Reproductive biology in *Cytisus multiflorus* (Fabaceae)

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We studied the reproductive biology of two morphs (LF, large flowers; and SF, small flowers) of *Cytisus multiflorus*, an endemic legume from SW Europe. Visits by pollinators are indispensable for fruit-set to take place. After hand self-pollination, all the individuals have a uniform behaviour with null fruit, in spite of self-penetration of the ovules. This and the fact that the abscission of initiated fruit occurs en masse at the same time seems to indicate the possibility of a late-acting self-incompatibility in this species. Intramorph crosses showed that the SF morph has a significantly greater reproductive success than the LF morph. Intermorph crosses were quite ineffective due to the lesser viability of the LF morph pollen, although we cannot discard the possibility that its ovules are also less viable. The situation is suggestive of the presence of a partial reproductive barrier between the two morphs that operates both before and after crossing.

Key words: breeding system, *Cytisus multiflorus*, Fabaceae, pollination, self-incompatibility

Introduction

In the family Fabaceae, Arroyo (1981) found that only 22.1% of the species studied of Papilionoideae were self-incompatible. This proportion was very low in comparison with that of Caesalpinioideae (62.3%) and Mimosoideae (66.7%). She noted that self-incompatibility (SI) largely correlates with the woody habit in this family, so it would be reasonable to expect SI to be present in the tribe Genisteae, with a majority of woody genera. However, only three of its constituent genera had been studied for SI (*Argyrolobium*, *Lupinus*, and *Ulex*), and it was detected in only one species of the last genus (*see* Arroyo 1981). Very few woody species of this tribe have been investigated in any depth since the review by Arroyo (1981). We can only cite the case of *Chamaecytisus palmensis* (endemic to the Island of La Palma, Canary Islands), which behaves as self-compatible (SC) in naturalized populations of New Zealand (Webb & Shand 1985), and that of *Cytisus striatus* (endemic to the W of Iberian peninsula) and *Retama sphaerocarpa* (endemic of the W of the Mediterranean Region) which behave as SI (Rodríguez-Riaño *et al.* 1999a).

Most of the woody representatives of the Genisteae have yellow flowers, a papilionaceous corolla, and a monadelphous androecium with dimorphic anthers (Polhill 1976, López *et al.* 1999, Rodríguez-Riaño *et al.* 1999b). Their flowers have a great production of pollen (López

et al. 1999, Rodríguez-Riaño *et al.* 1999c) and are clearly adapted to bee pollination. Also the most widespread pollen presentation mechanism in this tribe is of the valvular type, in which the reproductive column (androecium and gynoecium) is released from the keel, and a small cloud of pollen adheres to the ventral part of the insect. The column comes back to its initial position upon release from pressure by the insect (López *et al.* 1999, Rodríguez-Riaño *et al.* 1999b).

The scarcity of reproductive studies, both for this tribe and for the subfamily in general, may be due to the difficulty in handling the flowers, since anther dehiscence occurs very early. In some Leguminosae pollen is usually released while the flower is in the bud stage, and in this stage is viable (Asmussen 1993, Rodríguez-Riaño *et al.* 1999a, Rodríguez-Riaño *et al.* 2001). Nonetheless, although the stigma is receptive in this phase, its receptivity is inoperative due to the presence of a stigmatic surface that blocks the germination of the pollen grain. Only the rupture of this surface by a pollinator will allow the pollen to germinate (Shivanna & Owens 1989, Rodríguez-Riaño *et al.* 1999a).

In *Cytisus striatus* and *Retama sphaerocarpa*, studies of pollen-pistil interaction have indicated the existence of a certain degree of self fertilization, confirming the low fruit-set obtained after self-pollination (2.4%–3.5% in *Cytisus* and 3.5%–4.3% in *Retama*). Also, the high level of self-sterility in both species seemed to be due rather to an early action of inbreeding depression than to late acting self-incompatibility (= LAS; Rodríguez-Riaño *et al.* 1999a). It is really difficult, however, to distinguish between the two possibilities, and we must use clues such as: (1) in the case of the inbreeding depression, seed-set varies widely between selfed individuals, whereas in the LAS phenomenon the behaviour is uniform between individuals, with values of selfed seeds either zero or close to zero; (2) the blocking or rejection of embryos occurs over a wide range of stages (from the first cell divisions to germination) in the inbreeding depression, and more or less in the same state in the LAS; (3) the fall of flowers and young fruit after selfing occurs progressively over time in the inbreeding depression, and approximately at the same time

in LAS (Seavey & Bawa 1986, Seavey & Carter 1994, Gibbs & Sassaki 1998).

