

Fitness Traits and Dispersal Ability in the Herb *Tragopogon pratensis* (Asteraceae): Decoupling the Role of Inbreeding Depression and Maternal Effects

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Abstract: Inbreeding depression can decrease several fitness traits and maternal effects can strongly influence the amount of inbreeding depression. Understanding the effects of inbreeding depression on plant fitness is especially important in the context of habitat fragmentation, where plant populations become smaller and more isolated, exhibiting increasing levels of inbreeding depression. We examined the joint influence of inbreeding depression and maternal effects on life cycle traits and dispersal ability in the herb *Tragopogon pratensis* that grows in fragmented populations in Europe. We conducted experimental crosses to obtain selfed and outcrossed progeny in two contrasted environments. In particular, we produced a first generation of seeds and plants that were self-pollinated again to produce a second generation of seeds. Individual seeds were weighed and their pappuses measured to estimate the dispersal potential. Pollination treatment only had a significant effect on seed mass and dispersal ability. Coefficients of inbreeding depression did not differ between selfed and outcrossed plants. Seed mass had a significant effect on germination date. Environment had a significant effect on mass of the second generation of seeds and the interaction between pollination treatment and family was significant for six traits, indicating the existence of strong maternal effects in *T. pratensis*. Results suggest population differentiation. Overall, *T. pratensis* populations exhibited a good performance under selfing, in terms of life cycle traits and dispersal ability, which would allow the species to cope with problems associated with fragmentation.

Key words: Life cycle traits, mating system, seed size, self-compatibility, terminal velocity, trade-off.

Introduction

Inbreeding depression, the reduction in fitness of selfed compared to outcrossed progeny, can affect different fitness components throughout the whole life cycle of plants, including seed production, germination, juvenile survival, growth, reproduction, and long-term survival (Charlesworth and Charlesworth, 1987; Husband and Schemske, 1996). The genetics un-

derlying inbreeding depression have been extensively studied and it is widely accepted that inbreeding depression is largely caused by homozygosity for deleterious recessive alleles (Charlesworth and Charlesworth, 1999). Deleterious recessive alleles that remain masked in heterozygotes by dominant alleles in an outcrossing plant population become exposed to and eliminated by selection when the population inbreeds. Hence, mean fitness decreases at first, but after some generations, deleterious recessive alleles may have been purged from the population and mean fitness can equal or even exceed that of the initial outbreeding population (Lande and Schemske, 1985; Barrett and Charlesworth, 1991; Byers and Waller, 1999). However, the extent of purging depends on many population and genetic factors and general predictions are not straightforward (Byers and Waller, 1999).

The amount of inbreeding depression may be strongly influenced by maternal effects, given their influence on progeny performance (Roach and Wulff, 1987). Strictly speaking, maternal effects refer to those traits of the offspring that are not due to genetic factors, but rather to environmental or non-genetic factors affecting the mother and that ultimately act on the embryo (Futuyma, 1998: p.233). However, it must be emphasized that maternal effects can also have an important genetic component (Roach and Wulff, 1987). In plants, maternal plant qualities often influence seed size, which has been found to affect germination and other fitness traits (Maun and Payne, 1989; Leishman and Westoby, 1994; Meyer and Carlson, 2001). Strong maternal effects may, therefore, mask inbreeding depression (Roach and Wulff, 1987). In particular, beneficial maternal environments for particular genotypes could buffer the negative effects of inbreeding depression on some fitness components. This would allow inbred plants to persist in particular beneficial environments, and in the long term, could have implications for the evolution of the mating system. Despite the interest of the joint effect of inbreeding depression and maternal effects on plant fitness, empirical studies that have attempted to disentangle these two factors are still scarce (Kalisz, 1989; Wolfe, 1993; Montalvo, 1994; Helenurm and Schaal, 1996; Galloway, 2001).

Among all fitness traits that can be affected by inbreeding depression, seed dispersal has received little attention. Seed dispersal represents a strategy for colonizing new patches and escaping unfavorable conditions, which may considerably increase individual fitness of dispersed seeds. Hence, seed dis-

persal is a trait that is likely to be under strong selection (Thiede and Augspurger, 1996; Donohue, 1998). In wind-dispersed plants, seed dispersal distance is a function of terminal velocity, which is determined by the relationship between seed size and plume area (Johnson and Gaines, 1990). Traits involved in seed dispersal (i.e. the plume) are largely maternally controlled (Imbert and Ronce, 2001), but seed size is a trait typically affected by maternal genotype and inbreeding depression. Hence, variation in fitness in terms of variation in dispersal ability can be the result of both inbreeding depression and maternal effects.

The response of plants to increased selfing and the influence of maternal effects on different life cycle traits may ultimately determine the viability of plant populations. This is especially relevant in the context of habitat fragmentation; plant populations become smaller and more isolated from each other, leading to increasing levels of inbreeding. In the long term, the ability of plants in fragmented habitats to cope with inbreeding depression, maintain certain levels of gene flow between fragments, and colonize new suitable patches can prove fundamental to avoid local extinction (Young and Clarke, 2000). In this paper, we present the results of a hand-pollination experiment to examine how inbreeding depression and maternal effects jointly act on plant fitness in terms of performance (i.e. germination, growth, survival, and fecundity) and dispersal ability (i.e. seed mass: pappus size ratio) in the short-lived, wind-dispersed herb *Tragopogon pratensis* L. (Asteraceae). This plant, as with many others in Europe, is currently forced to live in small and isolated natural or semi-natural fragments due to human demands and activities. Hence, a better understanding of how inbreeding depression and maternal effects jointly contribute to plant fitness will help to assess, along with other complementary ongoing demographic studies, the species' vulnerability to extinction in fragmented habitats. We address the following questions. (i) What are the effects of inbreeding on different life cycle traits and fitness of *T. pratensis*? (ii) Are maternal effects also affecting plant traits and fitness? (iii) How do inbreeding depression and maternal effects jointly influence the dispersal ability of the species?

