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Quantitative Genetic Effects of Bottlenecks: Experimental Evidence from a Wild Plant Species, *Nigella degenii*

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Abstract

Understanding the genetic consequences of changes in population size is fundamental in a variety of contexts, such as adaptation and conservation biology. In the study presented here, we have performed a replicated experiment with the plant *Nigella degenii* to explore the quantitative genetic effects of a single-founder bottleneck. In agreement with additive theory, the bottleneck reduced the mean (co)variance within lines and caused stochastic, line-specific changes in the genetic (co)variance structure. However, a significant portion of the (co)variance structure was conserved, and 2 characters—leaf and flower (sepal) size—turned out to be positively correlated in all data sets, indicating a potential for correlated evolution in these characters, even after a severe bottleneck. The hierarchical partitioning of genetic variance for flower size was in good agreement with predictions from additive theory, whereas the remaining characters showed an excess of within-line variance and a deficiency of among-line variance. The latter discrepancies were most likely a result of selection, given the small proportion of lines (23%) that remained viable until the end of the experiment. Our results suggest that bottlenecked populations of *N. degenii* generally have a lower adaptive potential than the ancestral population but also highlight the idiosyncratic nature of bottleneck effects.

Key words: *bottleneck, evolutionary constraint, G matrix, genetic drift, Nigella degenii*

Many populations have undergone severe, temporary reductions in size as a consequence of habitat loss, domestication, environmental catastrophes, or founder events (Carson and Templeton 1984; Hewitt 1999; Friar et al. 2000; Lee 2002; Briggs and Goldman 2006). Such "population bottlenecks" can have pronounced effects on the genetic constitution of populations and lead to immediate loss of genetic variation (e.g., Nei et al. 1975). Yet, despite evidence for bottleneck-induced losses of marker gene diversity (Leberg 1992; Friar et al. 2000) and short-term population viability (Newman and Pilson 1997; Saccheri et al. 1998), it is still unclear how reductions in population size alter an organism's evolutionary potential to adapt to novel ecological conditions, as determined by the genetic variances and covariances for suites of phenotypic characters (Willi et al. 2006).

Stochastic processes such as genetic drift are expected to convert genetic (co)variance within populations into genetic differences between populations. Under a strictly additive model of gene action, the within-population (co)variance after a bottleneck is expected to be $1 - F$ times the genetic (co)variance in the base population, where F is the inbreeding generated during the bottleneck (Wright 1951). This quantity and the corresponding prediction for the between-population (co)variance ($2F$ times the original (co)variance; Wright 1951) serve as natural baselines against which to compare the observed within- and between-line (co)variance after a bottleneck.

When there are high levels of nonadditive genetic (co)variance in a character, the additive (co)variances behave differently and can even increase, as observed in some bottleneck experiments (e.g., Bryant et al. 1986; López-Fanjul

and Villaverde 1989; Fernández et al. 1995; Cheverud et al. 1999; Saccheri et al. 2001; Briggs and Goldman 2006; van Heerwaarden et al. 2008). Such effects may be attributable to chance increases in the frequencies of recessive or partially recessive deleterious alleles (Robertson 1952; Willis and Orr 1993; Wang et al. 1998) or to the release of additive (co)variance as the number of polymorphic loci—and possible interlocus interactions—declines during the bottleneck (Goodnight 1988; Barton and Turelli 2004; López-Fanjul et al. 2004). In this context, it must be emphasized that drift can cause considerable random variation around the additive expectations, so that some populations might experience an increase in the additive (co)variance, even if on average the (co)variance decreases (Avery and Hill 1977; Lynch 1988; Zeng and Cockerham 1991). Furthermore, if an increase in additive (co)variance is accompanied by reductions in short-term population viability (Newman and Pilson 1997; Saccheri et al. 1998), then it seems unlikely that a recently bottlenecked population will have a greater evolutionary potential than the ancestral population (Willis and Orr 1993; Barton and Turelli 2004; Willi et al. 2006). As yet, only a few bottleneck experiments have been carried out on a sufficient scale to account for the variability in extinction rates and quantitative genetic parameters among replicate lines derived from the same base population (Cheverud et al. 1999; Whitlock and Fowler 1999; Phillips et al. 2001; Saccheri et al. 2001; Swindell and Bouzat 2005; van Heerwaarden et al. 2008).

Although the additive (co)variances in finite populations decline to zero, it probably takes many generations before stochastic processes will cause a reduction in the entire genetic (co)variance (G) matrix, especially if there is sufficient independent genetic control of different characters for genetic drift to operate independently on them. Thus, a sudden bottleneck is expected to cause idiosyncratic, element-specific changes in the G matrix—and a resultant change in principal component (PC) structure—rather than a proportional reduction in all (co)variances (Phillips and Arnold 1999; Phillips et al. 2001; Jones et al. 2003). Given the strong influence of the G matrix on the short-term trajectory of evolution by natural selection (Lande 1979), bottlenecks therefore have the potential to alter the persistence of genetic constraints and the evolutionary potential of natural populations (Wright 1978; Carson and Templeton 1984; Whitlock 1995; Lee 2002).

