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## INBREEDING DEPRESSION IN *NIGELLA DEGENII* (RANUNCULACEAE): FITNESS COMPONENTS COMPARED WITH MORPHOLOGICAL AND PHENOLOGICAL CHARACTERS

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We have compared selfed and outbred offspring from individual plants of the annual plant *Nigella degenii* to examine patterns of inbreeding depression in two direct components of fitness (flower number and pollen viability) and a number of morphological or phenological characters for which the optimal phenotype may be habitat specific. Selfing lowered flower number, plant height, flower size, and pollen viability and caused a shift toward later germination and flowering dates. There was no significant difference in inbreeding depression between fitness components and characters reflecting morphology or phenology regardless of how inbreeding response was estimated. Family-level analyses revealed moderately strong correlated responses involving flower number and each of the nonfitness characters, whereas pollen viability showed an independent response to inbreeding. On the basis of these observations, we hypothesize that morphology and phenology could make a significant contribution to lifetime inbreeding depression in *N. degenii*, that inbreeding responses in different types of characters involve loci with both general and specific effects on the phenotype, and that morphological inbreeding depression has contributed to the evolutionary reduction of floral structures so prevalent in the *Nigella arvensis* complex.

**Keywords:** *Nigella degenii*, floral evolution, inbreeding depression, mating system, morphology, phenology.

### Introduction

Inbreeding depression, i.e., the decline in mean phenotype with increasing homozygosity (Falconer 1989), has received considerable attention in conservation biology (Hedrick and Kalinowski 2000; Keller and Waller 2002) and evolutionary studies of plant mating systems (Lloyd 1979; Lande and Schemske 1985; Charlesworth and Charlesworth 1987). According to the dominance hypothesis, inbreeding depression is caused by the expression of recessive or partially recessive deleterious alleles in homozygotes, whereas the overdominance hypothesis predicts a link between heterozygosity and gene interactions that cause increased performance relative to both homozygotes (Charlesworth and Charlesworth 1987; Falconer 1989). Although recessive deleterious alleles seem to be the most important source of inbreeding depression (Charlesworth and Charlesworth 1999), there are still too few empirical data to draw general conclusions about the genetic control of inbreeding depression. For example, relatively few attempts have been made to determine whether the heritable basis of inbreeding depression varies across the life cycle (Carr and Dudash 1995; Husband and Schemske 1996; Rao et al. 2002; Andersson and Waldmann 2003).

Much effort has been devoted to the estimation of inbreeding depression in primary components of fitness, particularly those related to vegetative vigor and female function (Charlesworth and Charlesworth 1987; Falconer 1989; Husband and Schemske 1996; Keller and Waller 2002). Only a few studies have considered measures of male fertility (e.g., Carr

and Dudash 1995; Jóhannsson et al. 1998; Willis 1999; Stephenson et al. 2001), and there is still a paucity of information on patterns of inbreeding depression in characters that might be weakly related to fitness or under stabilizing selection for a spatially varying optimum, e.g., characters describing morphology or phenology (Willis 1996; Andersson 1997; Shaw et al. 1998; DeRose and Roff 1999; Rao et al. 2002; Andersson and Waldmann 2003). For such characters, one would expect the relative fitnesses of selfed and outcrossed progeny to be habitat specific, a complication in studies that attempt to estimate total inbreeding depression on the basis of data from plants grown in standardized (non-selective) environments (see also Dudash 1990).

Inbreeding depression in morphology or phenology not only affects the relative fitnesses of inbred and outbred progeny but has also been shown to bias measurements of phenotypic selection (Lande and Arnold 1983) toward the detection of strong directional or stabilizing selection in partially inbred populations (Willis 1996). It is also possible to imagine situations in which certain inbreeding responses are advantageous—or at least not deleterious—and thus conducive to evolutionary change in the mean phenotype (Lynch et al. 1999; Rao et al. 2002; see also Charlesworth 1992). For example, there is growing evidence for inbreeding depression in floral size characters (Karoly 1994; Andersson 1996, 1997; Willis 1996; Shaw et al. 1998), a pattern that could facilitate the reduction of floral structures so prevalent in selfing plant lineages (Ornduff 1969). However, there should be little potential for inbreeding-mediated evolution when the phenotypic response to inbreeding represents a side effect of low vigor, given the potentially strong selection

against those alleles that cause a decline in fitness under inbreeding (Lande and Schemske 1985). To evaluate the potential role of inbreeding depression in phenotypic evolution, it is therefore important to determine whether observed inbreeding responses in morphology or phenology involve loci with general or specific effects on the phenotype (Rao et al. 2002).

