The role of floral biology and breeding system on the reproductive success of the narrow endemic *Petrocoptis viscosa* rothm. (Caryophyllaceae)

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Abstract

Morphological and functional characteristics of flowers may have major effects on their reproductive success. Here, we report a study on the characteristics of flowers of *Petrocoptis viscosa*, a herb species endemic to the northwest Iberian Peninsula, restricted to crevices in limestone outcrops, and currently occurring in only three populations within an area of less than 30 km². We also investigated the effects of pollen source on indicators of reproductive success. The results show that *Petrocoptis viscosa* flowers are well adapted to autogamy. Inbreeding depression (as determined by comparison of results obtained after selfing and outcrossing) was negligible for fruit production (δ = -0.05) and mean seed number (δ = -0.11), low for seed germination percentage (δ = 0.08), but relatively high for mean seed weight (δ = 0.23). The spatial structure and small size of population of *Petrocoptis viscosa* may mean that inbreeding is frequent in natural habitats. Adaptation of plants to autogamy may therefore be energetically beneficial (i.e. less wasted expenditure on rewards and flowers). © 2001 Elsevier Science Ltd. All rights reserved.

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1. Introduction

There is a widespread consensus that studies on the reproductive biology of endangered, rare or threatened species may be useful for understanding why they are endangered, rare or threatened (Schemske et al., 1994). Information obtained from such studies may be useful for evaluating alternative in situ and ex situ management strategies (Menges, 1986). Thus, in the last few years, the management of some endangered, rare or threatened species has been assisted by previous studies on the reproductive biology. Depending on the reproductive biology and population history of the species, reduced pollinator service may have several negative impacts on the plant population, including reproductive failure (Jennersten, 1988) or decreased effective population size through reduced gene flow and increased selfing (Bawa, 1990; Menges, 1991; Aizen and Feinsinger, 1994). These altered reproductive patterns may cause loss of genetic diversity and/or reduced progeny fitness due to inbreeding depression (Karron, 1989; Menges, 1991; Barrett and Kohn, 1991). However, self-fertile individuals may be at a selective advantage in some particular habitats if outcrossing is disfavored because plant density is low (e.g. recent colonization or periodic population bottlenecks) or if pollinators are scarce and cross-pollination is inadequate (Jain, 1976; Lloyd, 1980; Schemske and Lande, 1985; Wyatt, 1988; Barrett, 1989). In addition, in highly self-fertile plants, lethal genes that are expressed early in development may already have been purged (Latta and Ritland, 1994; Husband and Schemske, 1996).

Endemic species with restricted geographic distributions have become a central concern of biologists faced with the problem of preserving rare species endangered by habitat destruction and fragmentation. Among the endemic species most prone to such effects are those in which population size is small. The genus *Petrocoptis* A. Braun (Caryophyllaceae), composed of 11 rupicolous taxa (Montserrat and Fernández-Casas, 1990) is endemic to the Iberian Peninsula, and its distribution area is restricted to limestone outcrops in the Pyrenees and...
Cantabrian range, except for *Petrocoptis pardoi* which is located in the Province of Castellón.

*Petrocoptis viscosa* Rothm. (Caryophyllaceae) is a rare endemic, with a distribution area covering less than 30 km². It is listed as “threatened” according to IUCN categories (Izco and Guitián, 1987). However, the only three populations are in an unprotected area. Following Rabinowitz (1981), this species can be considered as “rare” on the basis of its high habitat specificity, reduced geographic range and reduced population size (Rabinowitz, 1981).

The aim of the present study was to investigate the floral and reproductive biology of *Petrocoptis viscosa*, examining the implications that both floral biology and breeding systems can have in its life cycle. Alternatively, we expect to give information which will be useful on management strategies for its endangered populations.

Specifically, we examined (1) flower measurements that might affect pollinator attraction; (2) floral nectar production; (3) mean flower lifespan; (4) pollen grain germinability and stigma receptivity; (5) breeding system, including pollen/ovule ratio; (6) fruit set, seed number, seed weight and seed germination with different pollen sources; and (7) pollinator activity.

