Sources of phenotypic variation in floral traits in wild radish, *Raphanus raphanistrum* (Brassicaceae)[1]

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Pollinator-mediated natural selection has been shown to act on phenotypic variation in floral morphology, and this variation has often been demonstrated to be heritable, but few details are available concerning the sources of floral variation. We examined phenotypic variation in seven floral traits in wild radish (*Raphanus raphanistrum*) at six levels: between two populations grown in a common garden, among plants within populations, among flowers measured on different weeks, between flowers on two flowering stalks measured on the same day, between adjacent flowers on a flowering stalk, and within individual flowers. There were no significant differences between plants derived from the two source populations, which were ~800 km apart. Most of the variance was within individual plants; repeatabilities were all <0.35. There were highly significant differences between flowers measured in different weeks and also highly significant plant by week interactions, indicating that the among-plant variation was not consistent over time. There was substantial variance among adjacent flowers on the same stalk, particularly in the gynoecium. This high within-plant variance is partly responsible for the low heritability of floral traits in the field and the weak selection on floral traits found in previous studies of wild radish.

Key words: Brassicaceae; common garden experiment; floral morphology; phenotypic variation; population differentiation; *Raphanus raphanistrum*; within-plant variance.

Animal pollinators cause natural selection on floral traits in many species (e.g., Darwin, 1877; Campbell, 1989; Galen, 1989; Schemske and Horvitz, 1989). This pollinator-mediated selection acts on phenotypic variation among individuals in a population. Since individual plants in many species produce several to many flowers, selection and the evolutionary response to selection will be weakened if there is more variation within an individual than among individuals. For example, if each plant produces flowers with a wide range of floral characteristics, pollinators are less able to select among the flowers of different plants. However, if different plants produce consistently distinct flowers, pollinators may choose one plant more frequently than another and thus will select for specific floral traits.

Within-plant floral variation can be partitioned into "spatial" variation, that is, variation among flowers open at the same time on a plant, and temporal variation, that is, variation among flowers open at different times across the flowering period of each individual. Spatial variation can be further partitioned into variation among inflorescences, among flowers within inflorescences, and within flowers for traits with multiple copies in a flower (e.g., petals and stamens). Temporal variation in floral traits may be due to ontogenetic changes as well as changes in the environment over time. Spatial and temporal variation can have different effects on selection on floral traits. For example, if there are temporal changes in floral size, but different individual plants maintain their relative ranking over time (i.e., no time by individual interaction), then

pollinators on each given day will tend to select the same plants and selection will not be weakened. In contrast, if flower size varies more within individuals than among them, or there is a strong time by individual interaction, then pollinators will not consistently choose one plant and selection will be weakened.

Therefore, determining the sources of variation in floral morphology is crucial to a full understanding of floral evolution. A number of studies have measured pollinator-mediated selection and the heritability of floral traits (e.g., Galen, 1989; Johnston, 1991; Conner and Via, 1993; Carr and Fenster, 1994; Campbell, 1996; Conner et al., 1996). There are also a number of studies examining variation in floral traits among populations and among plants (e.g., Waser and Price, 1984; Schwaegerle, Garbutt, and Bazzaz, 1986; Herrera, 1990). Fewer studies have additionally measured variation among flowers within plants (e.g., Campbell, 1992; Svensson, 1992; Dominguez et al., 1998), and fewer still explicitly examine floral variation within plants over time (Ellstrand and Mitchell, 1988; Armbruster, 1991; Herrera, 1993; Mazer and Delesalle, 1996). To our knowledge, no study has quantified variation within individual flowers or tested time by individual interactions.

A further motivation for understanding sources of floral variation within populations and individuals is to help explain differences between environments in heritabilities of floral traits. Previous studies have found that heritabilities of wild radish floral traits were much lower in the field than in a very similar greenhouse experiment and that this was due in part to increased environmental variation in the field (Conner and Via, 1993; Conner et al., unpublished data).