Species in the *Nigella arvensis* complex (Ranunculaceae) have undergone considerable phenotypic divergence, especially in the Aegean, where a number of morphologically distinct taxa have been recognized (Strid 1970). For example, flowers of two selfing species, *Nigella doerfleri* Vierh. and *Nigella stricta* Strid, are 50%–60% smaller than the insect-pollinated flowers of *N. arvensis* L. subsp. *arvensis*, the largest-flowered taxon in this species complex. The reduction in flower size and other characters correlated with flower size (plant height, leaf size; Andersson 1997) may be an adaptive response to drought or low pollinator abundance (Strid 1969; Andersson 1997, 2000), but it is also meaningful to consider the direct effects of inbreeding: floral and vegetative size characters showed a negative response to inbreeding in a hybrid population from a cross between two nonautogamous subspecies of *Nigella degenii* Vierh. (Andersson 1997). In this study, we have obtained family-structured data from one of these subspecies to verify the existence of such inbreeding responses in a natural (nonhybrid) population and to provide more detailed information on the relationship between inbreeding depression in different characters. Specifically, we asked, Do measures of morphology and phenology respond to inbreeding, and are these responses of the same magnitude as those recorded for direct components of male or female fitness? Do maternal families respond differently to inbreeding, and are the family-level estimates of inbreeding depression correlated across characters? Is the pattern of inbreeding depression conducive to evolutionary change in the mean phenotype?

## Material and Methods

### Plant Material

*Nigella degenii* is an annual, diploid ( $2n=12$ ), self-compatible, insect-pollinated plant that occurs in more or less disturbed habitats, e.g., roadsides, abandoned terraced fields, seashores, and phrygana vegetation, in Cyclades, the Greek archipelago. The species belongs to the *Nigella arvensis* complex and has been divided into four morphologically distinct subspecies, including subsp. *barbro* Strid, which is endemic to Mykonos and a few other islands in northwest Cyclades (Strid 1970). Plants of *N. degenii* are erect to ascending with pinnatisect leaves and a stem with determinate flowering, starting with the flower terminating the central stem. Each flower consists of five white, petal-like sepals serving as attracting organs; eight stalked, bilabiate nectaries; a variable number of stamens; and a central gynoecium consisting of 3–10 partially united carpels. Protandry coupled with spatial separation of anthers and stigmas prevents within-flower selfing. Fertilized flowers develop into capsules, each consisting of 20–40 seeds (Strid 1969, 1970; Andersson 1997).

The plants used in this study were derived from seeds collected in a population of *N. degenii* subsp. *barbro* on the is-

land of Mykonos (along a stone fence ca. 2.5 km NNW of the town) in the summer of 1993.

### Experimental Procedures

Four generations of random outcrossing involving ca. 150 plants per generation were used to establish a completely outbred population in (near) linkage equilibrium for a quantitative genetic analysis (S. Andersson, unpublished data). In this study, we used this plant material as a base population for an inbreeding-depression experiment even though only one generation of outcrossing would have been sufficient for this purpose. In 2000, ca. 150 seeds from the base population were sown in separate pots and arranged in a random pattern on three benches in an insect-free greenhouse. Two randomly chosen flowers on each plant were marked and subjected to one of two pollination treatments: self-pollination or emasculation followed by cross-pollination with pollen from a randomly chosen plant in the same population. In April 2002, five outbred and five selfed seeds from each of 89 plants in the base population ( $n = 890$  seeds) were sown individually into 25-cm<sup>2</sup> cells in a series of plastic flats on the same greenhouse bench. Seeds from a given full-sib family were randomized across the whole planting area. Water was supplied as needed, but no fertilizer was applied. Given the lack of inbreeding in the base population, the coefficient of inbreeding was assumed to be 0 and 0.5 for outbred and selfed seeds, respectively (Falconer 1989).