In this paper we study the reproductive biology of *Cytisus multiflorus*, an endemic shrubby Genisteae from the W of the Iberian peninsula, which is a good representative in Mediterranean woodlands or in the scrub substituting these woodlands. A knowledge of their breeding system is indispensable for decision-making relative to flora and vegetation conservation, to deforested zone recovery, the sustainable use of certain zones (e.g. for apiculture), or even for their practical use as ornamentals not requiring irrigation, especially in Mediterranean type climates where water is the limiting resource. *Cytisus multiflorus* is a winter-flowering species in the study population (Rodríguez-Riaño *et al.* 1999d) and produces a great number of white flowers per plant (range = 8259–15 228; Rodríguez-Riaño *et al.* 1999d). The flowers have a valvular type pollen presentation system (López *et al.* 1999), they produce no nectar and its longevity is 9.4 ± 1.6 days (Rodríguez-Riaño *et al.* 1999d). In a previously studied population (*see* Study site) two floral morphs (SF, small-flowered morph; LF, large-flowered morph) were detected. These differed by the size of the flowers (mean ± S.E., LF = 14.5 ± 0.10 mm, range 13.0–16.0 mm; SF = 10.7 ± 0.17 mm, range 9.0–12.75 mm) and the reproductive pieces (anther volume, size of the pollen grains, and size of the ovary), with significantly greater values in the LF morph than in the SF morph, but not in their morphology or colour. Also, the production of pollen per flower is significantly greater in the LF morph than in the SF morph, but the percentage germination of pollen *in vitro* is significantly greater in the SF morph than in the LF morph. With respect to female function, the production of ovules per flower does not differ significantly between the two morphs (for further details *see* Rodríguez-Riaño *et al.* 2001).

The two morphs presented a phenologically differentiated pattern, reaching their peak of maximum flowering with a difference of ca. 4 weeks, with the flowering of the LF morph earlier than that of the SF morph, and although in both cases the final crop of fruit and seeds was sparse, it was much smaller in the LF morph

(Rodríguez-Riaño *et al*. 1999d). The pollen–pistil interaction studies showed that a percentage of self pollen germinated on the stigma, and that in the SF morph the percentages of ovules penetrated by pollen tubes after both hand self-pollination (54.9%) and hand cross-pollination (56.8%) were similar; in the LF morph the percentage after hand cross-pollination (39.9%) was significantly greater than after hand self-pollination (20.9%). This indicated that the rejection of the self pollen tubes was greater in the LF than in the SF morph (Rodríguez-Riaño *et al*. 2001). Also, in the intermorph crossing experiments, the ovular penetration was found to be lower (although not significantly) when the LF morph was the pollen donor (SF × LF) than when the SF morph was the donor (LF × SF). This could be due to a lesser viability of the male function in the LF morph (Rodríguez-Riaño *et al*. 2001).

The objectives of the present work were: (a) to determine the reproductive biology of the two morphs of *Cytisus multiflorus*; (b) to determine the production levels of fruit and seeds in both intermorph pollination experiments and intramorph crosses in order to explain the results of the pollen–pistil interactions. Furthermore, we discuss the main biological processes that would be found in this species, the LAS or the inbreeding depression, given the complete self-sterility of both morphs. Also, we discuss the problems related to the intermorph incompatibility which might be turning into a partial barrier of reproductive isolation.

