Genetic variation and evolutionary trade-offs for sexual and asexual reproductive modes in *Allium vineale* (Liliaceae)[1]

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Populations of *Allium vineale* commonly include individuals with very different allocation patterns to three modes of reproduction: sexual flowers, aerially produced asexual bulbils, and belowground asexual offsets. If selection is currently acting to maintain these different allocation patterns there must be a genetic basis for variation in allocation to these three reproductive modes. In addition, negative genetic correlations between reproductive traits would imply evolutionary trade-offs among reproductive strategies. We evaluated the heritability of these allocation patterns by growing 16 clones from a single population in the greenhouse at two levels of fertilization. Bulb mass and the mass and number of bulbils, offsets, and flowers were used as response variables, in addition to the proportion allocated to each reproductive mode. We found evidence of substantial heritable variation in allocation to sexual reproduction and in allocation within the two modes of asexual reproduction, indicating high sensitivity of these allocation patterns to natural selection. We also found evidence of strong negative genetic correlations between bulbil and flower traits, as well as between bulbil and offset traits, with one group of genotypes allocating greater resources to aerial asexual bulbils and the second group allocating more resources to belowground asexual offsets and aerial flowers. Phenotypic plasticity in allocation to above- vs. belowground asexual reproduction and sexual vs. asexual aerial reproduction was limited, indicating that plants are unlikely to change reproductive mode in response to nutrient availability. Together, then, we have demonstrated strong heritability for, and trade-offs in, the reproductive allocation patterns within this plant population.

Key words: *Allium vineale*; genetic correlations; heritability; life history traits; Liliaceae; multiple reproductive modes; trade-offs.

Diverse modes of reproduction are often maintained within species and within single plant populations. For example, plant species can produce combinations of chasmogamous and cleistogamous flowers (e.g., *Impatiens,* Schemske, 1978; *Danthonia spicata,* Clay, 1982; *Viola mirabilis,* Mattila and Salonen, 1995), aerial and subterranean seeds (e.g., *Amphicarpum purshii,* Cheplick and Quinn, 1988; *Amphicarpaea bracteata,* Trapp and Hendrix, 1988; see Cheplick, 1987, for a review), seeds and ramets (e.g., *Tussilago farfara,* Ogden, 1974; *Fragaria virginiana,* Holler and Abrahamson, 1977), as well as heteromorphic aerial achenes (e.g., *Hypochoeris glabra,* Baker and O'Dowd, 1982; *Heterosperma pinnatum,* Venable and Burquez, 1989). Adaptive explanations for the maintenance of this diversity posit trade-offs among alternate components of fitness. For example, some propagule types may be favored in competitive situations near the parent, while others may serve a role in dispersal (Schoen and Lloyd, 1984; Venable, 1985). Different advantages may also exist for producing sexual vs. asexual progeny, as sexual progeny may be favored in the presence of pathogens or changing environments, and asexual progeny may be favored in static environments.

In addition, genotypes are known to vary their allocation to different modes of reproduction with environmental change. Allocation to vegetative and sexual reproduction changes with plant density in several species (e.g., Ogden, 1974; Abrahamson, 1975; Holler and Abrahamson, 1977; for a review see Loehle, 1987). *Hypochoeris glabra* produces a higher proportion of beaked to unbeaked achenes at lower plant density (Baker and O'Dowd, 1982). In general, higher light and nutrient levels increase the proportion of chasmogamous to cleistogamous flowers (e.g., Schemske, 1978; Trapp and Hendrix, 1988; Le Corff, 1993; but see Mattila and Salonen, 1995). In *Amphicarpum purshii,* aerial reproduction occurs under higher resource availability, while subterranean seeds are produced at all resource levels (Cheplick and Quinn, 1982). Selection would favor such phenotypic plasticity if different propagule types are favored when environmental conditions change (Schoen and Lloyd, 1984; Loehle, 1987).

In order for natural selection to currently operate to maintain these diverse reproductive strategies and phenotypic plasticity in allocation patterns, the variation in reproductive mode must have a genetic basis. In fact, the response of a population depends not only on the availability of genetic variation, but also on the existence of genetic correlations between traits (Reznick, 1985). In particular, negative genetic correlations between life history traits suggest trade-offs in reproductive strategies (Falconer, 1981; Stearns, 1992), as selection for increased allocation to one trait will cause a decrease in allocation to the other.

In general, reproductive traits have lower heritabilities than morphological traits (Mousseau and Roff, 1987), but significant genetic variation in allocation to different reproductive modes has been demonstrated (e.g., cleistogamy vs. chasmogamy, Clay, 1982; proportion of achene morphs, Venable and Burquez, 1989; allocation to aerial seeds, Cheplick and Quinn, 1988). Negative genetic correlations, which indicate trade-offs among life history traits, have also been detected. For example, Law (1979) found a negative correlation between first- and second-year reproduction in *Poa annua,* and Campbell (1997) observed a negative correlation between corolla width,

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which affects pollen export rates and potentially male fitness, and net female fitness. However, not all species demonstrate trade-offs in life history strategies (see Stearns, 1992, for a review). For example, Cain et al. (1995) found no evidence for genetic trade-offs among five reproductive traits in *Trifolium repens,* and Weis, Hollenbach, and Abrahamson (1987) found only indirect evidence for a trade-off between sexual and asexual reproduction in *Solidago altissima.* In addition, the presence of trade-offs may be dependent on resource availability, with stronger allocation trade-offs present when nutrients are more limiting (e.g., Reznick, 1985; Biere, 1995; Schlichting and Pigliucci, 1998)

Our study organism, *Allium vineale,* has three modes of reproduction, each with its own implications for genetic variability, dispersal, dormancy, and probability of successful establishment. Two of the modes are asexual in nature, one being aboveground (bulbils), the other belowground (offsets). Sexually produced aerial seeds are the third type of propagule. Within one population, it is possible to find individuals that are reproducing solely asexually, as well as individuals that are reproducing both sexually and asexually, in varying proportions. To determine whether there is genetic variation in allocation to these three reproductive modes and to test for trade-offs between sexual and asexual reproduction in *Allium vineale,* we asked the following questions: (1) Do genotypes demonstrate heritable genetic variation in allocation patterns to the three propagule types? (2) Are there negative genetic correlations between these reproductive traits? If so, are they stronger when resources are more limiting? (3) Do plants demonstrate phenotypic plasticity in their resource allocation patterns? Is there genetic variation in plasticity, such that genotypes differ in their allocation to each reproductive mode with changing environmental conditions? If so, how do their allocation patterns change?

