

MEASUREMENTS OF NATURAL SELECTION ON FLORAL TRAITS IN WILD RADISH (*RAPHANUS RAPHANISTRUM*). I. SELECTION THROUGH LIFETIME FEMALE FITNESS

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Abstract.—Although the role of natural selection in the evolution of floral traits has been of great interest to biologists since Darwin, studies of selection on floral traits through differences in lifetime fitness have been rare. We measured selection acting on flower number, flower size, stigma exsertion, and ovule number per flower using field data on lifetime female fitness (seed production) in wild radish, *Raphanus raphanistrum*. The patterns of selection were reasonably consistent across three field seasons, with strong directional selection for increased flower production in all three years, weaker selection for increased ovule number per flower in two years, and selection for increased flower size in one year. The causes of the selection were investigated using path analysis combined with multiplicative fitness components. Increased flower production increased fruit production directly, and increased numbers of ovules per flower increased the number of seeds per fruit in all three years; pollinator visitation did not influence either of these fitness components. Increased flower size was associated with increases in both the number of fruit and the number of seeds per fruit in one year, with the latter relationship being stronger. Total lifetime seed production was affected more strongly by differences in fruit production than by differences in either the number of seeds per fruit or the proportion of fertilized seeds that were viable, but all three fitness components were positively correlated with total seed production.

Key words.—Lifetime fitness, multiplicative fitness components, natural selection gradients, path analysis, *Raphanus raphanistrum*, wild radish.

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The enormous diversity of floral displays among plant species has long been interpreted by biologists as evidence of the important role of natural selection exerted by animal pollinators in floral evolution. One approach to understanding selection on floral traits is to obtain estimates of the strength of selection acting in present-day plant populations and to combine this with studies of pollinator behavior and effectiveness to understand the causes of selection (the selective agents). Some studies have used population-genetic models to estimate the strength of selection on floral traits that exhibit discrete polymorphisms such as floral color (e.g., Waser and Price 1981). However, most floral traits are continuously distributed, and measurements of selection on continuous traits in the field are difficult or impossible using population-genetic methods.

In the last decade or so, a variety of new quantitative-genetic techniques to measure selection on continuous traits in the field have been developed (Lande and Arnold 1983; Arnold and Wade 1984a,b; Mitchell-Olds and Shaw 1987; Schluter 1988; Crespi and Bookstein 1989; Crespi 1990; Rausher 1992; Schluter and Nychka 1994). These techniques use regression or related methods to quantify the relationship between fitness and the phenotypic traits of interest; they have been applied to floral traits in only a few species (Campbell 1989; Galen 1989; Schemske and Horvitz 1989; Campbell et al. 1991; Johnston 1991; Herrera 1993). Measurements of selection are most reliable when lifetime fitness data are used (Lande and Arnold 1983; Arnold and Wade 1984a; Endler 1986; Travis and Heinrich 1986; Clutton-Brock 1991), but to our knowledge only one study of selection on floral traits has used lifetime data (Campbell 1991).

There are two types of floral traits for which measurements of selection are needed. First, floral attractants such as flower number and size may be under selection because increased pollinator visitation may increase fitness (see reviews in Clements and Long 1923; Waser 1983; Bell 1985; Bertin 1988; Kearns and Inouye 1993, ch. 7). Second, spatial relationships among floral traits, particularly the relationships among the positions of anthers, stigmas, and corolla structures, may affect the efficiency of pollen transfer during pollinator visits. One such spatial relationship in flowers with tubular corollas is stigma exsertion, defined as the distance between the mouth of the corolla tube and the stigma. A few studies have suggested that stigma exsertion can affect pollen deposition (Campbell 1989; Murcia 1990; Campbell 1991; but see Campbell et al. 1994).

A specific hypothesis of selection on stigma exsertion was advanced by Conner and Via (1993). This hypothesis predicts that intermediate stigma exsertion results in maximum pollen receipt, because higher and lower stigmas are less likely to contact the pollinator's body (cf. Campbell et al. 1994). These hypothesized patterns of pollen receipt could result in stabilizing selection on exsertion to maintain the optimal stigma placement.

Here we report estimates of selection on flower number, flower size, stigma exsertion, and ovule number in wild radish, *Raphanus raphanistrum* (Brassicaceae). Selection estimates were based on measurements of lifetime female fitness (seed production) in two field seasons and six cross-sections or "snapshots" of seed production spread over most of the life of the plant in a third field season. Selection gradients (Lande and Arnold 1983) were estimated from this data for each trait in each season. To investigate the ecological causes of this selection, a new method combining path analysis and multiplicative fitness components was employed.

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METHODS

Field Methods

Three field studies on experimental populations were conducted in the years 1991–1993. All three populations were started from randomly selected seeds collected from a natural population near Binghamton, New York, that contained only the yellow flower color morph (Conner and Via 1993). The studies were conducted in a fenced plot at the University of Illinois Phillips Tract natural area near Urbana, Illinois. The New York seeds were used for two reasons. First and foremost, although *Raphanus raphanistrum* occurs naturally in the Urbana area (J. Conner, pers. obs.), we have never found local populations large enough for experiments. Second, genetic information was available for the floral traits in this population (Conner and Via 1993). Flowers were visited by the same species of pollinators in Illinois as those visiting wild radish in upstate New York (Conner and Rush 1996; G. Eickwort, pers. comm. 1993).