Populations in the *Nigella arvensis* complex (Ranunculaceae)—a group of 6 diploid ($2n = 12$) annual plant species with allopatric distributions in Greece and western Turkey—have diverged for a number of phenotypic characters, especially in the central Aegean (Cyclades). A substantial portion of the large-scale pattern seems to be of an adaptive nature, with taxa occupying the most arid islands having earlier flowering dates, shorter stems, and fewer, smaller, and more autogamous flowers than those on more mesic islands (Strid 1969, 1970). In regards to within-species variation, the available data for certain species, for example, *Nigella degenii* Vierh., indicate significant levels of population differentiation and no obvious relationship with local environmental conditions (Strid 1970). This and other considerations, for example, the disturbed nature of typical *Nigella* habitats and the capacity for some selfing even in the insect-pollinated species, led Strid (1970) to invoke random genetic drift due to bottlenecks as a major evolutionary force in this species complex. Indirect support for this scenario is provided by surveys of putatively neutral molecular markers in Aegean *Nigella* (Bittkau and Comes 2005; Comes et al. 2008) and the small difference in estimates of population divergence between phenotypic characters (Q_{ST}) and amplified fragment length polymorphism markers (F_{ST}) within 2 subspecies of *N. degenii* (Jorgensen et al. 2006).

In the present study on *N. degenii*, we performed a replicated experiment to explore the quantitative genetic effects of a single-founder bottleneck, with special emphasis on morphological and phenological characters that have diverged within the *N. arvensis* complex. The design of this study not only enabled us to separate between general and line-specific responses but also accounted for the possible effects of selection after the founder event, a potential source of error when inferring the quantitative genetic effects of small population size (Lynch 1988). As well as comparing means and survival rates, we utilized the common-principal-components (CPC) method (Flury 1988; Phillips and Arnold 1999) to compare G matrices. This method allows the (co)variance structure of 2 or more populations to be compared in a hierarchical fashion, starting from unrelated structure and progressing through partial CPC, CPC, proportionality, and equality. In addition, we contrasted the overall estimates of the within- and among-line genetic (co)variances with their additive expectations. Specifically, we asked: Is the bottleneck effect sufficiently strong to change the genetic (co)variance structure and the adaptive potential of *N. degenii* populations? And, does the quantitative genetic partitioning of (co)variance after the bottleneck conform to additive theory?

Materials and Methods

Plant Material

Nigella degenii occurs in dry, disturbed habitats (mainly in phrygana communities and abandoned fields) on the Cyclades (Greece), where 4 subspecies have been recognized (Strid 1970). The plants are erect to ascending, with a branched stem, pinnately dissected leaves and 15–25 mm wide bisexual flowers visited by bees. Each flower has a double perianth with an outer whorl of 5 whitish, petaloid sepals and an inner whorl of 8 stalked, bilabiate nectaries. Protandry, coupled with spatial separation of anthers and stigmas, enhances outcrossing, although the receptive styles sometimes become twisted around the dehiscing anthers, which results in some self-fertilization. Fertilized flowers develop into capsules with up to 100 seeds that lack any special dispersal mechanism. Seed viability declines significantly after 2–3 years (Strid 1969, 1970).

The plants used in this investigation originate from seeds representing 80 maternal plants scattered throughout a large population approximately 2.5 km north-northwest of the town on the island of Mikonos. Plants from this site belong to *N. degenii* Vier. ssp. *barbro* Strid, which is endemic to Mikonos and a few neighboring islands (Strid 1970). All crosses and cultivations were carried out under pollinator-free conditions in a greenhouse at the University of Lund, Sweden.

Experimental Procedures

Before initiating the bottleneck experiment, we used 4 generations of random outcrossing, involving a minimum of 150 plants per generation, to establish an outbred base population in (near) linkage equilibrium. In 2000, we sowed approximately 150 seeds from the base population in separate pots filled with peat soil and placed in a random pattern on 3 adjacent greenhouse benches. When the majority of the plants had reached anthesis, we subjected a number of flowers to 1 of 2 treatments: 1) self-pollination (ca. 10 flowers per plant) or 2) emasculation followed by cross-pollination with pollen from a randomly chosen plant in the same population (1 or 2 flowers per plant). We saved 5 selfed and 5 outcrossed seeds from each maternal plant for a separate analysis of inbreeding depression (Ellmer and Andersson 2004) and used the remaining selfed seeds for the bottleneck experiment.

