

Original article

## Inbreeding effects on fitness traits in the heterocarpic herb *Leontodon autumnalis* L. (Asteraceae)

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### Abstract

Heterocarpic plants are characterized by the production of distinct types of fruits that usually differ in their ecological behavior. In the Asteraceae, differences are mainly found between peripheral non-dispersal and central dispersal achenes (single-seeded fruits). Inbreeding depression is considered as an evolutionary force as it may reduce several fitness traits, and in the case of heterocarpic plants, it could influence fitness traits (e.g., seed set, germination rate, growth rate) of each fruit morph, which may have important ecological and evolutionary consequences. In particular, differential effects on fitness traits and dispersal of selfed and outcrossed progeny can strongly determine the viability of extant populations and the potential to colonize new habitats. We conducted a hand-pollination experiment in greenhouse conditions to test whether inbreeding affects the fitness of achene morphs in the heterocarpic herb *Leontodon autumnalis* (Asteraceae). Results show that achene morphs significantly differ in their ecological behavior, peripheral achenes germinating more and faster than central achenes. The significant interaction between pollination treatment and achene morph for germination probability might indicate a link between dormancy and mating system in *L. autumnalis*: germination was higher for outcrossed achenes in central achenes whereas the opposite pattern was exhibited by peripheral achenes. Selfing dramatically reduced seed set, probably as a consequence of strong self-incompatibility mechanisms rather than inbreeding effects. Inbreeding depression significantly affected late life-cycle traits, such as growth rate and biomass at flowering. Overall, results suggest that inbreeding depression seems to be an important selective force maintaining outcrossing in *L. autumnalis*.

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**Keywords:** Achene; Dispersal; Inbreeding depression; Mating system

### 1. Introduction

Fruit heterocarpy and seed heteromorphism (the production of different types of fruits and seeds by a single individual, respectively) are quite common in flowering plants and have been described in 18 families of angiosperms (Imbert, 2002). Fruit heterocarpy occurs more frequently in Asteraceae and differences are mainly found between peripheral and central achenes (single-seeded fruits), which differ in their ecological behavior. Achene morphs typically differ in dispersal ability due to the presence/absence of a pappus, dormancy and germination requirements due to changes in the thickness and structure of the pericarp, and seedling emergence, growth and survival due to differences in embryo size that in turn affects seedling fate (see for a

comprehensive review Imbert, 2002). Given these important ecological differences that may occur between morphs, it is accepted that heterocarpy represents an evolutionary strategy to reduce temporal variance in progeny fitness, improve competitive ability, or spread offspring in time and space (Lloyd, 1984; Venable, 1985; de Clavijo, 1995; de Clavijo and Jiménez, 1998; Mandak and Pysek, 2001).

Inbreeding depression, the reduction in fitness of selfed compared to outcrossed progeny, may affect several fitness traits (e.g. seed set, germination rate, seedling growth, fecundity) throughout the life cycle of plants (Charlesworth and Charlesworth, 1987; Husband and Schemske, 1996). Given these strong negative effects of inbreeding on fitness, inbreeding depression represents an important evolutionary force governing the evolution of mating systems that prevent self-fertilization (Charlesworth and Charlesworth, 1987; Husband and Schemske, 1996). Many empirical studies suggest that recessive deleterious genes are the main contribu-

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tors to inbreeding depression (i.e. the partial dominance hypothesis). Hence, outcrossed progeny perform better than selfed progeny mainly because recessive alleles tend to be masked in heterozygous condition (Johnston and Schoen, 1995; Dudash and Carr, 1998). Levels of inbreeding depression mainly depend on the underlying genetic mechanism and the breeding history of the populations (Charlesworth and Charlesworth, 1987).