### 2. Material and methods

#### 2.1. Plant and study area

*Petrocoptis viscosa* Rothm. is a perennial herb of 15–25 cm with opposite leaves and pentameric flowers arranged in dichasia. It has been recently synonymized to *Silene glaucifolia* subsp. *glaucifolia* (Mayol and Roselló, 1999). The corolla comprises five white petals, which enclose 10 stamens and five styles. The fruit is a unilocular capsule and the seeds, of about 1 mm diameter, are smooth, black and glossy, with the strophiole composed of hairs. The species grows exclusively in limestone crevices, overhangs and ledges. It occurs in three small scattered populations on dolomite outcrops in the Montes Aquilianos in the El Bierzo region of northwest Spain: Ferradillo (UTM grid reference = 29TPH9304, 1100–1400 m), La Chana (UTM grid reference = 29TPH8706, 650–750 m), and Cornatel (UTM grid reference = 29TPH8808, 700–850 m). Its total range barely exceeds 25 km². The number of individuals is probably less than 3,000. Flowering commences in mid April and ends in mid June. The first mature fruits appear at the beginning of June. By the end of July all capsules have undergone dehiscence (Navarro, 1996).

The El Bierzo region is characterized by a mosaic of habitats including cultivated land, small villages, limestone crags, holm oak woodland (*Quercus rotundifolia*, *Arbutus unedo* and *Quercus suber*) and Mediterranean-type scrub communities dominated by *Cistus ladanifer* on siliceous soils or *Cytisus* and spiny Rosaceae on calcareous soils. The natural vegetation of the region consists of holm oak woodland and various types of scrub community, which develop following woodland disturbance (Izco et al., 1989). Following the classification of Rivas-Martínez et al. (1984), climate is subhumid Mediterranean with Central European tendency. Annual mean temperature in Cornatel (2 km from the study area and at a similar altitude) is 14.2 °C, and mean annual precipitation is 649 mm.

Data for the present study were collected in the La Chana population, which contains more than 60% of the known individuals of this species. The Ferradillo and Cornatel populations were not studied, in view of their critical status and difficulty of access.

#### 2.2. Floral morphology

In spring 1993 we collected 60 flowers from the La Chana population (three flowers per plant). The flowers were immediately transferred to the laboratory and the following measurements taken with a micrometer to the nearest 0.1 mm (Fig. 1): (1) peduncle length (distance from the base of the most distal bract to the base of the calyx; potentially affecting the presentation of the flower to pollinators); (2) calyx length (distance from the base of the calyx to the most distal point of the calycine teeth; potentially affecting ease of access to nectar); and (3) petal limb length (potentially affecting pollinator attraction and acting as a platform for certain pollinators).

We also calculated “en-face planar projection” (sensu Dafni, 1991) on 30 flowers from 15 different plants.

In 200 randomly chosen flowers we noted the type of corolla symmetry (actinomorphic or zygomorphic),
stamen disposition in the corolla tube (radial or nototribic), and the angle formed by the corolla surface with respect to the horizontal. In those cases in which type of corolla symmetry was not immediately apparent, we defined as zygomorphic all corollas in which the angle between the central axis of the lower petal and any of the adjacent petals was less than 72°.

2.3. Floral duration

Between 23 April and 7 June 1993, a total of 114 flowers on 20 different plants were marked before opening, and then monitored daily. We also noted the position of the stamens and style during the flower lifespan.

2.4. Pollen and ovule number

The number of pollen grains was counted following preparation by the microacetolysis method (Avetissian, 1975), always using flower buds or closed anthers to avoid contamination. A total of 20 stamens (one per flower) were examined, all from different flowers (n = 20 flowers). Number of ovules (in the ovaries of the flowers used for pollen grain counting) was determined with the aid of a magnifying glass.

2.5. Pollen germinability

We collected all pollen from 1- to 5-day-old flowers which had been bagged before opening to prevent receipt of pollen from other flowers (n = 8 flowers per age class). All pollen from each flower was collected and immediately placed in Petri dishes on a sterile solid medium (as per Bar-Shalom and Mattson, 1977) containing 30% sucrose (this being the concentration which gave the highest germination rate in preliminary tests with 0, 10, 20, 30, and 40% sucrose), and incubated for 4 h. Germination counts were done under a stereomicroscope, all grains with a pollen tube of length greater than grain diameter being counted as “germinated”.

2.6. Stigma receptivity

To investigate stigma receptivity, we hand-pollinated 1-, 2-, 3-, 4- and 5-day-old flowers with pollen from other plants (n = 25 per age class). Bagged flowers on the recipient plant were carefully emasculated at anthesis, and then manually pollinated. Once the necessary time had elapsed, we recorded whether or not each flower set fruit.

