

Influence of selfing and maternal effects on life-cycle traits and dispersal ability in the herb *Hypochaeris radicata* (Asteraceae)

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Received February 2003; accepted for publication May 2004

The ecological and evolutionary implications of dispersal are many. Pollination type and maternal effects may affect plant fitness traits, including life-cycle traits as well as dispersal ability. This study investigated the joint influence of pollination type and maternal effects on both life-cycle traits and dispersal ability in the herb *Hypochaeris radicata*. We conducted experimental crosses to obtain selfed and outcrossed progeny. Individual seeds and their pappuses were measured to determine seed terminal velocity. Seed size was also used to assess maternal effects. Selfing dramatically decreased seed set, indicating that *H. radicata* is self-incompatible. However, the few selfed seeds produced outperformed outcrossed seeds in seed size and flowering probability, surely as a result of an effective reallocation of resources among selfed seeds. None of the life-cycle traits was affected by seed size, the estimate of maternal effects. Selfed seeds were larger and bore a smaller pappus than outcrossed seeds. As a result, dispersal ability was lower for selfed than outcrossed seeds. Several factors, such as the low proportion of plants that produced selfed seeds, the low number of selfed seeds produced per plant, and the lack of self-fertility mechanisms might act in concert to prevent the evolution of selfing in *H. radicata*. © 2004 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2004, 146, 163–170.

ADDITIONAL KEYWORDS: fitness traits – hand-pollination experiments – outcrossing – seed size – self-incompatibility system – terminal velocity – trade-off.

INTRODUCTION

Individual fitness is achieved through numerous components summed across the life cycle of an organism, such as survivorship, growth and reproduction. In plants, these components are closely related to each other through different trade-offs, e.g. reproduction vs. survivorship in the next generation, which represent targets of selection (Crawley, 1997). Other traits not included in the life cycle, such as dispersal ability, may also play a very important role in determining fitness, as dispersal away from the mother plant often proves crucial for successful recruitment, colonizing new patches and/or escaping unfavourable conditions

(Hutchings, 1997; Clobert *et al.*, 2001). For this reason, dispersal may also represent a target of selection (Murren *et al.*, 2001; Morgan, 2002).

One of the main effects of selfing is inbreeding depression, i.e. the lower success of selfed compared to outcrossed progeny, which is considered as a strong evolutionary force because of its negative effects on different components of individual fitness (Lande & Schemske, 1985; Charlesworth & Charlesworth, 1987; Holsinger, 1991). Moreover, the evolution of inbreeding depression in flowering plants affects the evolution of life histories and mating systems. For example, outcrossing and longevity show a significant positive correlation (Charlesworth & Charlesworth, 1987), and outcrossers exhibit greater inbreeding depression than selfers (Lande & Schemske, 1985; Carr & Dudash, 1995; Dudash & Fenster, 2001). The effect of recessive deleterious mutations that accumulate without purging in outcrossers and long-lived plants can

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explain these general trends (Charlesworth & Charlesworth, 1987), which have been the subject of many evolutionary studies. However, studies focusing on the effects of pollination type (selfing or outcrossing) on other important fitness components, such as dispersal ability, are rare (Donohue, 1998).

Traits involved in seed dispersal are largely maternally controlled, so maternal effects may mediate changes in seed dispersal (Donohue, 1999; Imbert & Ronce, 2001). Maternal effects mainly refer to those traits of the offspring that have been influenced by environmental or non-genetic factors affecting the mother and that ultimately act on the embryo (Futuyma, 1998), although maternal effects can also have an important genetic component (Roach & Wulff, 1987). Maternal plant qualities also influence seed size, which has been found to affect germination and other fitness traits (Maun & Payne, 1989; Leishman & Westoby, 1994; Meyer & Carlson, 2001; Picó, Ouborg & van Groenendael, 2003). It must be noted, however, that seed size is also a trait commonly affected by pollination type. Thus, the ultimate effects of selfing on dispersal ability, but also on many other life-cycle traits, may be strongly influenced by maternal effects, given their large influence on progeny performance (Roach & Wulff, 1987). The joint influence of pollination type and maternal effects on plant performance has been tested in some studies (Kalisz, 1989; Wolfe, 1993; Montalvo, 1994; Helenurm & Schaal, 1996; Galloway, 2001), but only a few have focused on the ultimate effect of pollination type and maternal effects on both life-cycle traits and dispersal ability (Donohue, 1998; Picó *et al.*, 2003).

