Measurements of selection on floral traits in black mustard, *Brassica nigra*

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Introduction

The large diversity of floral displays in angiosperms has been attributed largely to natural selection exerted by animal pollinators (Darwin, 1859; Grant and Grant, 1965; Waser, 1983). Quantitative measurements of selection on floral traits have only been made recently, however, and only on a handful of plant species (Campbell, 1989; Galen, 1989; Schemske and Horvitz, 1989; Campbell et al., 1991; Johnston, 1991; Herrera, 1993; Conner et al., 1996a, b). Even fewer studies have used lifetime fitness data for selection measurements (Campbell, 1991; Conner et al., 1996a, b), in spite of the fact that selection measurements based on only part of an organism’s reproductive life can be misleading (Lande and Arnold, 1983; Arnold and Wade, 1984; Endler, 1986; Travis and Heinrich, 1986; Clutton-Brock, 1991). Here we report measurements of selection on floral traits based on lifetime female fitness measurements in black mustard, *Brassica nigra* (Brassicaceae).

Several studies have reported directional selection for increased flower size and/or number (Galen, 1989; Campbell et al., 1991; Johnston, 1991; Herrera, 1993; Conner et al., 1996a, b); these traits are important in pollinator attraction (e.g., Clements and Long, 1923; Willson and Ratcliffe, 1974; Willson and Bertin, 1979; Thomson et al., 1982; Bell, 1985; Geber, 1985; Schmid-Hempel and Speiser, 1988; Thomson, 1988; Eckhart, 1991; Conner and Rush, 1996), and may also be important determinants of pollen and ovule production. Flower number is particularly important in determining both male and female fitness of plants (e.g., Schoen and Stewart, 1986; Broyles and Wyatt, 1990; Devlin and Ellstrand, 1990; Herrera, 1991, 1993; John-

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ston, 1991; Devlin et al., 1992; Campbell and Halama, 1993; Mitchell, 1994; Conner et al., 1996a, b; but see Meagher, 1991). Flower size has been hypothesized to be under stabilizing selection to match the sizes of pollinators (Berg, 1960; Conner and Sterling, 1996), but direct evidence for this is scarce (Schemske and Horvitz, 1989; Conner et al., 1996a, b; but see Herrera, 1993).

In a previous study, we reported selection for increased flower size and number through differences in female fitness in wild radish (*Raphanus raphanistrum*). The principal goal of the study reported here was to measure selection on flower size and number in black mustard, which is closely related to wild radish. The pollinator fauna of black mustard and wild radish are extremely similar in our area (Conner and Rush, unpubl.), but black mustard has much smaller flowers that are produced in far greater numbers than wild radish. Therefore, a comparison of the strength of selection on flower size and number in these two species should provide useful insights into past and current selection on these important traits.

**Methods**

Black mustard is a self-incompatible annual weed of roadsides and disturbed areas. This species is pollinated by a variety of bees and syrphid flies, and the taxonomic composition of this pollinator fauna varies greatly on short temporal and spatial scales (Conner and Neumeier, 1995).

This study was conducted at the Phillips Tract Natural Area near Urbana, IL, the site of our previous wild radish studies (Conner et al., 1996a). Black mustard seeds were collected in 1991 along transects in a population about 4.5 km from the study site. Seeds from 64 of these field mothers were planted on 13 July 1992 in an 8 x 8 grid with 1-m spacing. The first plant flowered on 2 August; a total of 42 plants had flowered by 23 September and were included in the study. Thirteen of the plants stopped flowering by 30 October, and the rest were killed by frost on 4 November. The peak flowering season for black mustard in our area is mid-June to early September. Therefore, this study was conducted late in the natural flowering period, but the pollinators were all taxa that naturally visit black mustard in our area.

Six floral traits were measured on from 1 to 3 flowers per plant. Measurements were conducted from 13 August to 13 October. The traits measured were the length and width of the outer portion of the petal (the limb), the length of the inner portion of the petal (the claw), the lengths of one of the long filaments and one of the short filaments, and the length of the entire pistil (see Conner and Via, 1993 for details of traits and measurements). The number of ovules was also counted on each flower measured. For plants in which more than one flower was measured, the averages for each trait across all flowers were used.

