorescence sizes were similar in the sample used for calibration and in the plants used for the experiment. The mean heights (±SD) were 52.3 cm (±15.9) in the former, and 47.7 cm (±13.3) in the latter; the mean values of maximum perimeter were 19.1 cm (±2.0) in the former, and 21.9 cm (±3.3) in the latter.

In the calibration experiment, the number of flowers per inflorescence varied between 84 and 199 and was positively correlated with the height (H) ($R = 0.68$, $n = 19$, $P < 0.001$) and with the maximum perimeter (P) ($R = 0.42$, $n = 19$, $P < 0.01$). The best correlation was obtained between the number of flowers and $H \times P^2$ (Fig. 3), which was proportional to the volume of the inflorescence ($R = 0.79$, $n = 19$, $P < 0.001$). The number of flowers (fl) in the inflorescences used for controlled crossings was thus estimated by the equation of the corresponding regression: $\text{fl} = 3.68 \times H \times P^2 + 57.4$ where H and P are expressed in
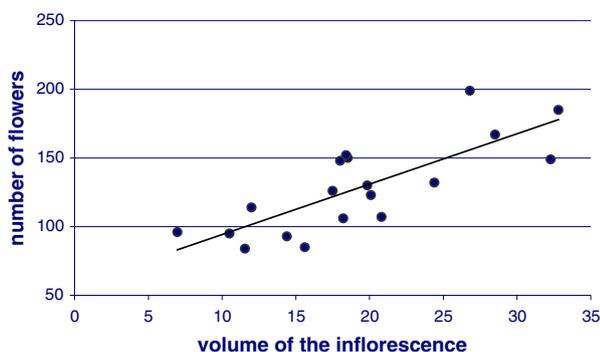

Fig. 3 Calibration of the numbers of flowers per inflorescence in *Pringlea antiscorbutica* R. Br: relation between the number of flowers (*F*) and *V*, a value proportional to the volume of the inflorescence (*V* = height × perimeter²). The correlation is highly significant ($N = 19$, $r = 0.78$, $P < 10^{-3}$)

dm. The number of flowers estimated from the above equation exhibited a highly significant plant effect ($F_{12,44} = 5.38$, $P < 10^{-4}$), but no experimental effect ($F_{2,12} = 2.53$, $P > 10\%$).

Fruit and seed set

All treatments resulted in fruit set and seed set at the inflorescence level. At the time of fruit and seed counting, four inflorescences had partially dispersed their seeds and two inflorescences showed imperfect seed ripeness. These were discarded, so that 54 inflorescences (18 per experiment) were included in statistical analyses. For the mean number of ripe fruits per flower, the plant effect was only significant in experiment III, and the treatment effects were not significant in any of the three experiments (Table 1a). For the mean number of ripe seeds per fruit, the plant effect and the inflorescence effect were higher than the treatment effect, but the treatment effects were significant in experiments II and III (Table 1b), and the plant × treatment interaction effects were highly significant in all three experiments. The absence of a significant difference between open pollination and bagged inflorescence (experiment I) revealed a low effect of bagging. The significant difference between bagged inflorescences without pollination assistance and bagged inflorescence with self-pollination assistance (experiment II) revealed self-pollen limitation, pollination assistance yielding 8.27% more seeds. The significant difference between bagged inflorescences with self-pollination assistance and bagged inflorescences with cross-pollination assistance (experiment III) revealed selfing depression, with hand cross-pollination yielding 20.0% more seeds than hand self-pollination.

Percentage of seed germination

The percentage of seeds germinated after 6 weeks was high (78%) but six of the 55 seed batches showed no germination at all. Although their seeds looked similar to those of the other batches, their inability to germinate may have resulted from incomplete maturation, rather than from genetic effects. When these batches were discarded from the analyses, the mean germination rate of the remaining batches reached 87%. Mean germination rates in the three experiments are presented in Fig. 4c. Neither the plant effects nor the treatment effects were significant in any experiment [Table 1(c)].

Insect fauna

The wingless fly *Calycopteryx moseleyi* is frequently found crawling on leaves inside the rosette of *P. antiscorbutica*.