2.7. Pollen transfer experiments

These experiments investigated the effect of pollen source on fruit set, number of seeds per capsule, seed weight, seed germination percentage, and seed germination speed (days to 50% germination). Five experimental treatments were applied, as follows:

1. Reproduction in the absence of insects: bagging with mosquito netting to prevent access by insects (n = 54 flowers on five plants).
2. Autogamy: pollination with pollen from the same flower followed by bagging (n = 21 flowers on three plants).
3. Geitonogamy: emasculation, pollination with pollen from a different flower of the same plant, followed by bagging (n = 97 flowers on five plants).
4. Xenogamy: emasculation, pollination with pollen from another plant, followed by bagging (n = 85 flowers on four plants).
5. Supplementary pollination: pollination with pollen from another plant, without bagging (n = 81 flowers on four plants).
6. Simultaneously, 20 plants which received no treatment were monitored as controls (n = 682 flowers).

In all cases, once the necessary time had elapsed, we recorded whether or not each flower set fruit, and if so the number and weight of seeds per capsule. Mean fruit set, overall mean number of seeds per capsule and mean seed weight were then calculated for each treatment. Two indices related to selfing were calculated for fruit set following Lloyd and Schoen (1992): (1) the self-fertility index, calculated as performance after spontaneous selfing/performance after hand outcrossing (i.e. xenogamy), which estimates the ability to set fruit and seed in the absence of pollinators; and (2) the self-compatibility index, calculated as performance after hand selfing, (i.e. autogamy)/performance after hand outcrossing (i.e. xenogamy).

To evaluate seed germination, seeds from each pollen transfer experiment were placed in a single Petri dish (30 seeds per dish) on dampened filter paper, and maintained at temperatures of 18–25°C in the dark. Previous experiments have shown that Petroscoptis viscosa seeds show good germination rates under these conditions (Navarro, 1996). On each day we counted and removed germinated seeds until all seeds had either germinated or decayed. At the end of each assay we assessed percentage germination and speed of germination, determining the latter as the number of days from the start of imbibition until 50% of seeds had germinated.

Inbreeding depression for fruit set (1), seed production (2), seed weight (3), and seed germination (4) was calculated as

\[ \delta = 1 - \left( w_s / w_o \right) \]

where \( w_s \) is the mean performance of selfed progeny (from autogamy) and \( w_o \) is the mean performance of outcrossed progeny. We also calculated a multiplicative estimate of inbreeding depression based on the above reproductive traits as one minus the product of the above ratios, i.e.
1 - \left[ \left( \frac{w_1}{w_1} \right) \times \left( \frac{w_2}{w_2} \right) \times \left( \frac{w_3}{w_3} \right) \times \left( \frac{w_4}{w_4} \right) \right]

2.8. Constancy of reproductive success

To examine whether or not fruit set levels are constant both between years and between plants, we monitored population-mean fruit set in the study population between 1993–1997. Population-mean number and weight of seeds per capsule were also determined in 1993 and 1994.

2.9. Daily nectar production

In spring 1993 a total of 86 flowers from 10 plants were bagged, and after 24 h the accumulated nectar was extracted and measured with a capillary micropipette.

2.10. Insect activity

Pollinator activity throughout the day was assessed by hourly censuses between 08:00–20:00 h Greenwich Mean Time (GMT), for a total of 36 h during 1993 and 1994. In each case we recorded (1) the type of visit (nectar extraction or pollen collection); (2) the number of visits to each plant; and (3) the duration of each flower visit.

2.11. Data analysis

To examine differences between the two types of corolla, symmetry data were compared by the chi-square test. The angle between the corolla surface and the horizontal was calculated by conversion of angles into trigonometric rates (Zar, 1996). The effect of flower age on stigma receptivity and pollen germinability was analyzed by logistic regression. Fruit set was compared between experimental pollen transfer treatments by a non-parametric Kruskal–Wallis test. The germination speeds of seeds from different pollen-transfer experiments were compared using Kolmogorov–Smirnov two-sample tests. Final percentage germinations were compared with a chi-square test.

3. Results

3.1. Floral morphology

The “en-face planar projection” of flowers of Petrocoptis viscosa was 0.8 ± 0.2 cm² (n = 30 flowers), while petal limb length was 4.7 ± 0.6 mm (n = 60), calyx length was 7.2 ± 0.8 (n = 60) and peduncle length was 4.7 ± 3.8 (n = 60). The peduncle length showed high variability in the study population (Coefficient of Variation = 81.4%). The least variable character was calyx length (CV = 11.7%), while petal limb length showed an intermediate coefficient of variation (CV = 13.0%). Stigma–anther separation did not exceed 1 mm.

Petrocoptis viscosa presented a zygomorphic corolla only in 34.5% of the flowers examined (n = 200), while stamens were located in radial position around the center of the corolla tube in 75.5% of flowers examined (n = 200).

The mean angle between the corolla surface and the horizontal was 75° 9’ ± 18° 1’ (range 10–125°; n = 200).