The three field seasons used somewhat different and complementary methods. The sample sizes chosen represent compromises between two conflicting goals. The number of plants need to be large enough to provide adequate statistical power for the selection analyses, while simultaneously being small enough so that there were not too many possible fathers in future paternity analyses for male fitness estimates (Conner et al. 1996). In 1991 and 1992, we measured total lifetime fitness in experimental populations of about 60 plants that we planted in the field. In 1991, the planting occurred late in the season after an earlier planting was destroyed by rabbits; therefore, the plants were flowering past the peak of wild radish flowering in our area but still within the normal flowering period. In 1992, we used the same basic design, but the plants were planted earlier, so that they were flowering during the normal peak flowering period. In 1993, we used potted plants that were brought to the field in groups throughout the peak flowering period. This design allowed us to increase our sample size and focus more on the effects of pollinators but did not result in estimates of total lifetime fitness.

In 1991, seeds were sown in pots in the greenhouse on August 5. On September 1, two days before the first plants flowered, 64 plants were transplanted into an 8 × 8 grid in the field with 1-m spacing. The study ended at the first killing frost on November 1. Fifty-seven plants flowered during this time and were included in the analysis. In 1992, seeds from 100 field plants were sown directly into the field plot on May 27 in a 10 × 10 grid with 1-m spacing. Sixty of these plants survived to flower and were included in the study. The bulk of flowering occurred in July and August, but some plants continued producing a few flowers through October; the last plant ceased flowering on November 4. To test for edge effects on the selection gradients, a categorical variable denoting whether the plant was on the edge of the plot was included in the analysis. In neither year did it affect the selection gradients. Thus, it was dropped from the final model.

Vertebrate herbivores were excluded by the fence, and severe outbreaks of lepidopteran larvae and aphids were controlled using *Bacillus thuringensis* (Dipel 2×; Abbott Lab-

TABLE 1. Eigenvectors and the total variance explained by the first principal component of the six morphological traits.

Trait:	Eigenvectors		
	1991	1992	1993
Petal length	0.43	0.37	0.37
Petal width	0.34	0.35	0.19
Corolla-tube length	0.46	0.48	0.48
Short-filament length	0.42	0.44	0.47
Long-filament length	0.46	0.47	0.48
Pistil length	0.32	0.32	0.38
Total Variance Explained:	65%	59%	59%

oratories, North Chicago, IL) and rotenone-pyrethrum (Red Arrow; Southern Mill Creek Products, Tampa, FL). These outbreaks were particularly serious in 1991, and insecticides were applied weekly. In 1992, rotenone-pyrethrum was only applied three times, all before the plants flowered. The insecticides were applied in the late evening, when pollinators were minimally active (P. Jennetten, pers. obs.). This allowed sufficient time for the insecticide to dry and degrade before pollinators returned in the morning. In both years, plants were fertilized with a top-dressing of 1 g of Osmocote (19-6-12; Grace-Sierra Horticultural Products, Milpitas, CA).

In 1993, 112 plants were grown in 2-liter pots in the greenhouse. When all plants had flowered, one flower was measured on each plant, and seven groups of 16 plants each were created such that the groups did not differ in mean or variance of floral traits (see below). Beginning on July 8, each group was transported to the field site and placed in a 4 × 4 array with 1-m spacing. Only one group was brought to the field per day; groups were left in the field for an average of four hours per day (range: 3–10 h). To provide a continuous display of wild radish flowers to pollinators, arrays of nonexperimental plants were left at the field site during all times that the experimental arrays were not present. After all the groups had been brought to the field once, the procedure was repeated five more times, ending on August 29. Thus, each plant was exposed to natural pollination for six days spanning the peak flowering period for wild radish in our area. The fitness estimates in 1993 are therefore not total lifetime fitness but rather the total of six days of reproduction spread over most of the natural reproductive life of the plant. Plant positions within the arrays were rerandomized each time the plants were brought to the field. Therefore, there should be no consistent position effects on fitness over the entire experiment.

Traits Measured

Six floral traits were measured (for details, see Conner and Via 1993): the length and width of the outer part of the petal (the “limb”), the length of the corolla tube (the “claw”), the lengths of one short and one long filament, and the length of the entire pistil. Because these traits are all correlated (Conner and Via 1993), two new morphological variables were created that were considered a priori to be likely targets of selection through differences in female fitness. First, a principal-components analysis was conducted on the six traits, and the first principal component was used as measure of overall flower size (Lande and Arnold 1983; Table 1). In

all three years, the first principal component explained about 60% of the variance in the traits, and all six traits loaded positively and approximately equally on this first principal component. The geometric mean of the six traits, another possible measure of overall size (Mosimann and James 1979), was highly correlated with the first principal component and gave the same results in the selection analyses.