In the case of heterocarpic plants in the Asteraceae, inbreeding may affect the fitness of progeny from different achene morphs (Gibson and Tomlinson, 2002). Hence, inbreeding depression may reduce the ecological advantages of each achene morph, a fact that may ultimately affect the population dynamics and distribution of heterocarpic plant species. Moreover, it has been shown that natural outcrossing rates significantly differ between inner and outer florets (setting central dispersal and peripheral non-dispersal achenes, respectively) within a flower head of an Asteraceae plant (Cheptou et al., 2001), indicating that mating system can play a very important role in determining fitness of progeny from different achene morphs. The main evolutionary implication of differential inbreeding effects on fitness of achene morphs is that the dispersal potential of the species may greatly vary as a function of outcrossing rates. In general, it has been shown that dispersal morphs often exhibit particular genotypes or phenotypic traits that may render them more efficient at colonizing or at integrating into a new population (Murren et al., 2001). As a result, it is accepted that dispersal may represent a way to escape the cost of inbreeding, which in turn is considered as one of the multiple causes of the evolution of dispersal (Gandon and Michalakis, 2001).

In this study, we conduct a hand-pollination experiment to determine whether selfing reduces fitness of both achene morphs in the heterocarpic herb *Leontodon autumnalis* L. (Asteraceae), which produces central dispersal and peripheral non-dispersal achenes. In addition, we analyze the effect of inbreeding depression on *L. autumnalis* life-history traits. To our knowledge, no data have been reported on the mating system, the proportion of achene morphs, or the effects of inbreeding depression on fitness for *L. autumnalis*. The ecological and evolutionary implications of results are discussed.

## 2. Materials and methods

### 2.1. Study species

*L. autumnalis* is a polycarpic, perennial herb that commonly occurs in a variety of grassy places such as meadows, pastures and road verges across Europe. The plant has a taproot and forms a basal rosette of deeply lobed leaves (10–15 cm length). Flowering may last for several months including summer and autumn seasons. Unlike many dandelions, *L. autumnalis* produces branched scapes (20–30 cm height) with two or more yellow flower heads (2.5 cm diameter). The species presents heterocarpy: all central achenes

bear a well-developed pappus facilitating wind dispersal, whereas some peripheral achenes are thicker, slightly darker, and without pappus or just with a very short crown of bristles.

### 2.2. Experimental design

In late summer 2001, central achenes from 15 widely separated plants (more than 5 m between sampling plants) were collected from a dry, semi-natural grassland in central Holland. Achenes were stored in paper bags at room temperature in a dry place. In early March 2002, 10–15 achenes per plant were planted in pots (15 × 15 cm) filled with standard soil mixture and placed in a conditioned greenhouse (20 °C day, 15 °C night, 16 h daylength, and constant high moisture) at the Botanical Garden of the University of Nijmegen. Three weeks after germination, one seedling per maternal plant (i.e. family) was individually potted. Plants flowered in early May 2002. To produce the selfed and outcrossed progeny from each family, 6–8 flower heads per plant were hand-pollinated. Half of them were self-pollinated while the other half were cross-pollinated. Hand-pollinations were carried out by gently rubbing one head over another one (two heads of the same plant for self-pollinations and two heads of different plants for cross-pollinations), and repeated during three consecutive days. This is the standard way to hand-pollinate composites as emasculation of single florets is unfeasible. One head always acted as a pollen donor whereas the other as a recipient head. In the case of cross-pollinations, pollen was pooled from heads of 2–3 donor plants (additional plants were also raised to act only as pollen donors) to increase the proportion of foreign pollen on each outcrossed recipient head, and to reduce paternal effects due to specific characteristics of donor plants. All hand-pollinated heads were bagged during treatments to avoid uncontrolled pollinations and remained bagged until seed harvesting.

Selfed and outcrossed achenes were collected in July 2002 from a total of 11 families that produced matched progeny, i.e. both selfed and outcrossed progeny. All achenes produced per flower, family and treatment were counted. Filled achenes were separated from the aborted ones, central dispersal and peripheral non-dispersal achenes sorted out, and all stored at room temperature in a dry place. Two plants only produced three and four selfed achenes, respectively. Hence, these plants were not included in the subsequent analyses, which were based on nine families. Peripheral achenes were not individually measured because their number per family and treatment was very low. Instead of that, in November 2002, all peripheral achenes produced per family and treatment were pooled and a sub-sample of seven selfed (all selfed peripheral achenes obtained in the experiment) and 15 outcrossed peripheral achenes per treatment were individually potted. In order to compare the performance of central and peripheral achenes, an additional sub-sample of 15 central achenes per treatment (overall 30 central achenes) pooled among families was also individually potted and kept under the germination conditions described above. Germina-

tion probability and germination date were recorded for each achene. Germination date was defined as the number of days between sowing and the emergence of the radicle.