By simultaneously examining the influence of pollination type and maternal effects on both life-cycle traits and dispersal ability, a comprehensive understanding of the effects of pollination type and maternal effects on a wide range of plant fitness traits can be achieved. It has been argued that dispersal evolved to avoid inbreeding, although dispersal might also have evolved as a response to other factors, such as competition for resources (Johnson & Gaines, 1990; Perrin & Goudet, 2001). Differences in maternal plant qualities in allocating resources to dispersal might also contribute to the evolution of dispersal. Hence, the evolutionary implications of maternal effects cannot be ruled out and also need to be investigated. In this study, we conducted a hand-pollination experiment with the perennial herb *Hypochaeris radicata* L. (Asteraceae) to analyse how pollination type and maternal effects jointly act on the species' life-cycle traits and its potential to disperse. Selfed and outcrossed progeny were produced and fitness traits throughout the whole life cycle analysed. Seed characteristics, including seed and pappus size, were individually recorded for each seed. The maternal effects

were analysed by including seed size as a covariate in the analyses, and the seed/pappus size ratio was used to determine the dispersal ability of each seed, which was estimated by using a specific terminal velocity function for *H. radicata* from the literature.

MATERIAL AND METHODS

PLANT SPECIES AND STUDY SITE

The long-lived perennial herb *Hypochaeris radicata* L. grows on dry grasslands and wasteland across Europe. This widespread plant has a fleshy taproot and forms a basal rosette of lobed leaves (around 15 cm long). The flowering season may last for several months (from May to October) and the plant produces several branched flowering stalks (around 40–50 cm height). Its seeds (achenes) bear a well-developed pappus facilitating wind dispersal.

In summer 2001, seeds from 15 widely separated plants (more than 5 m between sampled plants) were collected from two populations in the Netherlands with similar characteristics (dry grasslands) and area (0.2 ha each), population AK (Allemanskampje; 52°37'N, 6°08'E) and NH (Nijkampse heide; 52°01'N, 6°32'E) included about 100 and 125 flowering rosettes, respectively.

EXPERIMENTAL CROSSES

In March 2002, seeds (10–15 seeds per plant) were planted in pots (15 × 15 cm) filled with standard soil mixture and placed in a greenhouse (20°C-day, 15°C-night, 16 h day-length, and constant high moisture) in the Botanic Garden of the University of Nijmegen. One month after germination, one seedling per maternal plant (i.e. family) and population was individually potted. Plants flowered in May 2002. In order to produce selfed and outcrossed progeny for each family, four flower heads per plant were hand-pollinated (two self-pollinated and two cross-pollinated). Cross-pollinations were made among plants within each population. 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). One head always acted as a pollen donor and the other as a recipient head. In the case of cross-pollination, pollen was pooled from the heads of 3–4 donor plants to increase the proportion of foreign pollen on each outcrossed recipient head, and to reduce parental effects resulting from specific characteristics of donor plants. We raised a separate set of plants for each population only to act as pollen donors. All hand-pollinated heads were bagged during treatments to avoid uncontrolled pollinations and remained bagged until seed harvesting.

As a result of these experimental crosses, a total of nine and ten plants from population AK and NH, respectively, produced matched progeny (i.e. both selfed and outcrossed progeny).