Materials and methods

Study site

The study population was located in the El Hito country house, belonging to the municipal district of Alburquerque (Badajoz, SW Spain), between 390 and 395 m a.s.l., on a granitic substrate and subject to a typical Mediterranean climate. The vegetation is scrub consisting mostly of *Cytisus multiflorus* and, to a lesser degree, *C. striatus* (for more details *see* Rodríguez-Riaño *et al*. 1999d). The present study was carried out during 1996 and 2000.

Pollination and breeding system

The pollen/ovule ratio (P/O) for each morph (10 flower buds from 20 individuals in each morph) was estimated according to Cruden (1977). In the field, insect censuses were made, recording the number of visits received by a series of 20 selected plants in an area of some 20 m². The census was performed over 18 h spread out over 23 d throughout the blooming time (January, February, and March), at intervals of approximately three hours and for a period of 10 min/observation. The duration of each visit was recorded, as well as the type of pollinator, its behaviour on the flowers, the time of day, and the weather conditions at the study site. We considered those insects to be pollinators, which made contact with the flowers' sexual organs during a visit.

To determine the breeding system, a series of plants was selected and subjected to the following pollination treatments: (A) natural pollination (control); (B) spontaneous self-pollination (SSP, bagged plants); (C) hand self-pollination (HSP); and (D) hand cross-pollination (HCP). The last was further subdivided into two categories: intramorph hand cross-pollination (Intra-HCP) and intermorph hand cross-pollination (Inter-HCP), using either the SF morph as pollen receptor and the LF as donor (SF × LF) or vice-versa (LF × SF). Hereafter, in describing crosses within and between morphs the maternal parent is always listed first.

In 1996, two or three plants per treatment were manipulated and given the great quantity of flowers per branch produced by this species, and the impossibility of hand-pollinating all the flowers per plant, all the non-manipulated branches were cut before beginning the experiments. We wished thus to avoid a redistribution of resources by the plant towards the manipulated flowers relative to the unmanipulated flowers (*see* Zimmerman 1988). Also, all the flowers of a given plant were manipulated with a single type of treatment. Although many flowers per plant were manipulated (Table 1), the sample size (number of individuals studied) was very low (*N* = 2–3 plant per treatment). This prohibited any reliable statistical analysis, and obliged us to repeat the experiments in 2000 with appropriate sample

sizes (300–400 flowers per treatment and 13–17 plants per morph; Table 2).

In control, flower-bearing branches of different sizes were selected at random and tagged, with no further manipulation of any kind. In SSP, flower-bearing branches were also selected, and were bagged with nylon mesh until the time of fruit-set. In both, HSP and HCP, the flower-bearing branches were bagged after hand pollination and although all the selected flowers were virgin, their anthers had already dehisced, and therefore the stigma appeared generally covered by the flower's own pollen grains. Hence, in HCP, the staminal column and the gynoecium were carefully freed from the keel, the anthers removed and the stigma was brushed clean of pollen grains using a fine paintbrush. Simultaneously we provoked an artificial rupture of the stigmatic surface with the paintbrush. Pollen grains coming from different flowers distant by some 100 m were put in place with a fingernail to assure a sufficient pollen load. A judgment was made of the optimal pollen load after comparing the load on the stigmas of 20 flowers with natural pollination (mean ± S.E.; 83.33 ± 3.56) to that deposited artificially with a fingernail on another 20 emasculated flowers (143.67 ± 11.67), the load being greater in the latter case. Finally, each flower was tagged on the base of the calyx with plastic paint. In HSP the process was similar, but the stigma was rubbed with a fingernail on which a great quantity of pollen belonging to another flower of the same plant had been placed. In a previous series of trials, the stigma had been found to suffer no damage resulting from breaking its surface with a fingernail. Hand pollination was only done once, since further pollinations, rather than adding new pollen grains, would eliminate those already existing and germinated.

In all pollination treatments and years the fruit produced were counted, collected, and stored individually in opaque paper sachets for their later study in the laboratory. The fruit-set percentage, and the seed-set percentage were determined. We obtained the self-compatibility index as a function of the data relative to fruit-set and that relative to seeds — the average fruit or seed-set after self-pollination divided by the fruit or seed-set after cross-pollination (Zapata & Arroyo 1978, Lloyd & Schoen 1992). We also calculated the self-fertility index (Lloyd & Schoen 1992) for both of the reproductive parameters — the fruit or seed-set of isolated plants divided by that of artificial cross-pollinations.