Methods

Plant species and study site

Tragopogon pratensis L. (Asteraceae) is a widespread, short-lived, perennial herb that grows on dry grasslands, hedges, and ruderal places across Europe and North America. The plant has a taproot and produces long, narrow grass-like leaves (around 20 cm long). Flowering occurs in June/July and the yellow flowers are arranged on an erect, slightly branching stem (up to 90 cm in height). *T. pratensis* single-seeded fruits (achenes) show heteromorphism. In particular, peripheral achenes are darker and heavier than central achenes ($F_{1,50} = 6.39$, $P = 0.015$; one-way ANOVA for seed mass; F. X. Picó, unpublished material). All achenes present a well-developed pappus facilitating wind dispersal. Pappus length does not differ between central and peripheral achenes ($F_{1,50} = 2.10$, $P = 0.154$; one-way ANOVA; F. X. Picó, unpublished material).

In summer 2000, seeds from 12 widely separated plants were collected from each of two populations in central Germany. Population A (Kammerbach; 51°16'N, 9°55'E) is a large mea-

dow (ca. 250 flowering plants; 1500 m²), whereas population B (Großalmerode; 51°18'N, 9°47'E) is a small fallow field (ca. 50 flowering plants; 175 m²).

Experimental crosses

In autumn 2000, around 10 seeds/plant/population (overall 240 seeds) were planted in pots (20 × 17 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) in the Botanic Garden of the University of Nijmegen. Two weeks after germination, one seedling per plant and population (overall 24 seedlings) was individually potted and allowed to grow for 2 weeks in the same greenhouse. Plants were then transferred to an unconditioned greenhouse to overwinter. Plants flowered in spring 2001, but only nine and five plants from population A and B, respectively, produced matched progeny (i.e. both selfed and outcrossed progeny produced from the same plant).

To produce the selfed and outcrossed progeny on each plant, four flower heads per plant were hand-pollinated (two self-pollinated and two cross-pollinated). Two flower heads per treatment were used to produce a sufficient number of seeds to conduct the inbreeding depression experiment. Cross-pollinations were made among randomly chosen 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). Although *T. pratensis* tends to be protandrous, there is a clear overlap between pollen release and stigma receptivity. Thus, the two heads used for each selfing and outcrossing treatment were simultaneously pollen donors and recipient heads. In the case of outcrossing, pollen was pooled from heads of 2–3 different pollen donors to increase the proportion of foreign pollen on each outcrossed head, and to reduce parental effects due to specific characteristics of donor plants. Given that emasculation is not feasible in Asteraceae, self-pollination can accidentally occur in the outcrossing pollination treatment. To reduce this possibility, the outcrossing treatment was repeated over 2–3 days to overload flower heads with foreign pollen. All hand-pollinated heads were bagged during treatments to avoid uncontrolled pollinations and remained bagged until seed harvesting.

Inbreeding depression experiment

Ripe achenes (the first generation of seeds) were collected in early summer 2001. Filled achenes from the two flower heads per plant (i.e. family) and treatment were counted, put in paper bags and stored at room temperature for 3 days. A total of 40 achenes for each family and treatment ($N = 1120$ achenes; 2 treatments × 14 families × 40 achenes) were individually weighed to the nearest 0.1 mg. For each family and treatment, achenes were randomly selected but including both central and peripheral achenes to avoid a bias caused by different achene mass due to achene position in the head. Additionally, the pappus length of each achene was measured with calipers to the nearest 0.1 mm. All achenes were individually planted in small pots (5 × 5 cm), kept under the germination conditions described above, and monitored daily to record germinability and emergence date, which was defined as the number of days between sowing and the emergence of the radicle. In late

summer 2001, a total of 20 surviving seedlings per family and treatment ($N = 560$ seedlings; 2 treatments \times 14 families \times 20 seedlings) were transplanted into bigger pots (20×17 cm). Half of the pots were placed in the unconditioned greenhouse where they were maintained until flowering. The other half was placed outdoors to overwinter to evaluate the response of plants to inbreeding under harsher environmental conditions. In early spring 2002, just before flowering, surviving plants (49% of the plants) that were grown outdoors were brought back into the greenhouse with the other half of the plants. Up to 97% of the plants flowered in mid-spring 2002. Flowering date was recorded as the number of days between germination and the emergence of the flowering stalk. Flowering size was estimated as the product total number of leaves \times length of the longest leaf. The height of the flowering stalk was measured.

