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Early Inbreeding Depression and Pollen Competition in *Calluna vulgaris* (L.) Hull.

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We investigated whether partial self-sterility in *Calluna vulgaris* results from abortion of selfed offspring owing to inbreeding depression or a late-acting self-incompatibility mechanism, and whether self-pollen interferes with normal functioning of cross-pollen. Self-pollination resulted in 75% less seed set than cross-pollination. Self-pollen tubes reached ovaries and penetrated ovules as often as those of cross-pollen. Following self-pollination, examination of the size of undeveloped seeds showed that at least 70% resulted from ovule fertilization and arrest of development occurred at various stages. All self-pollinated plants produced seeds and self-fertility varied among plants. These results indicate that the reduced seed set observed in self-pollination is more likely the result of inbreeding depression rather than a late-acting self-incompatibility system. The fecundity component of inbreeding depression was high (0·762). Seed set was reduced by an average of 40% when self-pollen was mixed with cross-pollen, compared to pure cross-pollination. Using genetic markers, we found about 20% of seeds resulted from self-pollination in mixed-pollinated fruits. *C. vulgaris* is likely to experience self-pollination in nature and our data suggest this will reduce the number of ovules that might otherwise mature after cross-pollination.

Key words: *Calluna vulgaris* (heather), self-pollination, pollen tube, ovule fertilization, early inbreeding depression, pollen interference.

INTRODUCTION

Self-sterility, the reduction in seed set following selfing relative to that following outcrossing, is widespread among flowering plants. Self-sterility is often due to an active physiological self-incompatibility reaction operating in the maternal tissue. Typically, failure of pollen tubes occurs at the stigmatic surface or in the style, but some studies have also suggested true self-incompatibility reactions operate in the ovary or in the ovules before fertilization (Cope, 1962; Seavey and Bawa, 1986; Kenrick, Kaul and William, 1986). Incompatibility mechanisms operating after pollen tubes have entered the ovary are generally designated as late-acting self-incompatibility (late-acting SI; Seavey and Bawa, 1986). An alternative mode of self-sterility is fertilization of ovules by self-pollen followed by embryo abortion due to early inbreeding depression, that is, a process acting in the zygote as a function of its genotype (Krebs and Hancock, 1988; Seavey and Carter, 1994). Many reports of self-sterility in plants do not discriminate between prezygotic and postzygotic systems. Distinguishing between these two causes, however, is important in understanding the evolution of plant mating systems because self-incompatibility can evolve in response to cumulative inbreeding depression (Charlesworth and Charlesworth, 1987). Evidence in favour of one of the mechanisms may be gained from observations of pollen tube growth, ovule fertilization and seed development. Early inbreeding depression is expected to lead to embryo failure at various stages of development (Seavey and Bawa, 1986). Also, a hallmark of early inbreeding depression is variation in self-sterility across individuals (Waser and Price, 1991) whereas true self-incompatibility is expected to result in variation of cross-sterility across individuals.

Growth of the different types of pollen may be affected when both pollen types are present together on the stigma (Aizen, Searcy and Mulcahy, 1990). Furthermore, the presence of self-pollen on stigmas of predominantly self-sterile plants may have additional fitness consequences. Pollen deposited on the stigma of the plant of origin cannot be exported and is wasted, affecting male reproductive success (Waser and Price, 1991). Deposition of self-pollen may also lead to stigma clogging or reduction of cross-pollen tube number in the style (Ockendon and Currah, 1977; Shore and Barrett, 1983). In plants with late-acting self-incompatibility or early inbreeding depression, ovules may be preempted by self-pollen tubes and this may lead to ovule wastage by micropyle blocking or abortion of low quality embryos (Waser and Price, 1991; Krebs and Hancock, 1991; Seavey and Carter, 1994). Spatial (herkogamy) or temporal (dichogamy) separation of male and female functions is thought to have evolved to reduce pollen interference (Lloyd and Webbs, 1986; Webbs and Lloyd, 1986). Such mechanisms are nevertheless inefficient in reducing the overall level of self-pollination by way of geitonogamy experienced by plants with large floral displays (Broyles and Wyatt, 1993). Given the potential importance of pollen interference in the evolution of plant reproductive traits, empirical studies that assess the effect of self-pollen deposition on cross-pollination are needed.