Statistical analysis

We used the statistical program packages Statistica for Windows version 5.0 (StatSoft 1995), performing one-way analysis of variance and Tuckey test for multiple comparisons at $P < 0.05$ (Sokal & Rohlf 1979).

Table 1. Production of flowers (Fl), fruits (Fr), fruit-set percentage, seeds number per fruit (S), ovule number per ovary (O) and seed-set percentage in the LF (large flowers) and SF (small flowers) morphs of *Cytisus multiflorus* in the year 1996. For each treatment is given the mean value ± S.E. N = plant number, Control (natural pollination), SSP (spontaneous self-pollination), HSP (hand self-pollination), HCP (hand cross-pollination), ♀ = pollen receptor plant, ♂ = pollen donor plant.

		N	Fl	Fr	Fruit-set (%)	S	O	Seed-set (%)
Control	LF	2	630	13	2.08 ± 0.26	1.38 ± 0.05	9.51 ± 0.65	14.72 ± 1.67
	SF	2	850	200	23.24 ± 1.15	2.07 ± 0.20	9.70 ± 0.15	21.52 ± 1.63
SSP	LF	3	540	0	0	–	–	–
	SF	3	723	0	0	–	–	–
HSP	LF	2	474	0	0	–	–	–
	SF	2	272	0	0	–	–	–
HCP (inter- and intra-morph)								
	LF♀ × LF♂	2	331	20	6.05 ± 0.05	1.46 ± 0.04	8.25 ± 0.25	18.22 ± 0.13
	LF♀ × SF♂	2	375	23	6.09 ± 0.28	1.36 ± 0.14	8.40 ± 0.04	16.37 ± 1.71
	SF♀ × LF♂	2	294	3	1.01 ± 0.32	1.00 ± 0.00	10.75 ± 0.25	9.32 ± 0.23
	SF♀ × SF♂	2	341	218	63.92 ± 0.09	4.04 ± 0.09	10.21 ± 0.11	39.59 ± 1.52

Results

Cytisus multiflorus behaved as xenogamous according to the classification of Cruden (1977). The mean value ± S.D. of the P/O ratio was 6210.6 ± 1454.2 for the LF morph and 4064.5 ± 1001.1 for the SF morph, the difference being significant (one-way ANOVA: $F = 29.6$, $P < 0.001$, $N = 20$).

The total number of insects really visiting the flowers was 150, all belonging to the honey-bee (*Apis mellifera*), which made all their visits on sunny days. Other bees were not observed and two causes could explain this: (1) the bee inactivity at the flowering time of *Cytisus multiflorus* (winter) and (2) the study site was very degraded. The times of day with greatest intensity of pollination were between 09:00–10:00 and 13:00–14:00. The time employed per visit was 4 to 10 sec, with an average of 15 visits in 2 min (range 4–25 visits), either to different flowers of the same plant or to flowers of nearby plants (at distances of no more than 2 m). We observed that each flower could be visited several times in their life history, although new visits by bees lasted shorter than the first one. Nevertheless, on some occasions the insect aban-

doned its intention of browsing on previously visited flowers upon landing. With respect to its behaviour in the flower, the honeybee landed on the wing petals and the keel, with its head near the standard. It then exerted a certain pressure with its legs on the wing petals until these and the keel bent downwards, and then proceeded to collect pollen. After the visit, only the insect's abdomen appeared pollen smothered (sternotribic pollen deposition).

In 2000, the fruit-set of the control was low in the LF morph (4.7%), significantly smaller than that found in the SF morph (17.5%; Table 2). The situation was similar in 1996 (SF = 23.2% and LF = 2.1%, Table 1). The SSP led to no fruit-set in either year, which indicates that insect visits are indispensable for the pollination of this species (Tables 1 and 2).