Our experiment simulates a brief bottleneck involving a single founder that sets seed by autonomous or insect-mediated selfing, followed by an expansion of the population. Given the potentially low fitness after bottlenecks (Newman and Pilson 1997; Saccheri et al. 1998), we initiated 100 bottleneck lines (each founded from a separate individual) to account for possible loss of lines during the expansion phase. After the founder (selfing) event, we expanded the bottleneck lines using 3 generations of random outcrossing within lines (referred to as G_1–G_3). Each random-mating generation was initiated by sowing up to 45 (G_1) or 108 (G_2–G_3) seeds per line into 25-cm^2 cells (1 seed per cell) in 1 or 2 plastic trays per line placed in a random pattern in 1 or 2 adjacent greenhouse chambers. Water was supplied as needed, but no extra fertilizer was applied. Once plants began to flower, we assigned half the plants in each line as "males" and the remaining plants as "females" and mated each male to a distinct female within the same line (1 flower per cross). In a few cases, it was necessary to use the same plant as a male in 1 cross and as a female in another cross. Selfing and between-line pollinations were minimized by covering all females with fine-mesh nets before flowering and emasculating all recipient flowers before outcrossing. After flowering, we recorded the number of successful cross-pollinations (fruit set) for each line and mixed an equal number of seeds from every successful cross to form a bulk sample for the next random-mating generation.

Given the use of a single founder for each bottleneck line and the inferred lack of inbreeding in the base population, the coefficient of inbreeding (F) was assumed to be 0.5 immediately after the founder event (Falconer and Mackay 1996). To prevent further inbreeding during the expansion phase, we discarded lines with the lowest survival rate (<50%) and/or the lowest fruit set (<20 successful crosses) in each random-mating generation. The final F value of each remaining line was estimated by adding the new inbreeding in each generation (calculated as $1/2N$, where N is the number of parents involved in successful crosses) to the F value of the previous generation (Falconer and Mackay 1996).

The establishment of the control population was based on bulked seed samples from the original founders. To detect confounding effects of selection during the expansion phase, we established 2 control lines, 1 representing all 100 founders (C_{total}) and the other representing "successful" founders, that is, founders of bottleneck lines that remained viable 2 generations after the founder event (C_{subset}). Each founder contributed 10–15 outcrossed seeds to a given seed sample. Any difference between the 2 control lines would indicate that the surviving lines represented a nonrandom (selected) subset of the base population.

To minimize bias arising from differences in growth conditions and mating patterns, we subjected the control lines to 1 additional generation of within-line outcrossing, using the same cultivation and pollination designs as were used for the bottleneck group. The within-line outcrosses involved approximately 250 plants per control line planted at the same time and in the same greenhouse chamber as the G_3 plants in the bottleneck group.

In 2003, we established a large number of full sib progenies from the last outcrossing generation (G_3) to obtain phenotypic data for the quantitative genetic analyses. Seeds for this G_4 generation were derived from 110 families in the C_{total} line, 89 families in the C_{subset} line, and 19–21 families per bottleneck line. These were planted individually into 25-cm^2 cells in a series of plastic trays distributed across 5 adjacent benches in the same greenhouse chamber. Each family contributed 2 seeds to each bench (a total of 10 seeds per family), randomized across the whole planting area. The resulting plants were given supplementary light (12 h/day) and watered 2 or 3 times a week depending on weather conditions.

Measurements

We recorded whether or not a plant had died before flowering (survival status) and scored each flowering plant for 5 quantitative characters: first flowering date, flower number, plant height, leaf length, and sepal length. Data on leaf and sepal length were obtained by preserving the first flowering (terminal) flower and the uppermost leaf on the main stem in a microcentrifuge tube filled with 60% ethanol and then measuring each variable under a stereomicroscope. Sepal length is strongly positively correlated with the length of the other flower parts (Andersson 1997) and therefore provides a general measure of flower size. The quantitative characters exhibit both additive and nonadditive genetic

variance within *N. degenii*, as evidenced by parent–offspring comparisons (Andersson 1997) and data on inbreeding depression (Ellmer and Andersson 2004). They have also been found to define a major axis of differentiation in the *N. arvensis* complex, distinguishing early-flowering taxa with short, few-flowered stems and short leaves and small (selfing) flowers from those with the opposite features (Strid 1969, 1970). Ecological data strongly imply that the optimum phenotype differs between taxa, with more arid sites selecting for small-sized plants and more mesic sites favoring large-sized individuals (Strid 1969).

Phenotypic data were obtained for a maximum of 1837 plants in the control lines and 4542 plants in the 23 bottleneck lines that survived until the end of the experiment (mean 197.5 plants per line).

