

TESTING FOR CONFLICTING AND NONADDITIVE SELECTION: FLORAL ADAPTATION TO MULTIPLE POLLINATORS THROUGH MALE AND FEMALE FITNESS

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Received April 5, 2009

Accepted December 16, 2010

Although conflicting selection from different resources is thought to play a critical role in the evolution of specialized species, the prevalence of conflicting selection in generalists is poorly understood. Plants may experience conflicting selection on floral traits by different pollinators and between genders. Using artificial selection to increase phenotypic variation, we tested for conflicting and nonadditive selection on wild radish (*Raphanus raphanistrum*) flowers. To do this, we measured selection by each of the major pollinator taxa through both male and female fitness, and tested for a single-generation response to selection by a subset of these pollinators. We found some evidence for conflicting selection on anther exertion—sweat bees exerted stabilizing selection and larger bees selected for increased exertion. Stamen dimorphism was only under selection by honey bees, causing a response to selection in the next generation, and flower size was under similar selection by multiple pollinators. Selection differed significantly between genders for two traits, but there was no evidence for stronger selection through male fitness or for conflicting selection between genders. Our results suggest wild radish flowers can adapt to multiple pollinators, as we found little evidence for conflicting selection and no evidence for nonadditive selection among pollinators.

KEY WORDS: Floral evolution, generalization, natural selection on expanded variation, pollination, *Raphanus raphanistrum*, specialization, trade-offs.

Janzen's seminal paper, "When is it coevolution" (Janzen 1980), emphasized that selection on traits is likely more complex than simple pairwise interactions. Instead, evolution is diffuse when the selection on a given trait is dependent upon the broader community context in which a species is found (Hougen-Eitzman and Rausher 1994; Iwao and Rausher 1997; Inouye and Stinchcombe 2001). The majority of studies of diffuse evolution have measured mean fitness of the interacting species (Strauss and Irwin

2004); this does not provide any information about natural selection, defined as the slope of the relationship between fitness and a particular phenotypic trait (Strauss et al. 2005). Diffuse selection, where this slope changes depending on which species of selective agent are present, has been documented mainly in plant-herbivore systems (e.g., Pilson 1996; Juenger and Bergelson 1998; Stinchcombe and Rausher 2001) and in systems involving plant mutualists and antagonists (e.g., Gomez 2003; Cariveau et al. 2004; Irwin et al. 2004).

There are at least four nonmutually exclusive ways in which multiple selective agents may affect trait evolution. First,

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adaptation in a heterogeneous environment may be constrained if conflicting selection by different selective agents causes a trade-off, whereby an adaptation that is beneficial in the presence of one selective agent is deleterious in the presence of another (e.g., Irwin 2006; Lankau 2007; Gomez 2008). In an extreme example of conflicting selection, exactly opposing selection gradients could lead to no net selection on a trait (Fig. 1A). Second, multiple selective agents may select on the same trait in the same manner (Fig. 1B); thus, a trait may be adapted to multiple selective agents, and diffuse evolution does not occur. We refer to this as the common adaptive peaks hypothesis. Third, an organism may possess trait specializations, that is, traits that are adapted for a subset of the selective agents. This would occur if different selective agents exert selection on different traits (Fig. 1C). Therefore, a generalist could be well adapted to multiple selective agents simultaneously through different traits.

Fourth, selection by different agents may be nonadditive. Nonadditive selection occurs if the presence of one species alters the “selection” imposed by another species (Fig. 1D). In this case, selection is an emergent property of the community and cannot be predicted based on knowledge of pairwise selection gradients alone. Nonadditive selection is measured by comparing the selection imposed when multiple species co-occur with the selection imposed by each species when it occurs by itself. If selection is additive, the total selection exerted on a trait when multiple selective agents are present can be predicted by averaging the selection exerted by each selective agent (Strauss et al. 2005). Thus far, the few tests for nonadditive selection have all used plant-herbivore/seed predator systems (e.g., Pilson 1996; Juenger and Bergelson 1998; Stinchcombe and Rausher 2001, 2002; see Strauss et al. 2005 for a review).

