

Reproductive ecology of *Rhododendron ponticum* (Ericaceae) in relict Mediterranean populations

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In the southern Iberian Peninsula, *Rhododendron ponticum* occurs in restricted and vulnerable populations as a Tertiary relict. Population structure and the main phases of the reproductive process were examined in order to shed light on recruitment patterns and limitations. *Rhododendron ponticum* flowers are self-compatible and attract a diverse array of insects, which are responsible for a considerable number of seeds set in the populations. Nevertheless, only adults form populations, whilst seedlings are scarce and saplings virtually absent (only two juveniles out of 2489 adults sampled). Non-specialized vegetative multiplication by layering was observed. Recruitment failure seems to depend on the scarcity of safe microsites, which are free from drought, for seedling establishment. The observations contrast with *R. ponticum*'s reputation as an aggressive invader in temperate Atlantic areas. It is proposed that the species shows a variable balance between sexual reproduction and vegetative multiplication depending on environmental conditions. At present, only the latter seems to be prevailing in relict populations in the Iberian Peninsula. This flexible reproductive strategy is also discussed as a mechanism allowing persistence during geological climatic oscillations. © The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2002, 140, 297–311.

ADDITIONAL KEYWORDS: *Bombus* – breeding system – clonal plants – *Macroglossum* – plant extinction – pollination – recruitment – seedling survival – threatened plants – refugia.

INTRODUCTION

Two key tasks in plant conservation biology are to identify the factors that threaten plant abundance and evaluate the risks of population extinction. Although many factors influence plant abundance, most researchers have concentrated on either ecological or genetic aspects. However, these approaches are often incomplete because (i) ecological studies frequently turn out to be habitat descriptions of the rare species, and (ii) no clear differences are found between rare and widespread species in terms of genetic diversity pattern (Schemske *et al.*, 1994; Lewis & Crawford, 1995; Gitzendanner & Soltis, 2000; Podolsky, 2001), despite the theoretical relationship between population genetic diversity and probability of survival. In addition, few genetic studies have demonstrated prac-

tical outcomes for plant conservation (Schemske *et al.*, 1994; Hogbin, Peakall & Sydes, 2000). Some authors have stressed the importance of having knowledge of the reproductive biology and recruitment pattern in endangered species to be able to detect life-cycle stage(s) constituting the limiting phase(s) and to evaluate causes determining vulnerability (Karron, 1991; Schemske *et al.*, 1994).

The identification of current causes determining plant rarity and vulnerability has not only a practical use for implementing plant conservation policy, but could also shed light on the historical processes determining area restriction in relict plants. Relict species are supposed to have shown more competitive ability in the past, but have eventually faced changing, adverse environment conditions affecting critical stages of their life cycles, except in a number of sites where particular environmental conditions make persistence of these species possible (i.e. refugia). In the Mediterranean basin, a group of relict species represents vestiges of evergreen rain forests that thrived

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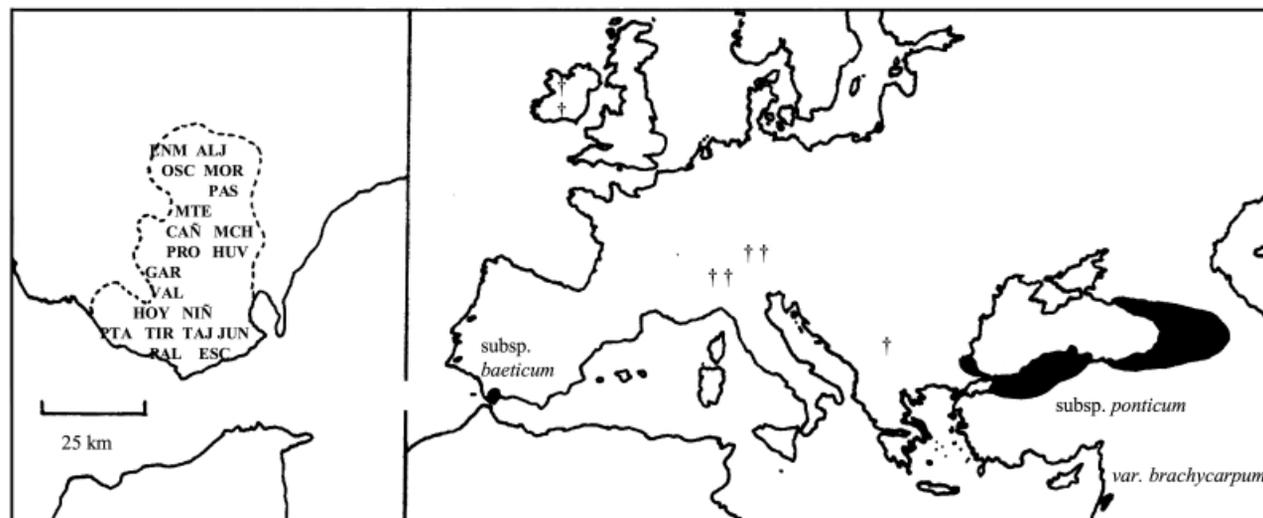


Figure 1. Geographical distribution of *Rhododendron ponticum* (after Cross, 1975; Chamberlain, 1982) and detailed map showing the location of the 20 study sites (for acronyms see Material and methods). (†) Fossil; (■) extant.

during the Tertiary in Europe (Quézel, 1985; Mai, 1989), before climatic changes and oscillations noted after the Upper Miocene resulting in the establishment of a Mediterranean-type climate, 3.2 million years ago (Suc, 1984). At present, these taxa are restricted to refugia where the warm, dry season, characteristic of the Mediterranean climate, is somewhat alleviated. *Rhododendron ponticum* L. is one of the best examples (Mai, 1989). It was widely distributed throughout Europe during the Tertiary, whereas it has, at present, a reduced, disjunct distribution including the Iberian Peninsula, the Black Sea coast and Lebanon (Cross, 1975, and references therein; Chamberlain, 1982; Castroviejo *et al.*, 1993; Fig. 1). Two subspecies have been recognized for the western (Iberian: *R. ponticum* L. ssp. *baeticum* (Boissier & Reuter) Handel-Mazzetti) and eastern (Black Sea: *R. ponticum* L. ssp. *ponticum*) plants, but some controversy remains about this taxonomic differentiation and nomenclature (Chamberlain, 1982; López González, 1995). Iberian populations are restricted to three remote areas (Castroviejo *et al.*, 1993), the largest being the well-conserved humid forests of the Aljibe Mountains (on the northern side of the Strait of Gibraltar, Fig. 1). Here, mild climatic conditions, and the orographical rainfall pattern derived from its maritime position, account for the presence of diverse relict taxa (Rivas Goday, 1968; Ojeda *et al.*, 1996). However, despite being locally abundant, only adults form populations of *R. ponticum*, and currently no effective recruitment seems to take place, although no proper demographic analysis has ever been done. Consequently, *R. ponticum* has been catalogued as an endangered species in Andalusia, southern Spain

(Blanca *et al.*, 2000). In contrast, *R. ponticum* is an aggressive invader in the British Isles and some other areas in temperate Atlantic Western Europe, following its introduction as a garden plant in the eighteenth century (Cronk & Fuller, 1995), most probably from south Spain (Milne & Abbott, 2000). This highlights the value of this species as an excellent study system to investigate reproductive plasticity under different environmental conditions. However, observational studies on the reproductive behaviour of *R. ponticum* are restricted to those of Cross (1981), who reported successful invasion and spreading of this species by effective seedling recruitment in Irish oak forests. Neither analytical nor observational study has been done so far in this species' natural range.