Initial Analyses

The survival data were pooled across families and analyzed with chi-square procedures to test for differences between control lines (C_{total} vs. C_{subset}) and between different lines in the bottlenecked population. The quantitative data were subjected to 1-way analyses of variance (ANOVAs, type III sums of squares) using "bench" as a categorical variable to provide residuals adjusted for spatial variation in the greenhouse chamber. Preliminary analyses of these data revealed approximately normal distributions; consequently, we used block-adjusted residuals in all analyses. Differences between the 2 control lines were tested for significance by univariate and multivariate ANOVAs (MANOVA) with line as a fixed factor and family (nested within line) as a random factor. Data for the bottleneck group were subjected to random-effects ANOVAs with line and family (nested within line) as group variables but also collapsed into line means to provide descriptive statistics on the among-line variation.

Matrix Analyses

To assess how the G matrix responded to the bottleneck event, we estimated covariance component matrices (based on 1-way analyses of covariance among full sib families) and used the CPC technique (Flury 1988; Phillips and Arnold 1999) to evaluate the type of differences between matrices.

As well as estimating the G matrix of the control population and each bottleneck line, we calculated the mean G matrix after the bottleneck based on data pooled across lines. To avoid confounding of within- and between-line (co)variation, we normalized the data by subtracting the line mean from the observed value of each individual (Whitlock and Fowler 1999). These analyses were performed using the program H2boot (Phillips 1998b), which uses a bootstrapping approach to estimate each parameter in the G matrix (5000 resamples).

Simultaneous CPC analysis of all lines was not computationally feasible; instead, we contrasted the control G matrix with the G matrix of each bottleneck line and the mean G across all bottleneck lines. We used the jump-up approach of Phillips and Arnold (1999) to determine the highest point in the hierarchy at which accumulated differences in the matrices became statistically significant ($P < 0.05$) and considered the model immediately below as the best-fitting model for the observed differences. These analyses were carried out with the program CPCrand (Phillips 1998a), which uses a resampling approach to test the Flury hierarchy (5000 resamples).

Differences in PC associated with large eigenvalues often cause the CPC technique to underestimate the degree of shared structure lower in the Flury hierarchy (Houle et al. 2002). To address this problem, we explored the consequence of switching the order of major and minor components in the partial models. As each group of major or minor components involved more than 1 PC, we repeated the analyses for all possible permutations of PCs within each category and recorded the greatest similarity, that is, the highest best-fitting model, observed for each matrix comparison.

In most cases, it was necessary to employ the bending option in CPCrand to eliminate negative eigenvalues, that is, to make the matrices positive definite. Although the validity of this approach remains uncertain (Phillips and Arnold 1999), we found no relation between the number of components shared between G matrices and the amount of bending required (Pearson $r = 0.02$, $P > 0.05$). Thus, we assume little or no consistent bias as a result of the bending procedure.

Comparison with Additive Predictions

As a final step, we contrasted the observed within- and among-line genetic (co)variance (V_{within} and V_{among}) after the bottleneck to the corresponding value for the control population and the additive predictions for these parameters, estimated as $E(V_{within}) = (1 - F)V_{g0}$ and $E(V_{among}) = 2FV_{g0}$, respectively, where F is the final inbreeding coefficient (see Results) and V_{g0} is the genetic (co)variance in the control population; Wright 1951). After the analyses of each (co)variance in the mean G matrix, we extended the analyses to the hierarchical partitioning of variance, as determined by both the within- and among-line genetic variance. The latter analyses were based on variance estimates obtained with restricted maximum likelihood (REML) procedures because of the nested experimental design (Lynch and Walsh 1998). To assess the significance of observed differences, we computed the approximate 95% confidence interval (CI) of each (co)variance estimate and its expected value based on the sampling variance obtained for each parameter.

All chi-square tests, ANOVAs, and REML analyses were carried out with SPSS for Windows (release 11.0.0).

Results

Patterns of (Co)variation before the Bottleneck

Plants in the C_{total} and C_{subset} lines had statistically indistinguishable survival rates ($\chi^2 = 0.74$, degrees of

Table 1 Means, broad-sense heritabilities (H^2), and the G matrix for the control population

			G matrix				
Character	Mean	H^2	1	2	3	4	5
1. Flowering date (May)	19.03	0.56*	29.52*				
2. Flower number	6.65	0.17*	−5.52*	1.23*			
3. Plant height (mm)	242.98	0.31*	−102.94*	15.64*	965.82*		
4. Leaf length (mm)	15.03	0.34*	−2.30*	0.06	9.06	1.93*	
5. Sepal length (mm)	11.84	0.52*	−0.31	0.02	2.22	0.55*	0.46*

Values followed by an asterisk (*) are significantly different from zero ($P < 0.05$) as determined by 95% CIs. Estimates of genetic parameters were obtained with the program H2boot (Phillips 1998b).

freedom [df] = 1, $P = 0.39$), means (ANOVA: $F < 2.7$, df = 1, 208–228, $P > 0.10$; MANOVA: $F = 0.62$, df = 5, 193, $P = 0.68$), and G matrices ($P_{EQUALITY} = 0.60$; CPC analysis), despite large sample sizes for both data sets (>790 individuals). For this reason, we pooled data over control lines where appropriate to provide a single data set against which to compare the lines in the bottleneck category.