The effects of inbreeding depression on fitness traits of the whole life cycle of the species were tested with a set of central achenes. In November 2002, the length, the diameter, and the pappus size of up to 15 central achenes per family and treatment ( $N = 252$  achenes) were individually measured to the nearest 0.1 mm with a stereomicroscope. The length and diameter were used to calculate the area, estimated as an ellipse, of each achene. All measured achenes were individually planted in small pots ( $5 \times 5$  cm) and kept under the germination conditions described above to record germination probability and germination date. In mid December 2002, a total of 3–7 seedlings per family and treatment ( $N = 106$  seedlings) were transplanted into bigger pots ( $15 \times 15$  cm) and placed in an unconditioned greenhouse where they were maintained until flowering. In mid February 2003, all plants were dried ( $65^\circ\text{C}$  for 24 h) and weighed as soon as the emergence of flowering stalks was detected. Total dry weight was divided by the number of days between germination date and harvesting date to obtain individual growth rate. All plants survived throughout the experiment and only four plants did not flower.

### 2.3. Statistical analyses

The effects of pollination treatment (selfing and outcrossing; fixed factor) and achene morph (central and peripheral; fixed factor) on the germination probability and germination date were tested with logistic regression and two-way ANOVA models, respectively. The replicate of these analyses was the individual achene germination traits per treatment and morph. Logistic regressions were based on the comparison between a null model including all main factors with their interaction and a model that lacked the term to be analyzed. For each factor, the difference in unexplained variance (deviance,  $D$ ) between models is approximately  $\chi^2$  distributed, with the number of degrees of freedom equal to the difference between models.

For the set of central achenes, the effects of pollination treatment (fixed factor) and family (random factor) on seed set, i.e. the proportion of ovules that set seed, were analyzed with a two-way ANOVA. In this analysis, the replicate was the seed set value obtained per flower head. The effects of pollination treatment and family on achene area and plume length were tested with a two-way ANOVA, where the replicate was the individual achene measurements per family and treatment. The effects of pollination treatment and family on germination probability were tested with a logistic regression model. This model included achene area as a covariate. The effects of pollination treatment and family on germination date, flowering date, growth rate, and dry weight at flowering were tested with a two-way ANCOVA, where individual achene area was also used as a covariate. The assumption of parallel slopes necessary for using covariates was examined with three-way interaction terms. Indeed, the triple interac-

tion covariate  $\times$  pollination treatment  $\times$  family was non-significant in all analyses ( $P > 0.05$  in all cases). When necessary, variables were arcsine-transformed for proportions and log-transformed for all others to normalize their distributions, and Levene's test was performed to check for homogeneity of variances. Transformed variables and type IV sums of squares were used in all analyses.

Inbreeding depression ( $\delta$ ) was calculated according to Ågren and Schemske (1993), so that  $\delta = 1 - (W_s/W_o)$  when  $W_s < W_o$ , and  $\delta = W_o/W_s - 1$  when  $W_o < W_s$ , where  $W_s$  and  $W_o$  are the mean fitness of selfed and outcrossed progeny, respectively. Positive  $\delta$  values indicate that outcrossed progeny is more fit than selfed progeny, whereas negative  $\delta$  values mean the opposite. The  $\delta$  value was only calculated for traits that showed significant differences between pollination treatments, and the mean measures of  $W_s$  and  $W_o$  were calculated as the mean of family values.