In addition to selection through different selective agents, selection through male and female fitness can differ for a given trait. This can occur with separate sexes, leading to sexual dimorphism, but conflicting selection across genders can be harder to resolve in hermaphrodites. Quantitative genetic theory suggests that selection on floral attraction traits in hermaphrodites should be of opposite signs through male and female function at equilibrium (Morgan 1992), that is, selection should be conflicting across genders. A related question is whether selection on floral traits in hermaphroditic plants is stronger through male versus female fitness; this question has been debated for more than three decades (Willson and Rathcke 1974; Willson and Price 1977; Willson 1979; Willson and Bertin 1979; Willson and Burley 1983; Bell 1985; Morgan and Schoen 1997; Burd and Callahan 2000; Ashman and Morgan 2004). Sexual selection theory suggests selection should be stronger through male fitness (Bateman 1948), but selection through female fitness can be strong if seed set is pollen limited (Wilson et al. 1994; Burd and Callahan 2000; Ashman and Morgan 2004), which it often is in na-

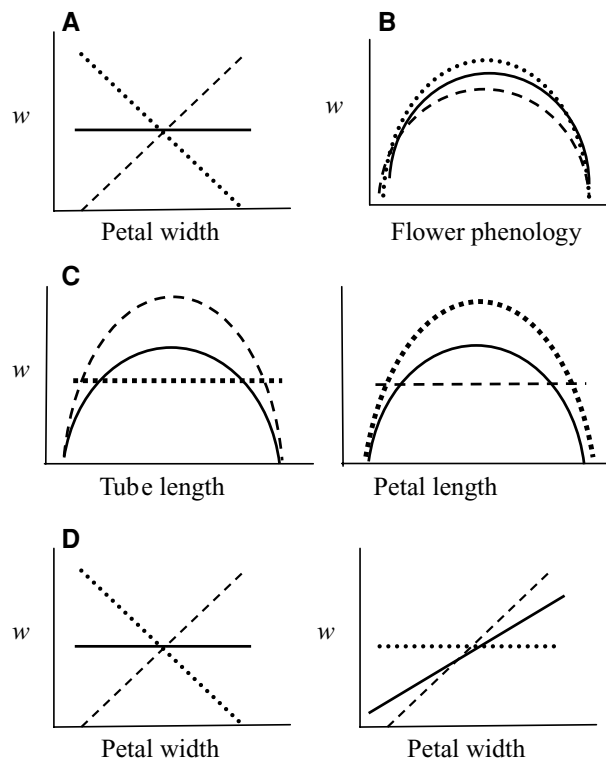


Figure 1. Four hypotheses on how different pollinators influence selection on floral traits. Dashed lines represent selection by individual pollinators and solid lines indicate total selection on the trait when multiple pollinators are present. (A) In the trade-off hypothesis pollinators differ in their selection on the same floral trait. This conflicting selection can lead to a trade-off, whereby an adaptation to one pollinator causes the plant to be maladapted for another pollinator. (B) In the common adaptive peaks hypothesis, a trait may be an adaptation for multiple pollinators if they all select on the trait in the same manner. (C) In the trait specialization hypothesis, an individual trait is only under selection by a subset of the pollinator assemblage, leading to a generalist plant with individual traits specialized for different pollinators. Thus, different plant traits may evolve in response to selection by different pollinators. (D) Selection may be nonadditive if the presence of one pollinator alters selection by another pollinator. The left panel shows selection by each pollinator when pollinator species are in isolation, and the right panel shows selection by each pollinator when pollinators co-occur. The presence of the dashed species alters selection by the dotted species when they co-occur, which is the definition of nonadditive selection. Net selection on the trait when both pollinators are present (right panel) is different from the expected net selection if selection by pollinators is purely additive (left panel).

ture (Burd 1994; Ashman and Morgan 2004). However, the dearth of studies examining selection using seed siring success to estimate male fitness has prevented general conclusions from being drawn (Conner 2006). By measuring selection through both male and female fitness we can estimate total selection on floral traits, test whether there is conflicting selection across genders (i.e.,

male and female fitness), and test whether selection is stronger through male than female fitness overall.

