

EVALUATION OF THE EXTENT OF AMONG-FAMILY VARIATION IN INBREEDING DEPRESSION IN THE PERENNIAL HERB *SCABIOSA COLUMBARIA* (DIPSACACEAE)¹

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Significantly different maternal line responses to inbreeding provide a mechanism for the invasion of a selfing variant into a population. The goal of this study was to examine the extent of family-level variation in inbreeding depression in the mixed-mating, perennial herb *Scabiosa columbaria*. Plants from one population were raised, and hand-pollinated to produce selfed and outcrossed progeny, and the effects of inbreeding depression on life-cycle traits were analyzed. Inbreeding depression significantly affected early life cycle traits. The pollination treatment by family interaction was significant for almost all traits, indicating a high family-level variation in inbreeding depression. The correlations between inbreeding depression values (e.g., percentage germination and flowering date, and flowering date and aboveground biomass) exhibited alternate signs, illustrating the type of association between inbreeding depression loci for different traits across the life cycle. Overall, it is concluded that the extent of among-family variation in inbreeding depression might allow a selfing variant of *S. columbaria* to invade an outcrossing population, though the pattern of correlations between inbreeding depression values might prevent effective purging of the deleterious genetic load.

Key words: family-level variation; inbreeding depression; mating system; outcrossing; purging; self-fertilization; selfing variant.

Inbreeding depression is the reduction in fitness of selfed progeny relative to outcrossed progeny. One of the most common results reported by the majority of inbreeding depression studies in plants is the important family-level variation (i.e., variation at the individual level) in inbreeding depression (Husband and Schemske, 1996; Dudash et al., 1997; Koelewijn, 1998; Mutikainen and Delph, 1998; Ouborg et al., 2000; Rankin et al., 2002; Rao et al., 2002; Picó et al., 2003), that is, families within a population respond differently to pollination treatment in such a way that some of them exhibit higher fitness under outcrossing whereas others perform much better under selfing. Family-level variation in inbreeding depression can be detected by significant pollination treatment by family line interactions in parametric models (e.g., ANOVA), though the graphical representation of inbreeding coefficients of fitness traits for each family also represents a good method to clearly identify families with different responses to pollination treatment. Significantly different maternal line responses to inbreeding provide a mechanism for the invasion of a selfing variant into the population through any maternal line exhibiting purging of its genetic load, which represent a mechanism for mating-system evolution (Dudash et al., 1997). Family values of inbreeding depression can largely differ from population-level estimates of inbreeding depression (Holsinger, 1988; Mutikainen and Delph, 1998), and as indicated by modeling work, population-level estimates of inbreeding depression alone are insufficient to predict whether a selfing var-

iant can invade a population (Holsinger, 1991; Uyenoyama et al., 1993; Shultz and Willis, 1995).

One of the explanations outlined as responsible for causing differences between families in their response to inbreeding depression is that families differ in the number of recessive alleles that they carry (Koelewijn, 1998), surely as a result of their different history of inbreeding or differences in the accumulation of mutations (Shultz and Willis, 1995). The influence of maternal effects in determining the final response of families to inbreeding cannot be ruled out (Roach and Wulff, 1987; Byers and Waller, 1999). Genetic and non-genetic plant qualities can interact with inbreeding in such a way that strong maternal effects can buffer or enhance the effects of inbreeding leading to among-family variation in inbreeding depression (Picó et al., 2003). Furthermore, family-level variation in inbreeding depression can also be caused by the action and/or 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). Such an interaction between inbreeding depression and mating system largely depends on the genetic basis of inbreeding depression (Charlesworth and Charlesworth, 1987, 1999; Keller and Waller, 2002). In general, it is accepted that selfing rates and inbreeding depression are negatively correlated, i.e., plants with high selfing rates should show low inbreeding depression (Holtsford and Ellstrand, 1990; Johnston and Schoen, 1995; Carr and Dudash, 1996; Husband and Schemske, 1996). Thus, if inbreeding depression is caused by deleterious recessive alleles (i.e., the partial dominance hypothesis; Charlesworth and Charlesworth, 1987, 1999), high fitness genotypes will become associated with highly selfing genotypes (Mutikainen and Delph, 1998).