Calluna vulgaris (L.) Hull (Ericaceae) is a branched,

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hemispherical, evergreen shrub widely distributed across Western Europe. Plants produce up to several thousand small flowers which are morphologically simple. Pollination in this species may involve either insects or wind, but insects are necessary for optimal seed set. Bumblebees, honeybees and syrphids are the most efficient pollinators (Mahy, De Sloover and Jacquemart, 1999). Bumblebees and bees usually visit many flowers before leaving a plant. There is thus a high probability that a flower will receive self-pollen by way of geitonogamy. Nevertheless, outcrossing rates measured at seedling stages range from 0·71 to 0·90 in natural populations (Mahy and Jacquemart, 1998). It has been suggested that *C. vulgaris* is protandrous because anthers dehisce in the closed bud (Faegri and van der Pijl, 1980). Nevertheless, protandry is probably not efficient in this species because stigmas are receptive very early after bud opening (Mahy, pers. obs.). In a preliminary study, we found that the mean number of well-formed seeds per fruit following self-pollination was 80 % less than that following cross-pollination. Also, 7 d after pollination, both self- and cross-pollen tubes reached the base of the style (Mahy and Jacquemart, 1998). Self-sterility in this species may thus be regulated by either late-acting SI or early inbreeding depression.

In this study we report results of pollen tube growth rate, ovule fertilization following self- and cross-pollination and comparisons of reproductive output following: (1) pure self-pollination; (2) pure cross-pollination; and (3) mixed pollination. Our goals are: (1) to distinguish between early inbreeding depression and late-acting self-incompatibility; and (2) to explore how self-pollen affects the success of cross-pollen in producing seeds.

MATERIALS AND METHODS

Study population

Ten individuals were randomly collected in a heathland in the Upper Ardenne, Belgium (50°15′00″ N, 5°15′00″ E, alt 652 m) in May 1996. Plants were potted in a greenhouse (16 h light, 20 ± 3 °C) and supplied weekly with a NPK solution.

Pollination treatments

Three sets of inflorescences with unopened flowers were arbitrarily chosen on each of the ten individuals. All flowers (five–20) on each inflorescence received the same pollination treatment. Flowers of the first set of inflorescences were self-pollinated with pollen collected from a flower of the same individual. Flowers on the second set of inflorescences were cross-pollinated with pollen from two donors collected 300 m from the sampling site. The cross-pollinated flowers were not emasculated because the anthers dehisce in bud and emasculation prior to anthesis causes very high levels of flower abortion (Dommée, 1968). The third set of inflorescences received mixed-donor pollen. To mix pollen, we collected pollen by pressing anther appendages with a small spatula successively on a flower of the same individual and on a flower of a donor individual. The operation was repeated twice and whether the first flower was self- or cross- was random. All individuals were genotyped at the first allozymic locus (*Mnr-1*) of the enzymatic system menadione reductase (MNR). Details of electrophoresis procedures are given in Mahy *et al.* (1997). The two cross-pollen donors used in this study were homozygous for a particular allele at *Mnr-1* (allele *Mnr-14*). We applied mixed-pollen to eight plants homozygous for an alternative allele at the same locus (allele *Mnr-12*). All pollinations involved flowers less than 4 d-old and the entire stigmatic surface was evenly covered with pollen.

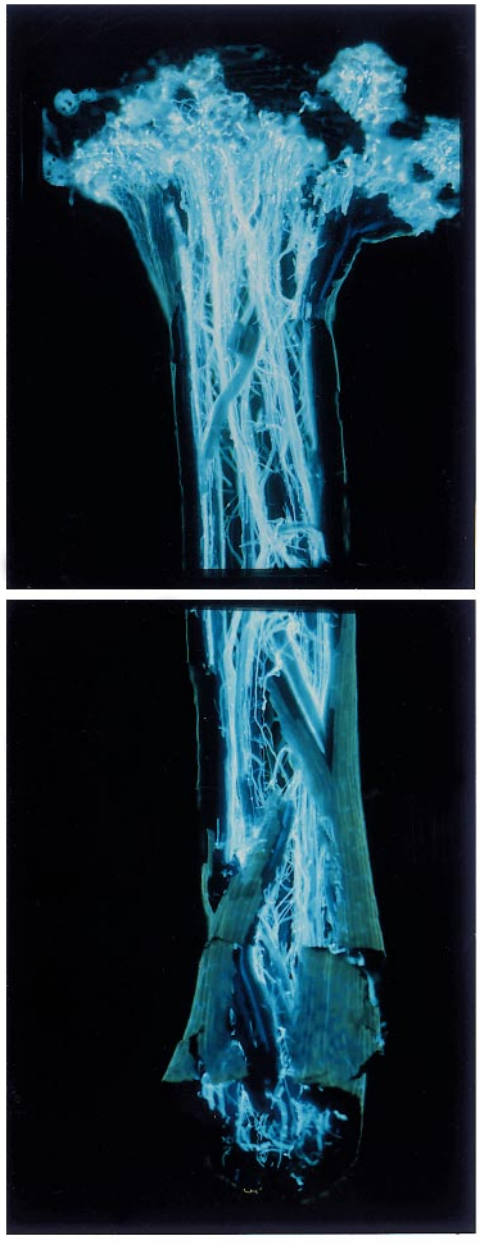

FIG. 1. Growth of pollen tubes across the style of *Calluna vulgaris* flowers 6 h after self-pollination. St, Stigmate; Bs, base of the style; PT, pollen tube (× 50).