Second, stigma exertion was calculated by subtracting pistil length from corolla tube length. Because filament length was highly correlated with tube length (Conner and Via 1993), stigma exertion was also a good estimate of stigma-anther separation: the correlation between stigma exertion and stigma-anther separation ranged between 0.91 and 0.93 for the three years.

In addition to the morphological traits, selection on ovule number per flower and overall flower production was estimated, using different measures of flower production in each season. In 1991, the total number of flowers produced was not directly counted. Thus, the number of days between the first and last flower produced (the flowering span) was used as an estimate; flowering span was positively correlated with number of flowers produced in the 1992 plants ($r = 0.42$, $P < 0.001$). In 1992, the total lifetime flower production by each plant was estimated by counting the pedicels borne on the plant at the end of its life. In 1993, the number of flowers open each time the plant was exposed to pollinators was counted, and these six counts were summed to give total number of flowers available for pollination.

In 1991 and 1992, the third or fourth flower to open on each plant was measured, and from one to four additional flowers were measured throughout the life of the plant. In 1993, in addition to the early flower measured (see above), one flower was measured each time the plants were taken to the field, for a total of seven flowers measured. In all three years, all floral measurements were averaged to provide one estimate of floral morphology per individual plant. Although there was considerable variability among flowers from the same plant, especially in 1991 and 1992, the selection analyses showed the same patterns regardless of whether they were performed using each set of measurements individually or on the average of all measurements.

Pollinator Observations

In all years, plants were observed and the taxon of visitors recorded in the following categories: honey bees, small bees (mainly *Ceratina* and *Dialictus*), bumble bees, syrphid flies (mainly *Allograpta*, *Sphaerophoria*, *Syrphus*, and *Toxomerus*), and butterflies. In 1991 and 1992, plants were observed for one-hour periods from September 5 to October 24, 1991 and July 16 to August 30, 1992. Each plant was observed from one to four times. In 1993, plants were observed for 10–40 min most days that they were in the field; 93% of the plants were observed five or six times during the six days in the field. On average, each plant was observed for 106 min total (range: 40–146 min). On each day, plants were observed in groups of two or three, with a single observer for each plant. The order in which plants were observed was randomized within days. Thus, each plant was observed on several different days at different times, so

that differences in visitation between plants were not caused by temporal variability in pollinator visitation.

Female-Fitness Measures

Several components of female fitness, as well as total lifetime fitness, were estimated. Total number of viable seeds produced was used as the total-fitness estimate, ignoring seed size or other possible determinants of early offspring growth and survival, because offspring success is considered an attribute of the next generation in the evolutionary models employing selection gradients (Arnold and Wade 1984b). All fruits produced by each plant were collected and counted. Next, the expanded fruit segments, which indicate the presence of a developing seed in *R. raphanistrum*, were counted. These counts were performed on all fruits in 1993 and on a random sample of 50 fruits or 20% of the fruits produced by the plant, whichever was larger, in 1991 and 1992 (fruit segments were counted on a total of 4315 fruits in 1991 and 6510 in 1992). An average number of seeds fertilized per fruit was then calculated from this sample. This is likely to be an underestimate of the number of fertilized seeds because it probably does not include seeds that aborted shortly after fertilization (Nakamura and Stanton 1987). To estimate the proportion of fruit segments that contained later-aborted seeds, a random sample of fruit segments (25 per plant in 1991 and 50 per plant in 1992, for a total of 1425 and 3000, respectively) were broken open and scored as filled (including partly filled) or aborted (no seed or unfilled seed coat only). These seeds were then planted in greenhouse germination trials; 2% of seeds scored as aborted germinated compared to 51% germination of seeds scored as filled. This resulted in an average percentage of fertilized seeds aborted per plant; this percentage was multiplied by the total number of seeds fertilized to estimate total fitness—the number of viable seeds produced over each plant's lifetime. Seed abortion rates were not measured in 1993. Thus, the fitness estimate is the total number of seeds fertilized (i.e., fruit segments, not counting early aborted seeds) over the six field days.

Selection Gradients

To estimate the strength of selection acting on floral traits, standardized selection gradients (Lande and Arnold 1983) were calculated by regressing relative total fitness on the five floral traits, after standardizing the floral traits to mean = 0 and variance = 1 (z -transformation). Linear-regression terms were included in the models for all traits to estimate directional selection, and quadratic terms were included for flower size and stigma exertion to estimate stabilizing selection (see above; the quadratic-regression coefficient was doubled to give the gradient [Lande and Arnold (1983)]). The number of flowers produced was ln-transformed to improve normality; all other floral traits were acceptably normal. Variance inflation factors (Neter et al. 1985) were less than 1.5 in all cases, indicating no multicollinearity problems. Residual plots from the 1991 and 1992 analyses showed heteroscedasticity. This problem disappeared and the overall fit of the model improved dramatically when relative fitness was ln-transformed. For these two years, untransformed relative fitness was used to estimate the selection gradients to pre-

serve the evolutionary interpretation of the gradients and because the estimates using untransformed fitness values are unbiased point estimates (Lande and Arnold 1983). Significance tests of these gradients are from the models using ln-transformed fitness, because the tests are sensitive to heteroscedasticity (Mitchell-Olds and Shaw 1987; T. Mitchell-Olds, pers. comm. 1994).