Pollinators are a critical resource for reproduction in many, if not most, angiosperms (Tepedino 1979) and most animal-pollinated plants have more than one species of pollinator. Therefore, diffuse selection on floral traits is likely to be common. Although many studies have measured spatio-temporal variability in pollinator visitation (Waser et al. 1996 and references therein, Price et al. 2005), and variation in the effectiveness of different pollinators (e.g., Schemske and Horvitz 1984; Herrera 1987, 1989; Stanton et al. 1991; Gomez and Zamora 1999; Sahli and Conner 2007), there are few empirical studies measuring selection on floral traits by each of multiple pollinator taxa (but see Aigner 2004; Castellanos et al. 2004) and even fewer in plant species with generalized pollination (but see Gomez et al. 2009).

In this study, we tested for conflicting and nonadditive selection among pollinators and genders in a generalist plant, wild radish (*Raphanus raphanistrum*), by measuring selection on floral traits by the five most important pollinators of this species worldwide—sweat bees (*Dialictus* sp.), syrphid flies (*Toxomerus* sp.), bumblebees (*Bombus impatiens*), honey bees (*Apis mellifera*), and cabbage butterflies (*Pieris rapae*) (Kay 1976, 1978; Stanton et al. 1986; Conner et al. 1996; reviewed in Conner et al. 2009), all of which are effective pollinators of wild radish (Sahli and Conner 2007). Selection by sweat bees and syrphid flies was estimated in the field and selection by the other three pollinators was measured using cage experiments. The sweat bees and syrphids forage for pollen on wild radish, the butterflies forage for nectar, and the honey bees and bumblebees forage for both. Although rare pollinators may be important selective agents, it was not possible for us to measure selection by all of the 14 genera visiting wild radish in our populations. To test whether selection is nonadditive, we measured selection through male and female fitness by bumblebees, honey bees, and cabbage butterflies individually and when all three were pollinating at the same time.

We focus primarily on two aspects of anther position: dimorphism and exertion. Wild radish is a member of the Brassicaceae and exhibits the family-diagnostic tetradynamous stamen condition with two short and four long stamens. We refer to the length difference between the long stamen and the short stamen as stamen dimorphism; note that this is a continuous trait, as all flowers have both “morphs,” unlike a typical dimorphism. We define exertion as the degree to which the long stamen anthers protrude above the opening of the corolla tube.

These floral traits have been well-studied in wild radish (reviewed in Conner et al. 2009). Previous studies have detected both stabilizing and directional selection (in different years) on both anther exertion and stamen dimorphism by the pollinator assemblage as a whole (Morgan and Conner 2001; Conner et al. 2003). Functional studies of pollen removal (Conner et al. 1995)

showed that honey bees and cabbage butterflies removed more pollen from wild radish flowers with increasing anther exertion, whereas small bees removed most from plants with intermediate anther exertion. Conner et al. (2003) also found selection on stamen dimorphism during one year when honey bees were common, but did not find selection on this trait when honey bees were rare. These previous findings suggest that floral traits of wild radish may be under differential selection by different pollinators.