The aims of this study are: (i) to determine the basic parameters of the reproductive biology in relict populations of *R. ponticum*; (ii) to assess the structure of populations in this range; (iii) to detect what stage(s) in the life cycle is acting as a limiting factor in the maintenance and expansion of populations; and (iv) to hypothesize which environmental factors are accounting for this limitation. This will provide a sound background to understand the processes that have forced this species to its relict condition and, from a practical point of view, it will help us to establish the baseline for the management and conservation of this Tertiary relict.

MATERIAL AND METHODS

STUDY PLANT

Rhododendron ponticum is an evergreen, lauroid-leaved shrub of up to 8 m, bearing a developed under-

ground lignotuber from which many axes or branches arise. Plants flower during two months in mid to late spring, but scattered flowers can also be seen during autumn–winter. Flowers are grouped in compact, corymboid racemes of 7–21 flowers. They have a small calyx and a conspicuous, slightly zygomorphic, funnel-shaped corolla up to 6 cm in diameter, composed of five pink–purple connate petals. The upper petal is larger and presents a narrow, longitudinal channel through which nectar rises from a broad, cushion-like nectary surrounding the ovary base. This petal is paler around the channel, showing abundant brown–orange spots as a nectar guide. Flowers present 10 stamens with long, curved filaments; the pistil comprises an oblong ovary with (4–) 5 (–6) carpels, and a long curved style topped by a small lobed stigma. The arrangement of

flower parts is consistent with sternotribic pollination (Fig. 2; see Faegri & van der Pijl, 1979). Capsules dehisce during autumn–winter and release many small seeds (0.4–1 mm, 0.05 mg). Seedlings bear small ovate cotyledons (up to 5 × 2 mm) and gradually produce several larger leaves. Seedling growth rate is slow and it usually takes more than two years for a seedling to become a juvenile showing a thin woody stalk that bears adult sized leaves (personal observations).

STUDY SITES

Fieldwork was carried out in the Aljibe Mountains, on the northern side of the Strait of Gibraltar. Twenty populations were sampled for age structure studies

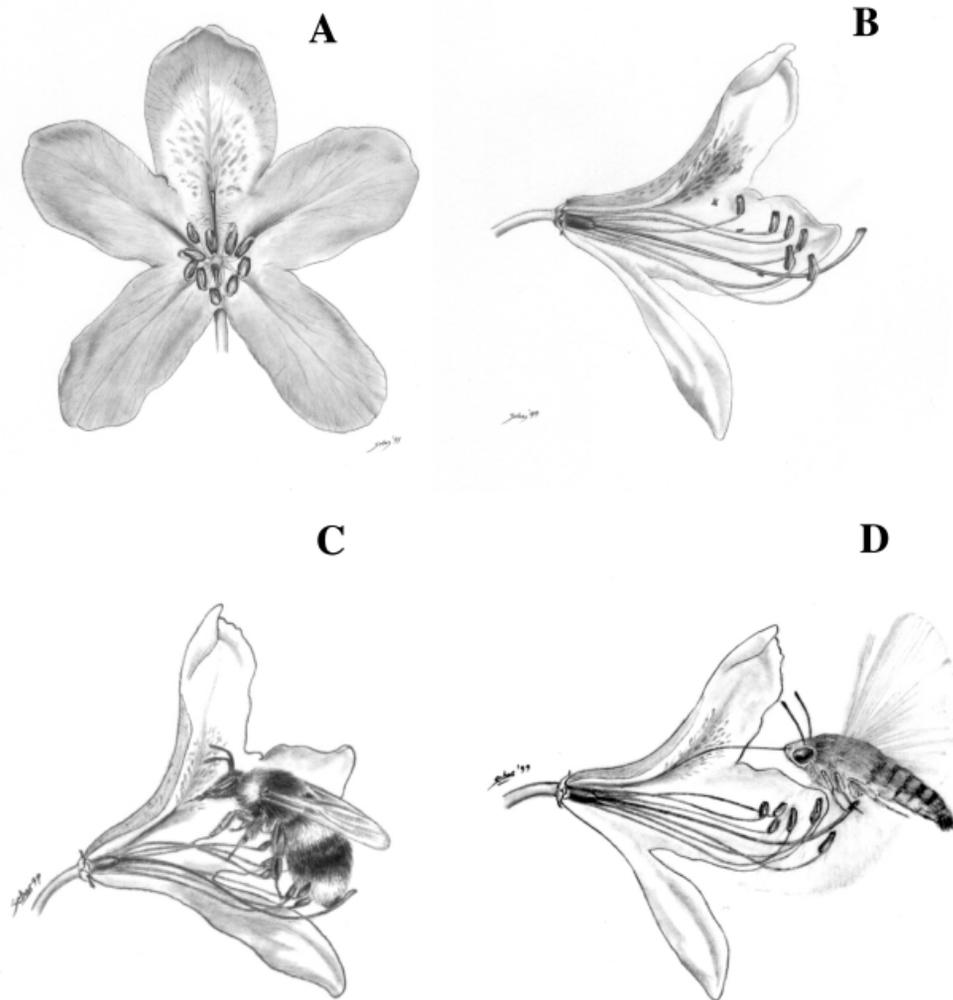


Figure 2. Flowers and insect visitors of *R. ponticum*. (A) Frontal view of a flower showing the nectar guide and the nectar channel. (B) Side section of a flower with six stamens and the pistil, showing some specimens of the small beetle *Meligethes aeneus* (Coleoptera). (C) Visit of *Bombus terrestris* (Hymenoptera). (D) Visit of *Macroglossum stellatarum* (Lepidoptera).

(see location in Fig. 1), most of them occurring in stream valleys and sources: (1) Garganta de Enmedio (ENM), (2) Garganta del Aljibe (ALJ), (3) Garganta de Puerto Oscuro (OSC), (4) Garganta del Moral (MOR), (5) Garganta de Pasadallana (PAS), (6) Affluent of Canuto del Montero (MTE), (7) Garganta de los Caños (CAÑ), (8) Garganta de la Moracha (MCH), (9) Garganta del Parroso (PRO), (10) Garganta del Huevo (HUV), (11) Arroyo de los Garlitos (GAR), (12) Arroyo de Valdeinferno (VAL), (13) Garganta de la Hoya (HOY), (14) Affluent of Garganta del Niño (NIÑ), (15) Stream in Sierra de la Plata (PTA), (16) Garganta del Tiradero (TIR), (17) Tajo del Espino (TAJ), (18) El Palancar Spring (PAL), (19) Tajo de las Escobas (ESC). Additionally, a rare, extensive population occurring under deciduous *Quercus canariensis* canopy was also sampled: (20) Llanos del Juncal (JUN).