Selfed and outcrossed seeds were collected in early July 2002. All achenes per head were counted and filled achenes were separated from the aborted ones. Because half of the plants from each population produced no selfed seeds, only four and five plants from population AK and NH, respectively, were used for the analyses. After a few days stored in paper bags at room temperature, the lengths of up to 25 achenes per family and treatment ($N = 365$ achenes) were individually measured to the nearest 0.1 mm under a stereomicroscope. Achene length can be considered as a good estimator of seed mass, as indicated by the positive significant relationship between these two traits in *H. radicata* ($N = 30$, $R^2 = 73.7$, $P < 0.001$; linear regression, C. Mix, unpubl. data). Additionally, the pappus length of each achene was also measured to the nearest 0.1 mm also under a stereomicroscope. All measured achenes were individually planted in small pots (5×5 cm) and kept under the germination conditions described above to record emergence date of each individual achene, which was defined as the number of days between sowing and the emergence of the radicle. In mid-August 2002, all surviving seedlings were transplanted into bigger pots (15×15 cm) and placed in an uncontrolled greenhouse where they were maintained until flowering. Flowering occurred in September 2002. Flowering date was recorded as the number of days between germination and the emergence of the flowering stalk. Plant size was measured for all surviving plants and estimated by the product between the total number of leaves and the length of the longest leaf, which is positively significantly correlated with total plant biomass expressed in dry weight ($N = 46$, $R^2 = 84.2$, $P < 0.001$; linear regression, C. Mix, unpubl. data). The growth rate of each plant was estimated by dividing plant size by the days since germination. The height of the flowering stalk was measured and the total number of flowers counted.

DISPERSAL ABILITY MEASUREMENTS

Terminal velocity was calculated with a function experimentally fitted for *H. radicata* (Soons & Heil, 2002). The function relates seed mass and pappus diameter with drop time, and can be used to estimate seed terminal velocity, which is proportionally related to seed weight and inversely related to pappus area (Greene & Johnson, 1993). The resulting linear regression (Soons & Heil, 2002) was applied to our data set to obtain the terminal velocity estimates of each individual seed:

$$\text{Terminal velocity} = 5.93 \times (\text{seed mass}^{1/2} / \text{pappus diameter}). \quad (1)$$

High terminal velocity values, resulting from high seed mass and/or low pappus area, imply low dispersal distances and vice-versa.

STATISTICAL ANALYSES

The response to pollination treatment and maternal effects of continuous plant traits were analysed with mixed-model ANOVAs and ANCOVAs. For seed set, i.e. the proportion of ovules that set seed, ANOVA included pollination treatment and population as fixed factors. For seed size, pappus length and terminal velocity, ANOVA included pollination treatment and population as fixed factors and family nested within population as a random factor. The relationship between seed size and pappus length was analysed with linear regression models for each family and treatment. For emergence date and growth rate, ANCOVA included the same main factors as in the second ANOVA above and seed size as a COVARIATE, which represented the maternal effect. The assumption of parallel slopes necessary for using covariates was examined with three-way interaction terms. Indeed, covariate by pollination treatment by population interaction terms were found to be not significant in all analyses. For the height of the flowering stalk and number of flowers, one-way ANOVA testing for pollination treatment was used by pooling families from the two populations of study and using family means as replicates for two reasons; (i) because the proportion of flowering plants was low or nil in some families, and (ii) because the population effect was never significant in previous analyses (see the Results section). 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.

The response of categorical variables, such as germinability, survivorship and flowering probability to pollination treatment and maternal effects was analysed using logistic regression models. In all cases, the analysis started with a null model including all main factors (pollination treatment, population and family), the covariate (seed mass) and interactions. Then, a new model lacking the term to be analysed was created. Subsequently, for each term, we tested whether the difference in unexplained variance (deviance, D) between models is approximately χ^2 distributed, with the number of degrees of freedom equal to the difference between models.

RESULTS

LIFE CYCLE TRAITS

Seed set significantly differed between selfed and outcrossed progeny (Table 1). Seed set of the outbred progeny was much higher than that of the selfed progeny (Table 4). Seed size and pappus length were also significantly affected by pollination treatment (Table 2). Selfed seeds were larger and bore a smaller pappus than outcrossed seeds (Table 4). Pappus length showed significant differences between families within populations whereas seed size varied little between families (Table 2). For seed size and pappus length, the population effect was nonsignificant

Table 1. Two-way ANOVA testing for pollination treatment (selfing and outcrossing) and population (AK and NH) for seed set of *Hypochaeris radicata*. The dependent variable was arcsine-transformed prior to analysis. Degrees of freedom (d.f.) and *F*-values are given

Factor	d.f.	<i>F</i>
Treatment (T)	1	422.56***
Population (P)	1	0.61 NS
T × P	1	0.09 NS
Error	34	

Significance: ****P* < 0.001; NS, nonsignificant.