### 3. Results

The proportion of peripheral non-dispersal achenes of *L. autumnalis* was low in both pollination treatments (mean proportion  $\pm$  S.E. of peripheral achenes per family =  $4.8 \pm 3.5\%$  and  $6.2 \pm 2.0\%$  for selfing and outcrossing, respectively). Germination probability did not differ between pollination treatments but significantly differed between achene morphs (Table 1). Central achenes had a higher germination probability than peripheral achenes (Fig. 1). The interaction between pollination treatment and achene type was also significant (Table 1), indicating that the effects of pollination treatment on germination probability differed between achene morphs (Fig. 1). Germination date significantly differed between achene types (Table 1). On average, central achenes germinated nearly 1 day before peripheral achenes (Fig. 2).

Selfing significantly and dramatically reduced seed set of *L. autumnalis* plants (Tables 2 and 3), while the other achene traits, such as achene area and plume length, were not significantly affected by pollination treatment (Tables 2 and 3). Only plume length varied significantly between families (Table 2). The interaction between pollination treatment and family was significant for seed set, achene area and plume length (Table 2), indicating that the effects of pollination treatment on these traits varied between families.

Table 1  
Logistic regression and two-way ANOVA models testing for the effects of pollination treatment (selfing and outcrossing) and achene morph (central and peripheral) on germination probability and germination date of *L. autumnalis* achenes. Degrees of freedom (df) and  $D$  and  $F$ -values for the logistic regression and the ANOVA, respectively, are given

Factor	Germination probability		Germination date	
	df	$D$	df	$F$
Treatment (T)	1	3.41 <sup>ns</sup>	1	0.01 <sup>ns</sup>
Achene morph (M)	1	19.20 <sup>*</sup>	1	5.10 <sup>**</sup>
T $\times$ M	1	5.10 <sup>**</sup>	1	0.00 <sup>ns</sup>
Error	–		30	

Significance: \*,  $P < 0.001$ ; \*\*,  $P < 0.05$ ; ns, non-significant.

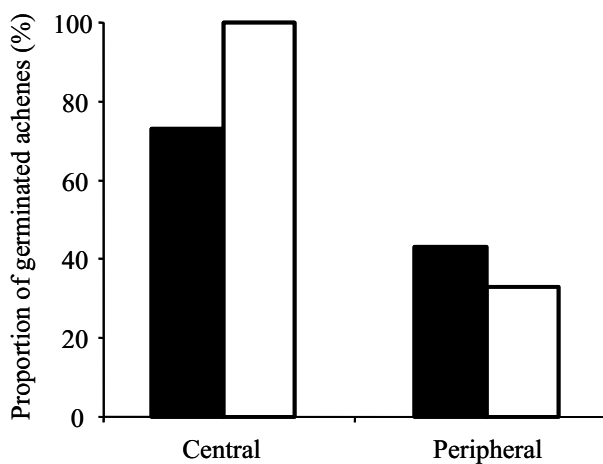


Fig. 1. Proportion of germinated achenes (%) for central and peripheral selfed (filled bars) and outcrossed achenes (hollow bars) of *L. autumnalis*.

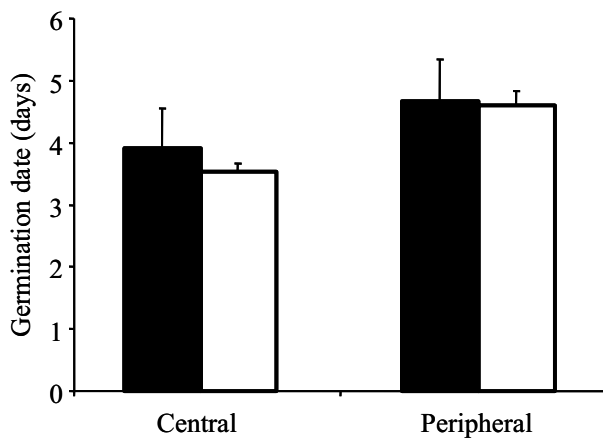


Fig. 2. Mean ( $\pm$  S.E.) germination date (days) for central and peripheral selfed (filled bars) and outcrossed achenes (hollow bars) of *L. autumnalis*.