Besides the implications of family-level variation in inbreeding depression on mating-system evolution, variability among families in the response to inbreeding can also have important ecological implications. In a case of dramatic reductions in population size (e.g., habitat fragmentation), theory

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predicts that the number of breeding individuals within a population and gene flow between populations may decrease, leading to increasing inbreeding rates and decreasing population fitness (Dudash and Fenster, 2000). The variability among individuals in the response to inbreeding depression might significantly buffer the effects of fragmentation on population persistence since some individuals would be able to cope with inbreeding depression. In fact, if a selfing variant can invade a population, it necessarily means that fitness of those particular selfing variants has to be high enough to prevent population extinction. The proportion of individuals within a population showing an increased fitness under selfing and the interaction of selfing variants with ecological conditions will ultimately determine the success of selfing variants in a population.

The goal of this study is to evaluate the extent of family-level variation in inbreeding depression in the perennial herb *Scabiosa columbaria* (Dipsacaceae). The species presents a mixed-mating system and the effects of inbreeding have been reported for different life cycle traits (van Treuren et al., 1993). We want to elucidate the effects of inbreeding depression on life cycle traits at the individual level and assess their ecological and evolutionary implications. We sampled a large number of plants from one population with a high natural outcrossing rate and performed self- and cross-pollinations in a greenhouse environment to produce selfed and outcrossed progeny, respectively. We address the following questions: (1) What is the extent of family-level variation in inbreeding depression in life cycle traits of *S. columbaria*? (2) What is the relationship between life cycle traits in their response to inbreeding depression at the individual level?

MATERIALS AND METHODS

Plant species and study site—*Scabiosa columbaria* L. is a perennial plant of dry, sunny grasslands, rocky hillsides, and open woods across Europe. Life expectancy of individuals ranges 2–5 yr (van Treuren et al., 1993). The plant grows on a basal rosette of lance-shaped leaves (5–15 cm long). The flowering season takes place between June and September, and the plant produces branched stalks (20–80 cm height) that bear several flowering heads (approximately 3.5 cm diameter). Each flower head contains numerous florets that produce single-seeded fruits. The species is protandric and insect-pollinated, and previous studies showed that *S. columbaria* is self-compatible (van Treuren et al., 1993). The species presents some degree of gynodioecy though we only used hermaphrodite individuals in this study.

We selected one large population in Southeastern Netherlands (Wrakelberg, 100 000 flowering individuals) in a calcareous grassland with the nearest *S. columbaria* population placed at 10 km. Wrakelberg has a very high outcrossing rate (100% estimated by electrophoresis; van Treuren et al., 1994). Hence, the population of study is likely to have a mean inbreeding coefficient of 0, and selfing increased the mean level of inbreeding by 0.5 relative to the outcrossed control group.

Experimental crosses—In summer 2001, single-seeded fruits (seeds hereafter) from 80 plants were randomly collected at Wrakelberg in an area of 1100 m². A total of 15 seeds 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) in the Botanical Garden of the University of Nijmegen. Three weeks after germination, one seedling per maternal plant (family hereafter) was individually potted and allowed to grow for 2 wk in the same greenhouse. Seedlings were then transferred to an unconditioned greenhouse to overwinter.