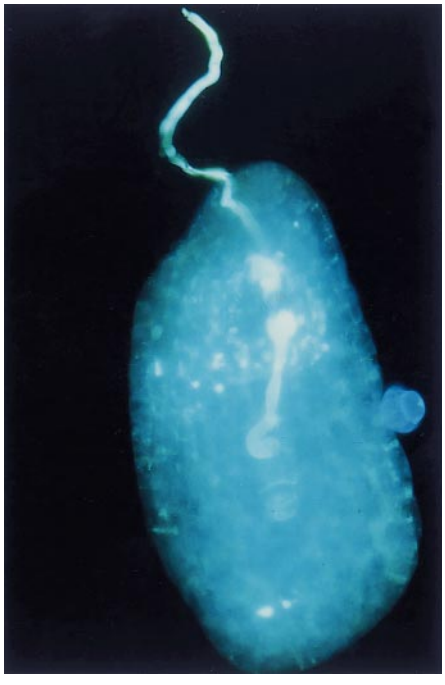

FIG. 2. Penetration of an ovule by a pollen tube 48 h after self-pollination. OV, Ovule; PT, pollen tubes.

Pollen tube growth and ovule fertilization

To investigate whether self- and cross-pollen tubes grow at different rates, a series of self-pollinated and cross-pollinated flowers were collected 6, 24 and 48 h after pollination on three individuals. These flowers were fixed in 1:1:3 formaldehyde:glacial acetic acid:ethanol for 24 h and stored in 70% ethanol. Prior to examination, styles were rinsed in water and stained in 0·1 M K_3PO_4 pH 9·0 with 0·1% aniline blue. Styles were crushed under a coverslip in a drop of aniline blue and examined under UV light. The very large number of overlapping tubes in most of the pistils examined precluded accurate counting (Fig. 1). We then examined the presence of pollen tubes at three levels: stigma (germination of pollen); mid-style; and at the base of the style just prior to the ovary. We used pistils collected 48 h after pollination to study penetration of ovules by pollen tubes. A total of eight pistils were randomly chosen on three individuals for each treatment. The four locules were separated and ovules were gently detached from the placenta under the stereomicroscope. The preparation was stained with aniline blue. We considered an ovule to be penetrated when a pollen tube (or part of it) was directly attached to the micropyle or when we detected a fluorescent channel across the superior part of the ovule by transparency (Fig. 2).

Two classes of seeds were distinguished in mature fruits: developed seeds (class 1) and undeveloped seeds (class 2). Developed seeds exhibited a clear brown colour, sometimes also light or darker brown. They were more-or-less bilaterally flattened with an egg-shaped or ellipsoidal outline and they were typically full. In contrast, undeveloped seeds were shrunken and empty, most of them being smaller than developed seeds. Undeveloped seeds may contain both aborted seeds and unfertilized ovules, but the distinction was not easily made *a priori*. We examined the size of undeveloped and developed seeds in selfed and crossed fruits: three fruits per treatment on each of eight randomly chosen individuals. Length and width of each seed were measured under a stereomicroscope to ± 25 μm. Also, the size of 50 ovules collected from unpollinated flowers was determined under a microscope at 100×. Comparison of the size of ovules collected in unpollinated flowers and undeveloped seeds in pollinated flowers allowed us to assess the minimum proportion of ovules that enlarged following pollination and thus were probably fertilized. Examination off the size distribution of undeveloped seeds allowed us to determine if rejection or seed abortion occurred at a single stage of embryo development.

Fruit set, seed set and paternity analysis

Fruit maturation was recorded for 20 flowers per individual and treatment. Mature fruits were harvested just before opening. When possible, ten fruits per individual for each treatment were examined for seed production. The total number of seeds (developed + undeveloped) detected in the fruits did not differ among the three pollination treatments (cross: $30{\cdot}4 \pm 8{\cdot}8$, self: $29{\cdot}3 \pm 5{\cdot}9$, mixed: $30{\cdot}3 \pm 7{\cdot}3$, one-way Anova, $F_{2,255} = 0{\cdot}59$, $P = 0{\cdot}555$) and was similar to the number of ovules detected in unpollinated flowers ($30{\cdot}6 \pm 4{\cdot}1$, $n = 12$). Thus the total number of seeds counted per flower was considered an estimate of the initial number of ovules. Seed set was then estimated as the number of developed seeds divided by the number of ovules [class1/(class1 + class2)]. Pollinated flowers that failed to mature fruits were not included in the determination of seed set because this component of self-sterility is already taken into account in the fruit set estimation. To determine the paternity of seeds resulting from the mixed-donor pollinations, we genotyped seedlings from a series of fruits at the *Mnr-1* locus. Because the pollen donors and the pollinated plants were homozygous for different alleles at the locus *Mnr-1* (*Mnr-14* and *Mnr-12*, respectively), heterozygous seedlings (*Mnr-12/Mnr-14*) resulted from cross-fertilization whereas homozygous seedlings (*Mnr-12/Mnr12*) resulted from self-fertilization.