MATERIALS AND METHODS

Study species—*Allium vineale* L. (Liliaceae) is an introduced species commonly found along roadsides and in fields. In North Carolina it is a winter perennial, which sprouts in early autumn and grows throughout the winter. The leaves and roots die back in May, with the bulb persisting underground until the following autumn. In early summer *A. vineale* produces a scape with an inflorescence containing either flowers (sexual reproduction), bulbils (asexual reproduction), or a combination of both. We refer to those plants that produce an inflorescence with bulbils but no flowers as ''non-flowering,'' although they have produced an aerial reproductive stalk. Seeds and bulbils ripen in early fall and disperse a mean distance of 34 cm away from the parent (Ronsheim, 1994). Seeds weigh ~1 mg, while bulbils range from 5 to 60 mg (mean mass = 19.8 mg; Ronsheim, 1996). Bulbils germinate that fall, while seeds require several months of cold, moist stratification. Overall, seeds have lower germination and survivorship rates than bulbils (Ronsheim, 1996). A third propagule type is produced underground. Offsets are underground asexual propagules analogous to the cloves of domesticated garlic. They are the largest of the propagule types (mean mass = 216.9 mg, ranging from 40 to 600 mg), may be dormant for up to 5 yr (Stritzke and Peters, 1972), and remain next to the parent plant. In addition to underground offsets, plants produce a new bulb each year. These bulbs do not have the thick protective coat of the offsets and are larger than offsets (mean mass = 2391 mg, ranging from 800 to 5000 mg).

Experimental design—Measurement of heritable variation and genetic correlations within *Allium vineale* is made difficult by the high phenotypic variation in reproductive modes among individuals. Since a high proportion of individuals produce bulbils but no flowers on their aerial reproductive stalk,

and others that do produce flowers have low seed set, measurement of heritability through maternal or paternal contributions would be difficult and not representative of the natural population. Instead, we have measured the broadsense or clonal heritabilities and correlations by using the aerially produced asexual bulbils to replicate genotypes. This approach has the obvious limitation that we are not able to separate the additive genetic variance components from the dominance, epistatic, or maternal components. Furthermore, in sampling the population we were not able to include individuals that produce inflorescences with flowers and no bulbils (a small minority in this population), which may reduce the genetic variation that we measure. We also purposefully collected bulbils from two groups of individuals: one that produced some flowers and a second that produced only bulbils. This resulted in a higher representation in our study than in the field of individuals that produce both flowers and bulbils in their inflorescence, and may inflate our estimate of genetic variance. While these sampling difficulties reduce the relevance of our measured values of heritabilities and genetic correlations to the population of their origin, these estimates are still valuable to address the issue of the evolutionary potential within *Allium* populations in general.

In this experiment we determined the reproductive allocation of replicates of 16 different genotypes when grown in two fertilization regimes. Infructescences were collected in September 1993 from 16 different plants at a field site in Durham, North Carolina (for details of the field site, see Fowler, 1978; Fowler and Antonovics, 1981). Each of the parents was considered a separate genotype, for a total of 16 genotypes. A range of infructescence phenotypes was included, varying from all bulbils to a combination of flowers and bulbils. Of the plants that produced flowers, flower production ranged from 14.8 to 86.2%. Numbers of flowers and bulbils were recorded for each infructescence. Individual bulbils from each of these genotypes were then weighed, planted in research mix (2:2:2:1 of sand, soil, peat moss and gravel) in 6×25 cm pots (Deepots, Stuewe and Sons, Inc., Corvallis, Oregon, USA), and randomized on a greenhouse bench under natural light and cool conditions (4°–21°C) in October 1993. Plants were fertilized either once or twice monthly with 150 mL of 20:20:20 Peter's soluble fertilizer at 300 ppm, creating low- and high-nutrient treatments. As this species grows both along roadsides and in highly fertilized agricultural fields, this range in nutrient availability is not unrealistic. There were five replicates for each of the 16 genotypes in each of the two nutrient treatments, with the exception of one genotype (M) that had ten replicates ($N_{total} = 170$).

Plants were harvested after their leaves died back during the summer of 1994, and the number of bulbils, flowers, and offsets produced was recorded. Bulb mass, total offset mass, total bulbil mass, and the mass of the flowers were also recorded. Fresh mass was used as these propagules were planted in further experiments.

Data analysis—The propensity of plants to produce an aerial reproductive stalk was analyzed with a loglinear model using the CATMOD procedure of SAS (SAS, 1986). To increase power for this analysis, genotypes were lumped by flowering type (inflorescences containing bulbils only or a mixture of bulbils and flowers). Only plants that produced an aerial reproductive stalk were included in analyses of patterns of biomass allocation, and their masses were analyzed with multivariate and univariate analysis of variance, using the GLM procedure in SAS (SAS, 1986). Initial mass of the bulbils before planting was used as a covariate. To be able to generalize over a range of environments, nutrient level was treated as a random effect in both the GLM procedure and in the calculation of heritability. Genotype was also treated as a random effect in the GLM procedure, with the effect of both nutrient level and genotype being tested over the interaction mean square. The interaction effect was tested over the residual error. Proportions were arcsine square-root transformed for normality. All other analyses were conducted using ranked data to improve normality, although analyses using log-transformed data gave very similar results. Post hoc comparisons of least squares means were made using Tukey's criterion to correct for multiple comparisons.

We tested for differences in allocation patterns among genotypes using two-way multivariate analyses of variance with profile contrasts (Repeated Profile option in GLM procedure in SAS; Morrison, 1976). Profile analysis provides an overall test of whether the slopes of lines connecting the means of each
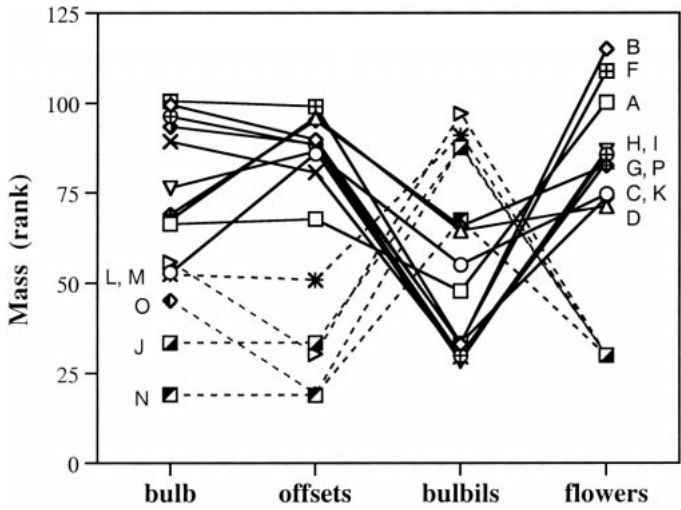
Fig. 1.   Genotypes differ in their relative biomass allocation to bulb, offsets, bulbils, and flowers (multiple profile analysis of biomass allocation, Profile × Genotype effect; $P = 0.0001$). Note the two major resource allocation patterns: genotypes that produce inflorescences with only bulbils (genotypes J, L–O, indicated by dashed lines) allocate more resources to bulbils, while genotypes that produce inflorescences with both flowers and bulbils (solid lines) allocate more resources to bulbs, offsets, and flowers. The vertical axis represents the mean rank for each genotype.

reproductive character differ between genotypes, thereby testing whether the response profiles of different genotypes are parallel (Schlichting, 1986). Thus a significant interaction of Genotype × Profile would indicate that genotypes differ in their allocation to different reproductive modes. The significance of these interactions was tested using Wilk's Lambda criterion because it is derived from a likelihood ratio approach (SAS, 1986); however, tests using Pillai's Trace and Hotelling-Lawley Trace gave similar results.