Preliminary analyses of the 1993 data showed that the seven groups (i.e., blocks) did not differ in the five floral traits measured (MANOVA: Wilks' lambda = 0.72; df = 30, 406; $P = 0.28$), but the groups did differ in fitness, that is, number of seeds produced (ANOVA: $F = 4.1$; df = 6, 105; $P = 0.001$). Therefore, group was included as a blocking variable in the regression analyses. There were no significant interactions between group and the floral traits with respect to fitness; this means that the slopes of fitness versus the floral traits did not differ between groups. The interactions were therefore not included in the final model.

Path Analysis

A combination of path analysis using structural equation modeling (Mitchell 1992, 1993) and multiplicative fitness-component analysis (Arnold and Wade 1984b) was used to investigate the causes of selection on floral traits and to determine the relative importance of different fitness components to total lifetime fitness. The number of variables that could be included in the path analyses were limited by sample size (see below). Thus, only floral traits for which selection gradients were significant at $P < 0.2$ were included in each year. Therefore, the path analysis was used to investigate the causes of selection only for those traits that were under selection based on the selection gradients. Including only these variables in the selection analysis did not change the gradients appreciably. Ovule number was also included in 1992, despite the fact that it did not meet this criterion, because it was significant in both other years, had a relatively large standardized selection gradient in 1992 (see Results) and seemed likely a priori to be related to at least some components of female fitness.

The multiplicative components of female fitness used were number of fruits produced, number of fertilized seeds per fruit, and number of viable seeds per fertilized seed. The last component was included only in the 1991 and 1992 analyses. Hypothesized causal paths (representing standardized partial-regression coefficients) were included from all floral traits in the path model to both number of fruit and fertilized seeds per fruit, but none of the floral traits measured was hypothesized to affect the number of viable seeds per fertilized seed (i.e., seed abortion rates) (Fig. 1). Correlations among all fitness components and total fitness were included to check for trade-offs among fitness components at the phenotypic level and to determine the relative importance of each fitness component in determining total fitness. Correlations between the fitness components and total fitness were used rather than causal paths because the components multiply to equal total fitness, making a multiple regression of fitness on the components invalid (Conner, in press). To explore the sources of variability in flower production in 1992, the flowering span and the total number of branches produced were also included

in the path diagram, with causal paths from these traits to total number of flowers produced. To correct for the effects of the seven groups (i.e., blocks) in 1993, six dummy variables were entered in the path model, with causal paths from each to the two pollinator variables (see below) and the two fitness components.

In 1993, two pollinator-visitation variables were also added to the path model: the total amount of time that small bees and syrphid flies spent at the plant over all six observation periods. The total amount of time spent integrates three aspects of visitation: the number of visits, the number of flowers probed per visit, and the time spent per flower probed. The path model included causal paths from flower number to visitation by both pollinator taxa (flower size was not included in the path analysis; see above), and from visitation to the two fitness components, number of fruit and number of seeds per fruit (Fig. 1). The relationship between pollinator visitation and fitness was examined only in 1993 because the 1993 plants were observed most days that they were exposed to pollinators (see above). In 1991 and 1992, individual plants were only observed on 1–4 days of a median of 53 and 115 days flowering, respectively. Thus, although these observations served to determine which pollinators were visiting the population, they did not adequately characterize lifetime-visitation patterns of individual plants.

The number of variables used in the path analyses exceeded the rule of thumb of 10 observations per variable (Mitchell 1993 and references therein), especially in 1991. However, reduced-path models (chosen a priori) with fewer variables were also run to check for possible problems. In no case were there any qualitative differences in the overall fit or the path coefficients between the reduced and full models. Harris (1985) recommends that the sample size minus the number of variables be greater than 50 for multiple regression. Our path analyses all meet this criterion. Some variables in each year were transformed to improve normality.

Regression analyses were performed using JMP (SAS Institute 1994) on a Macintosh computer, and the CALIS procedure in SAS was used for structural equation modeling (SAS Institute 1989).

RESULTS

Average values for the floral traits, fitness components, and total fitness in the three years of the study are shown in Table 2. The floral traits were very similar across the years, except that petals were longer in 1991 and pistils and stigma exertion were longer in 1993. Because the 1993 plants were each in the field for only six days, flower, fruit and total seed production were less in this year. Fruit production was less in 1991 than 1992, probably because of the 1991 plants growing late in the season, although the 95% confidence intervals overlapped. The number of fertilized seeds per fruit was greater in 1991 than the other two years, whereas the proportion of fertilized seeds that were viable was less in 1991 than 1992. These fitness components combined show lower total lifetime female fitness (seed production) in 1991 than 1992, although again the confidence intervals overlapped.