To increase our understanding of the sources of environmental variation in the field, we examined variation in seven floral traits at six levels in wild radish, *Raphanus raphanistrum.* We measured variation between two distant populations and among plants within populations. Within plants, we mea-
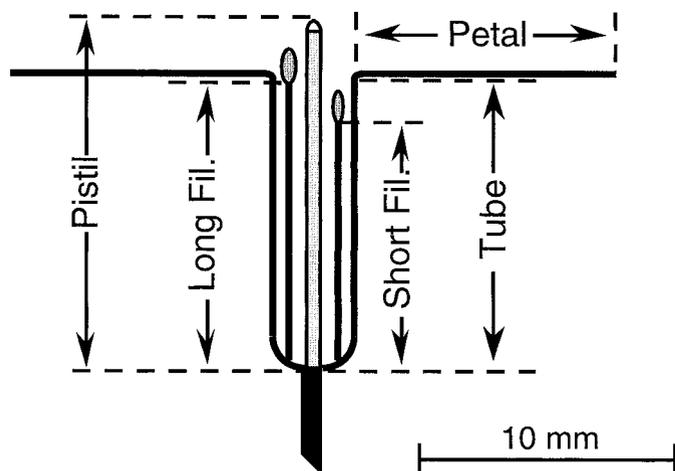
Fig. 1. Schematic cross section of a wild radish flower, showing the traits measured. Petal denotes the limb; only the length is shown, but the width was also measured. Only one long stamen and one short stamen are shown for clarity. Scale is approximate. Modified from Conner and Via (1993). Fil. = filament.

sured variation among flowers produced at different times, among inflorescences produced at the same time, and among adjacent flowers within inflorescences. Finally, we measured variation among the petals and stamens within individual flowers.

MATERIALS AND METHODS

Wild radish, *Raphanus raphanistrum* (Brassicaceae), is an annual weed that grows in disturbed areas. It has hermaphroditic, self-incompatible flowers pollinated by a variety of insects (Stanton, Snow, and Handel, 1986; Stanton et al., 1989; Conner, Davis, and Rush, 1995; Conner and Rush, 1996).

A field at the Kellogg Biological Station in Kalamazoo County, Michigan, USA, was tilled to simulate the highly disturbed sites in which wild radish are normally found. Field-collected seeds from two populations of wild radish, one from Kalamazoo County and one from a previously studied population from Binghamton, New York (Conner and Via, 1993), were used. Both populations were located in alfalfa fields. Seeds were planted in a 10×12 grid with 1-m spacing in April 1998. Sixty maternal plants from each population were represented in the grid, assigned randomly within each population to every other grid position. At each position, 12 seeds were planted from one of the maternal plants. At first flowering, one of the plants that germinated was randomly chosen to represent its maternal family. Only 67 of the plants germinated and survived to flowering, 60 of which were included in the study, 27 from Michigan and 33 from New York. Six plants flowered too late, and one died too soon for inclusion in the study.

Due to an unusually dry season, the field was watered roughly once every 2 wk after planting to encourage germination and flowering. The plots were partially weeded to prevent the dominant lamb's quarter (*Chenopodium album*) from completely shading the study plants, but it is likely that interspecific competition occurred.

We measured a total of 1059 flowers from all plants during a 7-wk period in June and July 1998. From an individual plant, the first samples consisted of the third and fourth flower on the central stalk the day after each opened. Every week following the initial sample of the fourth flower, we collected the two newest flowers from two randomly selected side stalks on the same plant. Within each flower, we measured the lengths and widths of the outer, showy parts (the limb) of two petals on either side of a haphazardly chosen short stamen (Fig. 1). We also measured the lengths of the inner parts, or claws, of these petals; we refer to this as the corolla tube length (Conner and Via, 1993). We measured the lengths of the two long stamens on either side of the

same short stamen, both short stamens, and the pistil. These traits have been studied extensively in wild radish and have been shown to be important to pollination success in wild radish and other species (e.g., Bell, 1985; Galen and Newport, 1987; Murcia, 1990; Harder and Barrett, 1993; Conner, Davis, and Rush, 1995; Conner and Rush, 1996). Measurements were taken with digital calipers using the same methods as Conner and Via (1993). The number of ovules was also counted.