In HSP no fruit-set was obtained in either morph in either year (Tables 1 and 2), which could indicate the presence of SI. A number of facts, however, pointed to the existence of a certain degree of self-fertilization in this species, with final rejection of the embryos: (1) many initiated fruit reached a considerable length, surpassing 15 mm (viable ripe fruit are ca. 21 mm in length); (2) initiated and then aborted fruit

Table 2. Production of flowers (Fl), fruits (Fr), fruit-set percentage, seeds number per fruit (*S*), ovule number per ovary (*O*) and seed-set percentage in the LF (large flowers) and SF (small flowers) morphs of *Cytisus multiflorus* in the year 2000. For each treatment is given the mean value ± S.E. Beneath the last treatment is given the test used to compare treatments: *F* (one way anova). *N* = plant number, Control (natural pollination), SSP (spontaneous self-pollination), HSP (hand self-pollination), HCP (hand cross-pollination), ♀ = pollen receptor plant, ♂ = pollen donor plant.

		N	Fl	Fr	Fruit-set (%)	*S*	*O*	Seed-set (%)
Control	LF	17	618	28	4.67 ± 0.76[ac]	1.5 ± 0.15[ab]	10.07 ± 0.16[ac]	14.91 ± 1.44[ab]
	SF	16	536	108	17.47 ± 1.99[b]	1.95 ± 0.16[b]	9.78 ± 0.12[abc]	19.88 ± 1.50[a]
SSP	LF	14	543	0	0	–	–	–
	SF	16	667	0	0	–	–	–
HSP	LF	13	300	0	0	–	–	–
	SF	16	375	0	0	–	–	–
HCP (inter- and intra-morph)								
	LF♀ × LF♂	17	395	32	7.97 ± 0.92[ac]	1.27 ± 0.09[a]	9.30 ± 0.19[b]	13.86 ± 1.11[bc]
	LF♀ × SF♂	17	400	38	10.52 ± 1.26[c]	1.35 ± 0.10[a]	9.43 ± 0.20[ab]	14.78 ± 1.38[ab]
	SF♀ × LF♂	15	325	13	3.33 ± 0.71[a]	1.00 ± 0.00[a]	9.90 ± 0.16[abc]	10.13 ± 0.17[ac]
	SF♀ × SF♂	16	400	178	44.19 ± 2.45[d]	3.76 ± 0.10[c]	10.20 ± 0.06[d]	36.89 ± 1.05[d]
					$F = 104.86$***	$F = 76.76$***	$F = 5.49$***	$F = 59.59$***

Means in each column followed by different superscript letters are significantly different at $P < 0.05$ (Tuckey post-hoc comparisons).
SSP and HSP not included in the statistical tests.
*** $P < 0.001$.

contained ovules with a gradient of sizes, as well as seeds at different stages of development (the smallest ovules were equal to those in a virgin ovary and had no aril, but the rest, as also the incipient seeds, did possess an aril, a typical characteristic of the seeds in this species). These results might suggest the existence of a mechanism of postzygotic SI, and, while we have no embryological results to indicate that the ovules possessing an aril are indeed embryos, it is nevertheless clear that the virgin ovules never possess an aril.

We also noted that the massive abscission of initiated fruit occurred approximately from the second week after the appearance of the first fruit.

In relation to intra-HCP trial, in 2000 the fruit-set of the LF morph was only 8%, while that of the SF morph rose to 44.2% (Table 2). Similarly, seed-set was much greater in the SF morph. These differences corroborated those found in 1996 (Table 1). With respect to inter-HCP, in 2000, if the LF morph acted as pollen receptor, fruit-set was only 10.5%. While low, this value was significantly greater than when the SF morph acted as a pollen receptor (3.3%). These data were similar to those obtained in 1996 (Table 1). Note that when the LF morph was the receptor, the fruit-set did not depend on the type of pollen donor used (1996: LF × LF = 6.1% or LF × SF = 6.1%; 2000: LF × LF = 8% or LF × SF = 10.5%). In contrast, if the SF morph acted as receptor, fruit-set was greatly affected by the used donor type (1996: SF × LF = 1% or SF × SF = 63.9%; 2000: SF × LF = 3.3% or SF × SF = 44.2%; Tables 1 and 2).