Table 1 Results of the analysis of the variance of the mean number of seeds per fruit (a), mean number of fruits per flower (b) and mean germination rate (c) in experiments I, II and III

Test	Source	df	MS	F	P
(a) Number of fruits per flower					
O vs. S	Plant	4	0.0429	2.97	0.08
	Treatment	1	0.0014	0.10	NS
	Plant × treatment	4	0.024	1.68	NS
	Residuals	9	0.014		
S vs. HS	Plant	4	0.072	2.96	0.081
	Treatment	1	0.0092	0.38	NS
	Plant × treatment	4	0.016	0.64	NS
	Residuals	9	0.024		
HC vs. HS	Plant	4	0.159	5.90	<0.05
	Treatment	1	0.001	0.01	NS
	Plant × treatment	4	0.008	0.29	NS
	Residuals	9	0.027		
(b) Number of seeds per fruit					
O vs. S	Plant	4	61.7	9.52	<0.001
	Treatment	1	7.1	1.09	NS
	Plant × treatment	4	199.6	7.68	<0.001
	Inflorescence (plant × treatment)	8	161.8	3.12	<0.01
	Residuals	321	2081.8		
HS vs. S	Plant	4	797.3	85.1	<0.001
	Treatment	1	37.6	4.1	<0.05
	Plant × treatment	4	71.9	7.7	<0.001
	Inflorescence (plant × treatment)	9	75.3	8.0	<0.001
	Residuals	345	9.4		
HS vs. HC	Plant	4	970.2	56.2	<0.001
	Treatment	1	92.3	5.34	<0.05
	Plant × treatment	4	135.2	7.83	<0.001
	Inflorescence (plant × treatment)	6	69.1	4.00	<0.001
	Residuals	300	17.3		
(c) Germination rate					
O vs. S	Plant	4	0.023	0.8140	NS
	Treatment	1	0.022	0.64	NS
	Plant × treatment	4	0.051	1.4832	NS
	Residuals	10	0.034		
S vs. HS	Plant	5	0.199	9.8439	0.09
	Treatment	1	0.003	0.1439	NS
	Plant × treatment	4	0.015	0.7386	NS
	Residuals	2	0.020		
HC vs. HS	Plant	4	0.089	1.2689	NS
	Treatment	1	0.005	0.0742	NS
	Plant × treatment	2	0.004	0.0550	NS
	Residuals	7	0.070		

O open pollination, *S* autonomous self pollination, *HS* hand assisted self pollination, *HC* hand assisted cross-pollination, *NS* not significant

No insects were ever observed on the flowering stalks. Werth (1911) did not observe any insects in the vicinity flowers either.

Only 39 insect species may be currently found at Kerguelen Islands (Table 2). Among them, 23 are endemic or subantarctic (59%). Among these indigenous insects, flightlessness is a very common feature (19 on 23, i.e. 83% of species). Among the 16 introduced species, the trend is reversed with only three flightless species (i.e. 19%). The winged indigenous species are weak flyers (e.g. *Listriomastax litorea*). Several introduced species may fly (e.g. Chironomidae or Trichoceridae), but only *Fucellia mari-*
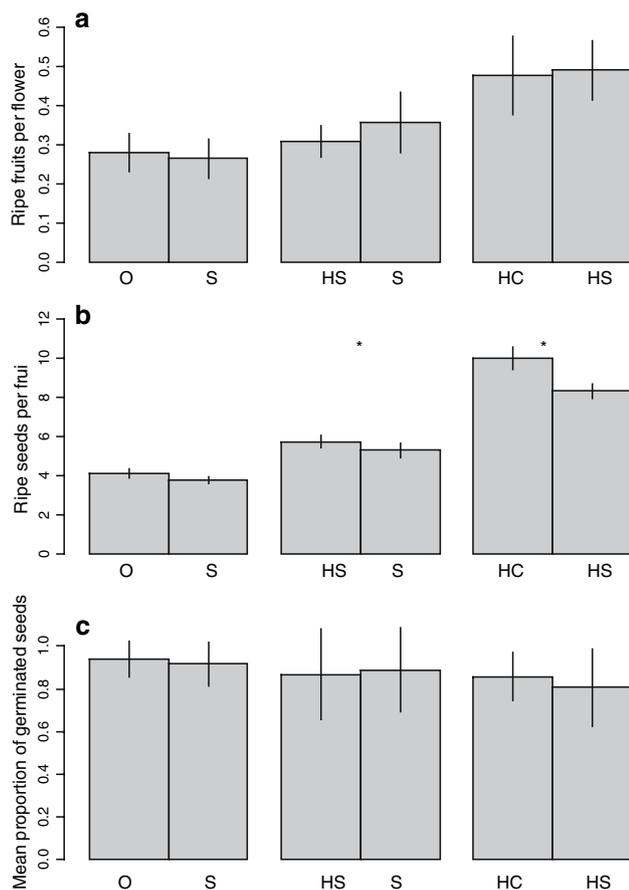
Fig. 4 Results of three controlled crosses experiments in *Pringlea antiscorbutica* R. Br.: two treatments are compared in each experiment (*O* open pollination, *S* obligate self pollination, *HS* hand self-pollination, *HC* hand cross-pollination). **a** Mean numbers of fruits per flower; **b** mean numbers of seeds per fruit and **c** mean seed germination rates, standard errors are indicated. (*Asterisk* represents significant at 5% level)

tima, a phycophagous fly, and above all the blue bottle fly *Calliphora vicina* are active flyers.