(Table 2). The treatment-by-family interaction was significant for both seed size and pappus length (Table 2) caused by the opposite pattern of seed size and pappus length between selfed and outcrossed progeny of a few families in both populations (Fig. 1).

Both selfed and outcrossed seeds exhibited very high germination percentages (Table 4) with no differ-

Table 2. Three-way ANOVA testing for pollination treatment (selfing and outcrossing), population (AK and NH), and family nested within population for seed size, pappus length and terminal velocity of *Hypochaeris radicata* seeds. Variables were log-transformed prior to analysis. Degrees of freedom (the same d.f. for the three dependent variables) and *F*-values are given

Factor	d.f.	Seed size <i>F</i>	Pappus length <i>F</i>	Terminal velocity <i>F</i>
Treatment (T)	1	18.50**	21.45**	36.43***
Population (P)	1	1.19 NS	0.01 NS	0.04 NS
Family (F)	7	1.95 NS	11.25**	7.95**
T × P	1	2.39 NS	6.45*	3.09 NS
T × F	7	28.17***	6.66***	8.41***
Error	347			

Significance: ****P* < 0.001; ***P* < 0.01; **P* < 0.05; NS, nonsignificant.

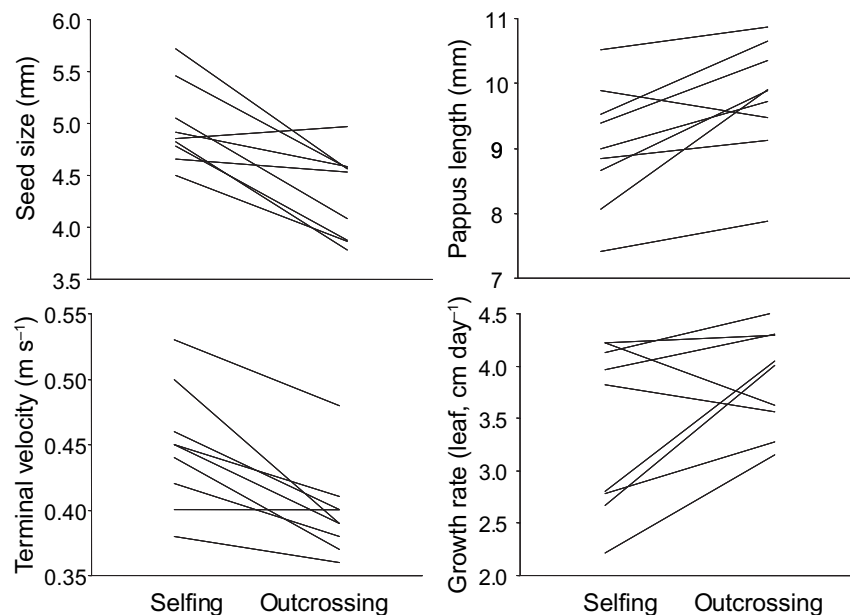


Figure 1. Mean values of seed size, pappus length, terminal velocity and growth rate for *Hypochaeris radicata* families in different pollination treatments. These traits showed a significant family-by-pollination treatment interaction in ANOVAs and ANCOVAs (see tables). Lines connect selfed and outcrossed progeny mean values of the same family. Populations are not differentiated because the population effect was nonsignificant in all analyses (see tables).