The first flower head of each plant and treatment was self-pollinated to produce a second generation of selfed seeds. By producing a second generation of selfed seeds, we analyzed the effects of inbreeding on the complete life cycle of *T. pratensis* (from seed to seed). Moreover, the second generation of seeds has two levels of inbreeding, so the effects of accumulated inbreeding on seed traits can also be assessed. Immediately after achene ripening and harvesting, the second generation of achenes was counted, weighed, and the whole plant was dried (65°C for 24 h) and weighed. Total biomass was divided by time to flowering to obtain the individual plant growth rate.

Statistical analyses

Pollination treatment and maternal effects on continuous plant fitness traits were analyzed with mixed model ANOVAs and ANCOVAs. For seed number, seed mass, pappus length, and the seed mass: pappus length ratio of the first generation of seeds, ANOVA included pollination treatment and population as fixed factors and family nested within population as a random factor. The seed mass: pappus length ratio is considered as the most important trait in determining terminal velocity, which is inversely proportional to dispersal ability (Greene and Johnson, 1993). The relationship between seed mass and pappus length was also analyzed with linear regression models for each family and treatment combination to test for a trade-off between traits related to establishment (seed mass) and dispersal (pappus length). The relationship between seed mass: pappus length ratio and growth rate was also analyzed for each population with linear regression models using family means as replicates to test for a trade-off between dispersal ability and plant performance.

For emergence date, flowering date, growth rate, flowering size, stalk height and number of flowers of the first generation of plants, ANCOVA included the same main factors as in the ANOVA above and seed mass as a covariate, which represented the maternal effect. For seed number and mass of the second generation of seeds obtained after the second self-pollination, we used the same ANCOVA model. The assumption of parallel slopes necessary for using covariates was examined with two- and three-way interaction terms. The interaction terms covariate \times pollination treatment, covariate \times population, covariate \times family and covariate \times pollination treatment \times population were all found to be non-significant in all analyses, indicating par-

allelism. All these interaction terms were not shown in the analyses.

The response of categorical variables, such as germinability and seedling and adult survival, to pollination treatment and maternal effects was analyzed with logistic regression models. In all cases, the analysis started with a null model including all main factors (pollination treatment and family), the covariate (seed mass) and all interactions. Then, a new model that lacks the term to be analyzed was created. Subsequently, for each term we tested whether the difference in unexplained variance (deviance) between models is approximately χ^2 distributed, with the number of degrees of freedom equal to the difference between models (Caswell, 2001: p. 326–335). Analyses were conducted for each population separately.

All plants that remained inside the greenhouse survived during the experiment, whereas around half of the plants left outside died. As only four families in population A were substantially represented in both inside and outside sets, to perform statistical analyses including site as another fixed factor, we only used the set of families outside the greenhouse to test the effects of pollination treatment and maternal effects on adult mortality. The rest of the analyses were performed with the dataset obtained from families that overwintered inside the greenhouse. However, we also performed a specific ANCOVA to test the effects of pollination treatment and environment (inside and outside the greenhouse) as fixed factors and family (the four above-mentioned families) as a random factor on seed mass of the second generation of seeds. Seed mass of the first generation of seeds was used as a covariate. With this analysis, we specifically tested for maternal effects on seed mass, a trait that is supposed to be under strong maternal effects.

Whenever the pollination treatment \times family interaction was significant in all ANOVAs and ANCOVAs, simple main effects were computed following Pedhazur (1982: p. 362–365) to test the significance of pollination treatment separately for the different families. By using the test of simple main effects, we analyzed the effects of inbreeding depression on a family basis and identified those families responsible for the significant pollination treatment \times family interactions. All analyses were based on 6–10 plants per family and treatment combination.

Inbreeding depression (δ) was calculated for each study population 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 $W_o < W_s$. The W_s and W_o are the mean fitness of selfed and outcrossed progeny, respectively. Negative δ values indicate that selfed progeny are more fit than the outcrossed progeny, whereas positive δ values mean the opposite. The δ value was calculated for all fitness traits measured during the life cycle, and the mean fitness measures of W_s and W_o were calculated as the mean of family values. Differences between W_s and W_o for each trait were compared with a *t*-test.

In all analyses, 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.

Table 1 Three-way ANOVAs testing for pollination treatment (selfing and outcrossing), population (A and B), and family nested within population for the number of seeds, seed mass, pappus length, and the seed mass: pappus length ratio of *T. pratensis* seeds obtained after the first hand-pollination experiment (first generation of seeds). Analyses were based on the set of plants inside the greenhouse. Variables were log-transformed prior to analyses. Degrees of freedom (*df*) and *F* values are given. Significance: *** $P < 0.0001$, ** $P < 0.001$, * $P < 0.05$; ns, non-significant

| Factor | No. seeds | | Seed mass | | Pappus length | | Seed/Pappus | |
|----------------|-----------|----------|-----------|----------|---------------|-----------|-------------|----------|
| | <i>df</i> | <i>F</i> | <i>df</i> | <i>F</i> | <i>df</i> | <i>F</i> | <i>df</i> | <i>F</i> |
| Treatment (T) | 1, 12 | 0.62 ns | 1, 12 | 15.57 ** | 1, 12 | 0.01 ns | 1, 12 | 8.29 * |
| Population (P) | 1, 12 | 4.31 ns | 1, 12 | 12.83 ** | 1, 12 | 58.80 *** | 1, 12 | 1.06 ns |
| Family (F) | 12, 12 | 1.25 ns | 12, 12 | 6.83 ** | 12, 12 | 1.27 ns | 12, 12 | 5.51 ** |
| T × P | 1, 12 | 0.94 ns | 1, 12 | 1.08 ns | 1, 12 | 0.07 ns | 1, 12 | 0.21 ns |
| T × F | 12, 28 | 1.40 ns | 12, 1092 | 3.26 *** | 12, 1092 | 18.30 *** | 12, 1092 | 4.59 *** |