Discussion

Self-pollination

Pringlea antiscorbutica produced a large seed set by selfing in the absence of any biotic or abiotic pollen vector. Obligate self-pollination in bagged inflorescences yielded a seed set comparable to open pollination in other inflorescences of the same plant (experiment I). This result demonstrates that *P. antiscorbutica* is self-compatible, whereas self-incompatibility is the ancestral state in Brassicaceae (reviewed in Mitchell-Olds et al. 2005), and still occurs in nearly half the species in this family (Nasrallah 1997). The

fact that fruit and seed set can be obtained without any biotic pollinator in many cases probably results from the location of dehiscent anthers just above the receptive stigma (Werth 1911), which allows autonomous pollination by gravity. The production of a high seed set from autonomous selfing in bagged inflorescences does however not mean that selfing is the main reproductive mode in the wild. Indeed, in hermaphroditic species, selfing is often delayed, which means that it is only allowed when outcross pollen is not available (Lloyd 1992; Sakai 1995). The protogyny of *P. antiscorbutica* flowers suggests that outcrossing may be advantageous in the species (Bertin and Newman 1993) and that selfing may be only promoted after the opportunity for outcrossing has passed (Lyon 1992).

Pollen limitation

Autonomous selfing may result in pollen limitation, which happens when an inadequate quantity or quality of pollen reduces plant reproductive success (Ashman et al. 2004). Limitation by pollen quantity was revealed by experiment II: in bagged inflorescences, supplementation of self pollen led to an increase in seed set of 8.27%. This is however moderate compared to the 42% average increase in seed set resulting from pollen supplementation reported by Ashman et al. (2004) in a review of 85 published cases. Limitation by pollen quality was revealed by experiment III: flowers produced 20% more seeds when they were hand-pollinated with outcross pollen than when they were hand-pollinated with self pollen. It is noteworthy that this protocol underestimates the advantage of cross-pollination, because anthers could not be removed, so that self-pollination was possible in both treatments. The quality of self pollen is thus lower than the quality of outcross pollen. This may result from inbreeding depression (Charlesworth and Charlesworth 1987) or partial self incompatibility (Charlesworth and Charlesworth 1979). Therefore, although possible and efficient, autonomous selfing has a cost in *P. antiscorbutica*, which suggests that it has evolved as a reproductive insurance. Such a reproductive insurance by selfing (delayed or not) is promoted by a poor pollination environment (Lloyd 1979, 1992), for plants growing isolated or in environments where pollination efficiency is uncertain (Porcher and Lande 2005). Colonizing species and/or species with long-distance dispersal are frequently selfing species (Baker's rule, Baker 1955). This is the case for *P. antiscorbutica*: the pioneer habit of *P. antiscorbutica* was demonstrated by Hennion (1992) and Chapuis et al. (2004), who showed that long-distance dispersal was due to seeds floating in sea water. In addition, *P. antiscorbutica* may also encounter poor pollination conditions in the Kerguelen Archipelago.