Variance component analyses were used to calculate the clonal heritabilities (Fry, 1992), averaged over the two environments. For traits that could be measured on the maternal parent in the field, we ran parent–offspring regressions. While this heritability estimate is rough (maternal parents were not grown in the same environment as each other or as their offspring), it does give a second heritability estimate for comparison. Genetic correlations among reproductive traits were calculated as follows: $r_{G_{ij}} = \text{COV}_{C_{ij}}/\sqrt{V_{C_i} \times V_{C_j}}$, where $\text{COV}_{C_{ij}}$ is the clonal covariance between traits i and j, and V_{C_i} and V_{C_j} are the clonal variances for traits i and j. A genetic trade-off was inferred when $r_G < 0$ (Platenkamp and Shaw, 1992; Cain et al., 1995). Genetic correlations were determined for each nutrient level separately, as allocation trade-offs may be stronger when nutrients are more limiting (e.g., Biere, 1995; Schlichting and Pigliucci, 1998). The Profile × Genotype × Nutrient Level interaction was used to test for plasticity in the overall genetic correlation structure across environments (Morrison, 1976). In addition, pairwise comparisons of the genetic correlations were made for high- vs. low-nutrient level for each set of reproductive traits. To do this, all correlation coefficients were z-transformed and pairs were tested for homogeneity between nutrient levels (Schlichting, 1989; Snedecor and Cochran, 1989; Roche and Fritz, 1997).

Results from the univariate analysis of variance were used to test for the presence of phenotypic plasticity (Schlichting, 1986; Scheiner, 1993; Via, 1993; Pigliucci, Diiorio, and Schlichting, 1997). Specifically, a significant effect of Nutrient Level for any of the characters measured would indicate phenotypic plasticity. In addition, a significant Genotype × Nutrient Level interaction would indicate that there is genetic variation for plasticity. We used post hoc comparisons of least squares means using Tukey's criterion to correct for multiple comparisons to determine the pattern of phenotypic plasticity. We tested for plasticity in allocation patterns among genotypes using profile analysis (Morrison, 1976; Schlichting, 1986). A significant Genotype × Nutrient Level × Profile interaction would demonstrate the presence of
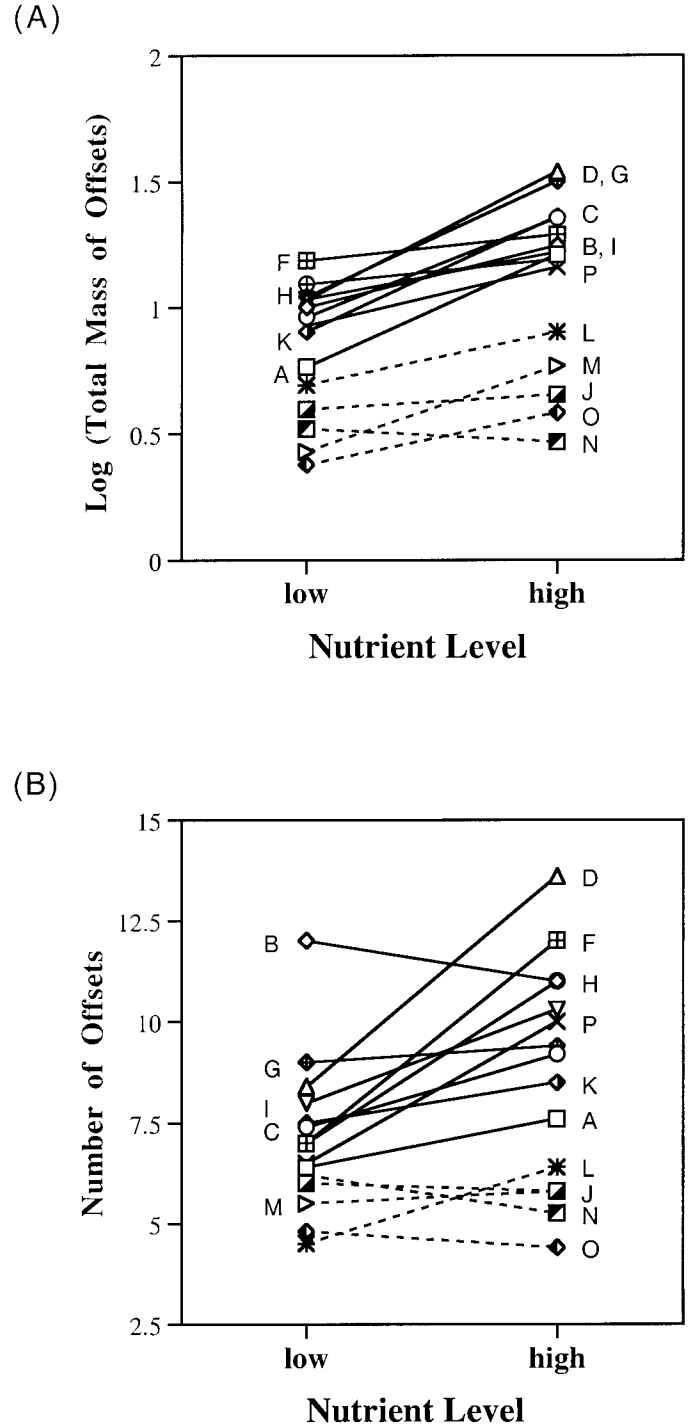

Fig. 2.   (A) The increase in total offset mass with nutrient availability is due to both increased numbers and increased size of offsets. Some genotypes respond less than others to increased nutrient availability (Genotype × Nutrient Level, $P = 0.022$). Genotypes that do not produce flowers produce lighter offsets than genotypes that do produce flowers (dashed lines). (B) Overall, more offsets are produced at the high-nutrient level than at the low-nutrient level. Genotypes that do not produce flowers also produce the fewest offsets (dashed lines).

TABLE 1. Analysis of variance results and heritabilities for all response variables. Significance levels are indicated as follows: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Initial mass was used as a covariate and was not significant in any of the analyses. Heritability estimates calculated from parent–offspring regressions are in parentheses in the clonal heritability column.

Response variables	Genotype (15 df)		Nutrient level (1 df)		Genotype × Nut. level (14 df)		Error (93 df)	Clonal heritability
	SS	F	SS	F	SS	F	SS	
Bulb mass	41523.10	2.67*	17064.12	16.44**	14527.97	1.87*	51617.98	0.282
Offset mass (total)	96516.35	13.27***	14363.64	29.69***	6789.20	2.04*	22093.97	0.585
Offset number	88894.17	10.77***	5517.93	10.03**	7705.33	1.42	35945.91	0.503
Ave. offset mass	78055.38	4.09**	7448.69	5.76*	17657.58	2.60**	45741.95	0.351
Bulbil mass (total)	73961.15	8.45***	21936.28	37.59***	8169.44	1.64	33045.25	0.503
Bulbil number	56177.96	4.11**	37160.79	40.80***	12750.97	1.82*	46615.81	0.263 (0.258)
Ave. bulbil mass	96916.69	11.27***	900.47	1.58	8025.86	2.47**	21541.11	0.743
Flower mass	100091.48	7.08***	12.26	0.01	13190.37	5.89***	14866.96	0.764
Flower number	98538.75	6.96***	3.87	0.00	13204.63	5.83***	15053.79	0.790 (0.910)
Bulbils/flowers	12.11	6.74***	0.14	1.15	1.68	5.23***	2.13	0.689 (0.820)
Aerial/subterranean asexual propagules	1.58	25.23***	0.01	2.46	0.58	1.28	0.30	0.800
Bulb/(bulb + offset) mass	1.03	11.79***	0.03	4.90*	0.08	0.72	0.75	0.358

genetic variation in plasticity for the pattern of resource allocation to the three reproductive modes.