Table 2 Mean (\pm SE) fitness measures of selfed (W_s) and outcrossed progeny (W_o) for different traits analyzed and for each *T. pratensis* population studied. Fitness measures were calculated as the mean of family values

| Fitness trait | Population A | | Population B | |
|---|------------------|------------------|------------------|------------------|
| | Selfed | Outcrossed | Selfed | Outcrossed |
| # Seeds (1st poll.) | 60.6 \pm 0.98 | 56.1 \pm 1.70 | 52.5 \pm 2.15 | 54.1 \pm 7.05 |
| Seed mass (mg) (1st poll.) | 6.2 \pm 0.16 | 6.8 \pm 0.23 | 7.6 \pm 0.33 | 8.1 \pm 0.44 |
| Pappus length (mm) | 15.9 \pm 0.17 | 16.0 \pm 0.19 | 18.0 \pm 0.30 | 18.0 \pm 0.56 |
| Seed/pappus (mg/mm) | 0.39 \pm 0.01 | 0.43 \pm 0.02 | 0.42 \pm 0.03 | 0.45 \pm 0.04 |
| Germinability (%) | 93.3 \pm 1.32 | 95.6 \pm 1.49 | 75.0 \pm 7.98 | 68.0 \pm 9.79 |
| Seedling survival (%) | 91.7 \pm 1.39 | 87.4 \pm 3.26 | 91.9 \pm 3.17 | 92.3 \pm 4.46 |
| Adult survival (%) ¹ | 46.7 \pm 12.91 | 52.2 \pm 9.54 | 32.5 \pm 13.77 | 47.5 \pm 14.93 |
| Germination date (days) | 6.3 \pm 0.26 | 6.0 \pm 0.22 | 13.0 \pm 0.71 | 14.1 \pm 0.97 |
| Flowering date (days) | 247.9 \pm 1.38 | 250.9 \pm 1.20 | 238.9 \pm 1.82 | 238.6 \pm 2.24 |
| Growth rate (mg/day) | 11.2 \pm 0.61 | 10.9 \pm 0.53 | 9.0 \pm 0.33 | 8.1 \pm 0.24 |
| Flowering size (leaf \times cm) | 480.2 \pm 10.3 | 448.5 \pm 14.0 | 380.1 \pm 17.1 | 374.0 \pm 12.8 |
| Stalk height (cm) | 62.4 \pm 1.53 | 61.5 \pm 1.07 | 50.3 \pm 3.37 | 50.7 \pm 2.86 |
| # Flowers | 2.2 \pm 0.08 | 2.2 \pm 0.07 | 1.8 \pm 0.06 | 1.9 \pm 0.06 |
| # Seeds (2nd poll.) ² | 45.1 \pm 0.76 | 45.1 \pm 0.57 | 42.8 \pm 1.47 | 42.5 \pm 1.32 |
| Seed mass (mg) (2nd poll.) ² | 6.9 \pm 0.11 | 6.8 \pm 0.13 | 8.6 \pm 0.15 | 8.1 \pm 0.17 |

¹ Adult survival was recorded from the set of plants outside the greenhouse. The rest of the data come from the set of plants inside the greenhouse.

² Data based on the second generation of seeds after the second self-pollination. The rest of the data are based on the first generation of seeds and plants.

Results

After the first hand-pollination experiment, seed set was greater than 97% for all families and treatments. The number of filled seeds produced per family did not differ between treatments, but outcrossed seeds were significantly heavier than selfed seeds (Tables 1 and 2). Seed mass significantly differed between populations, families and the treatment \times family interaction was also significant (Table 1). Pappus length significantly varied between populations and the treatment \times family interaction was also significant (Table 1). Dispersal ability, estimated as the seed mass: pappus length ratio, significantly differed between selfed and outcrossed progeny (Table 1). Seed mass: pappus length ratio also differed between families and the treatment \times family interaction was also significant (Table 1). Selfed seeds were lighter than outcrossed seeds whereas pappus length did not differ between treatments in both populations. As a result, selfed seeds showed lower seed mass: pappus length ratios (Table 2), and therefore higher dispersal ability, than outcrossed seeds.

Seed mass and pappus length were always negatively correlated but half of the correlation coefficients were only significant (r ranged between -0.30 and -0.66 , $P < 0.036$, in all significant linear regressions). The proportion of significant correlation coefficients did not differ between treatments ($N = 28$, $\chi^2 = 2.33$; G -test pooling the two populations). The relationship between seed mass: pappus length ratio and growth rate was positive ($r = 0.97$) and significant ($R^2 = 94.6$, $F_{1,4} = 52.45$, $P = 0.005$; linear regression) for the outcrossed progeny, and positive ($r = 0.86$) and marginally significant ($R^2 = 74.1$, $F_{1,4} = 8.57$, $P = 0.061$; linear regression) for the selfed progeny, in population B. Selfed and outcrossed progeny in population A exhibited no significant relationship between the seed mass: pappus length ratio and growth rate ($P > 0.45$ for both linear regressions).