Estimates of the genetic variance for the (pooled) control line (V_{g0}) were significantly greater than zero in all cases (Table 1). When expressed as the broad-sense heritability, the genetic variances were higher for first flowering date and sepal length ($H^2 = 0.52$–0.56) than for flower number (0.17), with plant height and leaf length being intermediate (0.31–0.34). The G matrix for the control line revealed negative associations between flowering date and each of the other characters and positive associations among some of the size variables (flower number vs. plant height and leaf vs. sepal length; Table 1).

Means, Survival Rates, and Levels of Inbreeding after the Bottleneck

More than 3 quarters of the bottleneck lines went extinct during the expansion phase, leaving a total of 23 lines that survived until the end of the experiment and provided data for the quantitative genetic analyses. Most of the losses were caused by low survival rates (40 lines) or low fruit set (16 lines) in the G_1 generation, the remainder reflecting low survival rates (3 lines) or low fruit set (18 lines) in the G_2 generation. No further losses occurred in the G_3 generation.

The surviving lines varied greatly in the fraction of plants that survived to flowering in the G_4 generation (range 63.6–98.2%; $\chi^2 = 243.7$, df = 22, $P < 0.001$). Sixteen lines had survival rates greater than 90%, resulting in an across-line mean (89.9%) similar to the survival rate of the control population (93.7%). For the 5 quantitative characters, there was extensive among-line variation in the overall mean ($F > 9.2$, df = 22, 483–490, $P < 0.001$ in all cases; nested ANOVAs), with across-line means close to the pooled control population (Tables 1 and 2). Correlation analyses on the line means revealed a significantly negative association between first flowering date and flower number and a significantly positive association between leaf length and sepal length (Table 2).

Based on the number of parents involved in successful crosses, the final F value of the surviving lines was slightly higher (mean 0.535, range 0.526–0.545) than the inbreeding attributed to the initial founder event (0.5).

Bottleneck-Induced Changes in the G Matrix

We found 3 major patterns in the line-specific G matrices (Supplementary material) and the mean G after the bottleneck (Table 3): 1) negative covariances generally involved flowering date, 2) flower number, plant height, and the lengths of the leaves and sepals showed predominantly positive covariance, and 3) different bottleneck lines had high or low (co)variance estimates for different characters.

Initial analyses of the CPCrand output enabled us to consider PC1 and PC2 for all G matrices as "major" (mean

Table 2 Means, ranges, and between-line correlations for the 23 lines that remained viable in the bottlenecked *Nigella degenii* population

			Line mean correlations[a]				
Character	Mean	Range	1	2	3	4	5
1. Flowering date (May)	17.90	9.86–24.11					
2. Flower number	6.65	5.13–7.99	−0.75***				
3. Plant height (mm)	250.96	202.44–315.28	−0.30	0.33			
4. Leaf length (mm)	15.24	12.24–17.53	−0.11	−0.09	−0.02		
5. Sepal length (mm)	11.77	9.74–13.10	0.12	−0.13	0.28	0.66***	

All statistics are based on line means.

[a] Entries are Pearson product–moment correlation coefficients (***$P < 0.001$).

Table 3 The mean G matrix (upper values) and its additive prediction (lower values) for the bottlenecked *Nigella degenii* population

Character	1	2	3	4	5
1. Flowering date (May)	19.62*				
	*13.73**				
2. Flower number	−3.56*	0.84*			
	*−2.57**	*0.57**			
3. Plant height (mm)	−59.62	6.65	602.76*		
	*−47.87**	*7.27**	*449.11**		
4. Leaf length (mm)	−3.18*	0.57*	5.09	1.38*	
	*−1.07**	*0.03*	*4.21*	*0.90**	
5. Sepal length (mm)	−0.41	0.08	−3.30	0.26	0.18*
	−0.15	*0.01*	*1.03*	*0.25**	*0.22**

Values followed by an asterisk (*) are significantly different from zero ($P < 0.05$) as determined by 95% CIs. Comparison of CIs (not shown) revealed no significant difference between observed and predicted values. Estimates of genetic parameters were obtained with the program H2boot (Phillips 1998b).