TABLE 2. Means and 95% confidence intervals (CI) for the floral traits, fitness components, and total fitness. Petal and pistil length are shown to represent floral size. Linear dimensions are in mm, the rest are counts. In 1993, values for flower production through total number of seeds are for the six field days only (see Methods).

	1991		1992		1993	
	Mean	95% CI	Mean	95% CI	Mean	95% CI
Petal length	8.7	(8.5, 9.0)	8.0	(7.8, 8.3)	7.9	(7.8, 8.1)
Pistil length	11.3	(11.0, 11.6)	11.3	(11.0, 11.6)	12.4	(12.1, 12.6)
Stigma exertion	1.5	(1.2, 1.8)	1.4	(1.1, 1.7)	2.2	(2.0, 2.4)
Ovule number	7.1	(6.7, 7.5)	7.8	(7.5, 8.2)	7.7	(7.5, 8.0)
Flower production	49.1*	(46.0, 52.0)	683†	(553, 843)	156	(149, 162)
No. fruit	265†	(196, 345)	418†	(331, 528)	98.8	(93.4, 104)
Fertilized seeds per fruit	4.0	(3.7, 4.3)	3.2	(3.0, 3.4)	3.5	(3.3, 3.6)
Viable seeds per fertilized seed	0.56	(0.50, 0.61)	0.70	(0.65, 0.75)	—	—
Total no. of seeds	625†	(428, 858)	867†	(650, 1157)	347‡	(323, 371)
N	57		60		112	

* Number of days from first to last flower.

† Due to non-normality, transformed and then back-transformed to original scale.

‡ Number of fertilized seeds.

Pollinator Visitation

Our experimental populations received high levels of visitation (Table 3). The lowest levels were in 1991, with an average of 20.4 visitors per plant per hour. The 1992 and 1993 visitation rates were similar to each other and two to three times higher than in 1991. The lower rates of visitation in 1991 were due largely to the fact that the observations took place late in the season. The taxonomic composition of the pollinators was also different in 1991 compared to the other two years. In 1992 and 1993 the pollinator assemblage was dominated by small bees and syrphid flies in similar numbers, whereas in 1991 the majority of pollinators were honey bees, with the remainder being small bees. Honey bees are not normally common in small plant populations such as ours (Schaffer et al. 1979; Sih and Baltus 1987; Sowig 1989; Stanton et al. 1992; Jennersten and Nilsson 1993; Conner and Neumeier 1995). The large proportion of honey bees in 1991 may have been due to reduced numbers of other plant species flowering in the fall.

Selection Gradients for Total Fitness

The selection gradients showed relatively consistent patterns of selection on the floral traits across all three years of the study (Table 4). The regression models were highly significant in all three years, with the five floral traits together explaining about 80% of the variance in lifetime female fitness in 1991 and 1992, and 43% of the variance in female fitness in 1993. In all three years, the strongest selection was directional selection for increased flower production. The standardized selection gradient for this trait was approximately two to three times greater than the next largest selection gradient. The gradient of 0.70 for flower production in 1991 means that an increase in flower production of 1 SD was associated with a 70% increase in relative fitness.

After flower production, the trait under the strongest selection was ovule number (Table 4). There was directional selection for increased ovule number in all three years, but the selection gradient for this trait was not significant in 1992. The only strong selection on floral morphology was directional selection for increased flower size in 1992. There was some evidence for directional selection for increased stigma exertion in 1992 and disruptive selection on flower size in 1991, but these were only marginally significant. The quadratic term for stigma exertion was close to significantly negative in 1993, but removal of one influential point (of 112) changed the significance of this selection gradient to 0.38. Therefore, there was no strong evidence for stabilizing selection on stigma exertion. The lack of selection on stigma exertion also meant that there was no selection on stigma-anther separation, because these traits were highly correlated (see Methods).

Path Analysis

The path analyses to help determine the causes of selection and the relative importance of different fitness components showed remarkably consistent results across the three years (Fig. 1). As expected from the selection gradient results, flower production was the most important trait in all years. Flower

TABLE 3. Rates of pollinator visitation and taxonomic composition of floral visitors in the three years of the study. In two cases, syrphid flies in 1991 and honey bees in 1993, the rates of visitation were less than 3% of all visits; thus, they are lumped in the Other category. The remainder of the Other category consisted mainly of Lepidoptera and bumble bees.

	1991		1992		1993	
	# visits per plant per hour (Mean \pm SD)	% of all visits	# visits per plant per hour (Mean \pm SD)	% of all visits	# visits per plant per hour (Mean \pm SD)	% of all visits
Small bees	6.4 \pm 10.8	32	23.6 \pm 19.3	40	26.6 \pm 21.2	55
Syrphid flies	—	—	23.5 \pm 20.0	40	18.4 \pm 18.3	38
Honey bees	11.3 \pm 17.4	55	3.2 \pm 4.9	6	—	—
Other	2.7 \pm 2.3	13	8.1 \pm 10.4	14	3.3 \pm 7.0	7
Total	20.4 \pm 20.2	100	58.4 \pm 32.9	100	48.3 \pm 27.7	100
Number of hr observed	151		78		198	

production was the main determinant of fruit set but had little effect on the number of fertilized seeds per fruit. In 1992, flowering span and the number of branches produced were highly positively correlated. After correcting for each other, only the number of branches was important in determining total flower production.