Achene germination probability did not vary significantly between pollination treatments or families ( $D < 7.92$ ,  $P > 0.05$  for both factors) and most of the achenes germinated within 2 weeks (Table 3). Germination date was not affected by pollination treatment (Table 4) and, on average, achene germination occurred 4 days after sowing (Table 3). Flowering date was not significantly affected by pollination treatment (Table 4), whereas selfing significantly reduced growth rate and dry weight of *L. autumnalis* plants at flowering (Table 4). Selfed plants grew slower and consequently reached smaller plant sizes at flowering than outcrossed

Table 2

Two-way ANOVAs testing for the effects of pollination treatment (selfing and outcrossing) and family on seed set, achene area and plume length in *L. autumnalis*. Degrees of freedom (df) and  $F$ -values are given

Factor	Seed set		Achene area		Plume length	
	df	$F$	df	$F$	df	$F$
Treatment (T)	1	40.73 *	1	1.29 ns	1	0.37 ns
Family (F)	10	0.60 ns	8	1.97 ns	8	13.29 *
T $\times$ F	10	8.97 *	8	8.46 *	8	7.56 *
Error	38		234		234	

Significance: \*,  $P < 0.001$ ; ns, non-significant.

plants (Table 3). The effect of family was never significant for germination date, flowering date, growth rate or dry weight at flowering (Table 4). The interaction between pollination treatment and family was only significant for growth rate and dry weight at flowering (Table 4). Achene area, the covariate, did not significantly affect germination rate, flowering date, growth rate or dry weight at flowering (Table 4), indicating that life-cycle traits were not determined by achene size.

Inbreeding depression ( $\delta$ ) for the traits that differed significantly between treatments (seed set, growth rate and dry weight at flowering; Table 3) indicated that the selfed progeny had reduced fitness relative to the outcrossed progeny in all these life-cycle traits. Seed set contributed much more to the fitness reduction than the other two later traits (Table 3).

#### 4. Discussion

The heterocarpic *L. autumnalis* produced a low proportion of peripheral non-dispersal achenes per flower head, as occurs in other seed heteromorphic and fruit heterocarpic plants of the Asteraceae family (Baker and O'Dowd, 1982; de Clavijo and Jiménez, 1998; Imbert, 2001). The explanation can be found in the ontogeny of the capitulum in Asteraceae due to the limited number of phyllotactic spirals within the capitulum that determine the number of outer florets, which produce peripheral achenes (Bachmann, 1983). The achene morph proportion in other plants of the Asteraceae family tends to change due to environmental stress, as peripheral and/or central achenes can be selectively aborted under stressful conditions (Baker and O'Dowd, 1982; de Clavijo and Jiménez, 1998; Imbert and Ronce, 2001).

Achene morphs of *L. autumnalis* significantly differed in important ecological characters, such as germination probability and germination date. The results agree with the accepted theory for Asteraceae that central dispersal achenes have higher and faster germination than peripheral non-dispersal achenes (Imbert, 2002). Peripheral achenes tend to have harder pericarps that seem to delay germination, by reducing water absorption rate, and even induce dormancy, due to chemical compounds of the seed coat (Mohamed-Yasseen et al., 1994). Differences in germination probability and germination date between achene morphs confer different ecological characteristics to the two *L. autumnalis* achene morphs. On one hand, dispersal and establishment of new populations is enhanced by central achenes with a high and fast germination, whereas differences in germination probability and germination date between achene morphs increase the probability of spreading individuals in time, which could reduce sib competition (Venable, 1985; Imbert, 2002). Both germination probability and germination date of *L. autumnalis* achenes were unaffected by pollination treatment as a main factor, but the interaction between achene morph and pollination treatment had a readily significant effect on germination probability. Outcrossed central achenes germinated more than selfed central achenes,

Table 3

Mean ( $\pm$ S.E.) measures of selfed and outcrossed progeny of *L. autumnalis* for different traits analyzed and obtained from the pollination experiment using a set of central achenes. Measures were calculated as the means of family values. The inbreeding coefficient for those traits that showed significant differences between selfing and outcrossing are shown