### Measurements

The following variables were scored on each selfed or outbred individual: the number of days from sowing to germination, the number of days from sowing to first flowering, plant height, leaf length, leaf-flower distance, flower size, pollen viability, and the total number of flowers initiated. Data on leaf length, leaf-flower distance, and flower size were obtained by preserving the first flower and the uppermost leaf in a microcentrifuge tube filled with 60% ethanol and measuring each character under a dissecting microscope equipped with an ocular micrometer. Leaf length refers to the uppermost leaf on the central stem, while leaf-flower distance refers to the distance between this leaf and the terminal flower (referred to as “peduncle length” in Andersson 1997). Flower size was quantified as the length of one randomly selected sepal per flower. This character is strongly positively correlated with the lengths of the nectaries and stamens (Andersson 1997) and therefore represents an overall measure of flower size. Pollen viability was determined by mixing a small amount of pollen from the first flower in a droplet of aniline blue lactophenol (cotton blue) on a glass slide and using a light microscope to determine the proportion of stained pollen grains (on the basis of 200 counted grains per sample). Cotton blue stains pollen grains that contain starch and is a fairly reliable measure of pollen viability (Stanley and Linskens 1974). The fate of nonflowering plants (germination failure, juvenile mortality) and the presence or absence of pollen in the anthers were also recorded.

Measures of vegetative or reproductive performance, such as germinability, survivorship, pollen viability, and flower number, were regarded as fitness components, while the

number of days to germination or flowering, plant height, leaf length, leaf-flower distance, and flower size represent characters describing morphology or phenology. The fitness components should be positively correlated with fitness irrespective of habitat, whereas the morphological and phenological characters might be subjected to genetic drift or spatially varying selection, as indicated by the extensive among-population variation recorded for most of these variables (Strid 1970; Andersson 1997). Comparative data from Strid (1969) strongly imply that the optimum plant height differs between populations, with more arid island sites selecting for plants with a short stature and more mesic island sites favoring tall individuals. For this reason, we consider plant height as a morphological character rather than as a fitness component.

Flower number is determined relatively late in the ontogeny and represents the most sensitive response variable in resource-manipulation experiments (Andersson 1997); hence, flower number also serves as an integrated measure of general plant vigor.

### Statistical Analyses

Frequency data were pooled across families and analyzed with  $\chi^2$  procedures to test for differences between selfed and outcrossed progeny. Differences between pollination treatments and maternal families in quantitative variables were tested for significance using mixed-model two-way ANOVA (type III sums of squares), with pollination treatment as “fixed” and family as “random.” To test for morphological and phenological inbreeding depression unconfounded by differences in overall plant vigor, we repeated the relevant analyses following the inclusion of total flower number as a covariate (ANCOVA). To normalize the residuals, germination date and leaf-flower distance were log transformed, and pollen viability was arcsine-square root transformed.

To quantify inbreeding responses, we first calculated the difference in the mean between selfed and outbred progeny (using least square means from two-way ANOVA or ANCOVA) and then standardized this quantity by the mean or standard deviation (SD) of the outcross progeny. The resulting parameters will be referred to as  $\delta_{\text{mean}}$  and  $\delta_{\text{SD}}$ , respectively. The first measure represents the conventional coefficient of inbreeding depression (Charlesworth and Charlesworth 1987) and allows direct comparison with estimates from previous inbreeding-depression experiments (Husband and Schemske 1996), whereas the second measure provides a scale-independent estimate of inbreeding depression (DeRose and Roff 1999), allowing comparison of characters for which the choice of scale is arbitrary. For example, the value of  $\delta_{\text{SD}}$  for a phenological character such as flowering date is the same regardless of whether day 0 is chosen to represent the emergence date or the start of the flowering period (Rao et al. 2002). Values of inbreeding depression for phenological parameters were presented as positive, even though selfed offspring had higher means for these variables (i.e., a larger number of days to germination and flowering; see “Results”) than outcrossed progeny.

Maternal estimates of inbreeding response—quantified as the difference in the mean of the selfed and outcrossed prog-

eny from each mother plant—were subjected to a product-moment correlation analysis to determine the correlated response to inbreeding for each pair of characters, followed by a principal components analysis (PCA) to summarize the pattern of variation in inbreeding response. Given the (apparent) lack of treatment effects for qualitative variables (see “Results”), we made no attempt to include them in the correlation analyses.