For reproductive biology studies, five populations were selected (ENM, ALJ, PAL, ESC and JUN), representing the variety of habitats occupied by *R. ponticum* in the area.

POPULATION STRUCTURE

Five sampling plots were regularly established (separated by 100 m from each other) along the stream course in each study site. Plots were 20 m long and the streamside width (range 3–28 m, exceptionally a 49-m width plot was found in MCH). Three sites (MOR, PAL and ESC) were too small to set up five plots, so four, three and two plots separated by 100 m were used, respectively. In TAJ, in the catchment area of Arroyo de la Miel River, five plots were established in five parallel small streams, beside a contour dirt-road, along a distance of 1250 m. For JUN, five 20 × 5-m plots were systematically established along a 500-m line in the forest, analogous to the above-mentioned streamside transects.

In each plot, the whole number of adults and juveniles (individuals no more than 0.5 m tall, showing a woody stalk and adult sized leaves) was counted. Additionally, in each plot, five 1-m-wide transects were set perpendicular to the stream-bed, each separated by 5 m. The length of these transects was the plot width. In these transects, the number of seedlings was recorded. Two classes were defined to reflect the development level: (i) seedlings without leaves ≥20 mm long, and (ii) seedlings with at least one leaf ≥20 mm. This classification is based on the short size of 1-year-old seedling leaves, which never reach 20 mm long (personal observations).

FLORAL PHENOLOGY

Flower longevity was determined in plants from ESC during May 1993. We selected randomly five racemes

and marked four floral buds that were about to open in each raceme, and checked them daily. In each flower we recorded (1) corolla aperture and lobe positions, (2) pollen exposure on the anthers (i.e. presence of pollen mass on the anther's terminal pores), (3) style position and vertical distance (D) of the stigma to the lowest anther, and (4) receptivity of the stigma (tested with hydrogen peroxide effervescence; Dafni, 1992).

FLORAL VISITORS

For diurnal observations, we selected one site at ESC, two sites at ENM and one site at PAS, all with abundant flowering. Observations were performed on all flowers in 5 m² for 5-min periods in ESC (13 and 15 May 1993, total 3 h), 10 min in ENM (19 May 1998, total 6 h), and 15 min in PAS (30 May 2000, total 3 h) from dawn to dusk on sunny days. We recorded the identity of each insect visitor, the number of flowers visited, and their activity (movements and rewards). Furthermore, some flowers were selected and observed more closely to account for the presence of very small insects and their foraging behaviour inside the flowers. We captured insect visitors to identify them and to count *Rhododendron* pollen tetrads attached to their bodies. Potential nocturnal visitors were collected by a light-trap placed in ALJ during a single, calm night (from 19:00 h to 05:30 h), and flower visitors were identified by the presence of pollen on their bodies.

We counted the *Rhododendron* pollen tetrads on the bodies of trapped insects by rubbing on them a small cube of glycerin jelly. This jelly was then mounted on a microscope slide and the pollen tetrads were counted under a light microscope (Kearns & Inouye, 1993).

NECTAR PRODUCTION

Racemes of six plants from ALJ and ENM were bagged (18 May 1998) with soft mesh bags to preclude nectar removal by nectar-feeding visitors. We removed the bags 36 h later and measured the volume of nectar accumulated in open flowers with 1-μL and 5-μL microcapillary tubes. Sugar concentration (of sucrose equivalents) in nectar samples was estimated using a temperature-compensated field refractometer (Bellingham & Stanley).

CONTROLLED POLLINATIONS

We hand-pollinated six randomly selected plants at each of ESC and JUN during May 1994. Between two and four racemes per plant were bagged before anthesis to exclude floral visitors. Individual flowers were randomly selected and tagged for each of the following treatments: (1) autonomous self-pollination (SP):

non-manipulated flower, (2) forced self-pollination (FSP), (3) intrapopulation cross-pollination (ICP), (4) between-population cross-pollination (BCP). In addition, 1–2 further racemes of exposed (non-bagged) flowers were used for two additional treatments: (5) hand-pollination with pollen of another plant from the same population (supplemental pollination; XFP) or (6) non-manipulated, freely pollinated flowers (FP), used as a control for non-bagged treatments. For three (ICP) and four (BCP) treatments, flowers emasculated prior to anthesis were always used. Due to flower availability, five plants were used for cross-pollination treatments (ICP and BCP), and in JUN three plants were tested for forced self-pollination (FSP). The number of flowers ranged from 102 to 146 flowers per population for non-manipulative treatments (SP and FP), and from 10 to 25 flowers per population for manipulative treatments (FSP, ICP, BCP, and XFP). During September, before seed release, we collected experimental racemes to estimate fruit- and seed-set in each treatment.

FLOWER, FRUIT SEED AND SEEDLING DENSITY

We estimated flower, fruit and seed density in the populations during autumn 1996 and 1997, in a 25×1 -m vertical plane, window-like transect at each site, except in JUN, where two such transects were established (JUN-1 inside the forest and JUN-2 along the forest margin which receives more sunlight). Racemes were counted in each transect. We collected all the racemes from a transect if fewer than 30 were included, otherwise we collected 30 racemes randomly. For each raceme we counted flowers (capsules plus peduncles and peduncle scars on the axis of the raceme), and capsules. We considered only capsules containing seeds as true fruits. We then calculated fruit-set as the ratio of capsules to flower production. Capsule length was measured and it was found to be highly correlated with seed number in most populations (JUN-1 96: $r = 0.85$, $P < 0.001$, d.f. = 10; JUN-1 97: $r = 0.89$, $P < 0.001$, d.f. = 8; JUN-2 96: $r = 0.71$, $P < 0.01$, d.f. = 8; JUN-2 97: $r = 0.90$, $P < 0.001$, d.f. = 8; ESC 96: $r = 0.92$, $P < 0.001$, d.f. = 8; ESC 97: $r = 0.58$, $P < 0.05$, d.f. = 11; PAL 96: $r = 0.92$, $P < 0.001$, d.f. = 10; PAL 97: $r = 0.59$, $P < 0.05$, d.f. = 10; ENM 97: $r = 0.86$, $P < 0.002$, d.f. = 8; ALJ 97: $r = 0.91$, $P < 0.001$, d.f. = 8). Therefore, we used capsule length to estimate seed number by means of linear regression equations established for each population.

The number of seedlings was also registered along the plot transects.

GERMINATION TESTS

We tested seed germination *in situ* at ESC and JUN-1 by means of the minute seed packet technique

described by Rasmussen & Whigham (1993). A total of 125 packets containing 20 seeds each were shallowly buried in February 1997, in three different habitats: under *Rhododendron* canopy (ESC, JUN-1), under *Quercus canariensis* canopy (ESC, JUN-1) and in forest gaps (JUN-1). Packets were collected in November 1997 and immediately examined in the laboratory for the presence of a radicle.

We also quantified germination tests with pooled seed in a growth chamber ($25 \pm 1^\circ\text{C}$, daylight period of 18–20 h). Eight groups of 50 seeds were sown on filter Petri dishes and soaked with 8 mL of tap water; four of these Petri dishes were kept under illumination conditions and the four remaining were covered by aluminium foil wrapping to simulate a darkness treatment. Additionally, 24 groups of about 30 seeds were sown in glass test tubes containing 8 mL of growth medium for *Rhododendron* shoot multiplication (Anderson, 1984). These latter trials did not include a darkness treatment. Germination was indicated by the production of well-developed cotyledons.