Ovule number was the next most important trait, also in agreement with the selection gradients. Ovule number had a consistently positive effect on the number of fertilized seeds per fruit but no consistent effects on fruit production. Increased flower size was associated with increases in both number of fruit and the number of fertilized seeds per fruit in 1992. The latter relationship was stronger than the former.

In 1993, increased flower number caused a significant increase in visitation by small bees but only a nonsignificant increase in syrphid fly visitation. Rates of visitation by the two taxa were uncorrelated with each other and had no significant effects on any of the fitness components.

The number of fruit produced was by far the most important of the fitness components in determining total seed production. Therefore, the principle path determining total female fitness in all three years was flower production to fruit production to seed production. Note, however, that the other two fitness components also had significant positive effects on total seed production in all cases, and the number of fertilized seeds per fruit was very important in 1993. Finally, fitness components were not strongly correlated

with each other, except for positive correlations between fruit production and number of fertilized seeds per fruit in 1991 and 1993, and between fruit production and the proportion of viable seeds in 1991. This indicates that there were no trade-offs among fitness components at the phenotypic level. The lack of correlation was due in part to how the multiplicative fitness components were defined, with the denominator of each being the numerator of the preceding component.

DISCUSSION

The main patterns of selection on floral traits in wild radish revealed by the selection gradients (Table 4) were relatively consistent across years: there was strong selection for increased flower production in all three years, selection for increased ovule number in two of the three years, and selection for increased flower size in one year. The causes of this selection and the determinants of fitness revealed by the path analysis were very consistent (Fig. 1): increased flower number increased fruit number, which in turn was the dominant cause of increased female fitness in all years. Increased ovule number increased the number of fertilized seeds per fruit in all years. This consistency existed in spite of a number of differences among the three study years. First, in 1991, the composition of pollinators was different, as were a variety of other environmental factors, because

TABLE 4. Standardized selection gradients for the floral traits. The results for each year represent a separate multiple regression of relative total fitness on the variables listed below. The fitness measure (dependent variable) was lifetime production of viable seeds in 1991 and 1992, and the total number of fertilized seeds produced in the 6 d of pollination in 1993. Significance levels and R^2 in 1991 and 1992 are from a regression using \ln -transformed fitness values, but selection-gradient values are from untransformed relative fitness. Ovule number was per flower. The flower production variable was number of d flowering in 1991, the total number of flowers produced over the plant's lifetime in 1992, and the total number of open flowers on the 6 d of pollination in 1993. The flower size² and stigma exertion² rows give the quadratic terms for stabilizing selection on these traits.

	1991		1992		1993	
	β or γ	P	β or γ	P	β or γ	P
Flower size	0.04	0.85	0.12	0.0007	-0.01	0.78
Flower size ²	0.10	0.03	0.19	0.53	0.03	0.61
Stigma exertion	-0.02	0.81	0.02	0.04	-0.03	0.29
Stigma exertion ²	0.10	0.44	-0.04	0.76	-0.07	0.09
Ovule number	0.23	0.0008	0.11	0.30	0.07	0.03
Flower production	0.70	< 0.0001	0.70	< 0.0001	0.17	< 0.0001
Total R^2	0.81	< 0.0001	0.84	< 0.0001	0.43	< 0.0001
N	57		60		112	