A total of 54 out of 80 families flowered in early summer 2002. We produced selfed and outcrossed progeny on each family by hand-pollinating two flower heads: one flower head was pollinated with pollen from the same plant

(self-pollination) while the other flower head was pollinated with pollen from another plant (cross-pollination). Cross-pollinations were made with a mixture of pollen from three of four different plants, and both self- and cross-pollinations were repeated during 3 d on each target flower. Prior to hand-pollination, target flower heads were emasculated to control the origin of the pollen in all fertilizations. Hand-pollinations were made by gently rubbing one flower head over another one. One head always acted as a pollen donor and the other as a recipient head. All treated recipient heads remained bagged during and after hand-pollinations until seed ripening and harvesting. In late summer 2002, seeds were collected and stored in paper bags at room temperature. A total of 42 out of 54 families produced matched progeny, that is, both selfed and outcrossed progeny.

Resulting seeds per treatment per family were collected and counted, and filled seeds were separated, counted, and weighed. The seed set (filled seeds/total seeds) and seed mass (total mass of filled seeds/number of filled seeds) were obtained. Afterwards, up to 25 seeds per family and treatment ($N = 537$ seeds) were randomly chosen and potted (15 × 15 cm) to record percentage germination 1 mo after sowing (in the same conditions as described above). A total of 9 out of 42 families produced less than five filled seeds from self-pollination. These families were included for seed set (based on 42 families) but excluded for germination (based on 33 families), as the number of seeds on which a germination rate had to be computed was too low. A total of 28 out of 33 families produced seedlings for both pollination treatments. In early winter 2002, five seedlings per family and treatment ($N = 280$ seedlings) were individually transplanted into pots (15 × 15 cm). Immediately after the transplant, the number of leaves and the length of the largest leaf of each seedling were recorded. The same measures were recorded 2 mo after transplanting to record juvenile size. Juvenile size was estimated as the product between the number of leaves and the length of the largest leaf, and juvenile relative growth rate (RGR) was computed as $[\ln(S_{t+1}) - \ln(S_t)] \div \Delta t$, where S_t is juvenile size at time t . Flowering occurred in late spring 2003. Flowering date was calculated as the number of days between transplanting date and the emergence of the first flowering stalk. Flowering plants were harvested in summer 2003 at the end of the flowering season. The total number of flowering stalks and flowers produced per plant were counted. Afterwards, the aboveground part of the plant was dried (65°C for 24 h) and weighed to obtain total aboveground biomass. Flower production rate was computed as the total number of flowers divided by the number of days between flowering onset and harvesting.

Statistical analysis—The effect of pollination treatment on seed set, seed mass, and percentage germination was analyzed with one-way ANOVA models using the mean value per family as a replicate. The effect of pollination treatment and family on juvenile RGR, flowering date, flower production rate, and aboveground biomass was analyzed with two-way ANOVA (for juvenile RGR) and ANCOVA (for flowering date, flower production rate, and aboveground biomass) models using the individual values of plants per family as replicates. The covariate was seedling size recorded immediately after transplantation to eliminate the effect of initial seedling differences on plant traits. The assumption of parallel slopes necessary for using covariates was examined with three-way interaction terms. The triple interaction between pollination treatment, family, and seedling size was found to be nonsignificant in all cases.

The response of a categorical variable, such as flowering probability, to pollination treatment was analyzed with a logistic regression model. The analysis started with a null model including all main factors (pollination treatment and family), the covariate (seedling size), and all interactions. Then, a new model that lacks the term to be analyzed was created. For each term we tested whether the difference in unexplained variance (deviance, D) between models was approximately χ^2 distributed, with the number of degrees of freedom equal to the difference between models (Koelewijn, 1998).

The effect of pollination treatment at the family level on life cycle traits was tested using two different statistical methods. For seed set and percentage germination, we used logistic regressions in which individual seeds were used as replicates. In the case of seed set, a 0 (aborted) or a 1 (filled) was assigned to each seed of the flower head, and in the case of percentage germination,

TABLE 1. One-way ANOVAs testing for the effects of pollination treatment (selfing and outcrossing) on seed set, seed mass, and percentage germination of *Scabiosa columbaria*. Seed set and percentage germination were arcsine-transformed whereas seed mass was log-transformed prior to analyses.