Table 4 The number of comparisons between the G matrix of the control population and the G matrices of the 23 surviving bottleneck lines that fit different models in the Flury hierarchy

	Number of pairwise comparisons	
Model	PC_{size}	$PC_{reordered}$
Equality	0	0
Proportionality	0	0
CPC	0	0
CPC3	0	0
CPC2	1	8
CPC1	2	13
Unequal	20	2

PC_{size} and $PC_{reordered}$ denote whether the partial models were based on size-ordered or reordered components, respectively (for details, see text). CPC indicates a full model with 4 common components, whereas CPC1, CPC2, etc. indicate partial models involving 1, 2, or more common components.

eigenvalue = 347.7) and the remaining ones as "minor" (mean eigenvalue = 3.4). The major PCs almost always represented variation in plant height and first flowering date, whereas the minor PCs had high loadings of the remaining characters (data not shown).

There was no support for the equality or proportionality model in CPC analyses that compared the G matrix of each bottleneck line with the G matrix of the control group (Table 4). When the partial models were tested with PCs ordered according to the size of their eigenvalues (PC_{size}), a majority of the CPC analyses also rejected the existence of common structure ($P_{CPC1} < 0.05$). Switching the order of major and minor components ($PC_{reordered}$) increased the level of similarity between matrices: the best-fitting model usually changed from no shared structure to a model involving 1 or 2 common components. In 2 comparisons, the matrices remained too different to support any of the models in the Flury hierarchy, regardless of how the PCs were ordered in the partial models (Table 4).

The bottleneck did not significantly influence the structure of the mean G matrix after the bottleneck ($P_{EQUALITY} = 0.36$; CPC analysis based on data pooled over lines), despite large sample sizes for both the control population (1837 plants in 199 families) and the bottlenecked population (4542 plants in 500 families). Evidently, the line-specific responses cancelled each other out in the pooled data set.

Comparison with Additive Predictions

Judging from the mean G matrix after the bottleneck (Table 3), the magnitude of most (co)variances declined relative to the control line (Table 1), but it always remained higher than the additive expectation, the most notable exception being the mean genetic variance for sepal length, which showed a slight deviation in the opposite direction (Table 3). Comparison of 95% CIs (not shown) revealed a significant decline in the variance for sepal length (no overlap between corresponding CIs). None of the other differences reached significance (overlapping CIs in all cases).

According to the REML-based variance estimates, the within- and among-line genetic variances for sepal length were in very good agreement with the values predicted under additive theory (Table 5). Although within-line variances for the other characters were lower than the corresponding estimates for the control group (Table 1), they always exceeded the expected values, albeit with overlapping CIs in all cases (Table 5). These characters also showed a deficiency of among-line genetic variance, with CIs excluding the CIs of the additive expectations in 2 cases (flowering date and flower number) (Table 5).

Table 5 Comparison of REML-based estimates of the within- and among-line genetic variance (V_{within} and V_{among}) with their additive predictions [$E(V_{within})$ and $E(V_{among})$] for the bottlenecked *Nigella degenii* population

	Genetic variance within lines				Genetic variance among lines			
Character	V_{within}	CI	$E(V_{within})$	CI	V_{among}	CI	$E(V_{among})$	CI
Flowering date	21.36	17.69, 25.03	14.39	10.75, 18.02	14.36	5.48, 23.24	33.11	24.75, 41.47
Flower number	0.93	0.65, 1.22	0.57	0.31, 0.82	0.43	0.14, 0.71	1.30	0.72, 1.88
Plant height (mm)	653.99	498.41, 809.57	435.15	295.30, 575.01	707.19	272.69, 1141.69	1001.3	679.5, 1323.1
Leaf length (mm)	1.49	1.16, 1.82	0.91	0.63, 1.19	1.51	0.58, 2.44	2.09	1.46, 2.73
Sepal length (mm)	0.20	0.15, 0.24	0.22	0.16, 0.28	0.53	0.21, 0.85	0.50	0.37, 0.63

Discussion

Although much attention has focused on the negative effects of bottlenecks on marker gene diversity and short-term population viability (e.g., Nei et al. 1975; Leberg 1992; Newman and Pilson 1997; Saccheri et al. 1998; Friar et al. 2000), there is still a paucity of experiments in which investigators have manipulated population size to determine the quantitative genetic effects of bottlenecks and so far few studies have used a wild plant species as their model system. Our results for the annual plant *N. degenii* not only suggest that bottlenecked populations of *N. degenii* generally have a lower adaptive potential than the ancestral population but also highlight the line- and character-specific nature of bottleneck effects.