In 2000 the level of fruit-set varied significantly between treatments (one-way ANOVA, $F = 59.59$, $P < 0.001$), with intra-HCP (SF × SF cross) significantly superior to the other treatments (Table 2).

The self-compatibility index (taking into account either fruit or seeds) is nil for the two years and both morphs, which is indicative of the presence of SI in both. Intermorph crosses were likewise fairly unsuccessful, even when the SF morph acted as pollen receptor, since, although some "fruit" may be formed, their seed counts were very low. This seems to indicate that some reproductive barrier isolating the two morphs

was operating, albeit only partially. Also, the self-fertility index was nil in all cases, whether it was the value of fruit-set taken into account or that of seed-set.

Discussion

Cytisus multiflorus possesses papilionaceous zygomorphic flowers, which are clearly adapted to bee pollination. Even though its flowers produce no nectar, they are attractive in their white colour, their softly perfumed scent, and bountiful flower production per plant (Rodríguez-Riaño *et al.* 1999d).

Although angiosperm breeding system studies are becoming ever more common in the botanical literature, the only in-depth studies of Genisteae shrubs are those on *Chamaecytisus palmensis* (Webb & Shand 1985), *Cytisus striatus* and *Retama sphaerocarpa* (Rodríguez-Riaño *et al.* 1999a). The first species is self-compatible, although it does present a very low fruit-set rate (12%; Webb & Shand 1985). The two last species are self-incompatible and they also present a very low fruit-set rate (*C. striatus*: 13.1%–21.6%; *Retama sphaerocarpa*: 5.3%–6.8%). The percentages of fruit-set in natural conditions of some Spanish Genisteae studied are also low: *Ulex minor* (5%), *U. australis* (16%), *C. grandiflorus* (12%), and *Stauracanthus genistoides* (40%; *see* Herrera 1987).

The only pollinator observed in the *Cytisus multiflorus* population that was studied, the honeybee (*Apis mellifera*), operates the pollination system of the flowers of *C. multiflorus* perfectly. It usually visits the flowers on sunny days, which is not in the best interests of *C. multiflorus* flowering mostly in winter, the season with the most cloudy or misty days in the year. The flowers may be visited on more than one occasion, as they have a valvular type pollen presentation system (López *et al.* 1999), although it was observed that the bees often abandoned their intention of revisiting the flowers probably because they had learnt that these flowers offered fewer rewards than virgin flowers. Visited flowers are somewhat different to virgin ones because the keel is not joined at its upper part and the yellow colour of the pollen and anthers can be

observed from above before landing. Also these insects visited with great intensity flowers of both the same plant and of nearby plants, which has the drawback that many of the pollinations will be with pollen from the same individual or from plants that are probably very closely related genetically.

Cytisus multiflorus behaves as xenogamous, requiring the visits of pollinators for the fruit and seeds to be produced. Agamospermy is absent since no fruit and/or seeds are formed after emasculation and later bagging of the flowers (Rodríguez-Riaño 1997). In none of the years of the study were fruits or seeds produced after hand self-pollination. This could be a clear indication of the presence of homomorphic SI in this species, but, as Gibbs and Bianchi (1999) observed, to rely simply on the results of fruit-set after self- and cross-pollinations constitute poor indicators of the existence of conventional SI. The germination capacity of its own grains of pollen on the stigma may mean that there can exist no SI of sporophytic type. Also, the fact that many pollen tubes stop growing between the style and the ovary suggests the existence of gametophytic SI (Rodríguez-Riaño *et al*. 2001), which is more general in leguminous species (Arroyo 1981, Weller *et al*. 1995, Newbigin 1996). However, after hand self-pollination, 20.9% of the ovules in the LF morph and 54.9% in SF were penetrated by their own pollen tubes, and that clearly indicated the possibility of self-fertilization (Rodríguez-Riaño *et al*. 2001). The latter was corroborated in the present work by the fact that initiated and aborted fruit presented large-size ovules and developing (aborted) seeds, in both cases showing the typical aril characterizing viable seeds (note that in this species virgin ovules never have an aril). Together, this evidence indicates that it is not conventional gametophytic SI, which is present in the two morphs of this species, since this category of SI has to be understood as a pre-zygotic self-recognition mechanism. Similar phenomena of SI have been reported in other species (Bawa & Beach 1983, Owens 1985, Seavey & Bawa 1986, Padrutt *et al*. 1992, Gibbs & Bianchi 1999) that were initially denoted by Seavey and Bawa (1986) as late-acting self-incompatibility, although in other cases they have been called