All analyses were performed with SPSS for Windows (release 11.0.0), except for the bivariate and multivariate procedures, which were carried out with SYSTAT on a Macintosh computer.

## Results

### Population-Level Analyses

Selfing had nonsignificant or marginally significant effects on the frequency of seeds that failed to germinate (10.6% vs. 7.9% for outcrossed progeny;  $\chi^2_1 = 1.93$ ,  $P = 0.164$ ), the frequency of plants that died before flowering (5.0% vs. 2.4% for outcrossed progeny;  $\chi^2_1 = 3.78$ ,  $P = 0.052$ ), and the frequency of flowering plants that produced sterile anthers (2.4% vs. 1.0% for outcrossed progeny;  $\chi^2_1 = 2.30$ ,  $P = 0.129$ ). The magnitude of inbreeding depression for these measures of fitness (measured by  $\delta_{\text{mean}}$ ) was 0.029, 0.027, and 0.014, respectively, and all changes were in the predicted direction (i.e., outcrossed progeny outperformed selfed).

Selfed progeny germinated later and had later flowering dates, fewer flowers, shorter stems, lower pollen viabilities, and smaller flowers than plants derived from outcrossing, whereas differences in leaf length and leaf-flower distance failed to reach significance (table 1). Judging from the conventional (mean standardized) estimate of inbreeding depression ( $\delta_{\text{mean}}$ ; table 2), there was a stronger effect of inbreeding on pollen viability (0.091), flower number (0.075), and plant height (0.074) than the other characters ( $<0.050$ ). Scaling by the standard deviation increased the difference between the estimate for pollen viability ( $\delta_{\text{SD}} = 0.708$ ) and the corresponding estimates for flower number (0.175) and all the remaining characters (0.105–0.305).

There was no significant difference in inbreeding depression between fitness components and characters reflecting morphology or phenology, regardless of whether the inbreeding response was quantified as  $\delta_{\text{mean}}$  (Mann-Whitney  $U_1 = 11.50$ ,  $P = 0.522$ ) or  $\delta_{\text{SD}}$  (Mann-Whitney  $U_1 = 4.00$ ,  $P = 0.505$ ). Classification of plant height as a fitness component had negligible effects on the results of these comparisons (data not shown).

Differences in pollen viability, germination and flowering date, plant height, and flower size remained significant in analyses accounting for variation in flower number ( $P < 0.001$ – $0.05$ , ANCOVA; data not shown). Adjusting for flower number had little effect on estimates of inbreeding depression for pollen viability but resulted in lower estimates for all the other characters (table 2). The reduction in  $\delta_{\text{mean}}$  and  $\delta_{\text{SD}}$  was 23%–25% for germination date, plant height, and leaf length; 17%–18% for flowering date and leaf-flower distance; and 13% for flower size.

**Table 1**  
**Effects of Pollination Treatment (Outcrossing vs. Selfing) and Maternal Parent on Each of the Quantitative Characters in the Inbreeding-Depression Experiment**

	F values			Means			
	Treatment (T)	Mother (M)	T × M	Outcrossing	n	Selfing	n
Fitness components:							
Flower number	4.77*	1.39 ns	1.41*	3.80	409	3.52	397
Proportion of viable pollen <sup>a</sup>	44.30***	1.08 ns	1.50**	0.965 <sup>b</sup>	389	0.925 <sup>b</sup>	358
Morphology/phenology:							
Days to germination <sup>c</sup>	8.05**	1.52*	1.70***	7.82 <sup>b</sup>	410	8.13 <sup>b</sup>	398
Days to flowering	10.73**	1.43*	1.60***	82.61	399	84.96	374
Plant height (mm)	11.50**	1.70**	1.58**	204.0	406	188.8	390
Leaf length (mm)	1.32 ns	1.97***	1.75***	12.41	390	12.20	368
Leaf-flower distance <sup>c</sup>	3.30 ns	2.94***	1.40*	3.74 <sup>b</sup>	390	3.39 <sup>b</sup>	368
Flower size (mm)	9.28**	2.12***	1.60***	12.11	390	11.78	368

Note. Data determined by using *F* values and least square means from two-way ANOVA; ns = not significant ( $P > 0.05$ ).