To analyze the effects of population (MI or NY), the week of measurement, and plant identity on these seven floral traits, mixed-model repeated-measures analysis of variance was performed using restricted maximum likelihood (REML; PROC MIXED; SAS, 1989). Population, week, and the population by week interaction were fixed effects, and the individual plant (nested with population) and the plant by week interaction were random effects. Z tests were used for significance testing of the random effects. The plant by week interactions were also tested by rerunning the models without plant by week and comparing the difference in $-2\times$ log-likelihood to a chi-square distribution with 1 df; all P values were very similar to those from the Z tests. Therefore, the results presented here are likely to be very robust.

To determine whether temporal changes in flower size were due to environmental changes vs. ontogeny, a simplified repeated measures model was fit also using REML in PROC MIXED. The geometric mean of the six linear dimensions (all traits except ovule number) was used as a measure of overall flower size (Mosimann and James, 1979). In our data the geometric mean was highly correlated ($r = 0.995$) with the first principal component of the six floral dimensions. Time was measured in two different ways: calendar week and plant age. Plant age was the number of weeks since flowering, with week 1 as the first week each plant flowered.

To examine the relative magnitudes of among- and within-plant variability in floral traits within each week, variance components from fully nested random models were estimated using REML (PROC MIXED; SAS, 1989). Variance components were estimated among plants, among flowering stalks within plants, among flowers within stalks, and within flowers. The within-flower component did not exist for pistil length and ovule number, because only one measurement per flower was possible. Since none of the tests of population or population by week interaction were significant (see RESULTS), populations were combined for simplicity. This analysis was not done for the first week because only the central stalk was measured for most plants that week, resulting in no replication of stalks, and because the flowers were measured the day after they opened that week only (see above). The proportion of among-plant variance from this analysis represents the repeatability within weeks; an overall repeatability was also calculated within each population as the proportion of total variance due to among-plant variation.

The repeatability (r) is the proportion of total phenotypic variance that is due to differences among individuals (Falconer and Mackay, 1996). The numerator of the repeatability consists of additive and nonadditive genetic variation and the "general environmental variance," which arises from permanent phenotypic differences among individuals caused by the environment. Therefore, the repeatability sets an upper bound on narrow sense heritability of the trait, because the numerator of the latter includes additive genetic variation but does not include nonadditive genetic variance or the general environmental variance. The complement of the repeatability ($1 - r$) is the proportion of population phenotypic variance that is due to within-individual variation (the "special environmental variance"), which is caused by the spatial and temporal environmental variation experienced by each individual as well as ontogenetic changes.

RESULTS

There were no significant differences between the two populations for any of the traits measured, nor were there any significant population by week interactions (Table 1). In contrast, week, plant, and the week by plant interaction were highly significant for all traits (Table 1, Fig. 2). Therefore, floral traits differed across weeks and among plants, but the among-plant variation was not consistent over time.

Plants had larger flowers at the beginning of the growing

TABLE 1. *P* values from mixed model repeated measures analysis. Population, week, and the population by week interaction were fixed effects, and the individual plant (nested within population) and the plant by week interaction were random effects. *Z* tests were used for significance testing of the random effects. *N* = 1047–1058.

Trait	Population (Pop)	Week	Pop × Week	Plant (Pop)	Plant × Week
Flower size	0.77	<0.0001	0.51	0.0001	<0.0001
Petal length	0.78	<0.0001	0.87	0.0002	<0.0001
Petal width	0.09	<0.0001	0.82	<0.0001	<0.0001
Corolla tube	0.25	<0.0001	0.12	<0.0001	<0.0001
Short filament	0.46	<0.0001	0.39	0.0001	<0.0001
Long filament	0.61	<0.0001	0.48	<0.0001	<0.0001
Pistil	0.29	<0.0001	0.53	<0.0001	<0.0001
Ovule number	0.33	<0.0001	0.34	<0.0001	<0.0001

season than at the end, with a sharp decrease in size in weeks three and four and a slight increase for the last three weeks (Fig. 3; week 4 vs. week 7, $F = 12.4$, $P = 0.0007$). When plotted by plant age, flower size declined linearly over the first 4 wk of the plants' flowering period and then increased slightly for the next 3 wk (week 4 vs. week 7, $F = 8.7$, $P = 0.004$). Calendar week explained over twice as much of the total var-