Table 3. Three-way ANCOVA and logistic regression models testing for pollination treatment (selfing and outcrossing), population (AK and NH), and family for germination date, growth rate, germinability, flowering probability, and overall survivorship of *Hypochaeris radicata*. Seed size was used as a covariate. Degrees of freedom (d.f.), *F*-values for ANCOVAs, and *D*-values for logistic regressions are given. For logistic regressions, all interactions between factors and the covariate were nonsignificant and are not shown. For logistic regressions, there is no error term because *D*-values were calculated by analysis of deviance of logistic regression model (see text for details)

Factor	Germination date		Growth rate		Germinability		Flowering probability		Overall survivorship	
	d.f.	<i>F</i>	d.f.	<i>F</i>	d.f.	<i>D</i>	d.f.	<i>D</i>	d.f.	<i>D</i>
Seed size	1	0.01 NS	1	2.45 NS	1	0.09 NS	1	0.12 NS	1	0.01 NS
Treatment (T)	1	1.32 NS	1	1.57 NS	1	0.01 NS	1	6.04 *	1	0.01 NS
Population (P)	1	3.26 NS	1	1.18 NS	1	0.04 NS	1	1.15 NS	1	0.78 NS
Family (F)	7	5.79 *	7	2.38 NS	7	5.40 NS	7	4.53 NS	7	7.06 NS
T × P	1	0.01 NS	1	0.01 NS	1	0.01 NS	1	2.53 NS	1	0.79 NS
T × F	7	1.34 NS	7	6.93 ***	7	1.11 NS	7	3.86 NS	7	2.06 NS
Error	309		290							

Significance: ****P* < 0.001; **P* < 0.05; NS, nonsignificant.

ences between pollination treatments or any other factor included in the model (Table 3). Germination date did not differ significantly between selfed and outcrossed progeny, and only significantly differed between families within populations (Table 3). Seed size was included in these models as a covariate but was nonsignificant for both variables (Table 3). Growth rate did not significantly differ between selfed and outcrossed progeny, and only the treatment-by-family interaction was significant (Table 3) due to several selfed plants outperforming outcrossed plants for some families and vice-versa for others (Fig. 1). Seed size, included as a covariate, did not significantly affect the growth rate (Table 3).

Pollination treatment had a significant effect on the flowering probability of selfed and outcrossed progeny and seed size had no effect on flowering probability (Table 3). The proportion of flowering selfed plants was greater than that of outcrossed plants (Table 4). Selfed and outcrossed plants differed neither in the number of flowers produced nor in the height of the flowering stalk (*P* > 0.06 for both cases; one-way ANOVA). Finally, survival rates were very high throughout the whole life cycle of *H. radicata* (Table 4), and overall survivorship did not significantly differ between selfed and outcrossed progeny (Table 3). Seed size did not affect the survival probability of plants (Table 3).

DISPERSAL ABILITY

Seed size and pappus size were positively significantly correlated for one of nine selfed families ($R^2 = 43.1$, $r = 0.66$, $F_{1,14} = 9.08$, $P = 0.011$; linear regression) and

Table 4. Mean (\pm SE) fitness measures and plant traits of selfed and outcrossed progeny of *Hypochaeris radicata* for all traits analysed. Values from plants of the two populations of study were pooled, as population factor was nonsignificant for all traits of study. Values were calculated as the mean of family values

Fitness trait	Selfed progeny	Outcrossed progeny
Seed set (%)	6.6 \pm 2.40	84.6 \pm 2.60
Seed size (mm)	5.0 \pm 0.14	4.3 \pm 0.20
Pappus length (mm)	9.0 \pm 0.33	9.8 \pm 0.33
Terminal velocity (m s ⁻¹)	0.45 \pm 0.02	0.40 \pm 0.02
Germination date (days)	6.4 \pm 0.22	6.8 \pm 0.19
Germinability (%)	90.3 \pm 2.79	90.2 \pm 2.76
Growth rate (leaf, cm day ⁻¹)	3.5 \pm 0.10	3.9 \pm 0.06
Flowering probability (%)	33.6 \pm 6.70	13.5 \pm 2.81
Number of flowers	7.8 \pm 0.64	5.8 \pm 0.77
Stalk height (cm)	50.5 \pm 2.87	47.5 \pm 4.70
Survivorship (%)	94.4 \pm 3.15	96.0 \pm 1.68

three of nine outcrossed families ($R^2 > 21.8$, $r > 0.47$, $F_{1,25} > 6.41$, $P < 0.019$; in all linear regressions). Terminal velocity of individual *H. radicata* seeds significantly differed between selfed and outcrossed progeny (Tables 2, 4). Terminal velocity also significantly differed between families within populations and the treatment by family interaction was also significant (Table 2), indicating that the dispersal ability greatly varied among plants and pollination treatments. The significant treatment-by-family interaction is explained by very similar terminal velocity values

between selfed and outcrossed progeny in two plants of one population (Fig. 1).