RESULTS

The probability of producing an aerial reproductive stalk generally increased with nutrient availability, with 88% of plants in the high-nutrient treatment and 70% in the low-nutrient treatment producing stalks ($P < 0.002$, $\chi^2 = 9.9$, df = 1). The genotypes also differed significantly in their probability of producing an aerial reproductive stalk, with 99% of genotypes that have only aerial bulbils and 59% of genotypes that have a bulbil-flower mixture producing a stalk ($P < 0.0004$, $\chi^2 = 12.3$, df = 1). The Genotype × Nutrient Level interaction was not significant.

Genotypes were strongly different in their allocation to bulb, offset, bulbil, and flower production (MANOVA Genotype effect; $F_{60,45} = 6.353$, $P < 0.0001$; Fig. 1). In fact, univariate analyses indicate significant genotypic variance for all reproductive characters measured (Table 1). These differences in reproductive allocation patterns were highly heritable, with eight out of 12 characters having clonal heritabilities >0.5 (Table 1). In particular, flower production, average bulbil mass, and the proportion of bulbils to flowers produced all had clonal heritabilities over 0.74. Average offset mass, number of bulbils, and allocation to belowground bulbs vs. offsets were the least heritable, being most affected by environmental conditions.

While one must be cautious in interpreting the clonal heritabilities presented here (see Mitchell-Olds and Rutledge, 1986), the heritability estimates obtained from the variance component analyses and parent–offspring regressions of bulbil and flower traits were similar (Table 1). Specifically, the clonal heritability calculated from a parent–offspring regression for flower number was 0.91 ($P < 0.0002$, $r^2 = 0.64$, $N = 16$), compared to 0.79 based on the variance component analysis. The clonal heritability of number of bulbils was 0.258 ($P < 0.265$, $r^2 = 0.09$, $N = 16$) compared to 0.263, and the clonal heritability of proportion of bulbils to flowers produced was 0.82 ($P < 0.0001$, $r^2 = 0.65$, $N = 16$) as compared to the estimate of 0.69 based on the variance component analysis.

Genotypes differed in their relative biomass allocation to bulb, offset, bulbil, and flower production (multivariate profile analysis of biomass allocation—Profile × Genotype effect; $F_{45,36} = 6.96$, $P = 0.0001$; see Fig. 1). Genotypes that produced a mixture of flowers and bulbils had different resource allocation patterns than did genotypes that produced only bulbils. While there was significant heritable variation within these groups, the difference between these groups captured the majority of the genotypic variation in the experiment. Genotypes that produce inflorescences with only bulbils produced fewer, smaller offsets (Fig. 2A, B) and fewer, larger bulbils (Fig. 3A, B) than genotypes that produced inflorescences with a mixture of flowers and bulbils. Thus nonflowering genotypes allocated more resources to aerial asexual reproduction relative to underground asexual reproduction than did flowering genotypes (Fig. 4). In addition, nonflowering genotypes allocated more belowground resources to bulb rather than offset production (Fig. 5).

These very different allocation patterns between the genotypes resulted in strong positive and negative genetic correlations among the reproductive traits (Table 2). Offset mass, offset number, bulb mass, and flower number were all positively correlated. Bulbil mass and number were also positively correlated with each other, but negatively correlated with the previous traits. Thus there seem to be two main reproductive strategies in this population of *Allium vineale*, with one group of plants producing primarily bulbils and fewer, smaller offsets and a second group allocating less to bulbils but producing flowers and greater numbers of larger offsets. However, these correlations are not perfect, reflecting our observations that heritable differences in allocation patterns exist within these two groups.

There was significant plasticity in the genetic correlation structure between environments (Profile × Genotype × Nutrient Level interaction, $F_{84,558} = 4.16$, $P = 0.0001$; Table 2). Overall, most genetic correlations were smaller in the low-nutrient level treatment (15 out of 21 correlations). Only one negative correlation, between number of offsets and bulbil mass, was stronger in the more resource-limited environment. There was one switch in sign, as the correlation between number of offsets and number of bulbils was positive in the high-nutrient level and negative (but nonsignificant) in the low-nutrient level. However, despite the overall significant difference in the genetic correlation structure between environments, the pairwise tests indicated that only one pair of characters (bulb mass and number of offsets) had significantly different
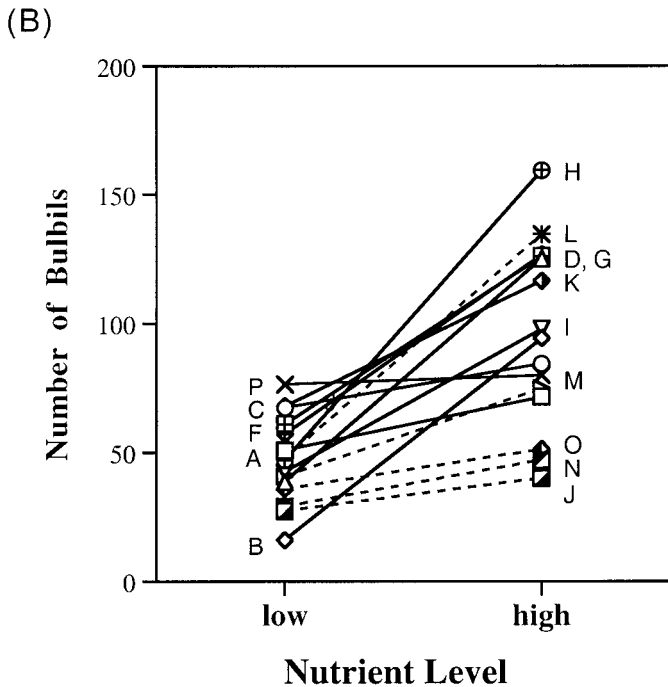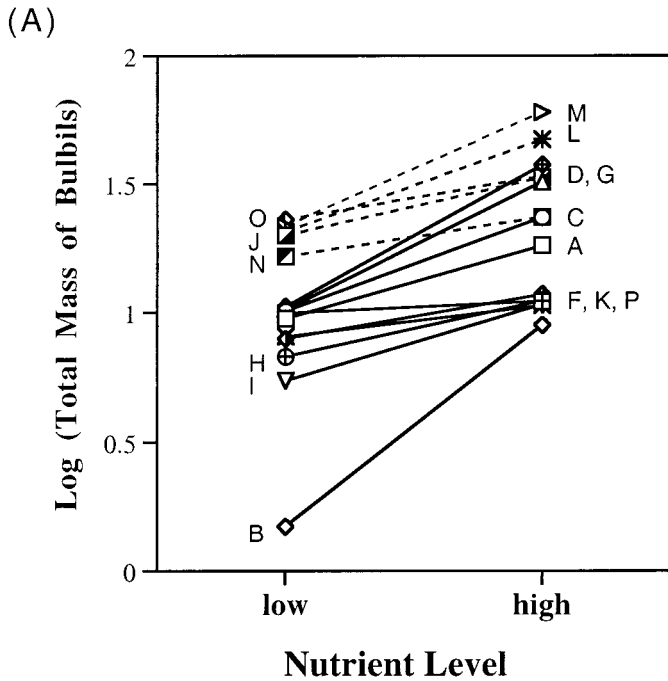
(A)