3.2. Floral duration, stigma receptivity and pollen germinability

Of the flowers monitored, the first to open did so on 2 May and the last on 10 June. Flower anthesis occurs early in the morning. The flowers of an inflorescence (dichasia) do not open simultaneously; the first to open is the central flower, followed by first-order lateral flowers and finally second-order lateral flowers. Mean floral duration was 5.4 ± 1.6 days (n = 114). Flowers seemed slightly protandrous, with the stamens slightly exerted and in all cases dehiscing on the first day of anthesis. The visual switch from the male phase to the female phase was signaled by a very slight elongation of the style from the second day post-opening onwards. However, 96% of stigmas were receptive as early as the first day after flower opening. Maximum receptivity was attained on the second day, when 100% of pollinated flowers initiated fruit (Fig. 2). Subsequently, stigma receptivity decreased markedly (Wald Chi-square = 29.1; P < 0.0001). By 5 days after anthesis, only 16% of stigmas remained receptive. Pollen dehiscence and the

Fig. 2. Variation in pollen germinability and stigma receptivity with flower age in Petrocoptis viscosa.
germination peak occurred on the first day. Subsequently, pollen germinability decreased steadily with days since anthesis (Wald Chi-square = 2606.9; P < 0.0001).

3.3. Pollen and ovule number

The mean number of pollen grains per flower was 4686 ± 2094, and mean ovule number was 13 ± 2. Thus, mean pollen/ovule ratio was 457 ± 128.

3.4. Pollen transfer experiments

Under natural conditions, fruit set in the year in which the breeding system study was carried out (1993) was 85.5% (n = 682 flowers). The mean number of seeds per capsule was 8.9 ± 2.5 (n = 320 capsules) and the mean seed weight 491 ± 105 μg (n = 50 seeds) (Table 1). Flowers that were unmanipulated and bagged showed similar fruit set to hand-pollinated flowers. As a result, the self-fertility index for fruit set was high (0.88). All other hand-pollination treatments, except geitonogamy, showed more than 81% fruit set, as did the population control. The self-compatibility index for fruit set was thus high (1.05). Although after geitonogamy fruit set was lower than after the other treatments, no significant among-treatment differences in fruit set were observed (H = 9.9; d.f. = 5; P = 0.0760). Mean seed number and mean seed weights for each treatment are shown in Table 1. No significant among-treatment variation was observed in mean seed number (F = 2.03, d.f. = 5; P = 0.0736). However, mean seed weight showed significant variation (F = 16.20, d.f. = 5; P < 0.0001). The seeds obtained after spontaneous autogamy, autogamy and geitonogamy were significantly lighter than seeds obtained after xenogamy (Table 1).

3.5. Constancy of reproductive success

Fruit set in the study population did not vary among the 5 years of study (F4, 744 = 0.7; P = 0.570; Fig. 3a). However, fruit set levels varied among plants (F78, 744 = 9.6; P < 0.002). On the other hand, no significant between-year differences (1993 versus 1994) were observed either in mean seed number (t = 0.4; P = 0.518), or in mean seed weight (t = 1.95; P = 0.1661; Fig. 3b, c respectively).

3.6. Germination test

The percentage germination of seeds of *Petrocoptis viscosa* is very high (Table 2). No significant between-treatment differences were found in percentage germination (χ2 = 24; P = 0.2424). Note that germination was ≥90% for all seed types. In contrast germination speed (days to 50% germination) showed significant differences between treatments. In fact, germination speed was about 36% faster for crossed seeds than for selfed seeds (Kolmogorov–Smirnov test, P < 0.005).

3.7. Inbreeding depression

Inbreeding depression (based on mean values after selfing and outcrossing, calculated over all plants) was negligible for fruit set (δ = −0.05) and mean seed number (9 ± 0.7). However, inbreeding depression was significant for mean seed weight (t = 4.82; P = 0.0001; Fig. 3b, c respectively).
number ($\delta = -0.11$), low for percentage germination ($\delta = 0.08$), but relatively high for mean seed weight ($\delta = 0.23$). These levels of inbreeding depression give a relatively low multiplicative value (Section 2) of $\delta = 0.18$.

### 3.8. Daily nectar production and insect activity

Daily nectar production by flowers of *Petrocoptis viscosa* was below the amount detectable by extraction with a 0.25 µl micropipette.

Visits during the 36 h of observation in 1993–1994 were by two diptera species only: *Melanostoma* sp. (Syrphidae) and *Bombylius major* (Bombyliidae) with only six and four flower visits, respectively.