DISCUSSION

MATING SYSTEM AND SELFING EFFECTS

The perennial herb *Hypochaeris radicata* exhibited a dramatic reduction in seed set after selfing in comparison with the seed set value obtained after outcrossing. These results clearly indicate that *H. radicata* is self-incompatible though a few plants presented some variability in the functioning of the self-incompatibility system and produced some selfed seeds. All outcrosses conducted on *H. radicata* plants exhibited very high seed set values (>80% in all cases), suggesting that there were a sufficient number of cross-compatible mates among all randomly selected plants from the two populations, as successful reproduction in self-incompatible species depends on the availability of cross-compatible mates (DeMauro, 1993; Byers, 1995; Morgan, 1999; Luijten *et al.*, 2002). It must be emphasized that around half of the plants initially sampled per population failed to produce selfed progeny after experimental pollinations because selfing led to the abortion of all ovules per flowering head. Early-acting inbreeding depression might have caused such a failure. However, other studies analysing the effects of inbreeding depression on fitness of other self-incompatible species suggest that reduced seed set is probably a consequence of the mating system and not inbreeding depression (Luijten *et al.*, 2002). This is supported by the fact that the incompatibility system in the Asteraceae is generally of the single-locus, multiallelic, sporophytic type (De Nettancourt, 1977), even though the incompatibility system in *H. radicata* has not yet been examined.

Other than seed set, and to a lesser extent pappus length, no negative effects of selfing were detected in any life-cycle trait of *H. radicata*. Selfed progeny even significantly outperformed outcrossed progeny in some traits, such as seed size and the proportion of flowering plants, selfed seeds being significantly larger than outcrossed seeds and selfed plants flowering more than outcrossed plants, respectively (Table 4). Given that seed size usually affects other life-cycle traits, the fact that selfed plants flowered more than outcrossed plants might be the consequence of increased seed size in the selfed progeny (i.e. a positive correlation between these two traits). Although *H. radicata* produced selfed plants that perform better than outcrossed plants, such selfed seeds are produced in very low numbers in comparison with the high production of outcrossed seeds. As a result, it seems unlikely that selfed plants outnumber outcrossed plants in a population, even though we do not know

how plant performance would change with increasing levels of selfing. In addition, plants in the wild bear several receptive flowers simultaneously, thus enhancing their outcrossing rates.

MATERNAL EFFECTS

One of the earliest traits in which selfing and maternal effects are expressed is seed size (Roach & Wulff, 1987). Seed size has been found to affect emergence date, seedling growth and/or seedling survival in the studies that have included individual seed size in their analyses (Kalisz, 1989; Wolfe, 1993; Helenurm & Schaal, 1996; Galloway, 2001; Picó *et al.*, 2003). In the case of *H. radicata*, none of the traits in either early or late stages of the life cycle was significantly affected by seed size. Montalvo (1994) also found that selfing affected seed size in *Aquilegia caerulea*, but there was no direct effect of seed size on any other fitness trait, and only leaf expansion rate (an indirect fitness trait) was marginally affected by seed size. It must be noted, however, that family was significant for pappus length and germination date, and that the family-by-pollination treatment interaction was also significant for seed size, pappus length and growth rate of *H. radicata*. These differences between plants could also be the result of maternal effects, as pointed out in other studies (Byers & Waller, 1999).