pistillate sorting or even post-zygotic ovarian gametophytic SI (*see* de Nettancourt 2001 and references therein).

Post-zygotic abortion is a phenomenon difficult to define (since it operates beyond the formation of the zygote) and to distinguish from inbreeding depression caused by the accumulation of deleterious or lethal recessive genes. Various facts indicate, however, that in this species, in contrast to the case for *Cytisus striatus* or *Retama sphaerocarpa* (Rodríguez-Riaño *et al*. 1999a), it is not inbreeding depression that is operating: (1) flower and young fruit abscission after self-pollination occurs en masse approximately two weeks after the formation of the first fruit; (2) all the individuals studied behaved uniformly with respect to selfing, with none of them producing ripe fruit. It does not, however, fit the pattern of LAS that, in the initiated and then aborted fruit, there was a gradient of ovule sizes as well as well-formed seeds at different stages of development. We have nonetheless assumed in the present work that ovules with an aril have been fecundated, although one must be cautious about this point since the aril may arise in unfertilized ovules following their growth. It has to be recalled that in certain species (e.g. *Epilobium obcordatum*) the unfertilized ovules keep growing in the presence of other fertilized ovules, with a very broad overlap in sizes (Seavey *et al*. 2000). Further investigation at the embryo level in *C. multiflorus* are currently in progress.

Intra-HCP trials carried out in 2000 led to levels of fruit-set more than 2.5 times greater in the SF morph and almost 2 times greater in the LF with respect to the control. For the seed-set, the increase was almost 1.9 times in the SF morph, but in the LF there was a decrease by a factor of 0.9. The case had been similar in 1996. These results indicated that there might possibly be some limitation involved in the pollination, both in a limitation of the number of pollinators and in their behaviour in depositing very light pollen loads, carrying out geitonogamous pollination or pollination between genetically closely related plants, as has been shown in other species (*see* review in Stephenson 1981, Burd 1994) and even in *Cytisus* (Parker 1997, Rodríguez-Riaño *et al*. 1999a, Suzuki 2000). The limitation might also be due to rainfall, which is abundant in the

study zone during the species' flowering, and would imply a reduction in the amount of available pollen, as has been observed in *Pistacia lentiscus* (Martínez-Pallé & Aronne 2000). Other causes such as resource limitations could also be present (Stephenson 1981, Suzuki 2000).

The two morphs recognized in the study population have flowers that are similar in colour and morphology. However, the reproductive success of the two morphs is quite different, since both in the control and in the intra-HCP the differences between the two are highly statistically significant in the production of both fruit and seeds. Since the two cohabit in the study population and their differences are more due to the size and the production of the reproductive attributes than to their morphology, it is natural to expect abundant intermorph pollinations when there is an overlap in flowering. When this occurred, the artificially performed intermorph crosses yielded quite surprising results: when the LF morph acted as pollen donor, there was very little fruit-set, less, although not significantly, than when it was crossed with itself. The fruit-set was also very low when that morph acted as pollen receptor in the LF × SF cross, without significant differences relative to the cross with itself (LF × LF). The case was similar with respect to seed-set. Two conclusions may be drawn from these results: (1) the LF morph possesses a lower reproductive capacity than the SF morph, and (2) the crosses between the two are quite ineffective, independently of which is the donor and which the receptor.