Selfed and outcrossed progeny of the first generation of plants exhibited no differences in any categorical variable, such as germinability and survival of seedlings and adult plants (Deviance < 0.82 , $P > 0.37$ for all three logistic regressions); germinability ranged 68–95%, seedling survival 87–92%, and adult

Table 3 Three-way ANCOVAs testing for pollination treatment (selfing and outcrossing), population (A and B), and family nested within population for germination date, flowering date and growth rate of *T. pratensis* plants (first generation of plants). Analyses were based on the set of plants inside the greenhouse. Variables were log- or arcsine-transformed prior to analyses. Seed mass was used as covariate. Degrees of freedom (*df*) and *F* values are given. Significance: *** $P < 0.0001$, ** $P < 0.001$, * $P < 0.05$; ns, non-significant

| Factor | Germination date | | Flowering date | | Growth Rate | |
|----------------|------------------|------------|----------------|-----------|-------------|-----------|
| | <i>df</i> | <i>F</i> | <i>df</i> | <i>F</i> | <i>df</i> | <i>F</i> |
| Seed mass | 1, 937 | 5.36 ** | 1, 248 | 1.90 ns | 1, 247 | 1.06 ns |
| Treatment (T) | 1, 12 | 0.09 ns | 1, 12 | 2.46 ns | 1, 12 | 4.27 ns |
| Population (P) | 1, 12 | 436.93 *** | 1, 12 | 22.93 *** | 1, 12 | 22.06 *** |
| Family (F) | 12, 12 | 0.62 ns | 12, 12 | 4.47 ** | 12, 12 | 1.72 ns |
| T × P | 1, 12 | 1.13 ns | 1, 12 | 2.96 ns | 1, 12 | 0.26 ns |
| T × F | 12, 937 | 3.01 *** | 12, 248 | 2.17 * | 12, 247 | 1.96 * |

Table 4 Three-way ANCOVAs testing for pollination treatment (selfing and outcrossing), population (A and B), and family nested within population for flowering size, stalk height and number of flowers of the first generation of plants, and number of seeds and mass of *T. pratensis* seeds obtained after the second self-pollination (second generation of seeds). Analyses were based on the set of plants inside the greenhouse. Variables were log- or arcsine-transformed prior to analyses. Seed mass was used as a covariate. Degrees of freedom (*df*) and *F* values are given. Significance: *** $P < 0.0001$, ** $P < 0.001$, * $P < 0.05$; ns, non-significant

| Factor | Flowering size | | Stalk height | | No. flowers | | No. seeds | | Seed mass | |
|----------------|----------------|-----------|--------------|-----------|-------------|----------|-----------|----------|-----------|-----------|
| | <i>df</i> | <i>F</i> | <i>df</i> | <i>F</i> | <i>df</i> | <i>F</i> | <i>df</i> | <i>F</i> | <i>df</i> | <i>F</i> |
| Seed mass | 1, 246 | 1.49 ns | 1, 242 | 1.27 ns | 1, 242 | 0.24 ns | 1, 242 | 1.01 ns | 1, 242 | 0.49 ns |
| Treatment (T) | 1, 12 | 4.54 ns | 1, 12 | 0.30 ns | 1, 12 | 0.28 ns | 1, 12 | 0.31 ns | 1, 12 | 10.30 ** |
| Population (P) | 1, 12 | 32.33 *** | 1, 12 | 19.68 ** | 1, 12 | 15.75 ** | 1, 12 | 5.77 * | 1, 12 | 51.93 *** |
| Family (F) | 12, 12 | 2.26 ns | 12, 12 | 10.94 *** | 12, 12 | 1.62 ns | 12, 12 | 3.79 * | 12, 12 | 3.91 * |
| T × P | 1, 12 | 0.73 ns | 1, 12 | 0.47 ns | 1, 12 | 0.50 ns | 1, 12 | 0.06 ns | 1, 12 | 2.89 ns |
| T × F | 12, 246 | 1.05 ns | 12, 242 | 0.67 ns | 12, 242 | 1.17 ns | 12, 242 | 0.91 ns | 12, 242 | 0.77 ns |

survival 32–52% across treatments and populations (Table 2). None of the other factors (seed mass as a covariate, population, family within population, and all interactions) of the logistic regression had significant effects on these three categorical variables ($P > 0.07$ for all cases).

Germination date, flowering date and growth rate of the first generation of plants did not show significant differences between treatments (Table 3). All three variables significantly differed between populations and the treatment × family interaction was also significant (Table 3). Only family was significant for flowering date (Table 3). Families from population A germinated a week earlier, had faster growth rates and their flowering took more days than plants from population B (Table 2). Maternal effects, mediated by seed mass of the first generation of seeds, were detected in only one life cycle trait of *T. pratensis*. In particular, germination date was significantly affected by seed mass of the first generation of seeds (Table 3). In population A, the larger the seed the sooner it germinated, whereas population B exhibited the opposite pattern (Table 2).