All the seeds for germination tests were collected in open pollinated fruits from ESC and JUN.

SEEDLING SURVIVAL

We established nine plots in ENM to monitor seedling survival in July 1997, a period during which considerable seedling emergence had been observed. As can be seen in Table 1, plots show variability in size and substrate since they fit to particular sites where regular seedling emergence had been detected. Periodically, we registered the number of seedlings in each plot until July 2001. As in populations' age structure sampling, we defined two classes: (i) seedlings without leaves ≥ 20 mm long and (ii) seedlings with at least one leaf ≥ 20 mm.

STATISTICAL ANALYSES

The existence of significant correlations between the reproductive parameters estimated in the populations (i.e. fruit, flower and seeds and seedling density) was evaluated by means of simple regression analyses. Regression analyses were also performed to explore the relations between (i) capsule length and seed number (see above), and (ii) nectar production and sugar concentration.

Two-way ANOVA was performed to test for differences in fruit-set, and number of seeds per capsule, between pollination treatments (fixed effect) in the two studied populations (random effect). Fruit-set was arcsine-transformed, and number of seeds log-transformed so as to satisfy ANOVA assumptions (Sokal & Rohlf, 1995). *Post-hoc* pairwise comparisons

Table 1. Size and ecological features of the nine plots containing seedlings established in ENM used to monitor seedling survival

Plot no.	Substratum	Size (cm)
1	Soil in grassland	40 × 25
2	Soil with moss and leafy liverworts	21–23 × 15–18
3	Soil with moss and leafy liverworts	26–29 × 21–24
4	Soil with moss and leafy liverworts	40–41 × 18–21
5	Soil on tree base with leafy and thalloid liverwort and moss	40–45 × 20–22
6	Soil on tree base with moss	36 × 28–34
7	Soil with moss and thalloid liverwort	60 × 35
8	Bare soil	200 × 100
9	Rock covered by moss	140 × 90

were implemented when appropriate by means of HSD Tukey's tests at $\alpha = 0.05$ significance level.

RESULTS

POPULATION STRUCTURE

Populations of *R. ponticum* were mainly composed of adults and small seedlings (largest leaf length <20 mm; Table 2). Adult density ranged from 0.9 to 38 individuals 100 m⁻². Greater variability was detected for small seedlings; these being virtually absent in seven of the 20 populations but reaching up to 129 individuals 100 m⁻² in Arroyo de los Garlitos (GAR, 11). The largest seedling class (largest leaf length ≥20 mm) was poorly represented, being virtually absent in 14 populations. Only two juveniles were authenticated in the 20 populations sampled, one from Arroyo de los Caños (CAÑ, 7) and another from Garganta del Niño (NIÑ, 14), against a whole of 2487 adults censused.

FLORAL PHENOLOGY

Flower lifespan lasted 5–11 days (mean ± 1 SE = 8.45 ± 1.54, $n = 20$). Three progressive phases were recognized during the floral cycle (Fig. 3). The first phase (phase α) usually lasts no more than one day. During this phase, the corolla is half open, anthers show abundant pollen through their apical pores, and the stigma, still non-receptive, lies among the whorl of stamens [D (mean ± 1 SE) = 1.32 ± 0.74 cm, $n = 18$; see Fig. 3]. The second phase (phase β) lasts 1–3 days during which the corolla is now more open, anthers are gradually emptied, the style elongates, and the stigma is exerted below the anthers ($D = -0.48 \pm 1.16$ cm, $n = 27$). The stigma becomes receptive at the end of this phase β . The third phase (phase γ) lasts 3–8 days; the corolla is wide open, sometimes showing reflexed lobes; the anthers contain no pollen and the stigma lies well beyond the

Table 2. Age class density estimated in 20 populations of *Rhododendron ponticum* from southern Spain. Two seedling classes were defined: seedlings without leaves ≥20 mm long (<20 mm), and seedlings with at least one leaf ≥20 mm (≥20 mm). Densities are given in number of individuals 100 m⁻²

Sites	Seedling density (<20 mm)	Seedling density (≥20 mm)	Juvenile density	Adult density
ENM (1)	94	3.9	0	11
ALJ (2)	51.2	1.5	0	8.4
OSC (3)	7.3	0	0	8
MOR (4)	14.8	1.6	0	38
PAS (5)	18.6	0	0	23.1
MTE (6)	0	0	0	15
CAÑ (7)	2.6	1.3	0.3	5.9
MCH (8)	70.8	6.5	0	14.8
PRO (9)	0	0	0	0.9
HUV (10)	0.2	0	0	0.9
GAR (11)	129.3	5.1	0	12.6
VAL (12)	0.3	0	0	9.2
HOY (13)	31.5	0.3	0	4.9
NIÑ (14)	14.8	0.6	0.3	3.3
PTA (15)	0	0	0	3.5
TIR (16)	1.3	0	0	0.9
TAJ (17)	14.9	0.7	0	17.1
PAL (18)	7.8	0	0	20.9
ESC (19)	0	0	0	14.4
JUN (20)	0	0	0	25

anthers ($D = -0.80 \pm 0.45$ cm, $n = 9$), being receptive until the flower withers.

NECTAR PRODUCTION

Flowers produce abundant nectar, which pollinators access through the upper petal channel (Fig. 2A). Production showed a wide range from 0.7 to 8.1 μ L (mean = 4.4 μ L, $n = 22$) in bagged flowers from ENM

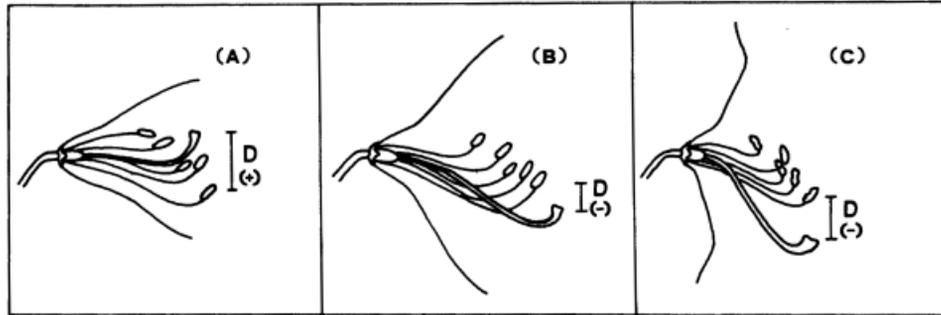


Figure 3. Diagram of the three stages in the flower cycle in *R. ponticum*. (A) Phase α , (B) Phase β , (C) Phase γ . D is the vertical distance from the stigma to the lowest anther. This parameter is positive when the stigma is above the lowest anther and negative when it is below.