Trait	df	F
Seed set	1, 83	8.06**
Seed mass	1, 65	5.67*
Percentage germination	1, 65	4.14*

* $P < 0.05$; ** $P < 0.01$.

a 0 (non-germinated) or a 1 (germinated) was assigned to each filled seed of the flower head. We could not analyze the within-family variation in seed mass because we only had a mean value per family. For juvenile RGR, flowering date, flower production rate, and aboveground biomass, we computed simple main effects (Pedhazur, 1982) to test the significance of pollination treatment separately for the different families. The test of simple main effects was applied whenever the pollination treatment by family interaction was significant in all ANOVAs and ANCOVAs.

Inbreeding depression (δ) was calculated for each family 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 mean inbreeding depression was calculated for seed set, seed mass, percentage germination, juvenile RGR, flowering date, flower production rate, and aboveground biomass, and the mean fitness measures of W_s and W_o were calculated as the mean of family values. We calculated the family multiplicative inbreeding depression using the product between inbreeding depression values of percentage germination and flower production rate of each family. We used flower production rate as a multiplicative fitness component because it integrates flowering time and reproductive effort, which have important implications for fitness in plants. We only used those families that were monitored during the whole life cycle from seed set to aboveground biomass (24 families). We finally calculated the mean multiplicative inbreeding depression of the population by averaging the family multiplicative inbreeding depression values.

The correlation between inbreeding depression values was examined with Pearson's correlations using family means as replicates. The Bonferroni correction was applied to limit the overall experiment-wise error and to avoid spurious correlations (Sokal and Rohlf, 1995). When necessary, variables were arcsine-transformed (for proportions) or log-transformed (for all others) to normalize their distributions. Type IV sums of squares were used in all analyses.

RESULTS

Selfed and outcrossed progeny of *S. columbaria* significantly differed between pollination treatments for seed set, seed mass, and percentage germination (Table 1). Outcrossed

TABLE 3. Logistic regression testing for the effects of pollination treatment (selfing and outcrossing) and family on flowering probability of *Scabiosa columbaria*. Seedling size was used as a covariate.

Factor	df	D ^a
Seedling size (cov)	110	6.73**
Treatment (T)	110	11.01***
Family (F)	84	21.86 NS
T × F	84	30.53 NS
T × cov	110	11.61***
F × cov	84	24.87 NS
T × F × cov	84	35.61 NS

^a D, deviance.

** $P < 0.01$; *** $P < 0.0001$; NS, nonsignificant.

progeny outperformed selfed progeny for seed set (mean ± SE; $41.0 \pm 4.1\%$ and $54.9 \pm 3.3\%$ for selfed and outcrossed progeny, respectively), seed mass (1.8 ± 0.1 mg and 2.0 ± 0.6 mg), and percentage germination ($53.5 \pm 3.7\%$ and $63.1 \pm 3.5\%$).

The effects of pollination treatment on juvenile RGR (mean ± SE among all families and treatments; 1.4 ± 0.1 leaf × cm), flowering date (190.7 ± 2.3 d), flower production rate (0.21 ± 0.01 flowers/d) and aboveground biomass (5.3 ± 0.4 g) were not significant (Table 2). Seedling size did not affect flowering date, flower production rate, or aboveground biomass, and family was only significant for aboveground biomass (Table 2). The pollination treatment by family interaction was significant for all these traits, indicating that the effects of pollination treatment significantly varied among families (Table 2).

Flowering probability significantly differed between pollination treatments, and seedling size significantly affected flowering probability (Table 3). A total of 73% and 83% of the selfed and outcrossed plants flowered, respectively. Large seedling sizes significantly led to flowering plants (mean ± SE seedling size; 23.4 ± 0.6 and 17.1 ± 0.9 leaf × cm for flowering and nonflowering plants, respectively). Neither family nor the pollination treatment by family interaction was significant for flowering probability (Table 3). The pollination treatment by seedling size interaction was significant (Table 3), indicating that the effects of seedling size on flowering probability differed between selfed and outcrossed progeny.