The lack of significance of seed size as a covariate might be explained by the low intraplant variation in seed size for all plants used in the experiment (the ranges of CVs among plants and populations are 0.8–46.3% and 2.3–10.4% for selfed and outcrossed progeny, respectively). In fact, the more important variation in seed size occurred between pollination treatments. These results suggest that pollination type mediates a trade-off between seed size and seed number. Under outcrossing, most of the ovules set seed, which were very similar in size, whereas under selfing, *H. radicata* would allocate more resources to the few seeds produced when allowed by the mating system. These differences between selfed and outcrossed progeny in the seed polymorphism of *H. radicata* could also have an important genetic component, as shown by many other studies (e.g. Baskin & Baskin, 1998). It remains to be seen, however, to what extent harsher abiotic conditions and competition among maternal plants affect selfed and outcrossed seed size variation and subsequent fitness traits in *H. radicata*. Other studies have clearly shown that the magnitude of genetic factors may be highly influenced by the environment in which selfed and outcrossed progeny are compared (Dudash, 1990; van Treuren *et al.*, 1993), and that the effects of seed size on plant traits increase under stressful competitive environments (Stanton, 1984; Wulff, 1986).

DISPERSAL ABILITY

Both seed and pappus size varied between pollination treatments such that selfed seeds were larger and bore a smaller pappus than outcrossed seeds. As a result, selfed seeds of *H. radicata* exhibited higher terminal velocities and therefore reduced dispersal abilities. Other studies focusing on the relationship between dispersal ability and seedling fitness found that there is a trade-off between these two fitness traits, i.e. increased achene mass implies higher plant fitness in terms of growth rate, but carries a cost in terms of dispersal ability (Meyer & Carlson, 2001; Picó *et al.*, 2003). Such a trade-off did not apply in the case of *H. radicata* for two reasons. First, the relationship between seed size and pappus size was positively significant in only one selfed and three outcrossed families, indicating that the general trend within families is that seed and pappus size vary independently in *H. radicata* or that larger seeds also bear larger pappuses. Second, seeds with lower dispersal potential (selfed seeds with larger size and smaller pappus) only outperformed seeds with higher dispersal potential (outcrossed seeds with smaller size and larger pappus) in the proportion of flowering plants. The negative relationship between dispersal ability and plant fitness should be observed within pollination treatments, but this relationship did not exist in *H. radicata*, because seed size included as a covariate in the analyses was never significant.

This study clearly showed that selfing affects the dispersal ability of *H. radicata* by (i) reducing seed set and therefore the potential number of achenes to be dispersed, and (ii) increasing seed size and reducing pappus length as a result of an effective reallocation of resources among selfed seeds. The corollary of this, that dispersal may influence the probability of selfing, remains an open question, because dispersal determines the spatial distribution among individuals, which could have an effect on outcrossing probability and relatedness among pollen donors (Donohue, 1998 and references therein). Outcrossed *H. radicata* seeds have an increased chance of dispersing further away from the mother plant than selfed seeds. Our data fit with the general idea that dispersal represents a means to enhance outcrossing, though it is accepted that this is not the ultimate cause of dispersal (Perrin & Goudet, 2001).

We conclude that several factors, such as the low proportion of plants per population that produce selfed seeds, the low number of selfed seeds produced per plant, and the lack of self-fertility mechanisms might act in concert to prevent the evolution of selfing in *H. radicata*. This is supported by recent studies on the effects of habitat fragmentation on the performance of *H. radicata* population in a fragmented habitat which

show that small *H. radicata* populations performed worse than large populations in terms of seed production, germinability, and dispersal ability (Soons & Heil, 2002; C. Mix, unpubl. data). Such a decrease in performance with decreased population size was attributed to higher biparental inbreeding resulting from increased relatedness among remnant individuals in small populations. In any case, small populations with high performance were found, suggesting that selfing variants cannot succeed in such populations. The evolution of selfing in *H. radicata* would require the coevolution of self-fertility traits, e.g. timing of pollen ripening and stigma receptivity, which would allow the species to increase the proportion of selfing variants in the populations after repeated generations of selfing.

ACKNOWLEDGEMENTS

We thank C. Mix, M. Soons and J. Retana for their comments on the manuscript, and also to C. Mix for providing unpublished data. We are grateful to the staff of the Botanic Garden of the University of Nijmegen for their valuable greenhouse assistance. This research was supported by TRANSPLANT (EVK2-1999-00042) EU project.

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