Fig. 3. Flower size vs. calendar week and plant age in weeks. Calendar week 1 began 7 June 1998, while plant age is defined as the number of weeks from first flowering. The error bars show one standard error of the mean, and sample sizes are given above the axis. Note that the sample sizes are small in the first week of calendar week and the last two weeks of plant age because not all plants flowered in the first week and therefore not many were measured for the full 7 wk. The sample sizes are almost always <60 because not all plants had sufficient flowers for measurement each week. Proportion of variance explained (R^2) and *P* values are from a one-way ANOVA.

iance in flower size than did plant age, despite the fact that calendar week and plant age were correlated ($r = 0.81$). When flowers that were measured in the first week of plant flowering were eliminated (because they may have been older than subsequent flowers, see MATERIALS AND METHODS) plant age explained only 14% of the variance in flower size, while calendar week explained 51%. Thus, changes in the environment had a greater influence on floral variation than did ontogenetic changes within the plant as it aged. This point, combined with the highly significant plant by week interactions (Table 1), suggests that individual plants responded differently to the variable environment.

The variance components for the random effects within weeks show fairly consistent patterns across the weeks except for week 2, but this variation was distributed differently in different traits (Fig. 4). For most traits the largest variance components within weeks were at the among-plant and among-flowers within-stalks levels; there was less variation among stalks or within flowers. The among-plant component represents repeatability within weeks. For the corolla traits the largest component was among plants (mean = 41%), while for the gynoecium traits the largest was among adjacent flowers (mean = 55%). The androecium (filament length) variation was more evenly distributed between the among plant (mean = 28%), among flower (mean = 30%), and within flower (mean = 26%) levels.

The three corolla measurements showed less overall variance than the androecium and gynoecium measurements (Table 2). As expected from the large amount of within-individual variation (especially across weeks), overall repeatabilities were low, ranging from 0.15 to 0.33 (Table 2).

DISCUSSION

The overall distribution of floral variation was different from our expectations. We expected that the among-plant and between-population variation would be the largest, based on
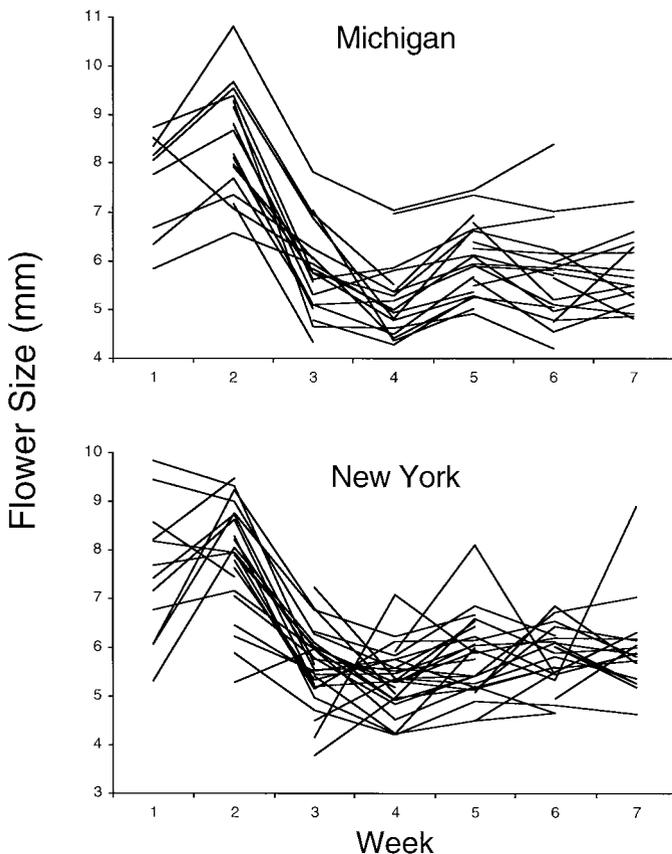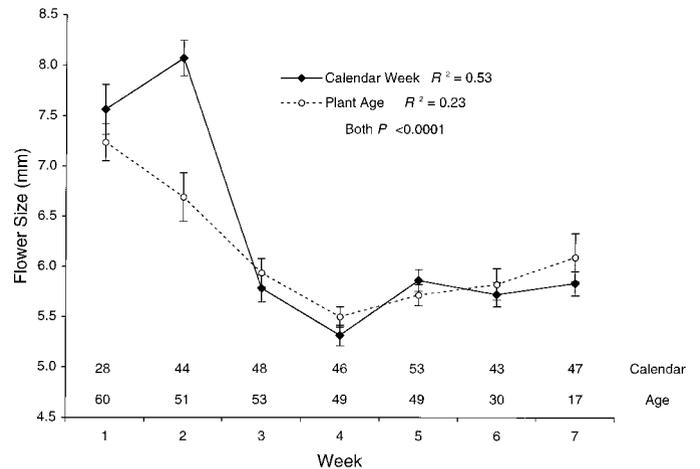