Flowering size, stalk height and the number of flowers of the first generation of plants basically showed significant differences between populations (Table 4). Plants from population A were bigger at flowering, produced taller flowering stalks and bore more flowers than plants from population B (Table 2). The number of seeds produced in the second generation did not significantly differ between treatments but the mass of the second generation of seeds did (Table 4). In partic-

ular, seeds from selfed families were heavier than those from outcrossed families in both populations (Table 2) indicating that repeated inbreeding had a positive effect on seed mass of *T. pratensis*. Both seed number and mass of the second generation of seeds significantly varied between populations (Table 4). In this case, families from population A produced more and lighter seeds than families from population B (Table 2).

For all significant treatment × family interactions (i.e. seed mass, pappus length, seed mass : pappus length ratio, germination date, flowering date and growth rate of the first generation of plants), simple main effects showed those families on which pollination treatment had a significant effect (Fig. 1). Within the same family, selfed progeny outperformed outcrossed progeny in some traits and vice versa in some others (Fig. 1), indicating that the effects of pollination treatment differed throughout the life cycle and that some families responded differently to the pollination treatments for these traits.

Although results from the set of families left outside the greenhouse were not included in the analyses due to the loss of families, surviving selfed and outcrossed families exhibited the same general pattern outlined above, that is, very low differences in fitness traits due to inbreeding (results not shown). Nevertheless, some differences were found between sets of families inside and outside the greenhouse. Basically, families outside the greenhouse had lower growth rates (overall mean over treatments and populations; 7.8 mg/day), lower flowering

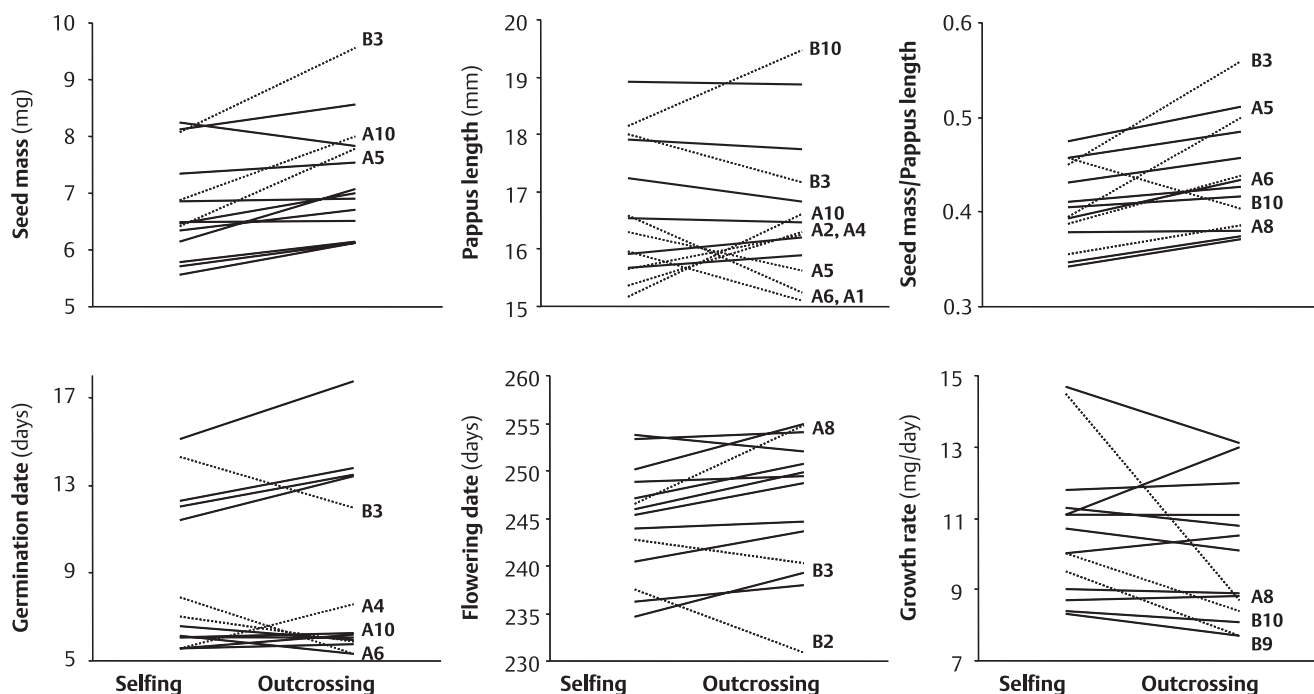


Fig. 1 Mean values of six different traits for *T. pratensis* families in different pollination treatments. These traits showed a significant pollination treatment \times family interaction in ANOVAs and ANCOVAs (see Tables 1 and 3). Lines connect selfed and outcrossed progeny mean values of the same family. Families with significant F values in the simple main effects test are indicated with dotted lines ($P < 0.05$ in all cases) and their respective code, which indicates whether they belong to population A or B. The degrees of freedom used in the simple main

effects to compute the F values are 1 in all numerators and 1092 in the denominator for seed mass, pappus length and seed mass:pappus length ratio, 937 for germination date, 248 for flowering date and 247 for growth rate. In a test of simple main effects, the degrees of freedom of the denominator correspond to the error degrees of freedom of the overall analysis of the factorial design (see further details in Pedhazur, 1982).

sizes (301.6 leaf \times cm), and lower reproductive output (1.6 flowers per plant) than families left inside the greenhouse. The effects of environment were significant for the mass of the second generation of seeds ($F_{1,3} = 62.06$, $P = 0.002$; three-way ANCOVA), clearly indicating that seed mass was influenced by maternal effects. In particular, seeds from families outside the greenhouse were lighter than seeds from families inside the greenhouse (mean \pm SE = 6.4 ± 0.2 and 6.9 ± 0.1 mg for seeds from outside and inside, respectively). None of the other factors included in the ANCOVA had a significant effect on seed mass ($P > 0.35$ in all cases).