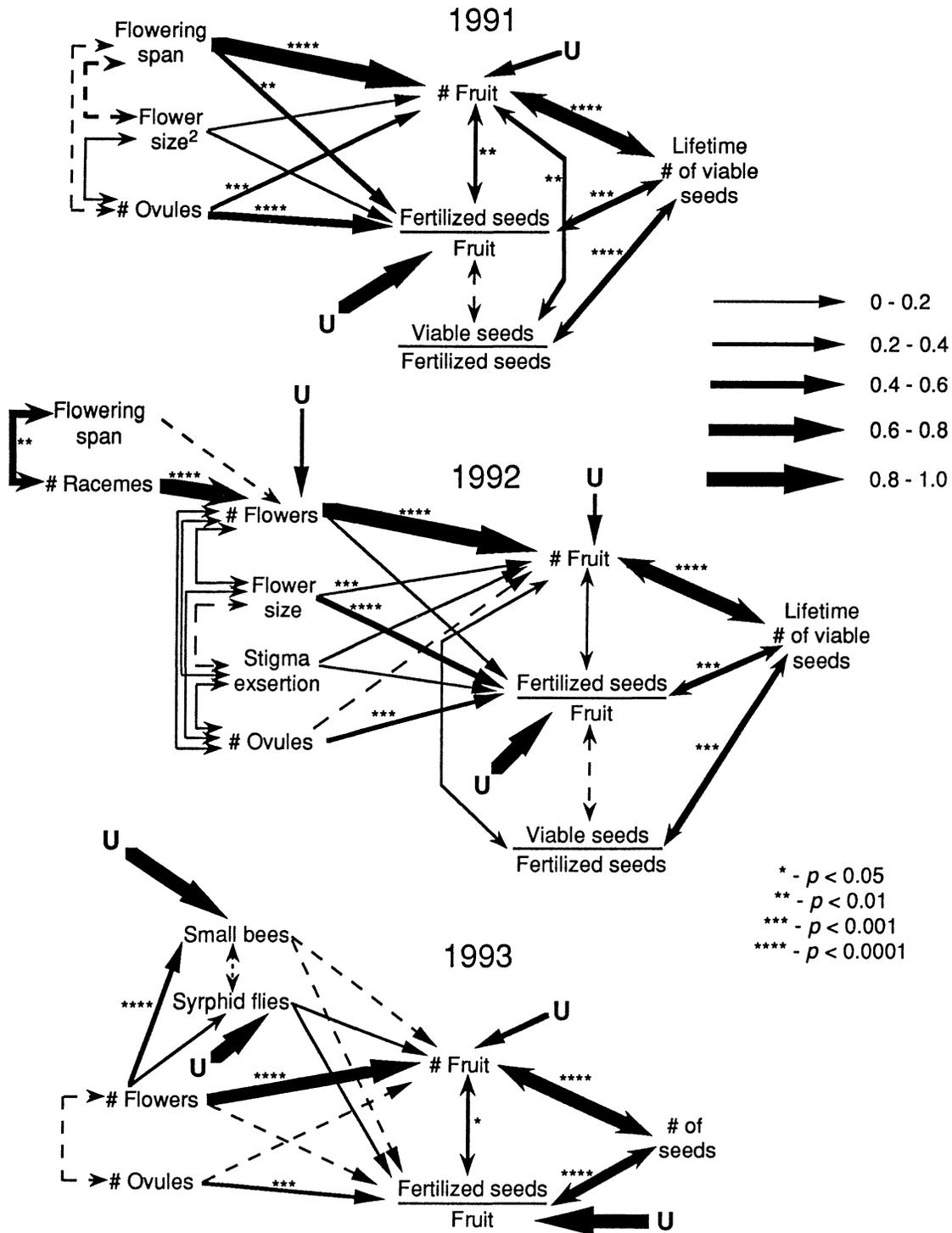


FIG. 1. Path diagrams for the three years of the study. Correlations are depicted as double-headed arrows, and causal relationships as single-headed arrows. Dashed arrows denote negative coefficients, and arrow width is proportional to the standardized coefficients (see scale). The U terms are unexplained variance. The 1993 path model included six dummy variables to correct for group membership (not shown for clarity); each dummy variable had a causal arrow leading from it to small bee visitation, syrphid fly visitation, number of fruit produced, and fertilized seeds per fruit. Sample sizes were 57, 60, and 112 for the three years, respectively.

the 1991 study was conducted in the fall rather than the summer. Second, 1992 was much wetter than 1991 (15.8 cm vs. 8.3 cm average monthly rainfall during the period of most growth and flower production in each year). Finally,

the 1993 study used potted plants that spent most of their lifetime in the greenhouse. Together, these points indicate that the patterns seen are robust and may be generalizable (see below).

Selection on Morphology

The cause of the selection for increased flower size in 1992 is not obvious. Larger flowers do attract more pollinators (Conner and Rush 1996), but female reproduction is probably not strongly limited by pollination success (Stanton et al. 1986; Rush et al. 1995; Pfennig and Conner, unpubl.; Fig. 1). The selection for larger flowers is not due to increased ovule number, because this was corrected for in the regression and path analyses. As in any phenotypic-selection analysis, this selection may be due to unmeasured correlations, including environmental correlations between flower size and fitness (Price et al. 1988; Rausher 1992; see below).

There was little other strong evidence for selection on floral morphology. The *P*-values were above 0.28 for two-thirds of the morphology selection gradients (Table 4). One of the exceptions to this was the quadratic term for stigma exertion in 1993, but this *P*-value of 0.09 was due to one influential point. Therefore, it seems safe to conclude that there was no stabilizing selection on stigma exertion, contrary to the hypothesis outlined above. This conclusion is in agreement with inferences made from patterns of correlations among floral traits in wild radish (Conner and Via 1993; Conner and Sterling 1995). There was some suggestion of weak selection for increased stigma exertion in 1992 and disruptive selection on flower size in 1991, but these were not corroborated by the path analyses (Fig. 1).

Other studies have found significant selection on floral morphology through female fitness (e.g., Campbell 1989; Galen 1989; Schemske and Horvitz 1989; Campbell 1991; Johnston 1991; Herrera 1993), and in agreement with our results, most of these studies did not find consistently significant selection across years or sites (Campbell 1989; Schemske and Horvitz 1989; Campbell 1991; Johnston 1991).