Fig. 2. Reaction-norm plot showing flower sizes (estimated as the geometric mean of the six floral dimensions) over time for each individual in the Kalamazoo, Michigan, USA, and Binghamton, New York populations. The arithmetic mean of all measurements of each of the six linear dimensions on each individual within each week was taken, and then the geometric mean of these six trait averages was calculated as a measure of flower size that week for each individual.
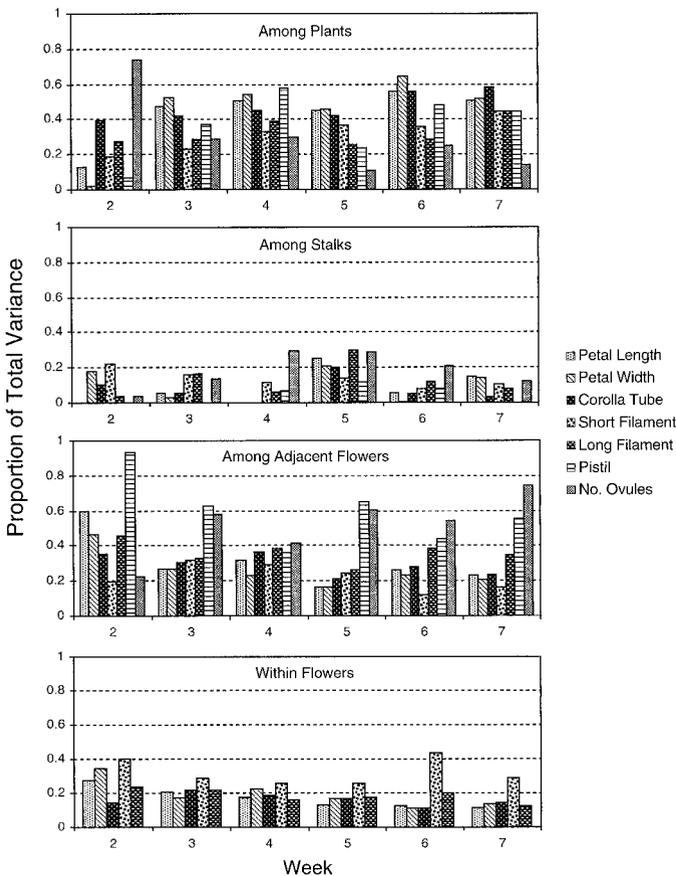
Fig. 4. Variance components from a nested ANOVA for the seven floral traits within each week. The two populations were lumped for simplicity and because they did not differ (Table 1). Note that the among-plants components represent the repeatabilities within weeks. Week 1 was excluded due to lack of replication for stalks. For weekly sample sizes see Fig. 3.

Table 2. Measures of floral variation. Total variance is the total phenotypic variation across all plants measured in the study. Repeatability is the among-plant variance component divided by the total variation within each population. These repeatabilities are all significantly greater than zero at $P = 0.006$ or less.