<sup>a</sup> Analyses based on arcsine-square root transformed data.

<sup>b</sup> Mean back transformed to original scale.

<sup>c</sup> Analyses based on log-transformed data.

\*  $P < 0.05$ .

\*\*  $P < 0.01$ .

\*\*\*  $P < 0.001$ .

#### Family-Level Analyses

The maternal parent had a significant influence on all but two variables—flower number and pollen viability—and there was a significant interaction between cross type and mother for all variables (table 1). Hence, our data indicate extensive between-family variation, both in the overall mean and in the response to inbreeding.

Correlation analyses demonstrated significant and moderately strong correlated responses involving flower number and each of the morphological and phenological characters ( $|r| = 0.265$ – $0.642$ ; table 3), whereas estimates of correlated response involving pollen viability failed to reach significance ( $|r| < 0.09$ ). Consistent with these patterns, we found high loadings for all characters except pollen viability on the first principal component, which extracted 46% of the between-family variability in inbreeding response (table 4). The second axis accounted for a supplementary 13% of the variation, contrasting families with high versus low inbreeding depression in pollen viability.

#### Discussion

Although much attention has focused on the negative effects of inbreeding on direct components of fitness (Charlesworth and Charlesworth 1987; Husband and Schemske 1996; Keller and Waller 2002), there is still a paucity of studies that document patterns and amounts of inbreeding depression in characters reflecting morphology or phenology. In this study, we have obtained family-structured data from an extensive crossing experiment with a self-compatible, nonautogamous population of *Nigella degenii* to compare the phenotypic effects of selfing or outcrossing. Next, we evaluate the results with particular emphasis on the magnitude of inbreeding depression in fitness components versus morphology and phenology, the pattern of correlated response to inbreed-

ing in the different types of characters, and the hypothesis that inbreeding effects have promoted the evolutionary reduction of flower size accompanying the evolution of selfing in the *Nigella arvensis* complex.

Consistent with the results from other wild plant species (Andersson 1996; Willis 1996; Shaw et al. 1998; Rao et al. 2002; Andersson and Waldmann 2003), inbred and outbred offspring of *N. degenii* subsp. *barbro* were found to differ in a wide variety of characters. Selfing depressed measures of

**Table 2**  
**Estimates of Inbreeding Depression for Different Characters, Quantified as  $\delta_{\text{mean}}$  and  $\delta_{\text{SD}}$**

	$\delta_{\text{mean}}$		$\delta_{\text{SD}}$	
	Non-adjusted	Adjusted <sup>a</sup>	Non-adjusted	Adjusted <sup>a</sup>
Fitness components:				
Flower number	0.075	...	0.175	...
Proportion of viable pollen <sup>b</sup>	0.091	0.091	0.708	0.704
Morphology/phenology:				
Days to germination <sup>c</sup>	0.019	0.014	0.252	0.193
Days to flowering	0.028	0.023	0.305	0.251
Plant height	0.074	0.056	0.288	0.216
Leaf length	0.017	0.013	0.105	0.081
Leaf-flower distance <sup>c</sup>	0.049	0.041	0.139	0.115
Flower size	0.027	0.023	0.273	0.237

<sup>a</sup> Values are based on least square means from analyses using flower number as a covariate.

<sup>b</sup> Analyses and means based on arcsine-square root transformed data.

<sup>c</sup> Analyses and means based on log-transformed data.

**Table 3**  
Product-Moment Correlations of Family-Level Estimates of Inbreeding Depression among Characters

Variable	1	2	3	4	5	6	7	8
Fitness components:								
1. Flower number	...							
2. Proportion of viable pollen	0.011	...						
Morphology/phenology:								
3. Days to germination	-0.372***	-0.046	...					
4. Days to flowering	-0.424***	-0.059	0.601***	...				
5. Plant height	0.384***	0.063	-0.493***	-0.597***	...			
6. Leaf length	0.356***	0.083	-0.423***	-0.442***	0.527***	...		
7. Leaf-flower distance	0.352***	-0.059	-0.326**	-0.326**	0.350***	0.413***	...	
8. Flower size	0.265*	0.078	-0.556***	-0.453***	0.642***	0.522***	0.305**	...

Note. Analyses based on 89 families.