Importance of Flower and Fruit Number

Another key result of this study was the strong selection for increasing flower number (Table 4). The overwhelming importance of flower number in determining female fitness in wild radish is mirrored in results from numerous studies (e.g., Herrera 1991; Johnston 1991; Campbell and Halama 1993; Herrera 1993; Mitchell 1994). Given this strong directional selection to increase flower number, the question of whether this trait is evolving in natural populations becomes important. Genetic variance for total flower production of natural populations has not been measured very frequently, but two recent field studies have shown low heritability for flower number (Mazer 1987; Schwaegerle and Levin 1991). Mazer (1987), however, found a significant sire effect on flower number in one of her crosses of *Raphanus raphanistrum*, suggesting that there was additive genetic variance for this trait. Although much of the variability in flower number is therefore due to the environment in field studies, even a small amount of genetic variability would result in a relatively rapid response resulting from the very strong selection.

The strong selection on flower number was due in large part to the overwhelming importance of fruit production in determining total seed set, as compared to the two other fitness components, number of seeds per fruit and number of viable seeds per fertilized seed (Fig. 1). The relative impor-

tance of fruit production is in turn due largely to the much greater variability in this fitness component compared to the other two components (Table 2). In 1993, there was not as much variability in fruit production, because the plants were raised in the greenhouse and only exposed to pollinators for a total of six days over their lifetimes. This is at least part of the reason for the lower proportion of variance explained in that year (Table 4) and the more equal importance of the two fitness components measured in that year (Fig. 1).

Our 1992 path analysis shows that flower production was closely related to plant size, measured as the number of branches. Thus plant size is an important determinant of female fitness through flower and fruit number in wild radish (see also Mazer 1987). Studies of other species have also found evidence for size as an important determinant of lifetime female fitness (e.g., Farris and Lechowicz 1990; Dudash 1991; Herrera 1991; Mitchell 1994 and references therein).

Pollinator Visitation

The path analysis for 1993 and the similarity between years with different pollinators strongly suggest that variability in pollinator visitation has little effect on variation in female fitness in our populations. Another study of *R. raphanistrum* reached a similar conclusion (Stanton et al. 1986), but studies of the closely related *R. sativus* have found relationships between visitation and female fitness (Young and Stanton 1990; Stanton et al. 1991).

Caveats

As with any study of selection on phenotypes, our results could be biased by two factors: selection on unmeasured traits that are correlated with the measured traits (Lande and Arnold 1983; Endler 1986; Mitchell-Olds and Shaw 1987; Crespi and Bookstein 1989) and environmental correlations between fitness and the traits (Price et al. 1988; Rausher 1992). Both traits found to be under consistently strong selection, flower and ovule number, set an upper limit on the fitness components that they were found to affect most strongly, fruit number and seeds per fruit, respectively. Thus, some causal relationship seems likely. However, on the average, only 63% of the flowers set fruit and from 42–57% of the ovules were fertilized. Thus, it is possible that factors other than flower and ovule number affected fruit number and fertilized seeds per fruit. It could be that flower or ovule production is influenced by some unmeasured environmental factor or phenotypic trait that is the true cause of differences in fitness. If the unmeasured variable is related to general health or resource availability to the plant, it seems likely that it would affect all fitness components. However, the fact that flower and ovule production were each related strongly to different fitness components, but not all fitness components (Fig. 1), argues against the presence of an unmeasured general health or resource factor. In addition, we randomized the positions of individuals over a relatively homogenous garden environment, reducing the likelihood of phenotype-environmental correlation (Mitchell-Olds and Shaw 1987).

Another potential source of bias is our use of artificial populations. Clearly, a complete understanding of evolution through natural selection requires studies of natural popu-

lations. In this context, however, it is all the more striking that there was much less evidence for selection on floral morphology than for selection on flower and ovule number. The latter are likely to be more sensitive to environmental variability than floral morphology (Schwaegerle and Levin 1991; Conner and Via 1993; Frazee and Marquis 1994). In our evenly spaced gardens with reduced herbivory, we probably reduced variation in fitness that was random with respect to genetic variation for floral morphology, potentially increasing our ability to detect selection on these traits.

Future Directions

Our finding of little selection on floral size and shape is surprising in light of the diversity of flowers in the Brassicaceae. One possibility that has often been suggested (e.g., Bell 1985; Stanton et al. 1986) is that selection on these traits is mainly through differences in male fitness. However, selection estimates based on male fitness (seeds sired) by these same plants revealed no selection on floral morphology (Conner et al. 1996). We also did not measure selection through differential survivorship before flowering. It is possible that indirect selection on a trait genetically correlated with flower size or shape could cause the evolution of these traits. Another possibility is that the floral traits have reached evolutionary equilibrium at an adaptive peak, such that there is little current selection on these traits. One way to test for this possibility would be to measure selection on plants that have been manipulated to produce floral trait values outside the current population range. If the equilibrium hypothesis is correct, then selection against the artificially produced extremes should be detectable.

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