Early inbreeding depression estimates

We estimated the fecundity component of inbreeding depression of the population as recommended by Husband and Schemske (1995): $\delta = 1 - ws/wo$, where ws and wo are, respectively, the mean fitnesses of selfed and outcrossed progeny calculated from the mean of each cross type for each maternal plant. We calculated the population means for selfed and outcrossed progeny by averaging the mean fitness values calculated at the family level. Inbreeding depression was estimated at three stages: fruit set, seed set and total fecundity. Total fecundity fitness for self- and cross-progeny from each maternal plant was calculated as the product of mean fitness for fruit set and seed set.

Statistical analysis

Prior to analysis, proportions were arcsin-transformed. When data met normality assumptions classical parametric tests were used: paired *t*-tests or Anova (SASInc, 1989). When normality assumptions and/or equality of variances could not be met following classical transformations, Kruskall-Wallis tests were used (SASinc, 1989). Details of statistical tests as well as particular procedures are given below. Means are presented with their s.d. unless otherwise stated.

RESULTS

Pollen tube growth and ovule fertilization

Examination of styles revealed no conspicuous differences in the rate of pollen tube growth following cross- and self-pollination. Twenty-four h after pollination, pollen tubes were observed at the base of all styles examined in both self- and cross-pollinations (Table 1). The number of pollen tubes reaching the base of the style at this time largely exceeded 50, except in two self-pollinated styles where fewer than ten pollen tubes were detected. Six h after pollination, pollen germination was detected on 86 and 100% of self- and cross-pollinated styles, respectively, and the proportion of styles with at least one pollen tube growing to its base was 60·0 and 47·6% for self- and cross-pollinations, respectively (Fig. 1, Table 1).

Forty-eight h after pollination, many pollen tubes were visible in the ovaries (eight observations per treatment) in the two pollination treatments. The average proportion of ovules penetrated by a pollen tube in an ovary (Fig. 2) following self- and cross-pollination was, respectively, 50·4% ($\pm$25·5) and 61·4% ($\pm$25·4); the difference was not significant (one-way Anova, arcsin transformed proportion of penetrated ovules, $F_{1,14} = 0{\cdot}884$, P = 0·362).

Mean length of ovules in unpollinated flowers was 223·7$\pm$24·2 μm ($n = 50$). The length and width of undeveloped seeds were significantly correlated in both self- and cross-pollinated fruits (Spearman's rank correlation; selfed: $n = 447$, $r = 0{\cdot}596$, $P < 0{\cdot}01$; crossed: $n = 50$, $r = 0{\cdot}853$, $P < 0{\cdot}01$). The same relationship was observed for developed seeds but with less acuity (Spearman's rank correlation; selfed: $n = 113$, $r = 0{\cdot}267$, $P < 0{\cdot}01$; crossed: $n = 564$, $r = 0{\cdot}453$, $P < 0{\cdot}01$). Thus we only considered the length of seeds. Figure 3 compares the size distribution of undeveloped and developed seeds, following self- and cross-pollination, with the size distribution of ovules collected in unpollinated flowers. Following self-pollination, 70% of undeveloped seeds belonged to length classes superior to the maximum length class of ovules collected in unpollinated flowers (Fig. 3A). This indicated that, following self-pollination, a minimum of 70% of undeveloped seeds resulted from ovule enlargement, most probably as a result of embryonic development following fertilization. By comparison, 84% of undeveloped seeds exhibited similar enlargement following cross-pollination (Fig. 3B). Following self-pollination, the range of sizes detected for undeveloped seeds larger than ovules was highly variable (from 300 to 700 μm) (Fig. 3A). Furthermore, following self-pollination, the range of sizes of undeveloped seeds was similar to the range of sizes of developed seeds (250–750 μm) but with variation among the proportion of each size class (Fig. 3A). This indicated that the arrest of selfed embryo development occurred at various stages. Nevertheless, the majority of undeveloped seeds occurred in the length classes between 300 and 400 μm. These are the length classes just superior to the maximum length of ovules collected in unpollinated flowers. Thus, following self-pollination, embryo failure seemed to occur mainly in the first stage of development.