(B)

Fig. 3. (A) The increase in total mass of bulbils in the higher nutrient level is due to the production of more, not heavier, bulbils, as the average mass of bulbils does not change (see Table 1). Genotypes that do not produce flowers have heavier bulbils (dashed lines). (B) More bulbils are produced at higher nutrient levels ($P = 0.006$), although some genotypes respond less (Genotype × Nutrient Level effect, $P = 0.047$). Genotypes that do not produce flowers tend to produce fewer bulbils (dashed lines).
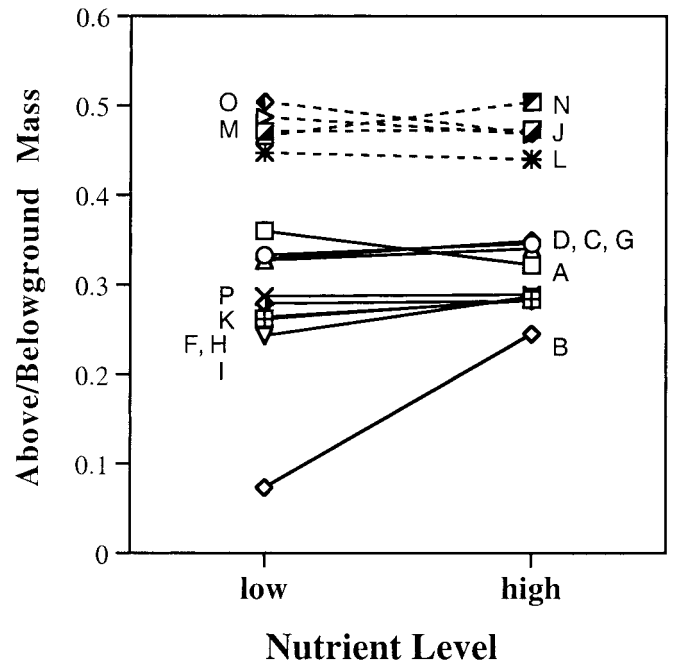

Fig. 4. There is a significant effect of genotype ($P = 0.0001$) on the relative allocation to aboveground asexual propagules (bulbils) vs. underground asexual propagules (offsets and bulbs). Genotypes that do not produce flowers allocate more to aboveground asexual bulbils (dashed lines). Nutrient availability does not affect this allocation pattern.

genetic correlations in high- and low-nutrient environments. Specifically, they had a significantly higher positive genetic correlation with greater resource availability ($t = 2.0159$, $P < 0.05$).

Phenotypic plasticity was found in seven out of the 12 characters measured (Table 1, significant effect of Nutrient Level). In general, plants produced greater biomass and greater numbers of propagules with increased nutrient availability. In contrast, no phenotypic plasticity was found in the relative allocation of biomass to above- and belowground asexual propagules. Average bulbil size was also relatively unaffected by nutrient availability, with only one genotype (B) producing bulbils that differed in size significantly (Fig. 4). Thus, the increase in total mass of bulbils in the high-nutrient treatment was due primarily to plasticity in the number of bulbils produced, not an increase in the average size of bulbils. This pattern is different from offset production, where the increase in total offset mass with nutrient availability was due to plasticity in both number and size of offsets.

Genetic variation in phenotypic plasticity was found in response to increased nutrient availability (MANOVA Genotype × Nutrient Level effect; $F_{56,352} = 2.263$, $P < 0.0001$). In fact, genetic variation for phenotypic plasticity was found for eight of the 12 traits analyzed using univariate analysis of variance (significant Genotype × Nutrient Level interactions; Table 1). In addition, their allocation of resources to different propagule types also varied with nutrient availability (Profile × Genotype × Nutrient Level effect; $F_{42,270} = 2.49$, $P = 0.0001$; see Fig. 6). While plants tended to produce greater bulb, offset, bulbil, and flower biomass in the high-nutrient treatment, this increase was not significant for all genotypes. Interestingly, most of the significant Genotype × Nutrient Level interactions were due to five of the 16 genotypes, with genotypes A, D,

TABLE 2. Negative genetic correlations between bulbil mass and bulb, offset and flower traits, as well as between bulbil number and flower mass, indicate trade-offs in resource allocation between these reproductive modes. Nonsignificant genetic correlations are in parentheses ($P > 0.05$, $r_{G_{ij}}$ not significantly different from zero). Genetic correlations calculated for the high-nutrient treatment are on top, genetic correlations calculated for the low-nutrient treatment are on the bottom. While the overall matrices are significantly different, pairwise tests for homogeneity of genetic correlations between nutrient levels were not significant, with the exception of the correlation between bulb mass vs. number of offsets which was significantly different between the two nutrient levels.

	Bulb mass	Mass of offsets	Mass of bulbils	Mass of flowers	Number of offsets	Number of bulbils
Offset mass	0.780					
	0.722					
Bulbil mass	−0.614	−0.410				
	−0.491	(−0.207)				
Flower mass	0.623	0.286	−0.859			
	0.351	0.592	−0.743			
No. offsets	0.787	0.890	−0.630	0.504		
	(0.251)	0.593	−0.804	0.479		
No. bulbils	(0.157)	0.489	0.331	−0.524	0.248	
	0.444	0.342	(0.225)	(−0.228)	(−0.257)	
No. flowers	0.794	0.635	−0.874	0.818	0.733	(−0.072)
	0.448	0.821	−0.745	0.778	0.727	(−0.081)

G, K, and M demonstrating greater plasticity in response to variation in nutrient availability.