Table 3. Diurnal and nocturnal visitors to flowers of *Rhododendron ponticum* and number of pollen tetrads attached to the bodies of captured individuals. When possible, upper and lower sides of insects were discriminated. Observations were made in four populations: ESC (in 1992), ENM-ALJ (in 1998), and PAS (in 2000)

Family or superfamily (order)	Species	Diurnal/nocturnal (D)/(N)	Pollen tetrads* upper side/lower side
Apoideae (Hymenoptera)	<i>Apis mellifera</i>	D	1/17, 2/111
	<i>Eucera</i> sp.	D	2/3, 8/3
	<i>Lasioglossum</i> sp.	D	0
	<i>Xylocopa violacea</i>	D	0
	<i>Bombus terrestris</i>	D	1/146, 0/0, 1/30, 0/2, 0/2, 3/9, 6/12, 3/0, 1/1, 0/0
	other medium-size bees	D	
Nymphalidae (Lepidopterae)	<i>Pararge aegeria</i>	D	0/0, 0/0, 1/19, 0/0
	<i>Euphydryas aurinia</i>	D	0/1
Sphingidae (Lepidoptera)	<i>Macroglossum stellatarum</i>	D	1/0
Noctuidae (Lepidoptera)	<i>Noctua pronuba</i>	N	0/41
Geometridae (Lepidoptera)	<i>Campaea honoraria</i>	N	1
Syrphidae (Diptera)	<i>Eristalis tenax</i>	D	7/74
Bombyliidae (Diptera)	<i>Bombylius</i> sp.	D	no data
Nitidulidae (Coleoptera)	<i>Meligethes aeneus</i>	D	2 in 5†

*Different numbers separated by commas indicate observations in different individuals.

†Data are pooled from five insects due to their small size.

and ALJ. The highest production was detected in developed flowers (phase γ). Young flowers, which opened inside the bags, contained little or no nectar. Sugar concentration in nectar ranged from 18 to 40% (mean \pm 1 SE = 27.9 \pm 6%, n = 23). No significant correlation (r = 0.18; d.f. = 20; P = 0.43) was found between nectar production and sugar concentration.

FLORAL VISITORS AND FORAGING BEHAVIOUR

Floral visitors included at least four insect orders and more than 13 species (Table 3). Among diurnal visitors, the large bees *Bombus terrestris* and *Xylocopa violacea* accounted for 65.3% of the number of visits

(Fig. 4). This global frequency mainly shows visiting activity of *B. terrestris* that carried out 59.8% and 79.3% of the visits registered in ENM and ESC, respectively; whereas in PAS, *B. terrestris* accounted for 26% of the visits and *Xylocopa violacea* accounted for 27.3%. Other medium-size hymenopterans have been registered, but accounted for 15% of the visits. Lepidopterans (moths and butterflies) and dipterans also visited *Rhododendron* flowers, particularly the hawk moth *Macroglossum stellatarum* that carried out 32.2% of the visits registered in ENM. The small coleopteran *Meligethes aeneus* (1.5–2.7 mm in length) was very frequent in most populations (up to 20 beetles in a single flower). Most insects visited *Rhododen-*

dron flowers to feed on nectar, although the small bee *Lasioglossum* sp. and the coleopteran *M. aeneus* also collected pollen.

Most hymenopteran specimens captured were detected to carry pollen tetrads on their bodies (Table 3), so they are assumed to be suitable pollen vectors. Ten individuals of *Bombus terrestris* were analysed, of which eight had pollen tetrads attached. When this species lands on *Rhododendron* flowers the ventral surface of its abdomen invariably contacts the anthers' tips and the stigma (Fig. 2). This bumblebee usually visits several flowers per raceme and makes short flights to neighbouring racemes; eventually, it makes long flights to keep on feeding in other groups of racemes. No pollen was found on the single individual of *Xylocopa violacea* captured, but the visit behaviour observed in PAS was very similar to those of *B. terrestris*, and suggests a similar role as pollen vector. A high number of pollen tetrads was also found on the body of the single dipteran specimen analysed (*Eristalis tenax*), but the few visits involved and the small size lessen the importance of dipterans as

pollinators. Little or no pollen was found in five out of six diurnal lepidopteran specimens captured (Table 3). Many moth species, of variable size, dipterans and some coleopterans were captured by the light-trap. Of these species, only the large moths *Noctua pronuba* and *Campaea honoraria* were found to carry *Rhododendron* pollen on their bodies.

CONTROLLED POLLINATIONS

Fruit set did not vary between the populations studied (JUN and ESC), but it differed consistently among treatments for both populations (Table 4, Fig. 5). Fruit-set for bagged, non hand-pollinated flowers (autonomous self-pollination treatment, SP) was low, but did not differ significantly from that recorded for open-pollinated flowers (FP). All the hand-pollination treatments (FSP, ICP, BCP, XFP) had equivalent fruit-set, which were significantly higher than those of the two former, non hand-pollinated treatments (Fig. 5)

Seed set in developed capsules was significantly higher in JUN than in ESC, but no significant differences were found among treatments (Table 4).

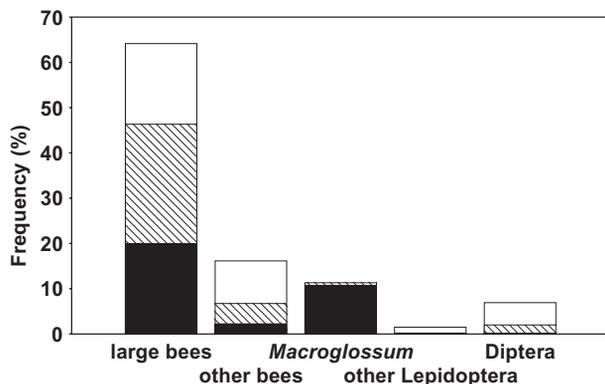


Figure 4. Frequency of number of visits of diurnal insects to *R. ponticum* flowers in ENM (filled section), ESC (shaded section) and PAS (open section) sites. Large bees' visits correspond to those of *Bombus terrestris* and *Xylocopa violacea*.

FLOWER, FRUIT, SEED AND SEEDLING DENSITY

Flower, fruit, seed and seedling density in five populations, one of them with two subpopulations (JUN-1 and JUN-2), over two years is summarized in Table 5. Fruit density correlated strongly and positively with flower density ($r = 0.90$; d.f. = 8; $P < 0.001$). Seed density was also significantly and positively correlated to fruit density ($r = 0.84$; d.f. = 8; $P < 0.005$).

We found small, 1-year-old seedlings in the transects, but neither saplings nor 2-year-old seedlings. This highlights the scarcity of juveniles in the wider population structure survey (see above). The density of seedlings ranged from 0 to 7 seedlings m^{-2} , being zero over the two years in three of the six sites studied (Table 5). Seedling density was not significantly correlated with either seed ($r = 0.28$; d.f. = 8; $P = 0.44$) or fruit ($r = 0.13$; d.f. = 8; $P = 0.73$) densities.