TABLE 1. *Growth of self- and cross-pollen tubes across styles of* Calluna vulgaris *examined at three levels: stigma (germination), mid-style and base of the style (just prior the ovary)*

Time (h)	Level	Self	Cross
6	*n*	20·0	21·0
	Stigma	85·0	100·0
	Mid	75·0	71·4
	Base	60·0	47·6
24	*n*	24	15
	Stigma	100·0	100·0
	Mid	100·0	100·0
	Base	100·0	100·0
48	*n*	17	14
	Stigma	100·0	100·0
	Mid	100·0	100·0
	Base	100·0	100·0

Time, Time since pollination. Proportion (%) of examined styles with pollen tubes reaching the level considered. *n* = number of styles examined.

Fruit set, seed set and inbreeding depression

Mean comparisons of self- and cross-fertility are given in Table 2. Average fruit set did not differ following self-pollination (87%) or cross-pollination (94%) (paired *t*-test, arcsin transformation of data, $t_9 = 1{\cdot}67$, $P = 0{\cdot}129$). In contrast, mean seed set following self-pollination (22%) is significantly decreased by 75% compared to mean seed set following cross-pollination (86%) (Kruskall-Wallis test, $H = 129{\cdot}2$, d.f. = 1, $P < 0{\cdot}001$). All plants examined in the population set seeds upon selfing, but variation in self-fertility was present among clones. Individual self-fruit set ranged from 61 to 100% and individual self-seed set ranged from 8 to 52% (Fig. 4). Variation in cross-fertility was also detected at the seed-set stage with individual seed-set ranging from 66 to 97% following cross-pollination (Fig. 4). The population inbreeding depression estimates at the fruit, seed and total fecundity stage were $\delta = 0{\cdot}073$, 0·737 and 0·762, respectively. The individual total fecundity component of inbreeding depression ranged from 0·626 to 0·929.

Fecundity and seed paternity following mixed-pollination

Mean fruit-set following application of mixed-pollen loads (95%, Table 2) did not differ significantly from mean