In the high-nutrient treatment, genotypes A, D, G, and M ($P = 0.0015$, $P = 0.0075$, $P = 0.0045$, $P = 0.0075$, respectively; all P values reported here are derived from least squares means and corrected for multiple comparisons) were the only genotypes with a significant increase in bulb size. Total offset mass also increased significantly in genotypes A, C, D, G, K, and M ($P = 0.0015$, $P = 0.0015$, $P = 0.0015$, $P = 0.0015$, $P = 0.0285$, $P = 0.0015$, respectively). Average offset mass also increased for genotypes A, G, and M ($P = 0.0195$, $P = 0.0075$, $P = 0.0015$, respectively). Genotypes D, I, L, and M ($P = 0.0015$, $P = 0.0330$, $P = 0.0030$, $P = 0.0015$, respectively) produced more bulbils in the high-nutrient treatment,

and genotype D produced bulbils with a larger average mass ($P = 0.0420$). Genotypes D and G produced less flower biomass and fewer flowers ($P = 0.0015$ for all four) in the high-nutrient treatment, while genotype K produced more flower mass ($P = 0.0240$) and greater numbers of flowers ($P = 0.0030$) in the high-nutrient treatment. The proportion of bulbils to flowers produced increased for genotypes D, G, and H ($P = 0.0015$, $P = 0.0015$, $P = 0.0150$, respectively) in the high-nutrient treatment.

DISCUSSION

Allium vineale is unusual in maintaining high frequencies of both sexual and asexual modes of reproduction in naturally occurring populations. This work demonstrates conclusively that this variation in modes of reproduction, as well as the
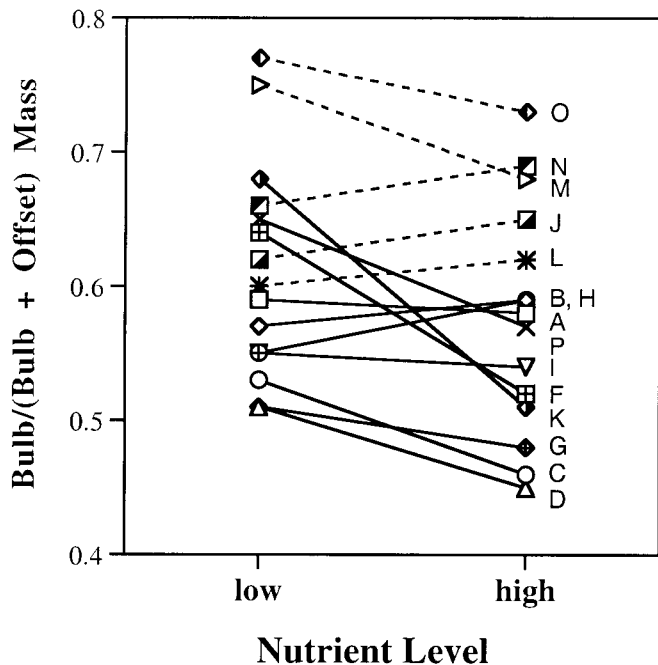

Fig. 5. Genotypes that do not produce flowers allocate more resources to belowground bulb production, rather than to offset production (dashed lines). Proportionally more resources are allocated to offsets with higher nutrient availability ($P = 0.044$).
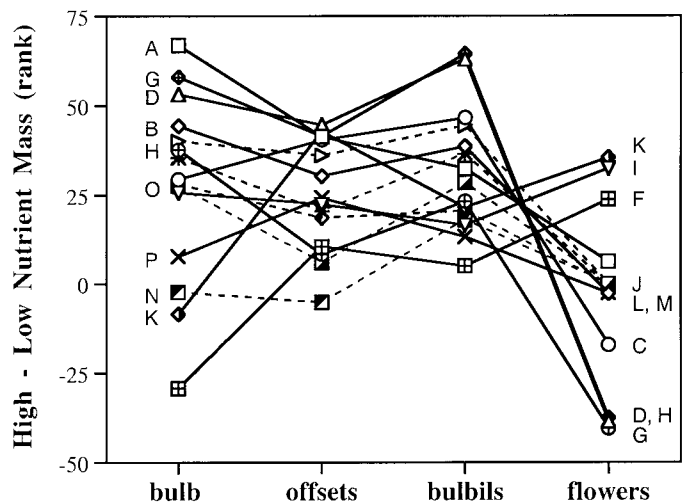

Fig. 6. Genotypes differ in how nutrient availability affects their relative biomass allocation to different propagule types (Profile × Genotype × Nutrient Level effect, $P = 0.0001$). This variation is demonstrated in this figure by the differences in slopes among genotypes. For example, genotypes I, F, and K allocate relatively more resources to flowers at high-nutrient levels, while genotypes D, H, and G allocate relatively fewer resources to flowers at high-nutrient levels. The vertical axis in this figure represents the difference in rank of each genotype in high- vs. low-nutrient levels (genotypes indicated by dashed lines did not produce flowers).

variation in allocation patterns within asexual reproduction, is highly heritable. As stated earlier, the fact that many individuals in this population never produce flowers makes it impossible to cross some genotypes, necessitating the use of clonal heritabilities for this species. Moreover, it is unlikely that our measurements of clonal heritabilities are caused by ephemeral maternal effects, since initial bulbil size was not a significant covariate in the analyses and the differences in allocation patterns in these genotypes persisted undiminished for several years (J. D. Bever, personal observation). Together these results suggest that any maternally inherited contributions to the clonal heritabilities are likely genetic factors, rather than ephemeral nutritional effects. While heritability estimates may be high because of their measurement with clones in a controlled greenhouse environment, allocation patterns within *Allium* should be able to change rapidly in response to selection. Because these divergent allocation patterns are maintained within populations of this species in general, and within this single population in Durham in particular, we infer that selection actively maintains this variation in reproductive systems. Previous studies have documented high heritabilities for reproductive traits in a wide variety of species (e.g., Cain et al., 1995; Delesalle and Mazer, 1995), including the allocation to different reproductive modes (e.g., Clay, 1982; Cheplick and Quinn, 1988; Venable and Burquez, 1989). It is perhaps particularly surprising that *A. vineale,* a predominantly asexual species, demonstrates substantial genetic variability, although high levels of genetic variation have also been found within populations of other asexual species (e.g., *Erigeron annuus,* Stratton, 1991; *Taraxacum officinale,* Lyman and Ellstrand, 1984; see Ellstrand and Roose, 1987, for a review). Understanding the selective forces maintaining these divergent reproductive systems represents a major challenge to evolutionary biology.

Most theory on the evolution of reproductive systems relies on trade-offs in allocation to different modes of reproduction (see references in the introduction). In this study, we found direct evidence for these trade-offs in the form of significant negative genetic correlations both between allocation to sexual and asexual reproduction and between modes of asexual reproduction. Such trade-offs prevent individual plants from maximizing their reproduction to both modes simultaneously. As one mode of reproduction will likely have higher fitness than the other in any one environment, maintenance of these divergent reproductive modes likely reflects spatial or temporal heterogeneity in their environment.

In contrast to predictions that trade-offs in resource allocation will be greater when nutrients are limiting (e.g., Reznick, 1985; Biere, 1995; Schlichting and Pigliucci, 1998), only two pairs of traits demonstrated stronger trade-offs (larger negative genetic correlations) when resources were more limited. Both of these pairs involved trade-offs in allocation to different forms of asexual reproduction (numbers and mass of bulbils and offsets). Trade-offs were actually weaker when resources were more limiting for the other negatively correlated traits (bulbil mass with bulb mass, offset mass, and flower mass and number, bulbil number with flower mass). However, based on pairwise tests, only one pair of correlations were significantly different between the two environments. While comparison of individual correlations show little difference in the genetic correlation structure between the two environments, the overall correlation structure between the two environments was shown to be significantly different within the profile analysis (the sig-

nificant Profile × Genotype × Nutrient Level interaction). Profile analysis provides a more powerful test for detecting overall differences in genetic structure by integrating all of the phenotypic measures into a single test.