Table 4. Two-way ANOVA results for the comparison of fruit-set ratio (arcsin-transformed data) and seed set in developed fruits (log-transformed data) among pollination treatments between both populations. Significance level at $P < 0.05$

Effect	Fruit set ratio			Seed set		
	d.f.	MS	F	d.f.	MS	F
Population (P)	1	0.384	2.86 ns	1	10.774	11.89 **
Treatment (T)	5	1.190	8.86 ***	5	0.822	0.91 ns
Interaction (P × T)	5	0.106	0.79 ns	5	1.640	1.81 ns

*** $P < 0.0001$, ** $P < 0.002$, ns not significant.

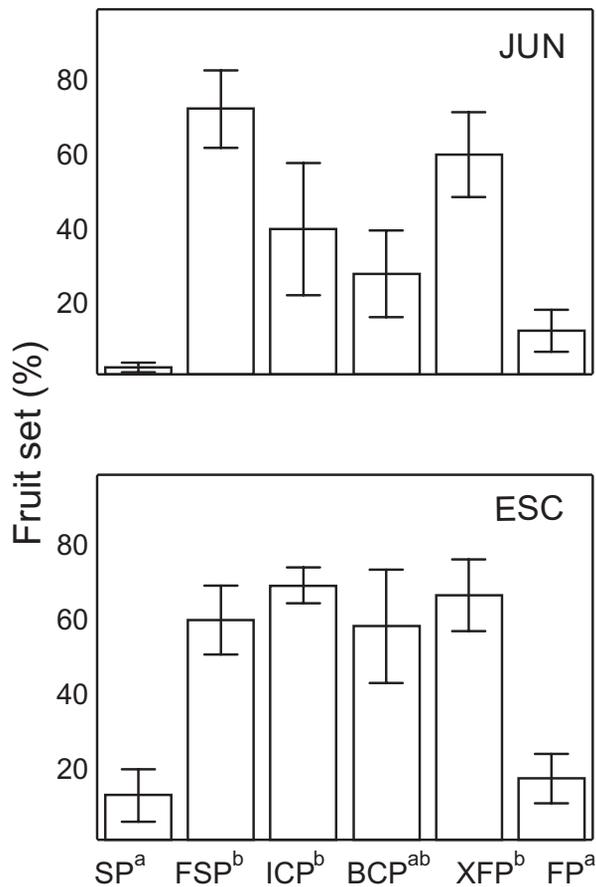


Figure 5. Fruit set in experimental pollinations of *R. ponticum* flowers in JUN and ESC populations during 1993. SP, autonomous self-pollination; FSP, forced self-pollination; ICP, intrapopulation cross-pollination; BCP, between-population cross-pollination; XFP, supplemented pollination; FP, freely pollinated flowers. Same letters as superscripts indicate non-significant differences between treatments after Tukey HSD *post hoc* paired comparisons for pooled data from both populations.

GERMINATION TESTS

The *in situ* germination tests carried out in ESC and JUN-1 produced no natural seed germination. Only 73 packets (1460 seeds) remained out of the 125 initially buried. In these packets only three seeds produced a radicle, but cotyledons were never developed, indicating a virtual 0% germination. Germination tests on 200 seeds in Petri dishes yielded 45% germination after 33 days for the illumination treatment, compared to a mere 3% for the darkness treatment. Light germination tests in Anderson growth medium of 720 seeds yielded 90% germination after 30 days.

SEEDLING SURVIVAL

Plots were established during an active period of seedling recruitment corresponding to the rainy previous year (October 1996 to September 1997: 1419 mm; weather station El Picacho; average: 1200 mm). Very young seedlings (without leaves ≥ 20 mm) were abundant at the beginning of the observation period (July 1997). All of these probably germinated the previous spring. This number of seedlings gradually decreased in the plots (Fig. 6). However, in the two plots on bare soil at ENM where seedlings were detected, the number of seedlings decreased drastically and finally reached zero during the next two dry years (October 1998–September 1999: 683 mm; October 1999–September 2000: 606 mm), although some recruitment took place later (Fig. 6). In contrast, the decrease of seedlings was considerably buffered in most plots on bryophyte carpets although sudden disappearance occurred in some of them (Fig. 6). The density of large seedlings (with at least one leaf ≥ 20 mm) was always lower compared to that of small seedlings and persistence of these large seedlings was clearly favoured in bryophyte plots. We observed an increase in them (Fig. 6) due to moderate rainy conditions towards

Table 5. Flower, fruit, seed and seedling number per square metre in six plots in two yearly observations (autumn 1996 and 1997)

Population (year)	Flowers m ⁻²	Fruits m ⁻²	Seeds m ⁻²	Seedlings m ⁻²
JUN-1 (1996)	10	3	458	0
JUN-1 (1997)	7	6	1 819	0
JUN-2 (1996)	64	38	2 042	0
JUN-2 (1997)	13	7	1 479	0
ESC (1996)	105	21	827	0
ESC (1997)	102	47	880	0
PAL (1996)	138	76	3 702	0
PAL (1997)	148	106	12 235	0.5
ALJ (1997)	78	46	5 354	7
ENM (1997)	12	9	865	0.8