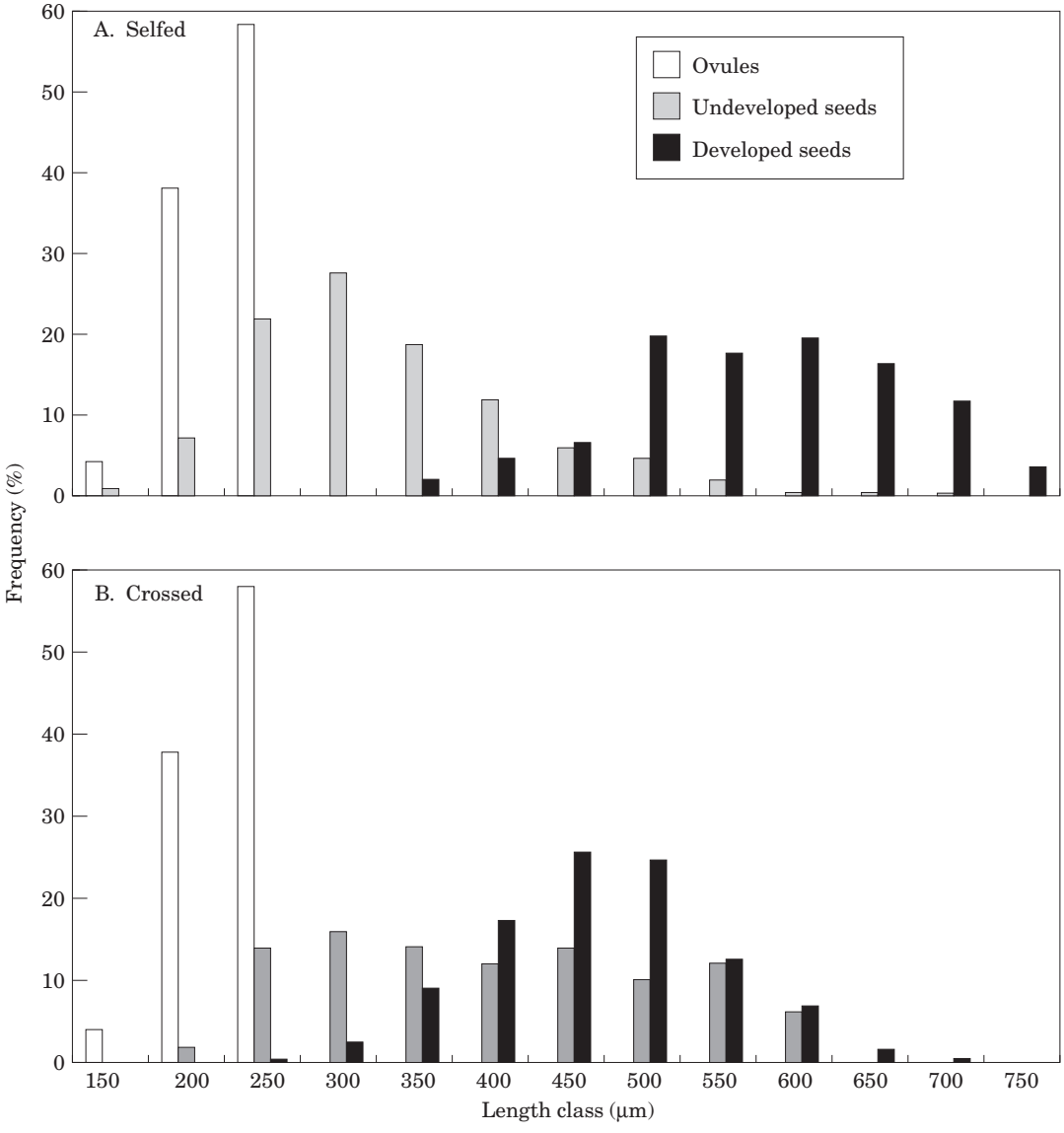

FIG. 3. Frequency distribution of length of undeveloped and developed seeds following self- (A) and cross-pollinations (B) of *Calluna vulgaris* flowers compared to ovule length in unpollinated flowers. 'Ovules' refer to ovules collected in unpollinated flowers; undeveloped seeds refer to both unfertilized ovules and aborted seeds following pollination treatments.

TABLE 2. *Mean* ($\pm$*s.d.*) *fruit set and seed set for three pollination treatments*

Pollination treatment	Mean fruit set	Ni	Mean seed set	Nf
Crossed	0·94 (0·08)	10	0·86 (0·16)	92
Selfed	0·87 (0·13)	10	0·22 (0·18)	94
Mixed	0·95 (0·09)	8	0·52 (0·27)	72

Ni, Number of individuals; Nf, number of fruits examined.

fruit set following either cross-pollination (paired *t*-test on arcsin transformed data, $t_7 = 0{\cdot}203$, $P = 0{\cdot}845$) or self-pollination (paired *t*-test on arcsin transformed data, $t_7 = -0{\cdot}81$, $P = 0{\cdot}446$). Mean seed set following mixed-pollination (51%) was reduced by 40% compared to pure cross-pollination and was 58% higher than following pure self-pollination, both differences being significant (crossed *vs.* mixed, Kruskall-Wallis test, $H = 61{\cdot}7$, d.f. = 1, $P < 0{\cdot}001$; selfed *vs.* mixed: Kruskall-Wallis test, $H = 49{\cdot}7$, d.f. = 1, $P < 0{\cdot}001$). Paternity of developed seeds was assessed in five fruits resulting from mixed-pollinations. All seedlings (from five to 19 per fruit) that had developed sufficiently were genotyped. From the 46 seedlings genotyped, nine (19·6%) were sired by self-pollen.

DISCUSSION

Evidence for early inbreeding depression

Seed set in this study was significantly reduced following self-pollination compared to cross-pollination. This may