Inbreeding depression coefficients (δ) were low for all fitness traits analyzed (Fig. 2). Mean fitness values for all traits analyzed in both populations did not differ between selfed and outcrossed progeny ($P > 0.06$ in all cases; t -test).

Discussion

Mating system and inbreeding effects

Apart from the significant effects of pollination treatment on seed mass and dispersal ability, the rest of the fitness traits did not differ between selfed and outcrossed progeny of *Tragopogon pratensis*. In addition, all coefficients of inbreeding depression for all fitness traits analyzed did not significantly vary between selfed and outcrossed progeny (Fig. 2). These results indicate an absence of segregating genetic load and that self-

ing does not reduce individual fitness in *T. pratensis*. The species would fit with the theory that the evolution of self-compatible mating systems has been accompanied by the evolution of mechanisms decreasing the effect of inbreeding depression (Lande and Schemske, 1985; Carr and Dudash, 1996; Dudash and Fenster, 2001), that is, effective purging of highly deleterious mutations that represent the main cause of inbreeding depression (Charlesworth and Charlesworth, 1987; Johnston and Schoen, 1995). Other studies also showed very low effects of inbreeding depression on all life history stages in selfing plant species (Goodwillie, 2000).

The strong selection that accompanies purging of deleterious mutations may increase population differentiation and decrease within-population variation (Byers and Waller, 1999). This theoretical prediction is supported by our results. Most of the fitness traits analyzed for *T. pratensis* exhibited significant differences between populations. In addition, variation between families within populations was significant for some of the fitness traits analyzed, but *T. pratensis* traits were always more variable between populations than within populations. The role of selfing in population differentiation has been examined by other studies (Lynch et al., 1999), and examples of population and subsequent genetic differentiation have also been found in other plant species in similar habitats (van Tienderen and van der Toorn, 1991; van Treuren et al., 1993; van Kleunen and Fisher, 2001).

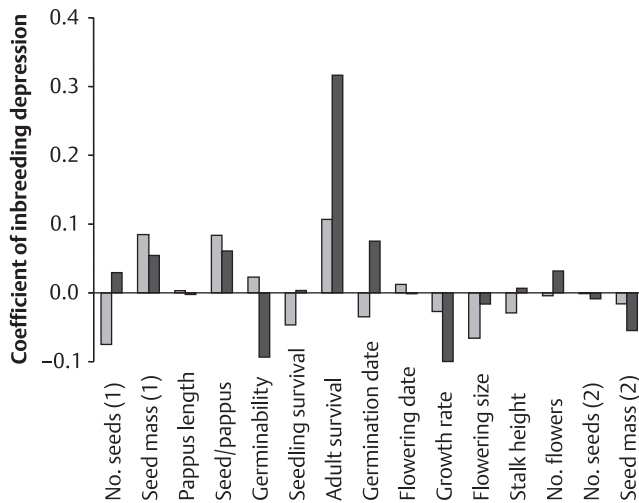


Fig. 2 Coefficients of inbreeding depression (δ) for different plant traits over the life cycle of *T. pratensis* (from the number of seeds after the first hand-pollination to the mass of seeds obtained after the second self-pollination). Values are given for each population separately (hollow bars, population A; filled bars, population B).

Although outcrossed seeds were significantly heavier than selfed seeds, after a second self-pollination, selfed seeds slightly increased their mass whereas outcrossed seeds maintained their mass constant (Table 2). In contrast, the number of seeds produced decreased 20% after the second self-pollination (Table 2). Other studies comparing two generations of selfing in flowering plants yielded contrasting results. Some studies did not show any difference in plant traits after one or two generations of selfing (Schoen, 1983; Levin, 1991; van Treuren et al., 1993; Wolfe, 1993), whereas others found a generalized decrease on fitness traits after several generations of selfing (Dudash et al., 1997; Koelewijn, 1998). Differences between first and second selfing generations of seeds might arise because of differences in the maternal environment. In fact, plants that come from natural populations produced the first generation of seeds while plants that completed their life cycle in the greenhouse produced the second generation of seeds. This would complicate the interpretation of results of studies including only two generations of selfing (Wolfe, 1993).

Maternal effects

Seed size represents one of the earliest traits on which maternal effects are expressed (Roach and Wulff, 1987). In *T. pratensis*, germination date was significantly affected by seed mass. An identical result was found for other species, such as *Collinsia verna* (Kalisz, 1989), *Aquilegia caerulea* (Montalvo, 1994), *Lupinus texensis* (Helenurm and Schaal, 1996), and *Campanula americana* (Galloway, 2001). In contrast, Wolfe (1993) found that seed mass did not influence germination time in *Hydrophyllum apendiculatum*. In *T. pratensis*, the effects of seed mass on germination rate varied between populations in such a way that in population A, the heavier the seed the sooner it germinated, while population B exhibited the opposite pattern. Interestingly, seed mass was a trait also affected by inbreeding in *T. pratensis*. Hence, life cycle traits of *T. pratensis* with a major role in determining fitness, such as emergence date, are influenced by both maternal effects and inbreeding depression.