The lower reproductive capacity of the LF morph, both with itself and with the SF morph, could be due to problems of viability of its pollen grains, as was indicated above. There is backing for this in the low percentage of formation of seeds/fruit found when that morph acted as pollen donor and the SF morph as receptor (Tables 1 and 2). Nevertheless, there may also exist some defect in the female part since when LF morph acted as receptor either with other LF individuals or crossed with the SF morph the levels of seed-set, while slightly greater, were also low (Tables 1 and 2). We may compare this with the seed set of the SF morph. While it has indeed been demonstrated that the LF morph pollen is less viable than that of the SF morph

(Rodríguez-Riaño *et al.* 2001), it is unfortunately unknown whether its ovules are less viable, although future work should decide the case.

We have found only two reports of the existence of morphs in other Fabaceae. In Australian populations of *Trifolium glomeratum*, Smith *et al.* (1995) differentiated two types basically by the number of flowers per inflorescence, the weight of the seeds, and the period of flowering, and considered to be genetically isolated (since there are no lines with intermediate features). In *Cytisus scoparius*, Baonza and Malo (1997) indicate that the flower size presents a clinal variation, with larger flowers at higher altitudes. These authors suggest that water is probably the resource with most influence on flower size variability, since in years of drought that size was found to be smaller. In *C. multiflorus*, however, the variability that is found cannot be explained by a clinal variation influenced by conditions of hydric stress, since the two morphs appear in the same population and under the same climatological conditions. Nevertheless, the microclimatic or edaphological conditions could be different in the sites occupied by the two morphs, although no analyses have been performed in this regard.

The differentiation of the two morphs in the study population could be related to the acquisition of a greater level of ploidy in the LF morph. Chromosome counts carried out in the two morphs do not, however, as of now support this hypothesis, since they show there to be similar, although quite variable, numbers of chromosomes. Thus, the counts for numerous individuals of the SF morph gave chromosome numbers of $2n = 44, 46, 48,$ and 52 with $2n = 46$ being the most frequent. The numbers in the LF morph case were $2n = 42, 46, 50, 70$ and 72, with $2n = 46$ also being the most frequent. A literature search yielded data of $2n = 46, 46 + 3B, 48, 54,$ ca. 96 and $n = 23, 33$, although with no discrimination between morphs, thereby supporting the variability that we found (*see* Cubas *et al.* 2001 and references therein). Even if it had been demonstrated, however, that the LF morph had a greater level of ploidy than the SF morph, it is quite usually found in angiosperms that polyploidy disrupts SI which was not the case in the LF morph. Nonetheless, there are many plants

in which SI coexists with polyploidy, in both monocots and dicots, including some Fabaceae (de Nettancourt 2001).

If we apply the formulae put forward by Zapata and Arroyo (1978) and Becerra and Lloyd (1992), the two morphs would be behaving as SI. But we also obtain zero values when the formulae are applied to the intermorph crosses, which points to the existence of a partial reproductive barrier between the two. The barriers which tend to isolate the two morphs might operate after crossing as well as before crossing, although in an incomplete fashion. Prior to crossing there are the temporal phenological divergence (although they present a certain degree of overlap, the peaks of maximum flowering occur with a major difference in phase; Rodríguez-Riaño *et al.* 1999d) and the size differences of the sexual organs (Rodríguez-Riaño *et al.* 2001). In regard to the latter, SF morph pollen is significantly smaller than LF pollen, and probably has problems in forming tubes that are long enough to propitiate fecundation when are deposited on the pistils of the LF morph, which has significantly longer styles. Various studies have shown that the stylar exudate is a nutritional source for growing pollen tubes (for a review *see* Heslop-Harrison 2000, Lord 2000), but there is also much evidence that some part is played by the nutritional content of the pollen grain itself, given the positive correlation between grain size and style length already demonstrated in this family (Vonhof & Harder 1995, Rodríguez-Riaño *et al.* 1999b and references therein) as well as in many others, such as the Asteraceae (*see* Torres 2000 and references therein).

Finally, the post-crossing barriers include the above cited intermorph incompatibility, with a post-zygotic mechanism that involves the abortion of a great number of embryos.

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