At the family level, seed set and percentage germination showed more families with significant effects of pollination treatment than for the rest of traits (Fig. 1). The number of families in which the outcrossed progeny outperformed the selfed progeny was greater for seed set (19 out of 25 families), percentage germination (10 out of 12), and aboveground biomass (six out of seven). The number of families exhibiting the

TABLE 2. Two-way ANOVA and ANCOVA testing for the effects of pollination treatment (selfing and outcrossing) and family on juvenile relative growth rate (RGR), flowering date, flower production rate, and aboveground biomass of *Scabiosa columbaria*. Variables were log-transformed prior to analyses. Seedling size was used as covariate.

Factor	Juvenile RGR		Flowering date		Flower production rate		Aboveground biomass	
	df	F	df	F	df	F	df	F
Seedling size	—	—	1, 157	1.36 NS	1, 157	0.19 NS	1, 157	3.57 NS
Treatment (T)	1, 27	0.35 NS	1, 23	0.06 NS	1, 23	0.44 NS	1, 23	1.69 NS
Family (F)	27, 27	1.65 NS	23, 23	1.25 NS	23, 23	0.95 NS	23, 23	2.02*
T × F	27, 221	2.01**	23, 157	1.65*	23, 157	2.19**	23, 157	3.14***

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.0001$; NS, nonsignificant.