Trade-offs between different reproductive modes were consistent in both environments, as both sexual seed production and asexual reproduction through offsets were negatively correlated with asexual bulbil production. The genotypes can generally be grouped into two major resource allocation patterns, with some genotypes allocating resources towards fewer, larger aerial asexual propagules, and other genotypes allocating resources to flowers and belowground asexual offsets. The fact that heritable variation in allocation patterns exists within these groups (and that the genetic correlations are not perfect) suggests that these two reproductive strategies do not result from an inherent constraint in allocation, but rather are themselves an outcome of selection. Understanding the selection pressures on these modes of reproduction is made difficult by the fact that these propagules differ not only in their genetic composition, but in their physiological state as well.

The genetic implications of the reproductive modes are clear. While seeds and bulbils have similar dispersal and dormancy patterns (Ronsheim, 1994), bulbils are genetically identical to the parent and seeds are genetically variable. While the genetic variability found among seeds might result in greater success in variable environments, there is no evidence for that in this population. In general, seeds suffer higher predation rates, lower germination rates, lower survival rates, show no dormancy in the field, and are dispersed no farther than bulbils (Ronsheim, 1994). In addition, evidence for facilitation among genetically identical siblings growing together would favor bulbil production (Ronsheim, 1996), as would the local adaptation found within the scale of seed and bulbil dispersal (Ronsheim, 1997). Evidence of positive feedback between genotypes through their changes in their soil community (Bever, Westover, and Antonovics, 1997) would also favor asexual propagules. It remains a possibility that rare events, such as pathogen attack, create strong selection for the sexual mode of reproduction. Such an event would abruptly raise the frequency of the sexual form, after which the asexual form would again increase in frequency. Data do not exist to evaluate whether such oscillations in frequency occur, though the frequency of reproductive types does differ between sites (M. L. Ronsheim, personal observation). However, the strong positive genetic correlation demonstrated here between flower and offset production coupled with selection for offsets could provide a nonadaptive mechanism for the maintenance of sexual reproduction.

Asexual reproduction through bulbils and offsets are not ecologically equivalent. Offsets do not disperse spatially but can remain dormant for up to 5 yr (Stritzke and Peters, 1972), therefore dispersing in time. In contrast, bulbils do not remain dormant and disperse up to a metre away from the parent (Ronsheim, 1994). Similar heteromorphic dispersal patterns have been found in other plant species, and a negative correlation between dormancy and dispersal has also been observed in *Heterosperma pinnatum* (Venable and Burquez, 1990). This pattern results in one propagule type dispersing through time and one through space (Venable and Lawlor, 1980). While spatial dispersal reduces the probability of sibling competition, dispersed propagules are more likely to experience heterogeneous environmental conditions. Ronsheim (1997) found evidence that an intermediate dispersal distance, which corre-

sponds to the dispersal distance of bulbils, is optimal in this population of *A. vineale*. Growth rates near the parent may be reduced as offsets experience competition with the parent or encounter parental pathogens, though this may be less of a problem after they are dispersed in time. As seen in many amphicarpic plants (see Cheplick, 1987, for a review), bulbils and offsets also differ in relative size and number produced. Offsets can be an order of magnitude larger than bulbils, though with this increase in average size comes a reduction in total offset number relative to bulbils. The subterranean propagules of many amphicarpic species have higher fitness than their aerial counterparts, as a consequence of both their size and burial underground (Cheplick, 1987).

Phenotypic plasticity in response to nutrient availability was found for several traits. In general, plants produced more offsets, larger offsets, and/or more bulbils, but average bulbil size was unaffected. However, no phenotypic plasticity was found in the relative allocation to above- vs. belowground asexual reproduction, and only three of the 16 genotypes changed their allocation to aerial sexual vs. asexual reproduction by producing more bulbils. This limited degree of plasticity indicates that most plants are unable to change their allocation patterns between different reproductive modes as nutrient availability increases. It is important to note that there was genetic variation in plasticity (as tested with a Genotype × Nutrient Level interaction for individual traits, and with a Profile × Genotype × Nutrient Level interaction for allocation patterns), with only five genotypes accounting for most of the plastic responses. The other genotypes had very limited plasticity in both number and mass for all the propagule types, with seven genotypes showing no significant plasticity at all, despite a doubling in nutrient availability. Thus there is limited potential for selection to increase the relative allocation of resources to asexual bulbils vs. flowers when more nutrients are available; however, the lack of genetic variation in plasticity for allocation to above- vs. belowground asexual reproduction will constrain selection to act to favor individual genotypes, regardless of nutrient level.

In summary, we demonstrate that the variation in resource allocation to reproductive mode has a genetic basis and that there are clear life history trade-offs between flower and bulbil production, as well as between offset and bulbil production. As the three propagule types vary widely in genetic variability, dispersal, and dormancy, these different allocation patterns are likely to affect plant fitness. In addition, with limited phenotypic plasticity in allocation to aerial asexual vs. sexual reproduction and to above vs. belowground asexual reproduction, allocation patterns are unlikely to change in response to nutrient availability.

LITERATURE CITED

ABRAHAMSON, W. G. 1975. Reproductive strategies in dewberries. *Ecology* 56: 721–726.

BAKER, G. A., AND D. J. O'DOWD. 1982. Effects of parent plant density on the production of achene types in the annual *Hypochoeris glabra*. *Journal of Ecology* 70: 201–215.

BEVER, J. D., K. M. WESTOVER, AND J. ANTONOVICS. 1997. Incorporating the soil community into plant population dynamics: the utility of the feedback approach. *Journal of Ecology* 85: 561–573.

BIERE, A. 1995. Genotypic and plastic variation in plant size: effects on fecundity and allocation patterns in *Lychnis flos-cuculi* along a gradient of natural soil fertility. *Journal of Ecology* 83: 629–642.

CAIN, M. L., B. KAHN, J. A. SILANDER JR., AND H. L. REYNOLDS. 1995.

Genetic variability and tradeoffs among reproductive traits in white clover (*Trifolium repens*). *Canadian Journal of Botany* 73: 505–511.

CAMPBELL, D. R. 1997. Genetic and environmental variation in life-history traits of a monocarpic perennial: a decade-long field experiment. *Evolution* 51: 373–382.

CHEPLICK, G. P. 1987. The ecology of amphicarpic plants. *Trends in Ecology and Evolution* 2: 97–101.

———, AND J. A. QUINN. 1982. *Amphicarpum purshii* and the "pessimistic strategy" in amphicarpic annuals with subterranean fruit. *Oecologia* 52: 327–332.

———, AND ———. 1988. Quantitative variation of life history traits in amphicarpic peanutgrass (*Amphicarpum purshii*) and its evolutionary significance. *American Journal of Botany* 75: 123–131.