However, selection through lifetime male and female fitness (seed siring and production) by individual pollinator taxa has not been estimated in radish or any other generalist plant species, to our knowledge. This is the most direct way to study diffuse selection by pollinators in generalist plants, because indirect measures of fitness like pollen removal and deposition are often not well correlated with actual seed production and siring success (e.g., Schemske and Horvitz 1984; Harder and Thomson 1989; Kobayashi et al. 1999; Conner et al. 2003). These measurements of selection by individual pollinator taxa through both male and female fitness are the novel contributions of this article, both to our understanding of the evolution of these traits in wild radish, and to our understanding of selection by multiple interacting species in general. We addressed three specific questions: (1) What role does conflicting selection across pollinators and genders play in adaptation in generalist organisms? (2) When multiple selective agents are present at the same time, is selection additive or are there interactions among pollinators that alter selection? (3) Is the strength and form of selection similar through male and female function?

Methods

We used outdoor flight cage studies to measure selection by honey bees, bumblebees, and cabbage butterflies individually and in combination, allowing us to test for differences in selection among pollinators as well as for nonadditive selection among these three taxa. We also measured a response to selection after one generation in each pollinator environment. We measured selection by syrphid flies and small sweat bees using a field study. In both the cage and field studies, we made direct comparisons of the strength of selection through male versus female fitness. For our main focal traits of anther exertion and stamen dimorphism, we estimated selection on expanded phenotypic variation resulting from artificial selection (see below). Increasing variation through artificial selection increases the power to detect selection on traits (Schluter 1988), as well as helps test whether populations in nature are at a fitness peak (Grafen 1988). All plants used in the experiments were grown in 15 cm pots in the greenhouse and fertilized with 7.1g Osmocote Plus 15–9–12 fertilizer (Scotts-Sierra, Marysville, OH).

Although we cannot say for certain that the selection estimated in the cage experiments reflects that found in nature in

any one year or population, note that the focus of this article is testing for differences in selection among pollinators and genders within the cage and field experiments. The comparisons can be extrapolated from the cage to the field unless there are environment by pollinator or environment by gender interactions. It is hard to imagine how the slope of the relationship of fitness on these pollination efficiency traits caused by single pollinator taxa would be altered by the cage environment, as the behavior of the pollinators on individual flowers is unlikely to be altered. It is possible that selection through female fitness could be stronger in our experiment than in the field due to reduced resource limitation in potted plants, but this should not affect selection through male fitness. It is also difficult to imagine how selection by individual pollinator species in a generalist plant could be directly estimated in a more natural setting than a large outdoor flight cage, except in rare cases where there are times or places in which only one of the taxa is present (see below and Gomez 2009). Similarly, the use of expanded variation also means that the selection we estimate may not be exactly the same as that in the field, although this should not affect the linear slopes or the comparisons across pollinators or genders, as all these used the expanded variation.

TRAITS STUDIED

Stamen dimorphism and anther exertion have little variation in natural populations due to a high phenotypic correlation (0.89) between the short and long filament lengths and between corolla tube and filament lengths (0.84) (Conner and Via 1993). However, variation in these traits can be increased through artificial selection, suggesting that one of the reasons for little variation in nature may be due to stabilizing selection eliminating extreme phenotypes from the population. Plants used in this experiment are from lines artificially selected to increase variation in anther exertion and stamen dimorphism over seven and five generations, respectively, with randomly mated control lines maintained over the same number of generations. Plants were selected either for increased or decreased anther exertion, or decreased stamen dimorphism. After artificial selection, variance in anther exertion ($\sigma^2 = 1.02$) was more than three times that in the control lines ($\sigma^2 = 0.31$), and variance in stamen dimorphism ($\sigma^2 = 0.41$) was almost twice that of control lines ($\sigma^2 = 0.23$). There were no correlated responses to selection on nontarget floral traits, as other floral traits did not differ from control lines (J. K. Conner and K. Karoly, unpubl. data).

We also estimated selection on natural variation in flower number and size, two traits that increase visitation rates in many species (e.g., Cruzan et al. 1988; Young and Stanton 1990; Campbell et al. 1991; Eckhart 1991). Furthermore, the effect of these traits on visitation differs among pollinator taxa in wild radish (Conner and Rush 1996), so conflicting selection by different pollinators on these traits is possible. Flower size was calculated

as the first principal component of six floral traits: petal length and width, long and short filament length, corolla tube length, and pistil length (see Conner and Via 1993 for details on these traits). All traits loaded positively and with similar magnitude on this first principal component (Appendix 1).