After the plants died, they were brought to the laboratory for estimates of total flower, fruit, and seed production. These numbers were estimated instead of directly counted because each plant produced thousands of flowers and fruits (Tab. 1). Forty racemes (flowering stalks) were randomly sampled from each plant, and the
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The total number of pedicels (number of flowers) and fruits on these 40 were counted. The lengths of each of the 40 racemes were also measured to the nearest mm with a ruler. These data were used to calculate an average number of flowers/mm of raceme and an average fruit set (=fruits/# flowers) for each plant. The lengths of all racemes on each plant were then measured, and the product of flowers/mm and the total length of racemes was used as an estimate of total flower production. This flower number estimate was multiplied by fruit set to give an estimate of total fruit production.

As a second estimate of fruit production, 100 fruits were randomly sampled from each plant. These fruits were air-dried and weighed to the nearest 0.0002 g on an analytical balance, and this number divided by 100 to give an average weight per fruit. All the fruits produced by each plant were then weighed, and this total fruit weight was divided by the average weight/fruit for the second estimate of fruit number. The correlation between the two estimates was 0.97, so the fruit number estimates are reliable. The direct count method was used in the analyses rather than the weight method, because the former should be more accurate at low fruit number than the latter (since most or all fruits were directly counted in that case).

To estimate seed number, all seeds from the 100 randomly sampled fruits were counted. The average number of seeds/fruit was then calculated, and then multiplied by the total number of fruits to produce an estimate of the total number of seeds produced by each plant.

### Analyses

Since the six floral dimensions measured were all positively correlated (Conner and Sterling, 1995), two new variables were created for selection analyses. First, the geometric mean of all six dimensions was used as a measure of overall flower size (Mosimann and James, 1979); the geometric mean was very highly correlated with the first principal component of the six traits (*r* = 0.998). Second, stigma exertion was calculated as the difference between the pistil and claw lengths (Conner et al., 1995).

<table>
<thead>
<tr>
<th>Trait</th>
<th>Mean</th>
<th>s.d.</th>
<th>c.v</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flower size (mm)</td>
<td>4.48</td>
<td>0.41</td>
<td>0.09</td>
</tr>
<tr>
<td>Stigma exertion (mm)</td>
<td>1.88</td>
<td>0.44</td>
<td>0.41</td>
</tr>
<tr>
<td>Ovules/flower</td>
<td>7.54</td>
<td>1.19</td>
<td>0.16</td>
</tr>
<tr>
<td>Flower number</td>
<td>5.357</td>
<td>3.752</td>
<td>0.70</td>
</tr>
<tr>
<td>Flowering time</td>
<td>20.1</td>
<td>12.5</td>
<td>0.61</td>
</tr>
<tr>
<td>Number of fruit</td>
<td>41,10</td>
<td>2,976</td>
<td>0.72</td>
</tr>
<tr>
<td>Seeds/fruit</td>
<td>110</td>
<td>176</td>
<td>0.41</td>
</tr>
<tr>
<td>Total seed number</td>
<td>14,759</td>
<td>13,461</td>
<td>0.91</td>
</tr>
</tbody>
</table>

*Table 1. Means, standard deviations, and coefficients of variation for all variables. Flower size is the geometric mean of six dimensions measured (see Methods), and flowering time is the number of days from planting until the first flower opened.*
Studies of other species have shown that maximum pollen deposition tends to occur with low or intermediate exsertion (see Conner et al., 1995 for a review). While black mustard does not have a tubular corolla, stigma exsertion still provides a good measure of the height of the stigma above the petal limb. Since the limb is used as a landing platform by pollinators, stigma exsertion may be important in successful pollen receipt from pollinators.