The Adaptive Potential of Bottlenecked Populations

The small proportion of lines that recovered after the founder event confirms previous observations from other organisms that indicate a relationship between small population size and increased extinction probability (e.g., Newman and Pilson 1997; Saccheri et al. 1998). Thus, our results for *N. degenii* provide no support for rejecting the conventional view that bottlenecks generally have negative effects on future evolutionary adaptation (Willis and Orr 1993; Barton and Turelli 2004; Willi et al. 2006). On the other hand, we also note the relatively high survival rate and extensive between-line heterogeneity in the (co)variance structure for the minority of lines that remained viable. Thus, it is premature to rule out the possibility that extreme bottlenecks have the potential to enhance the evolutionary lability of particular populations (e.g., Wright 1978; Carson and Templeton 1984; Cohan 1984; Whitlock 1995). Obviously, the relevance of this idea depends on the strengths and directions of selection in the natural habitat (Lande 1979) and whether the perturbed (co)variance structure could persist into future generations, as found in a large bottleneck experiment with fruit fly *Drosophila melanogaster* (Whitlock et al. 2002), or conversely, whether mutation, selection, and recombination would return the G matrices to their original state before the bottleneck.

Previous studies have shown conflicting results regarding the evolutionary potential of bottlenecked populations. A number of authors have reported bottleneck-induced release of additive variance for a broad variety of characters, including morphology in housefly *Musca domestica* (Bryant et al. 1986), viability in fruit fly (López-Fanjul and Villaverde 1989) and flour beetle *Tribolium castaneum* (Fernández et al. 1995), desiccation resistance in the fly *Drosophila bunnanda* (van Heerwaarden et al. 2008), egg hatching rate in the butterfly *Bicyclus anynana* (Saccheri et al. 2001), body weight in mouse *Mus musculus* (Cheverud et al. 1999), and cotyledon size in a rapid-cycling population of *Brassica rapa* (Briggs and Goldman 2006). For other study systems, the change in genetic architecture was in good agreement with additive theory (Wade et al. 1996; Whitlock and Fowler 1999; Saccheri et al. 2001; Swindell and Bouzat 2005). Results of the present investigation accentuate the advantage of performing large-scale experiments—accounting for among-line variation in both genetic and demographic parameters—before any broad generalizations are made regarding the adaptive potential of bottlenecked populations.

Changes in (Co)variance Structure

In agreement with population genetic theory of small, isolated populations (Avery and Hill 1977; Lynch 1988; Zeng and Cockerham 1991), we observed considerable variation in the response to the bottleneck treatment, with different lines showing high, or low, genetic (co)variance for different characters. Although this heterogeneity could be a reflection of the large error associated with the estimation of quantitative genetic parameters (Lynch and Walsh 1998), we emphasize that the pairwise CPC analyses—which compared the G matrix for each bottleneck line with the G matrix for the control (base) population—always rejected the equality and proportionality models, a result consistent with the generation of wide drift–induced variation in the orientation and magnitude of genetic variance and covariance (Phillips et al. 2001).

Differences in one or a few PC often prevent detection of shared structure lower in the Flury hierarchy (Houle et al. 2002). In the case of *N. degenii*, we found greater similarity between the G matrices of the control and bottleneck lines after switching the order of major and minor components in the partial models. In fact, the proportion of pairwise comparisons with similar components (ca. 90%) was somewhat higher than normally found in comparisons of natural or experimental populations of the same species (<80%; Arnold et al. 2008). This pattern indicates 1) that differences in the (co)variances for plant height and flowering time—the main determinants of the major components—had a disproportionately large influence on differences in the PC structure and 2) that the bottlenecked G matrices retained a nonnegligible portion of their (co)variance structure.

As expected from the presence of shared components, we observed a few consistent associations in the G matrices. First, most data sets showed a major trend distinguishing early-flowering genotypes with many flowers, a tall stem, and long leaves from those with the opposite features, indicating a close, persistent association between flowering time and vegetative size characters. Second, there was a consistent genetic correlation between leaf and sepal length in the control population and within and among different lines in the bottleneck group. This leaf–sepal size association has also been detected in a segregating hybrid population from a cross between our base population (*N. degenii* ssp. *barbro*) and a population of *N. degenii* ssp. *jenny* and in a comparison of different taxa in the *N. arvensis* complex (Strid 1969, 1970; Andersson 1997). Taken together, these findings indicate that some genes control the development of both leaves and flowers (Andersson 1997) and that it may be difficult for *Nigella* populations to escape this constraint, even after a severe bottleneck.

Results of this study not only suggest that simple, stochastic processes could make a significant contribution to the separation of G matrices observed in previous studies of wild species (e.g., Widén et al. 2002; Arnold et al. 2008) but also indicate the potential for bottlenecks to alter the trajectory of evolution by selection. In a parallel study, we translated the G matrices observed in the present study into predicted selection responses under a series of hypothetical though biologically relevant selection regimes (Andersson S, Ellmer M, unpublished data). The predicted shifts in mean phenotype after 1 generation of directional selection (calculated following Lande 1979) usually agreed in direction with the signs of the hypothesized selection pressures. Nevertheless, there was extensive heterogeneity in the bottleneck group, with some lines showing a greater response than the ancestral population, especially under a selection regime that simulated the transition to early-flowering populations with short, few-flowered stems and short leaves and small, selfing flowers—the major evolutionary trend within the *N. arvensis* complex (Strid 1969, 1970). Thus, despite evidence for stability in parts of the G matrix, it seems that bottlenecked populations of *N. degenii* would diverge, rather than converge, under a common force of selection, as a consequence of differences in genetic (co)variance structure (cf., Cohan 1984). Whether such divergence actually contributes to the structuring of quantitative variation observed in this and other *Nigella* species (Strid 1970; Jorgensen et al. 2006) remains to be investigated.