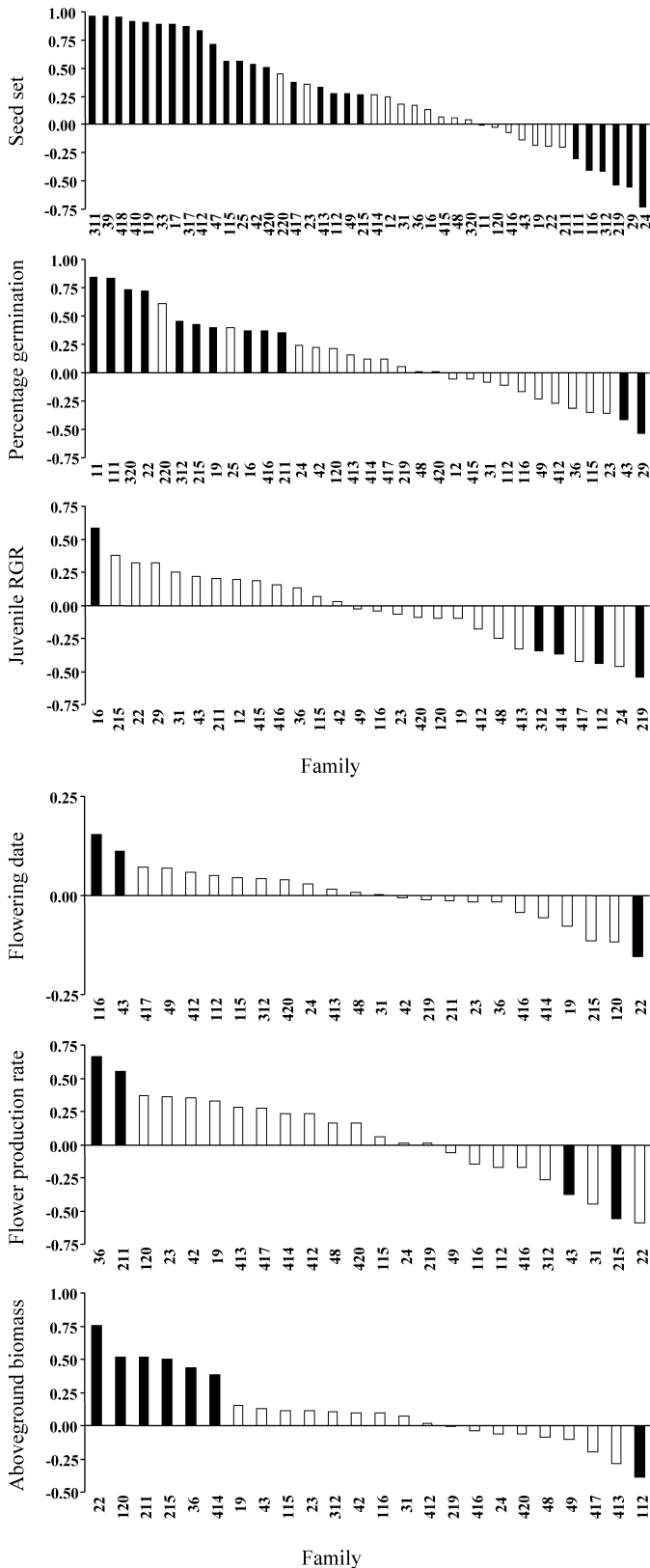


Fig. 1. Inbreeding depression for different life cycle traits of each *Scabiosa columbaria* family of study. Families are arranged in order of decreasing inbreeding depression value: positive values indicate that outcrossed progeny outperformed selfed progeny whereas negative values indicate the opposite. Family numbers are arbitrary. Black bars indicate that selfed and outcrossed

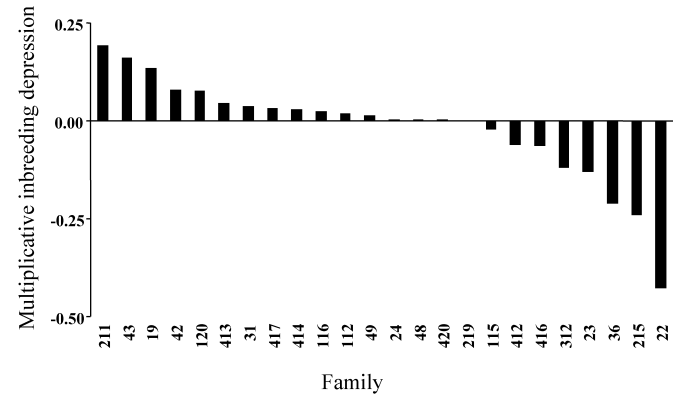


Fig. 2. Multiplicative inbreeding depression calculated as the product between inbreeding depression values of percentage germination and flower production rate for those families of *Scabiosa columbaria* monitored throughout their whole life cycle. Families are arranged in order of decreasing inbreeding depression value: positive values indicate that outcrossed progeny outperformed selfed progeny whereas negative values indicate the opposite. Family numbers are arbitrary.

opposite pattern was greater for juvenile RGR (four out of five) (Fig. 1). Both flowering date and flower production rate showed a low number of families with significant differences between pollination treatments but showing both patterns (Fig. 1). We did not analyze the effects of pollination treatment at the family level for flowering probability because the effects of pollination treatment on this trait did not significantly differ between families (Table 3).

The mean (\pm SE) inbreeding depression for each trait was 0.257 ± 0.073 for seed set, 0.095 ± 0.022 for seed mass, 0.142 ± 0.066 for seed germination, -0.026 ± 0.056 for juvenile RGR, 0.004 ± 0.015 for flowering date, 0.053 ± 0.071 for flower production rate, and 0.115 ± 0.056 for aboveground biomass. The mean multiplicative inbreeding depression for each family indicated that approximately half of the families had positive cumulative inbreeding depression whereas the other half had negative ones (Fig. 2). This apparent balance between families was supported by the mean multiplicative inbreeding depression of the population, which was approximately 0 (mean \pm SE = -0.02 ± 0.028).