SELECTION BY THREE POLLINATOR TAXA—CAGE STUDIES

To estimate selection by cabbage butterflies (*Pieris*), bumblebees (*Bombus*), and honey bees (*Apis*) separately and in combination, we constructed three $2 \times 4.5 \times 4.5$ m outdoor shade cloth cages containing either a small honey bee hive, a hive of *B. impatiens* (Koppert Biological Systems; Romulus, MI), or 15–20 wild caught cabbage butterflies. To measure selection when all three were present, the bumblebee hive and cabbage butterflies were temporarily moved into the cage with the honey bee hive. Cage or greenhouse studies are a common way of testing preferences or pollen-transfer efficiency of pollinator taxa (e.g., Hurlbert et al. 1996; Aigner 2004; Castellanos et al. 2004).

Three arrays of 20 plants each were constructed, with five plants from each of the artificial selection treatments (high exertion, low exertion, low dimorphism, and control) per array. Every week an array was exposed to a pollinator treatment, one flower from each plant was photographed for later measurement using NIH Object Image (2.12, Vischer 2004), and the total number of open flowers per plant was counted. Traits of flowers co-occurring on the same greenhouse-grown plant vary much less than those of flowers growing on different plants (repeatabilities greater than 0.85; J. K. Conner, unpubl. data).

All inflorescences with newly opened flowers were marked with small pieces (approx. 3 mm \times 15 mm) of uniquely colored tape for subsequent identification of the pollinator responsible for fruit production, and all buds remaining on the inflorescence were removed. Tape was removed after fruits matured so that tape did not accumulate. Array plants were placed in a cage in a 4 \times 5 m square, with plants from each line interspersed in a regular fashion. The number of visits to each plant during a 10-min period was recorded. When plants received an average of 10 visits per flower, the average daily visitation rate in nature (J. K. Conner, unpubl. data), the array was removed from the cage, returned to the greenhouse, and allowed to set seed. This procedure was repeated so each array was exposed to bumblebees, honey bees, cabbage butterflies, and all three together for three days each (with the exception of two days of exposure to bumblebees for one array and two days of exposure to all pollinators combined for two arrays). In the combined pollinator treatment honey bees made 55% of the visits, cabbage butterflies 30%, and bumblebees 15%; these percentages are well within the range of values reported for these taxa in field studies of wild radish (Conner et al., 2009 Table 1).

Table 1. Average fitness components for each pollinator treatment.

Treatment	Visit/flower/min	No. of open flowers/plant/day	Total seeds/plant	No. of seeds/flower (SE)
Sweat bees	0.004	51.1	31.2	0.78 (0.18)
Syrphid flies	0.02	34.6	85.3	2.5 (0.24)
Bumblebees	0.06	43.2	197.8	2.0 (0.14)
Honey bees	0.24	35.7	234.6	2.5 (0.18)
Cabbage butterflies	0.04	42.4	296.9	2.6 (0.16)
All three cage species combined	0.27	35.0	170.4	2.4 (0.15)

In this repeated-measures design each plant is exposed to each of the four pollination treatments multiple times. However, the flowers were different in each trial because wild radish flowers last a maximum of two days (J. K. Conner, unpubl. data) and the minimum time between subsequent uses of the same array was three days. The pollinator individuals differed each time an array was used as well because the minimum time between exposure to the same pollinator treatment was 10 days (mean of 18 days), yet honey bee and bumblebee workers usually forage for one week and at most two weeks (Rodd et al. 1980; Goldblatt and Fell 1986; Winston 1987), and cabbage butterflies were replaced on a daily to weekly basis.