\*  $P < 0.05$ .

\*\*  $P < 0.01$ .

\*\*\*  $P < 0.001$ .

male and female fertility (pollen viability, flower number) and caused a shift toward later germination and flowering dates, shorter stems, and reduced flower size. Most of these responses were also observed in a previous comparison of F1 hybrids and inbred F3 genotypes from a cross between *N. degenii* subsp. *barbro* and *N. degenii* subsp. *jenny* Strid, the most notable exception being flowering date, which showed the opposite response in the hybrid population (Andersson 1997). We have no explanation for the latter discrepancy; however, it is possible that the results from the previous study were confounded by factors related to the use of hybrid genotypes (heterosis effects, breakup of coadapted gene complexes, etc.).

Judging from the absolute values of the conventional inbreeding-depression coefficient ( $\delta_{\text{mean}}$ ), flower number and pollen viability showed greater responses to selfing (0.075–0.091) than characters related to morphology or to phenology ( $< 0.075$ ). However, the difference in inbreeding depression between the two types of characters was too small to reach significance in a comparison that also included the three qualitative fitness components (germinability, survivorship, anther fertility;  $\delta_{\text{mean}} < 0.03$ ). Furthermore, there was no consistent difference in the level of inbreeding depression between the two quantitative fitness components and the remaining group of morphological and phenological characters when all inbreeding responses were expressed in standard deviation units to account for differences in measurement scale (DeRose and Roff 1999; Rao et al. 2002). Thus, our results for *N. degenii* provide no support for the notion that direct components of fitness are more sensitive to inbreeding depression than characters that are less directly related to fitness (Falconer 1989; DeRose and Roff 1999).

Our results for pollen viability—a component of male fertility—extend similar findings of other recent studies. Carr and Dudash (1995) found inbred offspring of *Mimulus guttatus* (Scrophulariaceae) to produce 28%–33% fewer pollen grains than plants derived from outcrossing. In another study of this species, recessive male-sterility alleles were found to account for 26% of the inbreeding depression in lifetime fitness (Willis 1999). In *Cucurbita texana* (Cucurbitaceae), outcrossed individuals not only initiated more male flowers

but also produced pollen that sired more seeds than pollen from selfed plants under conditions of pollen competition (Jóhannsson et al. 1998; see also Stephenson et al. 2001). On the basis of these findings, it seems that male fertility could make a significant contribution to lifetime inbreeding depression, stressing the necessity of considering both sex functions before any broad generalizations are made regarding the negative consequences of inbreeding.

Lifetime inbreeding depression represents the multiplicative effects of all variables that influence fitness. In this investigation, it is possible to obtain a rough measure of total inbreeding depression on the basis of the frequency of seeds that germinated, the frequency of plants that survived to flowering, the frequency of flowering plants producing fertile anthers, the proportion of viable pollen, and the total flower production. The resulting estimate,  $\delta_{\text{mean}} = 0.217$ , is lower than the level of inbreeding depression needed to prevent the invasion of a selfing mutant, according to traditional models ( $\delta_{\text{mean}} > 0.5$ ; Lloyd 1979; Charlesworth and Charlesworth 1987). However, our estimate is almost certainly lower than the true level of inbreeding depression: all progenies were raised under favorable growth conditions, a factor that has been shown to reduce the detectable effect of inbreeding in other studies (Dudash 1990), and no attempt was made to account for inbreeding responses

**Table 4**  
Character Loadings for the First Two Components in a PCA of Family-Specific Estimates of Response to Inbreeding

	PC1	PC2
Fitness components:		
Flower number	0.599	-0.206
Proportion of viable pollen	0.085	0.915
Morphology/phenology:		
Days to germination	-0.760	-0.038
Days to flowering	-0.776	-0.029
Plant height	0.811	0.077
Leaf length	0.734	0.034
Leaf-flower distance	0.575	-0.364
Flower size	0.761	0.154
Variance explained (%)	45.6	13.1

in morphology and phenology, whose fitness effects may be habitat specific. It would be interesting to obtain data on the relationship between each character and fitness in the natural habitat to determine whether inbreeding depression in morphology and phenology affects the relative fitnesses of selfed and outcrossed progeny and thus contributes to lifetime inbreeding depression in *N. degenii* subsp. *barbro*.