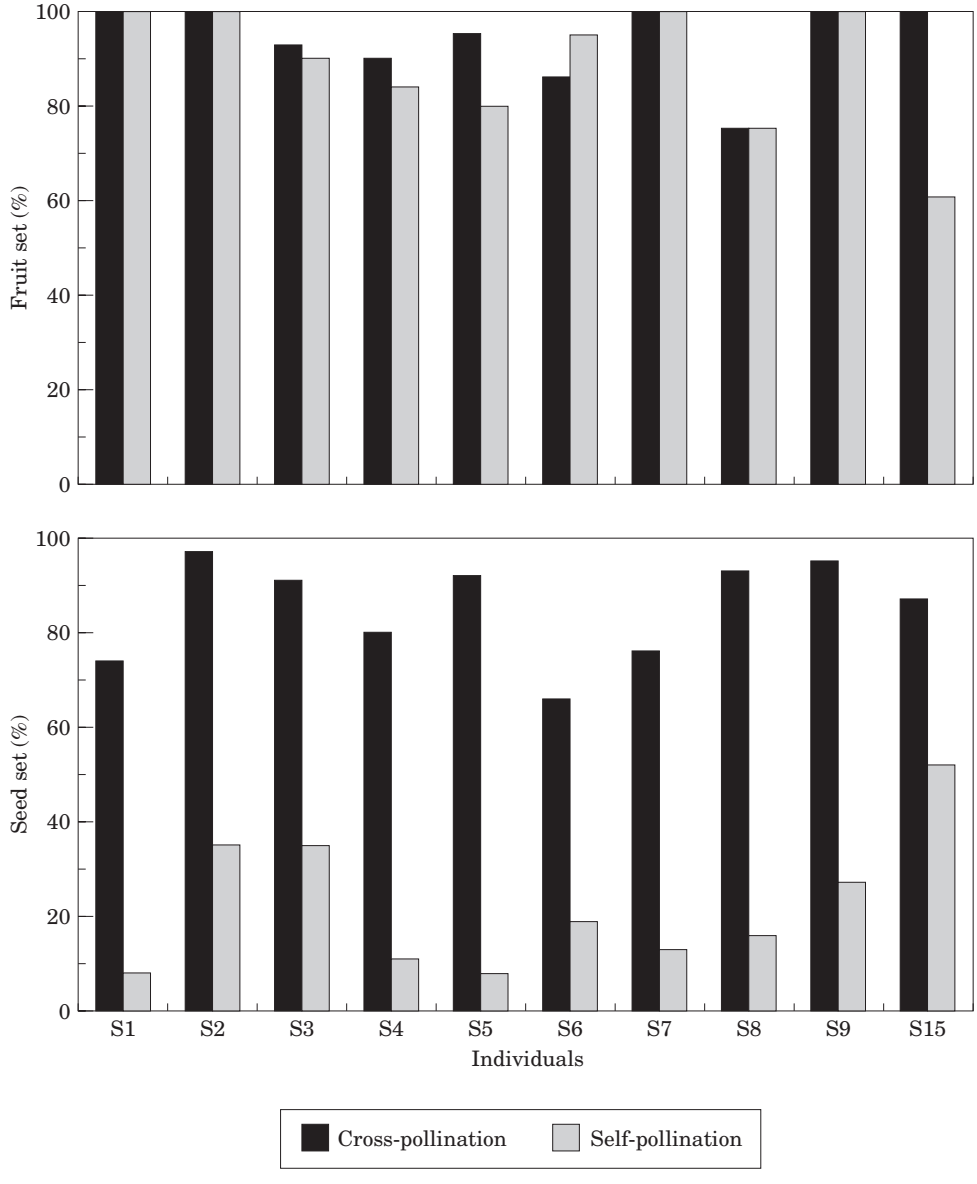

FIG. 4. Variation of fecundity following self- and cross-pollinations of ten *Calluna vulgaris* plants collected in the same population. A, Variation in fruit set; B, variation in seed-set.

result either from self-incompatibility reactions preventing the pollen tube from reaching the female gamete or from a higher rate of postzygotic abortion of self-fertilized ovules. It is important to distinguish between these alternatives before interpreting our findings with regard to inbreeding depression. In the present study, no evidence was found for stigmatic, stylar or an ovarian incompatibility reaction. Pollen tube growth and ovule penetration by pollen tubes were very similar following pure self- and cross-pollinations. An estimation of a minimal fertilization rate following self-pollination may be obtained by taking into account the seed-set (the ovules that developed into well-formed seeds and thus were fertilized i.e. 22% of all ovules) and the proportion of aborted seeds larger than the ovules (i.e. 70% of undeveloped seed, the latter representing 78% of all ovules). Thus, the minimum fertilization rate following self-pollination was 80% (22% seed set $+ 0{\cdot}7 \times 78\%$ undeveloped seeds). This represents the minimum proportion of ovules that were fertilized following self-pollination and strongly suggests that selfing is prevented at the level of ovules by a post-zygotic mechanism.

Seed set phenotypes for true SI systems generally fall into two categories, compatible and incompatible, and are typically based on one or a few loci with many alleles (de Nettancourt, 1977; Seavey and Carter, 1994). Thus, if an active rejection reaction was postzygotic, fertilized ovules would be routinely rejected (Seavey and Bawa, 1986; Seavey and Carter, 1994) and uniform rejection of selfed flowers should be observed (Gibbs and Bianchi, 1993). On the other hand, inbreeding depression may result from the action of many genes with varying detrimental effects on embryogenesis and, as a result, variation in self-sterility should be

observed across individuals (Krebs and Hancock, 1991; Waser and Price, 1991; Seavey and Carter, 1994; Husband and Schemske, 1996). The individual variation in seed set following self-pollination found in this study is consistent with the hypothesis that early inbreeding depression regulates self-sterility in *C. vulgaris*. As well, late-acting SI should lead to early embryonic failure at a fixed stage of development. In contrast, inbreeding depression is expected to act at a variety of stages (Seavey and Bawa, 1986; Husband and Schemske, 1995). Experimental studies on *Arabidopsis thaliana* have demonstrated that embryo lethality may be expressed at any stage of embryo development from the first cell division to seed maturation (Meinke, 1991). In our study, the presence of undeveloped seeds of various sizes in selfed fruits may be an indication that embryos fail at various stages, and thus give additional support to the action of early inbreeding depression. Finally, all plants of *C. vulgaris* examined produced selfed seeds. Consequently, the proportion of self-fertile plants appears to be too high to be attributed to incomplete SI due to chance mutation(s) at the S locus (loci).