Selection gradients (Lande and Arnold, 1983) were calculated by regressing relative fitness (seed production) on flower number, flower size, stigma exsertion, ovule number, and flowering time (defined as the number of days from planting to opening of the first flower). Linear terms were fit for all variables to test for directional selection, and quadratic terms were fit for flower size and stigma exsertion to test for stabilizing selection on these traits (see Introduction). Type III SS were used for all significance tests. All predictor variables were standardized to mean = zero and variance = one before analysis, so the resulting partial regression standardized selection gradients. All variance inflation factors (Neter et al., 1985) were less than two, indicating no collinearity problems, and inspection of the residuals revealed no serious heteroscedasticity. The sample size was smaller than the rule of thumb of at least ten observations per variable (Mitchell, 1993); however, re-analysis using only the three significant variables (see Results) gave virtually identical results.

To examine possible causes of selection, a path analysis (Wright, 1968; Kingsolver and Schemske, 1991; Mitchell, 1993) was performed that related the floral traits to two multiplicative components of fitness, and in turn related the components to total fitness (Conner, 1996). Only floral traits for which selection gradients had P-values < 0.20 were included because of the limited sample size; 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. Adding the non-significant traits did not alter the results appreciably. Two multiplicative components of fitness were examined: number of fruit and number of seeds per fruit. These multiply to equal total fitness, that is, total seed production.

Causal paths were fit using multiple regression between all floral traits and the two fitness components to see which of the fitness components was affected by each trait. Paths between the two fitness components and total fitness were estimated using separate simple regressions to determine which component was a stronger determinant of total fitness (Conner, 1996).

Results

Descriptive statistics for the measured variables are given in Table 1. Plants in our study produced thousands of flowers and seeds. On average, 77% of flowers produced a fruit but only 41% of the ovules in these flowers produced a seed. Flower, fruit, and total seed number exhibited the highest coefficients of variation of all the variables, with flower size and number of ovules per flower showing the lowest variation.
Table 2. Standardized selection gradients for the floral traits, calculated from a multiple regression of relative female fitness (lifetime seed production) on the traits listed. $\beta$ refers to the directional selection gradients from the linear terms in the regression. Squared terms represent quadratic terms testing for curvature in the fitness function and possible stabilizing or disruptive selection ($\gamma$).

<table>
<thead>
<tr>
<th>Trait</th>
<th>$\beta$ or $\gamma$</th>
<th>s.e.</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flower size</td>
<td>-0.01</td>
<td>0.05</td>
<td>0.83</td>
</tr>
<tr>
<td>Flower size$^2$</td>
<td>0.04</td>
<td>0.06</td>
<td>0.52</td>
</tr>
<tr>
<td>Stigma exsertion</td>
<td>0.09</td>
<td>0.05</td>
<td>0.06</td>
</tr>
<tr>
<td>Stigma exsertion$^2$</td>
<td>0.02</td>
<td>0.07</td>
<td>0.83</td>
</tr>
<tr>
<td>Ovules/flower</td>
<td>0.05</td>
<td>0.05</td>
<td>0.30</td>
</tr>
<tr>
<td>Flower number</td>
<td>0.75</td>
<td>0.05</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Flowering time</td>
<td>-0.16</td>
<td>0.05</td>
<td>0.004</td>
</tr>
</tbody>
</table>

$R^2$ = 0.91

The five traits together explained 91% of the variance in total female fitness (Tab. 2). Extremely strong directional selection for increased flower number occurred; the standardized selection gradient of 0.75 indicates that an increase of one standard deviation in flower number was associated with a 75% increase in relative fitness. Weaker directional selection for decreased flowering time was detected. There was no evidence for selection on the other traits, with the possible exception of weak directional selection for increased stigma exsertion.

The path analysis revealed that the selection for increased flower number was mainly caused by increased fruit production rather than increased number of seeds per fruit (Fig. 1). Increases in flower number strongly increased fruit number, which

Fig. 1. Path diagrams relating the floral traits, multiplicative fitness components, and total female fitness (no. of seeds). 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 represent unexplained variance in dependent variables. $N = 42.$
in turn was the main determinant of total female fitness (number of seeds). Increased stigma exsertion was also related to fruit production rather than seeds per fruit. Conversely, flowering time was only related to the number of seeds per fruit; plants that flowered later had lower numbers of seeds per fruit, but did not have significantly decreased fruit production.