Fitness trait	Selfed progeny	Outcrossed progeny	Inbreeding coefficient ( $\delta$ )
Seed set (%) <sup>a</sup>	10.20 $\pm$ 2.52	58.89 $\pm$ 7.27	–
Achene area (mm <sup>2</sup> )	1.94 $\pm$ 0.06	1.85 $\pm$ 0.08	–
Plume length (mm)	6.81 $\pm$ 0.14	6.77 $\pm$ 0.21	–
Germinability (%)	74.20 $\pm$ 7.50	94.07 $\pm$ 3.03	–
Germination date (days)	4.19 $\pm$ 0.21	3.84 $\pm$ 0.14	–
Flowering date (days)	82.4 $\pm$ 0.75	80.4 $\pm$ 0.67	–
Growth rate (mg/day)	9.11 $\pm$ 0.52	12.24 $\pm$ 0.49	0.26
Dry weight at flowering (g)	0.75 $\pm$ 0.04	0.99 $\pm$ 0.04	0.24

<sup>a</sup> The  $\delta$ -value was not computed because inbreeding depression did not cause the reduction in seed set (see Section 4 for details).

Table 4

Two-way ANCOVAs testing for the effect of pollination treatment (selfing and outcrossing) and family on germination date, flowering date, growth rate and dry weight at flowering in dispersal offspring of *L. autumnalis*. Achene area was used as a covariate. Degrees of freedom (df) and *F*-values are given

Factor	Germination date		Flowering date		Growth rate		Dry weight at flowering	
	df	<i>F</i>	df	<i>F</i>	df	<i>F</i>	df	<i>F</i>
Achene area	1	1.15 <sup>ns</sup>	1	0.15 <sup>ns</sup>	1	0.19 <sup>ns</sup>	1	0.12 <sup>ns</sup>
Treatment (T)	1	2.74 <sup>ns</sup>	1	5.08 <sup>ns</sup>	1	7.02*	1	5.92*
Family (F)	8	2.12 <sup>ns</sup>	7	1.28 <sup>ns</sup>	7	0.96 <sup>ns</sup>	7	1.26 <sup>ns</sup>
T $\times$ F	8	1.64 <sup>ns</sup>	7	1.23 <sup>ns</sup>	7	2.94**	7	3.04**
Error	196		85		85		85	

Significance: \*,  $P < 0.05$ ; \*\*,  $P < 0.001$ ; ns, non-significant.

whereas the opposite pattern was exhibited by peripheral achenes. This result might indicate a link between dormancy and mating system. Studies on other heterocarpic Asteraceae suggest that pollination type may have different effects on fitness of progeny from different achene morphs, though results usually indicate an unclear relationship between ecological characters of each achene morph and its genotype (Cheptou et al., 2001; Gibson, 2001; Gibson and Tomlinson, 2002).

Due to the shortage of peripheral achenes obtained with the experimental crosses, the experiment to assess the effects of inbreeding depression on fitness traits throughout the whole life cycle of *L. autumnalis* was conducted using a set of central achenes. The results show that selfing reduced seed set and late life-cycle traits, such as growth rate and biomass at flowering. The dramatic reduction in seed set after selfing (the selfed progeny was 83% less fit than the outcrossed progeny; Table 3) suggests that *L. autumnalis* has a strong self-incompatibility system. Hence, mating system, rather than early acting inbreeding depression, was more likely to be responsible for the failure of seed set after selfing. The sharp reduction in seed set after selfing is a common result of studies focusing on the effects of inbreeding depression on fitness traits of self-incompatible species (Luijten et al., 2002). This is supported by the fact that the incompatibility system in Asteraceae is generally of the single-locus, multi-allelic, sporophytic type (De Nettancourt, 1977). Additional studies are needed to determine the specific incompatibility system of *L. autumnalis*.

Inbreeding depression differed widely among the life-cycle traits examined. No effects were found at early stages,

such as achene germination, whereas late life-cycle traits, such as growth rate and biomass at flowering, were significantly affected by inbreeding depression. Such a reduction perhaps occurred because the combined effects of inbreeding depression are manifest most at the end of the life cycle (see Dudash, 1990). The effects of inbreeding depression on late life-cycle traits of *L. autumnalis* might be the result of mildly deleterious mutations that cannot be purged from the population. Furthermore, our results partially fit the general conclusion that outcrossing plants express substantial inbreeding depression at both early and late life-cycle stages (Husband and Schemske, 1996), assuming that the effects of inbreeding on seed set are due to mating system and not to inbreeding depression.