Results of this study confirm previous observations from other plant species (Carr and Dudash 1995; Husband and Schemske 1996; Rao et al. 2002; Andersson and Waldmann 2003) that families within natural populations vary in their response to inbreeding. Such among-family variation can be attributed to differences in the occurrence of newly risen deleterious mutations and/or variation in past inbreeding history (Schultz and Willis 1995) and has been proposed to facilitate the evolution of selfing or the maintenance of a mixed mating system, involving both selfing and outcrossing (Uyenoyama and Waller 1991). In fact, some authors consider genetic variation in inbreeding response to be a more important factor in mating-system evolution than the average inbreeding depression of a population (Holsinger 1988; Schultz and Willis 1995). To evaluate this hypothesis in *N. degenii*, it is necessary to determine whether the heritable differences in inbreeding depression can be expressed in the field with normal levels of environmental variation.

Our family-level analyses also provided evidence for correlated responses to inbreeding for groups of characters: the magnitude of inbreeding depression in total flower number showed a significant relationship with the magnitude of inbreeding depression in each of the morphological and phenological characters, whereas correlated responses involving pollen viability were too weak to reach significance. Taken together, these results suggest that inbreeding depression in *N. degenii* involves loci with both general and specific effects on the phenotype, contrasting with the more strictly character-specific expression of inbreeding depression seen in previous studies (Carr and Dudash 1995; Husband and Schemske 1996; Rao et al. 2002; Andersson and Waldmann 2003). It is interesting that the matrix of correlated response was strongly correlated ( $r = 0.90$ ) with the family-mean correlation matrix for outbred progeny (data not shown), indicating a close similarity between the pleiotropic effects of those alleles that cause inbreeding depression and those that influence the phenotype of noninbred individuals.

Despite evidence for correlated responses involving flower number, there is no reason to invoke differences in general plant vigor as the principal cause of the observed inbreeding responses in morphology and phenology. The estimates of correlated response were moderate ( $|r| < 0.65$ ), and differences in resource status (measured by flower number) contributed little to the treatment effects in the other characters. For example, estimates of inbreeding depression for flower size,

adjusted for flower number, were only 13% lower than those based on “nonadjusted” data. On the basis of this observation, we attribute most of the inbreeding depression in flower size to loci expressed during flower development and not solely to loci influencing overall vigor, an interpretation that also applies to other plant systems (Rao et al. 2002; Andersson and Waldmann 2003).

Comparative and genetic studies of the *N. arvensis* complex suggest that differences in flower size evolved in response to drought and/or low pollinator abundance (Strid 1969), either as a result of direct selection for small flower size as plants became less dependent on pollinators or as a correlated response to selection for reduced plant size at sites occupied by the selfing taxa (Andersson 1997). In addition, experimental data from *N. degenii* indicate that the attracting structures (sepals, nectaries) represent an important sink for assimilates, a factor that should enhance the selective advantage of small flowers under conditions in which large, conspicuous flowers are unnecessary (Andersson 2000). When combined with the negative effects of inbreeding on flower size observed in this investigation, available evidence leads to the hypothesis that the direction of selection and the direction of inbreeding response sometimes converge for this character.

Observed inbreeding responses in this and previous studies of *N. degenii* (Andersson 1997) indicate that alleles for small flowers are recessive (Falconer 1989). The increased expression of recessive alleles under inbreeding not only causes an immediate (nonadaptive) decline in flower size as the proportion of recessive homozygotes increases in the population but also enhances the potential for further adaptive reduction in flower size by exposing these alleles to selection (Charlesworth 1992; Andersson 1997). However, the 2%–3% reduction in flower size after one generation selfing is small relative to the more than 50% decline accompanying the evolution of autogamy within the *N. arvensis* complex (Strid 1970). Thus, it is necessary to invoke a continuous supply of (recessive) mutations suppressing flower size—and a long history of inbreeding—to propose floral inbreeding depression as a persistent force in the evolution of small, autogamous flowers (see also Rao et al. 2002). To provide further insights into the role of inbreeding in facilitating floral evolution, it is necessary to know more about mutation rates and newly risen mutations (magnitude of effects, degree of dominance, pleiotropic side effects, etc.) at loci affecting flower development.

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