### 4. Discussion

The floral morphology of *Petrocoptis viscosa*, together with the lack of floral nectar, is as expected for a self-compatible species. An important feature of the floral biology of *Petrocoptis viscosa* is the close stigma–anther proximity and the only slight temporal separation between the pollen grain germinability peak and the stigma receptivity peak, so that (as we found in our pollen transfer experiments) autonomous self-pollination is enabled, providing reproductive assurance in the absence of pollinators. In fact, the lack of nectar secretion suggests that “pollinator uncertainty” (Primack, 1985) may be a frequent feature of the reproductive biology of this species.

Selfing ability presumably evolves as a result of the advantages of reproductive assurance, but these advantages are of course counteracted by the increased risk of inbreeding depression (Charlesworth et al., 1990; Hollings, 1992; Lloyd, 1992; Uyenoyama et al., 1993; Lande et al., 1994). *Petrocoptis viscosa* is self-compatible: flowers that have not received pollen from other sources can pollinate themselves by reflexing their stigmas to contact their own anthers. This mechanism is probably facilitated both by the suitable flower morphology (i.e. disposition of stamens radially around styles) and by the relatively high winds of the study site.

In the absence of insect visits, wind may increase pollination indirectly by forcing reflexed stigmas against anthers of the same flower.

We did not find any evidence of inbreeding depression of fruit set or mean seed number. However, the seeds obtained following xenogamous pollen transfer were significantly heavier than those obtained following autogamous pollen transfer. Previous studies of this species have shown that seed weight has significant effects on seedling growth and survival. Percentage seedling survival after 4 months was 84% lower for seeds weighing less than 450 micrograms than for seeds weighing more than 650 µg (Navarro, 1996). In addition, speed of germination was greater for outcrossed seed than for selfed seed. Note, that speed of germination can be of vital importance, because seeds that fall into a crevice must be able to respond to short-term increases in water availability (Navarro, 1996).

Selfing, however, may have selective advantages over outcrossing during colonization or population bottleneck when access to mates is limited or when outcross pollen is limited because pollinators are scarce or unreliable (Ramsey and Vaughton, 1996). In the year of the present study, there was no pollinator limitation in the study area. Plants of *Petrocoptis viscosa* must establish in the crevices of limestone walls, because they are outcompeted by other plants in more favorable sites (Navarro, 1996). In such adverse environment, the dispersion system of this species does not favor genetic variability. The seeds fall to the ground once the capsule is open, and have a hairy strophiole with hygroscopic properties (myxospermy sensu Grubert, 1974): this facilitates their adherence to the wet crevices in the limestone surfaces, and in fact seeds typically adhered to the substratum near the mother plant. This seed morphology may be a strategy for keeping plants in the microsite previously colonized by the mother plant. However, it is also true that the lack of a good mechanism of seed dispersion does not favor genetic variability. The seeds fall to the ground once the capsule is open, and have a hairy strophiole with hygroscopic properties (myxospermy sensu Grubert, 1974). In the absence of insect visits, wind may increase pollination indirectly by forcing reflexed stigmas against anthers of the same flower.

Historically, plants of *Petrocoptis viscosa* may have experienced periods of intense inbreeding, perhaps during glaciation, which might have purged deleterious alleles, reduced inbreeding depression, and relaxed selection against self-pollination (Lande and Schemske, 1985). In any event, the ability to produce fruits and seeds by autonomous self-pollination may reduce the dependence of *Petrocoptis viscosa* on insect pollen vectors. Because autonomous self-pollination is facultative, the option of outcrossing, which improves seed “quality”, is still preserved in *Petrocoptis viscosa*. Such reproductive flexibility may pre-adapt species like *Petrocoptis viscosa* for the colonization of harsh habitats such as limestone crevices.
In conclusion, our results indicate that neither fruit nor seed production are limiting factors in the survival of the populations of *Petrocoptis viscosa*. The rarity of this species may be attributable to its short dispersal distances and to low habitat availability. However, given its high rates of fruit set and seed germination, and the ability of the plant to produce seeds in the absence of pollinators, *Petrocoptis viscosa* may in fact be rare but not intrinsically endangered. Indeed it is possible that, thanks to its breeding system, this species may be more resilient than others to habitat fragmentation. Nevertheless, its narrow distribution makes it very vulnerable to *habitat destruction*; as a result, the current proliferation of limestone quarries in its area of distribution clearly constitutes the most important short-term threat to this species. The survival of the three remaining populations of this Tertiary relict is thus entirely dependent on the commitment and effective responses of the relevant local, regional and national authorities.

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References