The correlations between inbreeding coefficients indicated negative and positive interactions between life cycle traits (Table 4). Inbreeding depression of seed mass was negatively significantly correlated with inbreeding depression of seed set. Inbreeding depression of percentage germination was also negatively significantly correlated with inbreeding depression of flowering date, while inbreeding depression in flowering date was also negatively significantly correlated with inbreeding depression of aboveground biomass. Finally, the inbreeding depression of juvenile RGR was positively significantly correlated with inbreeding depression of aboveground biomass.

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progeny significantly differed from one another ($P < 0.05$). For seed set and percentage germination, logistic regressions were applied to each family, whereas for juvenile relative growth rate (RGR), flowering date, flower production rate, and aboveground biomass, simple main effect tests were performed on each family.

TABLE 4. Correlations for inbreeding depression values between life cycle traits (seed set, seed mass, percentage germination, juvenile relative growth rate (RGR), flowering date, flower production rate, and aboveground biomass) of *Scabiosa columbaria*. The correlation coefficients are given. The Bonferroni adjustment (a significance level of $P < 0.003$) for multiple comparisons was applied.

	Seed set	Seed mass	Percentage germination	Juvenile RGR	Flowering date	Flowering rate	Aboveground biomass
Seed set	—						
Seed mass	-0.68 †	—					
Percentage germination	-0.16 NS	0.14 NS	—				
Juvenile RGR	0.06 NS	-0.03 NS	0.01 NS	—			
Flowering date	0.07 NS	-0.27 NS	-0.65 †	-0.30 NS	—		
Flowering rate	0.25 NS	-0.41 NS	-0.22 NS	-0.30 NS	0.04 NS	—	
Aboveground biomass	-0.17 NS	0.19 NS	0.37 NS	0.60 †	-0.66 †	-0.04 NS	—

† $P < 0.003$; NS, nonsignificant. Significant correlation coefficients are in boldface type for the sake of clarity.

DISCUSSION

Among-family variation in inbreeding depression—We detected several families where selfed progeny significantly outperformed outcrossed progeny for many life-cycle traits of *Scabiosa columbaria*, as also shown by inbreeding depression studies on other plant species (Schemske, 1983; Schoen, 1983; Sakai et al., 1989; Ågren and Schemske, 1993; Husband and Schemske, 1996; Dudash et al., 1997; Koelewijn, 1998; Mutikainen and Delph, 1998; Ouborg et al., 2000; Rao et al., 2002; Picó et al., 2003). Such a high among-family variation in inbreeding depression could partly be explained by the high outcrossing rate of the population of study (van Treuren et al., 1994). Studies that compared the variability in inbreeding depression between populations of the same plant species with contrasting mating systems found that the among-family variation in inbreeding depression was lower in selfing populations than in mixed mating populations (Parker et al., 1995; Fishman, 2001). This pattern may arise from the long history of inbreeding in selfing populations in which lineages continuously purge new deleterious mutations or from the homogenization of the selfing population by recent genetic bottlenecks (Fishman, 2001).

The pattern of among-family variation in inbreeding depression dramatically changed throughout the life cycle of *S. columbaria*. For seed set and percentage germination, the proportion of families in which outcrossed progeny outperformed selfed progeny was higher than for the rest of traits. This could be attributable to the combination of inbreeding depression and maternal effects, as both factors may have strong effects on early life cycles in plants (Kalisz, 1989; Wolfe, 1993; Picó et al., 2003). Juvenile relative growth rate, flowering date, flower production rate, and aboveground biomass presented a lower proportion of families whose progeny differed between treatments. A general trend was that most families presented an alternating pattern in inbreeding depression throughout the life cycle (Fig. 1). These results indicate that the effects of inbreeding at the family level differ between life cycle traits, supporting the idea that the action and/or interaction of genetic mechanisms operating at different life cycle stages can largely be responsible for the family-level variation in inbreeding depression (del Castillo, 1998).