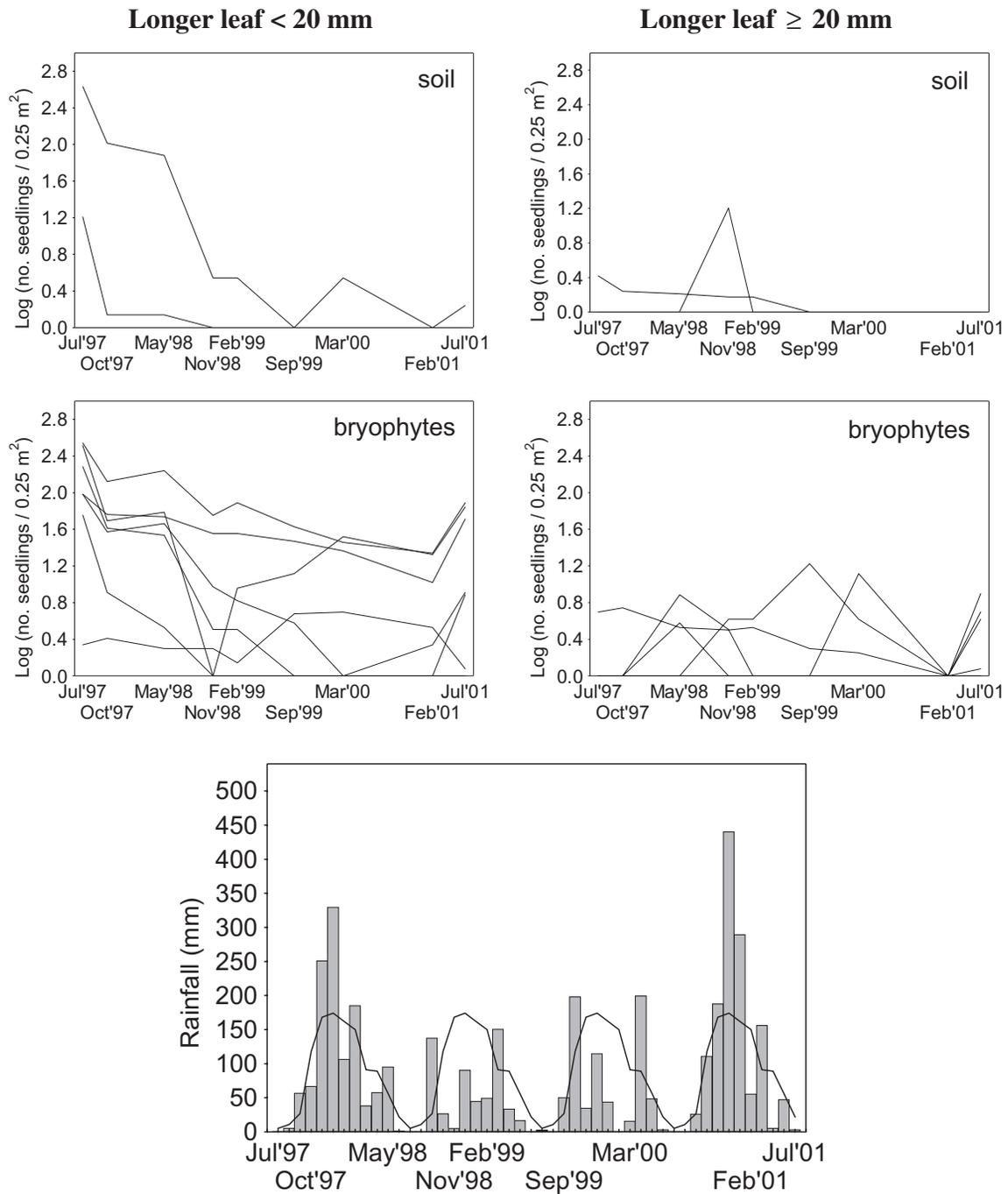


Figure 6. Seedling density across time in the nine plots established in ENM site. Two plot classes were considered: bryophytes (on moss and liverworts carpets, $n = 7$) and soil (not on these carpets: bare soil, $n = 2$) (see Table 2). Two seedling classes were represented: seedlings with longer leaf < 20 mm, and seedlings with longer leaf ≥ 20 mm. Lower figure: monthly rainfall; the line shows the mean monthly rainfall.

the end of the study (October 2000 – June 2001: 1293 mm). There was a high temporal increment of large seedling (≥ 20 mm) density in one of the soil plots, due to the growth of the many recruits following a wet period (July 1997–May 1998). However, a later dry period (1998–1999) rendered effective recruitment to be zero.

DISCUSSION

POPULATION STRUCTURE

Rhododendron ponticum populations in southern Spain contain a conspicuous scarcity of seedlings and a general lack of juveniles. There is a virtual absence of sexually derived recruitment in the area. The biological significance of this must be carefully interpreted, since sexual regeneration of populations formed by long-lived plants could be accomplished through sporadic recruitment (Watson *et al.*, 1997; Pierson & Turner, 1998). The age of individual plants is extremely difficult to determine due to vigorous resprouting from lignotubers, even after complete loss of aerial biomass. Some branches have shown up to 120 annual growth rings (unpublished data), and so individual plants may be much older. Notwithstanding this, our observations contrast sharply with *R. ponticum*'s reputation as an aggressive invader in temperate Atlantic areas of Western Europe (mainly Britain and Ireland; Cronk & Fuller, 1995), following introduction probably from southern Spain (Milne & Abbott, 2000). The main invasion process in these areas takes place by effective seedling recruitment (*R. ponticum* lacks any special vegetative dispersal propagules), and the species has become a serious threat to biodiversity in natural woodland, heathland and peatland communities (Cross, 1981; Mitchell *et al.*, 1998).

FLORAL BIOLOGY AND VISITORS

Rhododendron ponticum flowers show incomplete protandry and a wide separation of anthers and stigma during stigma receptivity ('approach herkogamy'; Webb & Lloyd, 1986), which acts to prevent autonomous self-pollination. Flowers can be considered long-lived, lasting approximately eight days (Primack, 1985). They are functionally female (phase γ) for more than two-thirds of their life-span, and the highest nectar production takes place in mature flowers, a common feature within the genus *Rhododendron* (Philipson, 1985).

Plants flower in spring and populations are in bloom for a period of about two months, and are particularly attractive to pollinators (Arroyo, 1988). Scattered flowers can be seen also during autumn–winter, but fruit-set corresponding to this late flowering is

extremely reduced (personal observations). Flower production can reach high densities that approaches 150 flowers m^{-2} in sunny sites (e.g. PAL population, see Table 5), but areas showing low flower density can often be found, generally in shady locations. Sunlight is probably an important factor promoting flowering in this species (Cross, 1981). A considerable diversity of insects, whose body lengths range from 1.5 to 30 mm, has been detected foraging on *R. ponticum* flowers. The attractive floral display, and the wide funnel-shaped corolla allows a generalistic pollination system (Arroyo, 1988), as seems to be common in the genus (Stevens, 1976; Argent, 1985; Williams *et al.*, 1990; Ng & Corlett, 2000).

Most *R. ponticum* insect visitors (hymenopterans, lepidopterans and dipterans) seek nectar. They get pollen tetrads attached mainly on the abdominal side of their bodies (sternotribic pollinators: Faegri & van der Pijl, 1979). The amount of nectar is large enough to fulfil the energy needs of the large insects. Sugar concentration is variable and probably related to individual features of the flowers (e.g. flower age, position in the raceme and the plant) given the lack of correlation with nectar volume. This concentration is also adequate for a variety of insects (Baker & Baker, 1983), such as those that visit *R. ponticum*. This array is even wider since it also includes pollen-feeding insects such as the opportunistic pollen beetle, *Meligethes aeneus*, which is usually considered a flower pest (Thies & Tschardt, 1999; although no damage in *R. ponticum* flowers was observed).

According to the pollen tetrads found on their bodies (Table 3), pollination efficiency of *R. ponticum* visitors seems to be very variable, probably with large and medium hymenopterans and dipterans being the most effective pollinators, as has been proposed for some other species (Ng & Corlett, 2000). *Bombus terrestris* was found to be the most regular visitor on *R. ponticum* flowers, even though the other large bee, *Xylocopa violacea*, an irregular flower visitor, displaces *B. terrestris* during warmest periods on sunny days (personal observations). However, both taxa showed a similar foraging behaviour, usually visiting many flowers in a raceme, which probably promotes geitonogamy. Nocturnal insects were also found to visit flowers and the moth *Noctua pronuba* might be an efficient pollinator, according to the pollen tetrads found on the underneath part of a captured specimen (see Table 3). Unfortunately, the method used (capture by light-trap) did not allow observations of its foraging behaviour.

BREEDING SYSTEM

Fruit and seed set are strongly reduced under pollinator exclusion (SP), but hand self-pollination (FSP)

yielded abundant fruit; therefore *R. ponticum* is fully self-compatible, but not agamosperous. Palser *et al.*, (1992) found similar results using garden plants. Protandry and herkogamy most probably account for low autonomous selfing. This reproductive behaviour is common within the genus (Palser *et al.*, 1992; Kudo, 1993; Escaravage *et al.*, 1997) but an important number of species seem to show a post-zygotic mechanism for self-sterility (Williams *et al.*, 1984; Padrutt *et al.*, 1992; Ng & Corlett, 2000) for which no evidence has been found here. All hand cross-pollinations produced similar fruit-set, irrespective of the origin of pollen (within- or between-population crosses). This is consistent with theoretical predictions of the loss of deleterious effects of inbreeding in selfers (Charlesworth & Charlesworth, 1987). Although the actual selfing rate of the populations is unknown, the breeding system and the pollinator behaviour suggest high levels of geitonogamy. Low fruit-set (less than 20%) after natural, non-manipulated pollination (FP), similar to that of flowers under pollinator exclusion (SP), contrasted with fruit-set after hand-pollinated (up to 70%; see Fig. 5), and suggests pollen limitation for fruit-set. This result is surprising given the large floral display and apparent high insect activity in populations. Pollen limitation is probably very variable across years and sites (Table 5), as found in other Mediterranean areas and plants (e.g. Baker, Barrett & Thompson, 2000). The low variability in seed number in developed fruits, irrespective of experimental pollination treatment, contrasted with the considerable variation in fruit-set (Table 5). In both hand- and natural-pollinated flowers, the stigma received enough pollen grains to sire all possible seeds.