Level and timing of inbreeding depression

In this study, we examined only the fecundity component of inbreeding depression. Taking into account both fruit and seed production, the average level of inbreeding depression at this early stage of development was high ($\delta = 0{\cdot}76$) compared to the mean level reported by Husband and Schemske (1996) for 30 outcrossed angiosperm species (mean δ at seed production stage = 0·19). The high level of early inbreeding depression detected in our study is similar to the level commonly observed in gymnosperms (reviewed in Husband and Schemske, 1996). Nevertheless, a similarly high level of inbreeding depression at the fecundity stage has been reported for some angiosperm species e.g. *Costus* spp. (Schemske, 1983), *Allium schoenoprasum* (Stevens and Bourgourd, 1988), *Epilobium angustifolium* (Husband and Schemske, 1995). It has been suggested that high levels of a lethal equivalent may not be rare in perennial clonal plants such as *C. vulgaris* because somatic mutations may accumulate with ageing and may be passed on to the reproductive tissues, increasing the number of loci with recessive lethal alleles (Klekowski, 1988; Klekowski and Godfrey, 1989). Inbreeding depression may be expressed at any life-history stage (reviewed in Husband and Schemske, 1996), but lethality is likely to be concentrated in embryonic stages for many plants because this is the time when a large number of essential genes are first expressed (Meinke, 1991; Seavey and Carter, 1994; Husband and Schemske, 1996). Nevertheless, in *C. vulgaris*, we might expect inbreeding depression in further growth stages. Indeed, despite a significant proportion of seeds produced in natural populations resulting from autogamy (Mahy and Jacquemart, 1998), in a survey of the allozymic variation of 30 adult populations of *C. vulgaris* we did not find a significant heterozygote deficit (Mahy *et al.*, 1997; Mahy, 1998). This suggests that selfed seeds in natural populations contribute less to adult populations than crossed seeds, most probably because of the expression of inbreeding depression during plant development.

Pollen interference and consequences for breeding system

In *C. vulgaris*, applying self-pollen at the same time as cross-pollen reduced seed set by 40% compared to pure cross-pollination, reducing it to a level intermediate between pure self- and cross-pollination as expected for pollen tubes of similar competitive ability. Similar results have been reported for other self-sterile angiosperms (Bertin and Sullivan, 1988; Waser and Price, 1991; Broyles and Wyatt, 1993). Results of the present study also indicated that a substantial proportion (20%) of developed seeds in mixed-pollinated fruit resulted from self-fertilization. Previous studies of the effect of self- and cross-pollen in single *vs.* mixed pollen loads have not been consistent in their findings. In *Campsis radicans*, up to 33% of viable seeds produced by mixed-pollination resulted from self-fertilization, whereas following pure self-pollination only 0·7% of flowers produced mature fruits (Bertin and Sullivan, 1988). In contrast, in the self-incompatible *Asclepias exaltata*, less than 1% of viable seeds were produced by self-pollen when mixed pollinations were realized (Broyles and Wyatt, 1993). In the self-compatible *Aquiligia caerulea*, the proportion of selfed seeds from mixed-pollination was predictable from pollen performance in single-donor fruits (Montalvo, 1992). The situation in *C. vulgaris* seems similar to that reported for *Aquilegia caerulea*, i.e. the proportion of selfed seeds in the mixed donor fruit (0·20) is similar to the ratio of seed set following pure self- and cross-pollination ($0{\cdot}22/0{\cdot}86 = 0{\cdot}26$). Because of the low number of fruits examined we must stress that these results should only be considered preliminary.

Our results imply that self-pollination in *C. vulgaris* will incur an immediate fecundity cost by reducing the number of developed seeds and the number of ovules available for cross-pollen. In two populations of *C. vulgaris* we found significant selfing rates by means of allozymic variation screening at the seedling stage (up to 34%) (Mahy and Jacquemart, 1998). Also, it is likely that populations of *C. vulgaris* exhibited high levels of primary selfing rates (the selfing rate at the time of pollination) because many flowers are open simultaneously on the same plant and bumblebees and bees tend to visit many flowers on the same individual. In theory, pollen interference of the magnitude found in this study would select for traits minimizing the intrafloral deposition of self-pollen (Lloyd and Webbs, 1986; Webbs and Lloyd, 1986). Examination of the floral morphology in the studied population indicated a spatial separation of male and female functions as the mean distance among stigmas and anthers (1·52 mm) represents 51% of the total style length (2·96 m) ($n = 12$ flowers) (Mahy, 1998). Marked separation of stigma and anthers helps to preclude intrafloral self-pollination, but is inefficient in precluding deposition of self-pollen by way of geitonogamy (Proctor, Yeo and Lack, 1996). Further work is thus needed to assess the impact of geitonogamy on fecundity variation in natural populations of *C. vulgaris*.

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