Trait	Total Variance	Repeatability	
		Michigan population	New York population
Petal length	1.81	0.15	0.20
Petal width	1.54	0.21	0.22
Corolla tube	1.91	0.33	0.24
Short filament	3.12	0.18	0.15
Long filament	3.98	0.24	0.15
Pistil	3.18	0.18	0.28
Ovule number	3.89	0.23	0.21

ings for pollinator visitation and fitness would also be likely to change over time, leading to a weakening of selection.

Previous work has suggested the presence of long-distance gene flow in wild radish, perhaps through contamination of agricultural seed with wild radish seeds, which could reduce genetic differentiation over long distances (Kercher and Conner, 1996). This may account for the lack of population differentiation for floral traits found in this study. It is also possible that the generalist suite of pollinators causes similar selection on floral traits in both locations. A final possibility is that the low levels of germination (only about half the maternal families germinated; see METHODS) caused strong selection so that the plants that successfully germinated from the two populations were similar to each other. This possibility requires a correlation between germination and floral traits; however, in an earlier study (Conner and Via, 1993) all phenotypic and genetic correlations between germination time and floral traits were 0.22 or less, and few were significant.

The vast majority of flowers measured within the same week on the same plant were measured the same day. This fact provides a possible explanation for why there was more variation among adjacent flowers on the same stalk than among stalks (Fig. 4). Since wild radish flowers open acropetally (sequentially moving up the stalk), two adjacent flowers on the same stalk are almost always different in age. However, the average floral age likely did not differ greatly between stalks, because on each stalk the newest and second newest flower were measured. Therefore, the greater variation among flowers within stalks may have been largely due to differences in floral age.

Our study sheds light on the sources of phenotypic variance in floral morphology and suggests that changes in the environment over a growing season may often be important determinants of floral variation. The plant by week interaction (Table 1, Fig. 2) further suggests that there may be genotype by environmental variation for floral traits, although our design could not address this directly. Our study was also not designed to determine what biotic or abiotic environmental factors caused this variation, but we can speculate on what factors are most likely. There were no detectable effects of rainfall or temperature on floral variation in this study (Williams and Conner, unpublished data). The within-plant variation could be caused by changes in inter- or intraspecific competition over the season. However, Mazer and Schick (1991) found no significant effect of population density on among-plant differences in petal area in *Raphanus sativus* and Wolfe (1992) reported no effect of resource status on flower size in *Hydrophyllum*

most previous studies (Schwaegerle, Garbutt, and Bazzaz, 1986; Herrera, 1990; Armbruster, 1991; Dominguez et al., 1998). Instead, most of the variation was within plants, similar to Campbell's (1992) results. Genetic variation is included only in the among-population and among-plant levels, so the vast majority of the phenotypic variation in floral traits is due to the environment and plant ontogeny (especially the former; Fig. 3). The low repeatabilities of all the traits indicate that the low heritabilities in the field are mainly due to large amounts of special environmental variance. Although floral morphology is often less plastic than vegetative parts (Bradshaw, 1965; Schlichting and Levin, 1984; Frazee and Marquis, 1994), these results demonstrate a strong environmental effect.

These results also suggest that selection on these traits would be weakened by this within-plant variation, and indeed little selection on floral morphology was found over 3 yr in a previous study (Conner, Rush, and Jennetten, 1996; Conner et al., 1996; but see Morgan and Conner, 2001). The overall repeatabilities for floral traits in this study were all <0.35 (Table 2). While the repeatabilities within weeks were higher, between 0.3 and 0.6 for most traits in most weeks (among plant variation in Fig. 4), the highly significant plant by week interactions (Table 1) suggest that plants do not maintain their relative rankings for floral traits over time. Therefore, the rank-

appendiculatum. Another possible biotic environmental factor is herbivory, since Strauss, Conner, and Rush (1996) found that herbivory affects variation among plants in petal size; a variety of herbivores attack wild radish at our site (personal observations; A. Agrawal, University of Toronto, personal communication). A challenge for future work is to discover the environmental factors underlying this within-plant variation and determine whether these are similar to the environmental factors that cause among-plant floral variation. This knowledge will provide a comprehensive understanding of the genetic and environmental sources of floral variation.

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