Our data provide no direct evidence as to the relative importance of different random factors that could contribute to the large, line-specific changes observed in this bottleneck experiment (linkage disequilibrium, simple fluctuations in allele frequencies, sampling variability, etc.). However, it seems that the initial genetic variance among the founders could account for a substantial portion of the variation. The coefficient of variation (CV) in the within-line genetic variance caused by this factor alone (estimated as $[2/N_eL]^{1/2}$, where N_e is the effective population size and L is the number of lines; Lynch 1988) is expected to be 0.29 for this experiment. This quantity represents a sizeable proportion (31–58%) of the observed CV for the 5 characters measured in this study (CV = 0.50–0.93, calculated from data in Supplementary material).

Comparison with Additive Predictions

The results from the matrix analyses and the hierarchical partitioning of genetic variance generally conformed to the predictions from additive theory: the bottleneck reduced the mean within-line genetic (co)variance (although not significantly so in most cases) and had a diverging effect on the mean phenotype for all the characters. In the case of sepal length, the partitioning of (co)variance after the bottleneck event was in good quantitative agreement with the additive predictions for this experiment. The remaining characters showed a deficiency of among-line (co)variance and an excess of within-line (co)variance when compared with the predicted values. These patterns imply that "too little" within-line (co)variance was converted into among-line (co)variance for some variables, presumably contributing to the relatively small difference between the control G matrix and the mean G matrix of the bottlenecked population.

Comparisons of observed and predicted (co)variances must be interpreted with care when many lines go extinct before the measurements (Lynch 1988) as was the case in the present investigation. For example, the deviating variance estimates for characters other than sepal length could be a manifestation of environmentally induced selection against lines with extreme means or unusually low genetic (co)variance for these characters. This possibility was evaluated by comparing 2 control lines, one representing all the initial founders and the other representing founders of lines that remained viable until the end of the experiment. Neither the means nor the G matrices significantly differed between the 2 control lines, as would be expected if the surviving lines represented genotypes better able to survive and reproduce in the greenhouse environment. Nevertheless, we note that the loss of lines followed a temporally decreasing trend during the expansion phase, with 56 lost lines in the first generation, 21 in the second, and none in the third. Such patterns are consistent with the selective removal, or "purging," of lines that suffer from severe inbreeding depression (Lynch 1988) and have the potential to attenuate the quantitative genetic effects of bottlenecks if the characters considered are genetically correlated with fitness.

Quantitative genetic data indicate that both additive and nonadditive genetic effects were segregating in the base population. As expected with a strong additive component of variance, there were no consistent differences between the broad-sense heritabilities for plant stature, flowering time, leaf length, and sepal length in the control population (H^2 = 0.31–0.56) and previously estimated narrow-sense heritabilities from a factorial crossing experiment with the same base population (h^2 = 0.27–0.64; Palmé A, Andersson S, unpublished data). As for the nonadditive component, the base population contained sufficient dominance variance for inbreeding to cause significant inbreeding depression in almost all the characters considered in this study (Ellmer and Andersson 2004). However, given the low estimates of inbreeding depression for these characters (<2% decrease in the mean phenotype per 10% increase in F; Ellmer and Andersson 2004), it seems reasonable to assume that the genetic (co)variances in the base population were mainly due to segregation at nearly additive loci. Thus, there is no reason to invoke bottleneck-induced conversion of nonadditive (co)variance into additive (co)variance (Robertson 1952; Goodnight 1988) to explain why there was an excess of within-line (co)variance for a majority of the characters after the bottleneck.

Although each parent contributed a similar number of seeds to the next random-mating generation, it is conceivable that the final inbreeding coefficient—estimated from the number of parents involved in successful crosses—was

underestimated. Many plants failed to produce seeds after outcrossing, and there is no guarantee that these losses were randomly distributed across families in the progeny generation. Therefore, the effective number of parents contributing to the next generation was probably lower than the number of parents contributing to the seed samples. The "extra" inbreeding resulting from these differences would reduce the expected within-line genetic variance and increase the expected among-line variance, leading to even larger differences between observed and expected values in the comparative analyses.

Supplementary Material

Supplementary material can be found at http://www.jhered.oxfordjournals.org/.

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