Discussion

The strongest selection found in our study was the strong directional selection for increased flower number. The importance of flower number is in agreement with results from our study of wild radish and studies of a variety of other species (reviewed in Conner et al., 1996a). In fact, the standardized selection gradient of 0.75 was very similar to the standardized selection gradient of 0.70 for wild radish in the same year at the same site (Conner et al., 1996a). There was absolutely no evidence for selection for increased flower size, in contrast to our results for wild radish in which strong selection for increased flower size was found in one of three years. It is possible that selection for increased flower size in black mustard might be detected if the study was repeated in additional years or in other locations.

It is tempting to speculate that this pattern of selection for increased flower size in wild radish but not in black mustard may have also occurred in the past, and was at least partly responsible for the current differences in flower size between these species (the geometric mean of the same floral traits is almost twice as great in wild radish). However, current patterns of selection cannot explain the differences between the two species in flower number: the selection gradients for flower number were very similar across the two species, while the average number of flowers produced by black mustard was almost eight times that produced by wild radish. Note that these conclusions might be altered if selection through differences in male fitness was measured in black mustard, as was done in wild radish (Conner et al., 1996b).

The path analyses of the selection for increased flower number show similar patterns in the two species. In both wild radish and black mustard, flower number had strong effects on fruit number but little or no effect on seeds per fruit, and fruit number was the main determinant of total seed production (Fig. 1; Conner et al., 1996a). The strong relationship between flower and fruit number could be due to two non-mutually exclusive causes: increased flower number could increase pollinator visitation, and/or limited flower number could limit fruit production directly. Pollinator visitation does not seem to affect female fitness in wild radish (Stanton et al., 1986; Conner et al., 1996a). Black mustard plants in our study were heavily visited by pollinators (Conner and Rush, unpubl.), suggesting that seed production was not pollen-limited, but our pollinator observations were not extensive enough to enable us to relate visitation directly to total fitness. On average, over 75% of flowers set fruit, suggesting that flower number may have directly limited fruit production in our study.

There was weak evidence for directional selection for increased stigma exsertion (Tab. 2, Fig. 1), in contrast with predictions based on pollen deposition in other
Measurements of selection on floral traits in black mustard, *Brassica nigra* species (see Conner et al., 1995). Weak evidence for selection for increased stigma exsertion was also found in wild radish in 1992 (Conner et al., 1996a). Stigma exsertion was highly correlated with stigma-anther separation in our study population ($r = 0.72$, $P < 0.0001$), so the positive selection on stigma exsertion could be due to reduced stigma clogging by self pollen (Galen et al., 1989). Alternatively, this selection could be due to increased outcross-pollen deposition due to better contact of the stigma with pollinators, or to a combination of both, i.e. a higher ratio of outcross to self-pollen (e.g., Thomson and Stratton, 1985). Studies of pollen deposition patterns that focus on these alternatives in black mustard would be useful. If either of these hypotheses are true, they suggest that pollen deposition is more important to fruit production than to the number of seeds per fruit, because the path analysis showed that increased stigma exsertion affected the former but not the latter fitness component.

We found no evidence for selection for increased ovule number per flower, in contrast to our results in wild radish (Conner et al., 1996a). It is somewhat surprising that ovule number per flower had no effect on total seed production. This result might be explained by the fact that variance in ovule number per flower was much less than the variance in flower number (Tab. 1); consequently, the principal determinant of the total number of ovules produced by a plant is flower number rather than the number of ovules per flower.

The directional selection for earlier flowering time (Tab. 2) was mainly due to the increases in the number of seeds per fruit rather than increased fruit production (Fig. 1). The inability of plants that flowered later to produce as many seeds per fruit may have been due to deteriorating environmental conditions late in the season, which were exacerbated by the study being done late in the normal flowering season.

In summary, we found strong selection for increased flower number in black mustard, in agreement with our earlier work on wild radish. In contrast to the wild radish results, however, there was no selection on flower size in *B. nigra*. Increased flower number increased fruit production but had no significant effect on the number of seeds per fruit. Future studies could further examine the causes of the relationship between flower number and fruit production, and determine whether additive genetic variance for flower number exists.

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