SEED AND SEEDLING BIOLOGY

Considerable numbers of seeds were produced as a result of the high number of flowers per plant and the breeding system, even in those populations where no seedlings were detected. However, important differences were found among sites and years (458–12235 seeds m⁻², see Table 5). Little fruit or seedling damage by herbivory was observed, suggesting that a substantial seed bank is created every season, which should be adequate to promote some sexual regeneration. Seeds germinate readily in the laboratory without any particular treatment to break dormancy (although they need light), indicating that recruitment failure does not result from seed non-viability. Seedlings have mainly been found on humid rocks or soils covered by bryophyte carpets, as Cross (1981) also indicated for Irish populations. In these particular sites, seeds can find buffered moisture and temperature conditions, and light levels suitable for germination and persistence (i.e. effective recruitment). Although virtually no

seeds germinated in the buried packets, all were located in bare soil, under or close to adult plants. Technical difficulties prevented us from performing this experiment in the thin bryophyte carpets, where we only could observe seedlings, but seed germination should be frequent in these microsites.

Effective recruitment is most probably jeopardized by the slow growth rate of seedlings and young plants under field conditions (personal observations). This is illustrated by the scarcity of large seedlings in monitored plots from ENM (Fig. 6) and the virtual lack of juveniles (only two juveniles from a whole of 2487 individuals censused throughout all the populations sampled; see Table 2). We hypothesize that drought episodes, particularly the characteristic Mediterranean-climate summer drought, but also the low rainfall reliability of the rainy season proper of this region (Ojeda, 2001), constitute the most important limiting factor for seedling establishment in the area. Cross (1981) reported high seedling mortality in Irish populations in exceptional dry years, while we saw seedling emergence in exceptional wet periods in southern Spain.

Relict populations of *R. ponticum* likely persist in the area despite a virtual lack of sexual recruitment, because prostrate branches have been observed to casually produce new ramets by layering. In fact, in all cases when plants were suspected to be saplings, excavation revealed a layering origin from a bigger plant, even when plants were 7–8 m apart. The ability to perpetuate by means of non-specialized layering is probably not rare among *Rhododendron* species (Peterson & Jones, 1997; Naito *et al.*, 1999). This mechanism allows persistence in populations of the closely related *R. maximum*, which has limited sexually derived recruitment (Plocher & Carvell, 1987) and in other species such as *R. metternichii* (Naito *et al.*, 1999) and *R. ferrugineum* (Pornon *et al.*, 1997; Escaravage *et al.*, 1998). Nevertheless, the occurrence of sporadic sexually derived recruitment events in southern Spain populations, sufficient to maintain sustainable population dynamics and genetic diversity (van Groenendael *et al.*, 1997), should not be dismissed. *R. ponticum* is a long-lived species, and these factors should be explored by population matrix models; there may, however, be some problems because of the difficulties in age estimation and a lack of correlation between age and size due to resprouting.

A CASE OF RELICTNESS IN THE MEDITERRANEAN

By examining the whole life cycle of *R. ponticum* in southern Spain populations, we have shown that its various life history stages cope differently with the current environment of the species in this region. Its

floral biology is typical of a generalist species: spring flowering phenology, many different pollinators, and no genetic incompatibility system limiting seed set. Therefore, pollination does not seem to be a limiting factor and huge numbers of viable seeds are released. Seed dispersal has not been studied, but there is no reason to infer that it is environmentally limited: seeds are abundant, minute, and wind-dispersed. By contrast, seed germination and seed establishment are strongly limited in all the studied populations. As in many plant species, this possible demographic bottleneck in sexual recruitment may be overcome by vegetative propagation, assuring population persistence.

Considering both natural (southern Spain) and alien (British Isles) populations, *R. ponticum* shows a variable balance between sexual and vegetative reproduction, depending on particular habitat features (see Eriksson, 1993, for review). According to molecular evidence, it should be noted that invasive populations from Britain have originated mainly from southern Spain populations (Milne & Abbott, 2000), which indicates that differences in recruitment patterns between native and alien populations may be explained as environmentally driven differences rather than genetically determined differences. However, the effect of some selective pressure favouring some genotypes during the invasion (about two centuries) should not be neglected. Moreover, Milne & Abbott (2000) found in British plants evidence of introgression from *R. catawbiense* and other species, which could confer some cold tolerance favouring its spread at least in the coldest area of the British range of the species.

Rhododendron ponticum is a relict endemic whose distribution has been considerably reduced by climate changes and fluctuations during the late Tertiary and Pleistocene (Cross, 1975, and references therein). Global cooling through the Tertiary would have forced its retreat to southern Europe, and the establishment of a Mediterranean-type climate with long summer droughts would have determined its present disjunct distribution (Fig. 1). Changes in distributional range would have taken place by means of repeated extinction processes over large parts of its range, and expansions from refugia, depending on climatic oscillations (Comes & Kadereit, 1998; Hewitt, 2000). During favourable, temperate periods, populations would mainly perpetuate and expand by effective sexual recruitment, as shown currently by alien populations in the British Isles (Cross, 1975, 1981) and New Zealand (Webb *et al.*, 1988). It can be postulated that the most recent favourable period in southern Spain populations for expansion took place during the humid Holocene optimum 9000–6000 years BP (Pons & Reille, 1988; Huntley & Prentice, 1993). Seasonal, non-favourable periods may have hampered the recruit-

ment process, most likely because of seedling establishment failure particularly during the dry season (e.g. summer under Mediterranean-climate regime). Under such conditions, populations would persist only if adult individuals are able to endure them, and perpetuate by vegetative means (e.g. layering). This seems to be the prevailing mechanism in present Iberian populations, which has prevented them from extinction. Nevertheless, if environmental conditions became too severe (e.g. changes derived from global climate warming), the perpetuation of these populations would be jeopardized (McCarty, 2001). Moreover, drastic disturbance events before the return of favourable climatic conditions for the species might also seriously endanger the future of these relict populations, which have no possibilities for natural regeneration under current climate conditions. *Rhododendron ponticum* is an ideal model system for integrative studies of both invasion and relictness, since the current species range covers large native areas with wide ecological amplitude, at least on elevation (Black Sea region; Denk *et al.*, 2001), narrow, relict areas of precise ecological conditions (Iberian Peninsula), and present invasive ranges (e.g. British Isles, where the species was even native in former geological periods, Fig. 1).

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