Achene area and plume length, surrogates of achene size and dispersal ability, respectively, were not affected by inbreeding depression. Moreover, achene area did not affect subsequent life-cycle traits, as indicated by the lack of significant effect of achene area when it was introduced as a covariate in the analyses (Table 4), so we were unable to detect maternal effects (mediated by achene area) on life-cycle traits. This is an accepted way to test for maternal effects in inbreeding depression experiments (Kalisz, 1989; Wolfe, 1993; Helenurm and Schaal, 1996). It must be noted, however, that family had a significant effect on plume length, indicating that maternal effects might mediate changes in seed dispersal (Donohue, 1999; Imbert and Ronce, 2001). This is not surprising as traits involved in seed dispersal are largely maternally controlled (Imbert and Ronce, 2001). Finally, the interaction between pollination treatment and family was significant for most of the traits analyzed in this study.

This interaction is typically found in studies dealing with the effects of inbreeding depression on fitness. It has been suggested that differences between plants in their response to inbreeding could also be the result of maternal effects (Byers and Waller, 1999). However, the effects of individual breeding history or differences between maternal plants in the accumulation of mutations cannot be ruled out as an additional explanation (Koelewijn et al., 1999).

Overall, results suggest that inbreeding depression represents an important selective force maintaining outcrossing in *L. autumnalis*. The dispersal ability of the species is determined by maternal plant qualities, whereas the germination ability of central and peripheral achenes strongly depends on the pollination treatment. Hence, population factors, such as reductions in population size, with an important influence on inbreeding and outcrossing rates can chiefly determine the performance and fate of central and peripheral achenes. Further studies are needed to fully assert the ecological and evolutionary effects of cross type on fitness of different morphs in heterocarpic plants, as the amount of studies dealing with the genetics of heterocarpy are still very rare in the literature (Cheptou et al., 2001; Gibson, 2001; Gibson and Tomlinson, 2002).