Theoretical work has demonstrated that determining among-family variation in inbreeding depression is crucial to assess whether a selfing variant can invade a population (Holsinger, 1991; Uyenoyama et al., 1993). Classical models on the relationship between mating system evolution and inbreeding depression predicted that complete selfing or outcrossing (the two evolutionary stable outcomes) evolve when inbreeding de-

pression is below or above 0.5, respectively (Lloyd, 1979). However, recent models have predicted that inbreeding depression below 0.5 does not necessarily lead to the selection of selfing given that inbreeding depression fluctuates in a stochastic manner among generations (Cheptou and Schoen, 2002). The reason is that the ecological factors that contribute to fitness, and consequently to inbreeding depression, also vary in time. Hence, attempts to predict mating system evolution should take into account the effects of varying ecological conditions on relative fitnesses of selfed and outcrossed progeny (Dole and Ritland, 1993; Cheptou and Schoen, 2002).

In the case of *S. columbaria*, it is worth noting that at the population level the effects of inbreeding depression are very low ($\delta = -0.02$), but at the individual level, the population presents a remarkably high among-family variation with some families exhibiting a strong negative multiplicative inbreeding depression (Fig. 2). Hence, families that perform better under selfing than under outcrossing could potentially invade an outcrossing population if loci increasing selfing become associated with loci responsible for such a high performance under selfing (Dudash et al., 1997; Mutikainen and Delph, 1998). Studies focusing on repeated inbreeding have addressed this issue by increasing the inbreeding load of families with controlled self-pollinations and comparing the performance of progeny differing in inbreeding level. Overall, results from multigenerational inbreeding studies suggest that although some families present improvement under continued inbreeding, the selection against the homozygotes seems to be strong enough to maintain inbreeding depression within these families (Dudash et al., 1997; Koelewijn, 1998). Other studies clearly showed that the extent of inbreeding depression is strongly influenced by the environment in which selfed and outcrossed progeny are compared (Dudash, 1990; van Treuren et al., 1993). Hence, it is reasonable to believe that a particular combination of environmental conditions enhancing self-fertilization might favor selfing variants in a population.

Correlation between inbreeding depression values—The positive and significant correlation between inbreeding depression values of juvenile relative growth rate and aboveground biomass suggested that plant growth at different plant stages is controlled by the same genetic mechanisms. Inbreeding depression between seed set and seed mass was negatively correlated, which could indicate that maternal effects override inbreeding depression in *S. columbaria*, as a result of the trade-off between seed number and seed size commonly found in plants (Crawley, 1997). Interestingly, inbreeding depression values of percentage germination and flowering date and of

flowering date and aboveground biomass were significantly negatively correlated. This result indicates that for *S. columbaria* individuals negatively affected by inbreeding depression on germination, the effect of inbreeding turns out to be positive for flowering time and becomes negative again for plant size. These chained effects of inbreeding depression on different traits throughout the life cycle suggest an association between inbreeding depression loci affecting important life cycle traits. The alternating sign of the relationships between inbreeding depression values of life cycle traits suggests that deleterious alleles with small selection coefficients might be difficult to purge (Lande and Schemske, 1985; Byers and Waller, 1999; Charlesworth and Charlesworth, 1999) and that effective maternal line improvement does not take place easily (Dudash et al., 1997; Koelewijn, 1998; Rankin et al., 2002). A recent study on another *Scabiosa* species (*S. canescens*) also conducted correlations between inbreeding depression estimates for life cycle traits (Andersson and Waldmann, 2002). Although the two studies are not fully comparable, results on *S. canescens* also indicated that the genetic basis of inbreeding depression varied across the life cycle, providing additional support for the findings reported in our study.

This study revealed that family-level variation in inbreeding depression in *S. columbaria* is an important process that may also occur in several other plant species. Although purging of mutations and maternal line improvement seems to be limited, results indicated that the potential of a selfing variant to invade the population exists and that it can be high. Hence, we believe that the effects of inbreeding on population viability might be, to some extent, buffered by among-family variation in inbreeding depression. Further studies are called on to search for empirical evidence.

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