Because life cycle traits are expected to be positively or negatively correlated, variation in traits (and in their correlation) under genetic and/or maternal influence may have an effect on several other fitness traits (Kalisz, 1989). If selection acts on variation in particular life cycle traits, then such correlations may speed up population differentiation events, as suggested for *T. pratensis*.

The test performed to examine the effects of pollination treatment and environment effects on seed mass of the second generation of seeds clearly indicated that seed mass is strongly affected by maternal effects in *T. pratensis*. The mass of seeds produced by the same families after the second self-pollination significantly differed between sites; seeds produced inside the greenhouse being heavier than seeds produced outside, surely due to the weakening effect of the harsher weather conditions outside. Although this result shows the influence of maternal environment on seed mass, that is, a non-genetic factor, the genetic maternal effects cannot be ruled out as an important source of variation. In fact, the effect of inbreeding on a character subject to a maternal effect, such as seed mass in *T. pratensis*, is two-fold: part being attributable to the inbreeding of the individuals measured and part to the inbreeding in the mothers (Falconer and Mackay, 1996: p.259).

Most of the analyses yielded a significant pollination treatment \times family interaction, indicating that the way in which families respond to inbreeding varies. Simple main effects indicated in what families inbreeding had a significant effect. It has been suggested that such interactions could also be the result of maternal effects due to individual characteristics, such as between plant differences in resource allocation patterns that may alter the response of plants to inbreeding (del Castillo, 1998; Mutikainen and Delph, 1998; Byers and Waller, 1999). The graphical representation of family level variation in fitness traits of *T. pratensis* showed that the effects of pollination treatment appear to be complex and diverse depending on the life cycle trait and maternal family considered (Fig. 1). Nevertheless, three patterns can be outlined: (1) most of the families analyzed (11 of 14) showed significant inbreeding effects at least for one trait, indicating that between family variability in inbreeding effects on fitness traits is a common trait in *T. pratensis*. (2) The trait that showed more families with significant inbreeding effects was pappus length, perhaps because dispersal-related traits are largely maternally controlled and differences between families in the response to inbreeding can be seen more clearly. (3) Families with significant inbreeding effects for seed mass and germination and flowering dates (e.g. A10 and B3) showed a coherent pattern in the effects of inbreeding on these traits. In particular, outbreeding led to increasing seed mass, which in turn led to faster germination and flowering (Fig. 1).

Different arguments can be found to explain the between family variation in inbreeding effects on different life history traits. For example, variation might result as a consequence of the action and interaction of different mechanisms and genes operating at different stages of the life cycle (del Castillo, 1998), or the associations that develop between loci determining mating system and loci determining different fitness traits (Dudash et al., 1997; Mutikainen and Delph, 1998). Theoretically, random mutations at these loci may suffice to produce considerable variation in inbreeding effects among families (Shultz

and Willis, 1995). Population historical effects, including genetic drift, founder events, and fixation of different alleles might also explain the family level variation in inbreeding effects (Ouborg et al., 2000 and references therein).

Dispersal ability

One of the most important aspects of dispersal ability in plants is the seed mass : pappus area ratio, which largely determines the individual seed terminal velocity, the inverse measure of dispersal ability (Greene and Johnson, 1993; Soons and Heil, 2002). In *T. pratensis*, the seed mass : pappus area ratio was affected by pollination treatment, with outcrossed seeds having lower dispersal ability than selfed seeds (Table 2). Hence, selfing, through its effects on seed mass, affected the dispersal ability of *T. pratensis* seeds. However, the existence of maternal effects, which would also be mediated through seed mass, on dispersal ability of *T. pratensis* cannot be ruled out. Despite the significant effect of pollination treatment on dispersal ability, differences in the seed mass : pappus length ratio were small between selfed and outcrossed progeny (Table 2), so it remains to be seen whether selfed seeds would have a clear advantage over outcrossed seeds in terms of dispersal ability in the field.

Interestingly, seed mass and pappus length were negatively significantly correlated in many *T. pratensis* families. Such a negative relationship may indicate the existence of a trade-off between plant performance and dispersal ability. Few studies have shown that increased seed mass implies higher fitness in terms of growth rate, but carries a cost in terms of dispersal ability (Ganeshaiyah and Shaanker, 1991; Meyer and Carlson, 2001). In *T. pratensis*, the relationship between seed mass : pappus length ratio and growth rate was positive and significant for selfed and outcrossed progeny in population B. Given that seed mass : pappus length ratio is the inverse of dispersal ability, such positive linear regressions in population B indicate the existence of a trade-off between plant performance and dispersal ability in *T. pratensis*. No significant relationship between the seed mass : pappus length ratio and growth rate was found in population A, perhaps as a result of specific-population characteristics that may mask the expression of the trade-off.

Overall, we conclude that both maternal effects and inbreeding depression were present in early life cycle stages of *T. pratensis*. The species exhibited a good performance under selfing, which would allow the species to cope with reduced population sizes. Albeit small, dispersal ability was also enhanced in selfed seeds, but the important trait is the high performance shown by selfed plants that would enhance chances for successfully colonizing suitable new habitats or increasing gene flow between nearby populations.

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