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## References

- Ågren, J., Schemske, D.W., 1993. Outcrossing rate and inbreeding depression in two annual monoecious herbs, *Begonia hirsuta* and *B. semiovata*. *Evolution* 47, 125–135.
- Bachmann, K., 1983. Evolutionary genetics and the genetic control of morphogenesis in flowering plants. *Evolutionary Biology* 16, 157–208.
- Baker, G.A., O'Dowd, D.J., 1982. Effects of parent plant density on the production of achene type in the annual *Hypochoeris glabra*. *Journal of Ecology* 70, 201–215.
- Byers, D.L., Waller, D.M., 1999. Do plant populations purge their genetic load? Effects of population size and mating history on inbreeding depression. *Annual Review of Ecology and Systematics* 30, 479–513.
- Charlesworth, D., Charlesworth, B., 1987. Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics* 18, 237–268.
- Cheptou, P.O., Lepart, J., Escarré, J., 2001. Differential outcrossing rates in dispersing and non-dispersing achenes in the heterocarpic plant *Crepis sancta* (Asteraceae). *Evolutionary Ecology* 15, 1–13.
- de Clavijo, E.R., 1995. The ecological significance of fruit heteromorphism in the amphicarpic species *Catananche lutea* (Asteraceae). *International Journal of Plant Sciences* 156, 824–833.
- de Clavijo, E.R., Jiménez, M.J., 1998. The influence of achene type and plant density on growth and biomass allocation in the heterocarpic annual *Catananche lutea* (Asteraceae). *International Journal of Plant Sciences* 159, 637–647.
- De Nettancourt, D., 1977. *Incompatibility in Angiosperms*. Springer-Verlag, Berlin.
- Donohue, K., 1999. Seed dispersal as a maternally influenced character: mechanistic basis of maternal effects and selection on maternal characters in an annual plant. *American Naturalist* 154, 674–689.
- Dudash, M.R., 1990. Relative fitness of selfed and outcrossed progeny in a self-compatible, protandrous species, *Sabatia angularis* L. (Gentianaceae): a comparison in three environments. *Evolution* 44, 1129–1139.
- Dudash, M.R., Carr, D.E., 1998. Genetics underlying inbreeding depression in *Mimulus* with contrasting mating systems. *Nature* 393, 682–684.
- Gandon, S., Michalakis, Y., 2001. Multiple causes of the evolution of dispersal. In: Clobert, J., Danchin, E., Dhondt, A.A., Nichols, J.D. (Eds.), *Dispersal*. Oxford University Press, Oxford, pp. 155–167.
- Gibson, J.P., 2001. Ecological and genetic comparison between ray and disc achene pools of the heteromorphic species *Prionopsis ciliata* (Asteraceae). *International Journal of Plant Sciences* 162, 137–145.
- Gibson, J.P., Tomlinson, A.D., 2002. Genetic diversity and mating system comparisons between ray and disc achene seed pools of the heterocarpic species *Heterotheca subaxillaris* (Asteraceae). *International Journal of Plant Sciences* 163, 1025–1034.
- Helenurm, K., Schaal, B.A., 1996. Genetic and maternal effects on offspring fitness in *Lupinus texensis* (Fabaceae). *American Journal of Botany* 83, 1596–1608.
- Husband, B.C., Schemske, D.W., 1996. Evolution and the magnitude and timing of inbreeding depression in plants. *Evolution* 50, 54–70.
- Imbert, E., 2001. Capitulum characters in a seed heteromorphic plant, *Crepis sancta* (Asteraceae): variance partitioning and inference for the evolution of dispersal rate. *Heredity* 86, 78–86.
- Imbert, E., 2002. Ecological consequences and ontogeny of seed heteromorphism. *Perspectives in plant ecology. Evolution and Systematics* 5, 13–36.
- Imbert, E., Ronce, O., 2001. Phenotypic plasticity for dispersal ability in the seed heteromorphic *Crepis sancta* (Asteraceae). *Oikos* 93, 126–134.
- Johnston, M.O., Schoen, D.J., 1995. Mutation rates and dominance levels of genes affecting total fitness in two angiosperm species. *Science* 267, 226–229.
- Kalisz, S., 1989. Fitness consequences of mating system, seed weight, and emergence date in a winter annual, *Collinsia verna*. *Evolution* 43, 1263–1272.
- Koelewijn, H.P., Koski, V., Savolainen, O., 1999. Magnitude and timing of inbreeding depression in Scots Pine (*Pinus sylvestris* L.). *Evolution* 53, 758–768.
- Lloyd, D.G., 1984. Variation strategies of plants in heterogeneous environments. *Biological Journal of the Linnean Society* 21, 357–385.
- Luijten, S.H., Kéry, M., Oostermeijer, J.G.B., den Nijs, J.C.M., 2002. Demographic consequences of inbreeding and outbreeding in *Arnica montana*: a field experiment. *Journal of Ecology* 90, 593–603.
- Mandak, B., Pysek, P., 2001. Fruit dispersal and seed banks in *Atriplex sagittata*: the role of heterocarpy. *Journal of Ecology* 89, 159–165.
- Mohamed-Yasseen, Y., Barringer, S.A., Splittstoesser, W.A., Constanza, S., 1994. The role of seed coats in seed viability. *The Botanical Review* 60, 426–439.
- Murren, C.J., Julliard, R., Schlichting, C.D., Clobert, J., 2001. Dispersal, individual phenotype and phenotypic plasticity. In: Clobert, J., Danchin, E., Dhondt, A.A., Nichols, J.D. (Eds.), *Dispersal*. Oxford University Press, Oxford, pp. 155–167.
- Venable, D.L., 1985. The evolutionary ecology of seed heteromorphism. *American Naturalist* 126, 577–595.
- Wolfe, L.M., 1993. Inbreeding depression in *Hydrophyllum appendiculatum*: role of maternal effects, crowding, and parental mating history. *Evolution* 47, 374–386.