CLAY, K. 1982. Environmental and genetic determinants of cleistogamy in a natural population of the grass *Danthonia spicata*. *Evolution* 36: 734–741.

DELESALLE, V., AND S. J. MAZER. 1995. The structure of phenotypic variation in gender and floral traits within and among populations of *Spergularia marina* (Caryophyllaceae). *American Journal of Botany* 82: 798–810.

ELLSTRAND, N. C., AND M. L. ROOSE. 1987. Patterns of genotypic diversity in clonal plant species. *American Journal of Botany* 74: 123–131.

FALCONER, D. S. 1981. Introduction to quantitative genetics, 2nd ed. Longman House, Burnt Mill, UK.

FOWLER, N. L. 1978. Competition and coexistence in an herbaceous plant community. Ph.D. dissertation, Duke University, Durham, North Carolina, USA.

———, AND J. ANTONOVICS. 1981. Competition and coexistence in an North Carolina grassland. I. Patterns in undisturbed vegetation. *Journal of Ecology* 69: 825–841.

FRY, J. D. 1992. The mixed-model analysis of variance applied to quantitative genetics: biological meaning of the parameters. *Evolution* 46: 540–550.

HOLLER, L. C., AND W. G. ABRAHAMSON. 1977. Seed and vegetative reproduction in relation to density in *Fragaria virginiana* (Rosaceae). *American Journal of Botany* 64: 1003–1007.

LAW, R. 1979. The cost of reproduction in annual meadow grass. *American Naturalist* 113: 3–16.

LE CORFF, J. 1993. Effects of light and nutrient availability on chasmogamy and cleistogamy in an understory tropical herb, *Calathea micans* (Marantaceae). *American Journal of Botany* 80: 1392–1399.

LOEHLE, C. 1987. Partitioning of reproductive effort in clonal plants: a benefit-cost model. *Oikos* 49: 199–208.

LYMAN, J. C., AND N. C. ELLSTRAND. 1984. Clonal diversity in *Taraxacum officinale* (Compositae), an apomict. *Heredity* 53: 1–10.

MATTILA, T., AND V. SALONEN. 1995. Reproduction in *Viola mirabilis* in relation to light and nutrient availability. *Canadian Journal of Botany* 73: 1917–1924.

MITCHELL-OLDS, T., AND J. J. RUTLEDGE. 1986. Quantitative genetics in natural plant populations: a review of the theory. *American Naturalist* 127: 379–402.

MORRISON, D. F. 1976. Multivariate statistical methods. McGraw-Hill Book Company, New York, New York, USA.

MOUSSEAU, T. A., AND D. A. ROFF. 1987. Natural selection and the heritability of fitness components. *Heredity* 59: 181–197.

OGDEN, J. 1974. The reproductive strategies of higher plants II. The reproductive strategies of *Tussilago farfara* L. *Journal of Ecology* 62: 291–324.

PIGLIUCCI, M., P. DIIORIO, AND C. D. SCHLICHTING. 1997. Phenotypic plasticity of growth trajectories in two species of *Lobelia* in response to nutrient availability. *Journal of Ecology* 85: 265–276.

PLATENKAMP, G. A. J., AND R. G. SHAW. 1992. Environmental and genetic constraints on adaptive population differentiation in *Anthoxanthum odoratum*. *Evolution* 46: 341–352.

REZNICK, D. 1985. Costs of reproduction: an evaluation of the empirical evidence. *Oikos* 44: 257–267.

ROCHE, B. M., AND R. S. FRITZ. 1997. Genetics of resistance of *Salix sericea* to a diverse community of herbivores. *Evolution* 51: 1490–1498.

RONSHEIM, M. L. 1994. Dispersal distances and predation rates of sexual and asexual propagules of *Allium vineale* L. *American Midland Naturalist* 131: 55–64.

———. 1996. Evidence against a frequency-dependent advantage for sexual reproduction in *Allium vineale*. *American Naturalist* 147: 718–734.

———. 1997. Distance-dependent performance of asexual progeny in *Allium vineale* (Liliaceae). *American Journal of Botany* 84: 1279–1284.

SAS. 1986. SAS user's guide: statistics. SAS Institute, Cary, North Carolina, USA.

SCHEINER, S. M. 1993. Genetics and evolution of phenotypic plasticity. *Annual Review of Ecology and Systematics* 24: 35–68.

SCHEMSKE, D. W. 1978. Evolution of reproductive characteristics in *Impatiens* (Balsaminiaceae): the significance of cleistogamy and chasmogamy. *Ecology* 59: 596–613.

SCHLICHTING, C. D. 1986. The evolution of phenotypic plasticity in plants. *Annual Review of Ecology and Systematics* 17: 667–693.

———. 1989. Phenotypic plasticity in *Phlox* II. Plasticity of character correlations. *Oecologia* 78: 496–501.

———, AND M. PIGLIUCCI. 1998. Phenotypic evolution: a reaction norm perspective. Sinauer, Sunderland, Massachusetts, USA.

SCHOEN, D. J., AND D. G. LLOYD. 1984. The selection of cleistogamy and heteromorphic diaspores. *Biological Journal of the Linnean Society* 23: 303–322.

SNEDECOR, G. W., AND W. G. COCHRAN. 1989. Statistical methods, 8th ed. Iowa State University Press, Ames, Iowa, USA.

STEARNS, S. C. 1992. The evolution of life histories. Oxford University Press, New York, New York, USA.

STRATTON, D. A. 1991. Life history variation within populations of an asexual plant, *Erigeron annuus* (Asteraceae). *American Journal of Botany* 78: 723–728.

STRITZKE, J. F., AND E. J. PETERS. 1972. Dormancy and sprouting cycles of wild garlic. *Weed Science* 18: 112–114.

TRAPP, E. J., AND S. D. HENDRIX. 1988. Consequences of a mixed reproductive system in the hog peanut, *Amphicarpaea bracteata,* (Fabaceae). *Oecologia* 75: 285–290.

VENABLE, D. L. 1985. The evolutionary ecology of seed heteromorphism. *American Naturalist* 126: 577–595.

———, AND A. BURQUEZ M. 1989. Quantitative genetics of size, shape, life-history, and fruit characteristics of the seed heteromorphic composite *Heterosperma pinnatum*. I. Variation within and among populations. *Evolution* 43: 113–124.

———, AND ———. 1990. Quantitative genetics of size, shape, life-history, and fruit characteristics of the seed heteromorphic composite *Heterosperma pinnatum*. II. Correlation structure. *Evolution* 44: 1748–1763.

———, AND L. LAWLOR. 1980. Delayed germination and dispersal in desert annuals: escape in space and time. *Oecologia* 46: 272–282.

VIA, S. 1993. Adaptive phenotypic plasticity: target or by-product of selection in a variable environment? *American Naturalist* 142: 352–365.

WEIS, A. E., H. G. HOLLENBACH, AND W. G. ABRAHAMSON. 1987. Genetic and maternal effects of seedling characters of *Solidago altissima* (Compositae). *American Journal of Botany* 74: 1476–1486.