Table 2 Provisional list of insect species recorded on Kerguelen Islands

Insects	Status	Flightlessness
Coleoptera		
Carabidae		
Oopterus soledadinus Guérin-Menneville 1830	Int.	Yes
Curculionidae		
Bothrometopus angusticollis Waterhouse 1875	End.	Yes
Bothrometopus brevis Jeannel 1940	Sub.	Yes
Bothrometopus gracilipes Waterhouse 1875	Sub.	Yes
Bothrometopus sulcatus Jeannel 1953	End.	Yes
Canonopsis sericea Waterhouse 1875	Sub.	Yes
Disker tenuicornis Dreux and Voisin 1987	End.	Yes
Ectemnorrhinus drygalskii Enderlein 1909	End.	Yes
Ectemnorrhinus viridis Waterhouse 1853	End.	Yes
Palirhoeus eatoni Kuschel 1971	Sub.	Yes
Hydraenidae		
Meropathus chuni Enderlein 1901	Sub.	Yes
Ptinidae		
Ptinus tectus Boieldieu 1856	Int.	No
Staphylinidae		
Antarctophytosus atriceps Kiesenwetter 1877	Sub.	Yes
Diptera		
Anthomyiidae		
Fucellia maritima Haliday 1838	Int.	No
Calliphoridae		
Calliphora vicina Robineau-Desvoidy 1830	Int.	No
Chironomidae		
Halirytus amphibius Eaton 1875	Sub.	Yes
Limnophyes minimus Meigen 1818	Int.	No
Smittia sp. Brundin 1962	Sub.	No
Ephydridae		
Amalopteryx maritima Eaton 1875	Sub.	Yes
Micropezidae		
Calycopteryx moseleyi Eaton 1875	Sub.	Yes
Psychodidae		
Psychoda parthenogenetica Tonnoir 1940	Int.	No
Sciaridae		
Bradysia aubertii Séguy 1940	Int.	No
Sciara jeanneli Séguy 1940	Sub.	No
Sciara womersleyi Séguy 1940	End.	No
Sphaeroceridae		
Anatalanta aptera Eaton 1875	Sub.	Yes
Leptocera curvinervis Stenhammar 1854	Int.	No
Tethinidae		
Apetenus litoralis Eaton 1875	Sub.	Yes
Listriomastax litorea Enderlein 1909	Sub.	No
Trichoceridae		
Trichocera maculipennis Meigen 1818	Int.	No

Table 2 continued

Insects	Status	Flightlessness
Homoptera		
Aphididae		
Aulacorthum solani Kaltenbach 1843	Int.	No
Myzus ascalonicus Doncaster 1946	Int.	No
Myzus ornatus Laing 1932	Int.	No
Myzus persicae Sulzer 1776	Int.	No
Rhopalosiphum padi Linnaeus 1758	Int.	No
Hymenoptera		
Eucoilidae		
Kleidotoma icarus Quinlan 1964	Sub.	Yes
Lepidoptera		
Hyponomeutidae		
Embryonopsis halticella Eaton 1876	Sub.	Yes
Tineidae		
Pringleophaga kerguelensis Enderlein 1905	Sub.	Yes
Psocoptera		
Psoquillidae		
Rhyopsocus eclipticus Hagen 1876	Int.	Yes
Thysanoptera		
Thripidae		
Apterothrips secticornis Trybom 1896	Int.	Yes

End. endemic species, *Sub.* subantarctic species, *Int.* alien species

High level of inter individual variation

All observers have reported high degrees of phenotypic variation in *P. antiscorbutica* (Werth 1911; Hennion 1992; Chapuis et al. 2000). Here, the highly significant plant effects revealed by the ANOVAS of fruit and seed sets [Table 1(a, b)] evidence high phenotypic variation for traits linked to reproduction. Hennion and Martin-Tanguy's (2000) results showed that this species has a high developmental plasticity. More must be known about the links between genetic and phenotypic diversity in this case, in order to asses the status of endangered species of *P. antiscorbutica* in the contemporary context of rapid climate change. Indeed, as reviewed by Frankham et al. (2002), high quantitative genetic variations are the major determinant of evolutionary potential, and low genetic diversity represents a threat for the survival and adaptation of species.

Highly significant interaction terms

Results concerning numbers of seeds per fruit [Table 1(b)] revealed highly significant interactions between plants and treatments. This means that all plants did not respond the same way to a given treatment. This may be part of the high level of individual variation discussed above and

therefore comparisons between the three experiments become hazardous, because the different experiments did not concern the same sets of plants. Nevertheless, the values of ''HS'' (Fig. 4a, b), seem to vary depending on whether the other treatment was ''S'' (experiment II) or ''HC'' (experiment III), as higher fruit and seed sets were obtained on plants submitted to enhanced outcrossing. This could be related to the classical, yet still largely unexplained result showing that increasing pollen diversity can enhance plant fecundity and offspring fitness (reviewed, e.g. in Kron and Husband 2006).

Potential pollen vectors of *P. antiscorbutica*

Only 23 indigenous insects species are known from Kerguelen Islands (7,200 km^2), a very low number even for such a geographically isolated oceanic archipelago, as 44 species, for example, are known from Possession Island (150 km^2) in the Crozet Archipelago (Southern Indian Ocean) (Chown et al. 1998). This autochthonous insect fauna is not only poor, but also unbalanced (two orders, Coleoptera and Diptera, account for 87% of the specific richness), as in other subantarctic islands (Vernon et al. 1998). The indigenous fauna does not include any active flyers, a feature probably resulting from the low temperature and the harsh wind conditions in this archipelago, and it is highly unlikely that *P. antiscorbutica* was pollinated by a flying insect in a recent past. Flightless ants have sometimes been described as efficient pollinators of Brassicaceae (Gómez 2000), but ants are absent from the Kerguelen fauna, and anyhow, no indigenous creeping insects have ever been observed in the inflorescences, not even those commonly found on its foliage such as the wingless fly *C. moseleyi*. It is thus probable that none of the indigenous insects could be efficient pollinator of *P. antiscorbutica*. Among introduced insects, two species, the phycophagous fly *F. maritima*, and the blue bottle fly *C. vicina* are active flyers. These species were not observed on *P. antiscorbutica* flowers, but their implication in pollen transfer cannot be excluded. The same reasoning may be applied to the observed presence of some alien Thysanoptera in the Kerguelen cabbage inflorescences. Thrips are known to be able to pollinate some flowers (e.g. *Erica tetralix*) (Hagerup and Hagerup 1953) and may even be considered as being amongst the oldest pollinators (Terry 2001), but visits of insects must not be confused with pollination, and evidence for thrips pollination in *P. antiscorbutica* remains to be provided. Anyhow, if it were so, this would constitute a recent evolution, and could not explain the present breeding system and flower morphology of *P. antiscorbutica*.

While insect mediated pollination seems unlikely, conditions for wind pollination are partially met in this species.

On the one hand, it has tall inflorescences located in the air stream and has small pollen (about 14 μm in diameter, A. Atlan, personal observation), which favours pollen dispersion by wind (Faegri and Pijl 1971). On the other hand, habitats typically associated with wind pollination are characterized by low to moderate wind speeds, low humidity and infrequent precipitations (Culley et al. 2002). This is very unlike the climatic conditions in the subantarctic islands, which are dominated by strong winds and high atmospheric humidity. It is therefore likely that wind may be a poor pollen vector in the species range, except on occasional mild days. Autonomous self-pollination, as observed here in bagged inflorescences, is most likely to be the best insurance for fertilization.

Scenario for the evolution of *P. antiscorbutica*

The presence of showy inflorescences with numerous flowers, and the persistence of nectar glands in *P. antiscorbutica* seem to indicate an adaptation to insect pollination. However, we hypothesize that none of the indigenous insects currently present in the Kerguelen Archipelago could be an efficient pollinator; in addition, *P. antiscorbutica* is indeed able to set seeds in the absence of any biotic pollen vectors. Thus, adaptations to insect pollination must have been inherited from its ancestor. *Pringlea*'s ancestor is unknown, as is the reproductive systems of its closest relatives, *Sisymbrium* species (Warwick et al. 2002). However, the primitive Brassicaceae were probably insect-pollinated (Mitchell-Olds et al. 2005), and insect pollination is common among *Sisymbrium* species (e.g. Denisow 2004), which may thus also have been the case for *P. antiscorbutica*'s ancestor. Furthermore, the protogyny of *P. antiscorbutica* suggests that out-crossing may have been advantageous for that ancestor (Bertin and Newman 1993), as confirmed by the selfing depression underscored in this study. Although wind nowadays appears as the only available vector for outcross pollen in Kerguelen, the weather is probably often too wet and the wind too strong to allow for effective wind-pollination. Wind-pollination in this island may rely on occasional favourable weather conditions.

Finally, *P. antiscorbutica* probably evolved from an insect-pollinated out-crossed species into a wind and/or autonomous pollinated species, by adapting its flower morphology (the presence of petals in some flowers suggests that the loss of corolla is recent, as may be the position of the anthers above the stigma). It is now able to produce a high seed set by autonomous self pollination, and this ultimate degree of reproductive autonomy has probably played an important role in its success under the harsh conditions of subantarctic islands.

Conclusion

Bergstrom and Chown (1999) point out the particular interest of the southern ocean islands for examining the responses of species and systems to climate change. The present study of *P. antiscorbutica* opens several opportunities to address other evolutionary and conservation problems. Here we discuss the way a probably insect-pollinating species adapted to a new environment where pollination conditions were impoverished. The recent occurrence of some good flying introduced insects (mainly the fly *C. vicina*) might also be an opportunity for investigating a possible resurgence of insect pollination. One of the important issues of contemporary global climate change is the disturbance of biotic pollination. Habitat loss and fragmentation have been identified as major threats to species. Being an insular endemic species, *P. antiscorbutica* can also be used as a model to improve the understanding of evolution of continental species restricted to isolated patches by fragmentation of their habitat.

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