Female fitness was estimated by counting the total number of seeds produced on each plant during each exposure to pollinators. To estimate male fitness (seed siring success), leaf tissue was collected from each array plant for later genotyping, and leaf and bud tissue was collected from 288–313 offspring produced from each pollinator treatment for a total of 1217 offspring across all four treatments. Offspring (seeds) were sampled across all days of exposure to pollinators from as many fruits as possible, with the number of seeds haphazardly chosen from maternal plants in proportion to the total number of seeds produced by each plant.

Response to selection

To measure a response to selection in each of the four treatments, we measured the above floral traits on the third flower of approximately 60 offspring from each pollinator treatment (234 total) with roughly equal representation from each array. The offspring from each pollinator treatment were chosen by haphazardly sampling seeds from each plant in proportion to each plant's total relative female fertility within each array under each pollinator treatment. We tested for differences in anther exertion, stamen dimorphism, and flower size among offspring produced in each of the pollination treatments using one-way ANOVAs.

SINGLE TAXON DAYS

As part of a separate experiment measuring total selection on anther exertion without separating selection by different pollinator taxa, three artificial arrays of 24 plants each were constructed using eight plants from each of the anther exertion artificial se-

lection lines (high exertion, low exertion, control). Plants from the stamen dimorphism selection lines were not used in this experiment. Array plants were placed in a square grid in an old field at Kellogg Biological Station in Hickory Corners, Michigan, with plants from each selection line interspersed regularly. Each array was placed in the field for five to nine hours per day for several days throughout the growing season; on each day 10-min pollinator observations were conducted on all plants. On two days during the experiment, the majority of the total visits were made by a single pollinator taxon, either the small sweat bee, *Dialictus* (97% of all visits), or the small syrphid fly, *Toxomerus* (89%). We measured selection during these two days to estimate selection by these pollinator taxa. Floral traits were measured on one flower from each plant in the array on both days. Tissue was collected from array plants and from 11% of offspring produced by *Toxomerus* visits (230 seeds total) and 16% of offspring produced by *Dialictus* visits (123 seeds total) for paternity analysis.

PATERNITY ANALYSIS

Total genomic DNA was extracted from each array plant and each offspring using Qbiogene's FastDNA Kit and the Fast-Prep Instrument (Carlsbad, CA) following the kit protocol. Individuals were genotyped at four microsatellite loci derived from *Brassica*: Bn35d, Brms 005, Na14-E08, and Ra2-E11 (radish.plantbiology.msu.edu). Microsatellites were PCR amplified as described in Sahli et al. (2008). Alleles were scored using FMBIO Analysis 8.0 (Hitachi Software Engineering 1991–1999) and placed in bins using Allelogram 1.2 (Manaster 1998).

Fractional male fertilities were estimated as follows (Morgan 1998): Let X_{ij} be the Mendelian genetic probability of observing the genotype of the i th offspring, given the genotypes of the maternal parent and the j th putative paternal parent. Male j received a fraction $X_{ij} / \sum_j X_{ij}$ of the paternity of offspring i . Over all offspring, the j th male fertility is $\sum_i (X_{ij} / \sum_j X_{ij})$.

RELATIVE FITNESS AND STANDARDIZED TRAIT MEANS

For the cage experiments, each plant's fitness in each pollinator environment was estimated by summing the fitness from the two to three days the plant was exposed to a particular pollinator.

Similarly, floral traits were averaged across those days plants were exposed to pollinators. Standardized selection gradients (Lande and Arnold 1983) were calculated by regressing relative fitness on the floral traits after standardizing the traits within each array and treatment to mean = 0 and variance = 1. For the single taxon days study, the total number of seeds sired and produced by each plant during that one day were used as our estimate of fitness. Fitness was relativized and the traits were standardized within each pollinator treatment.

By relativizing fitness (i.e., dividing by the mean) and standardizing traits within arrays and treatments, all mean differences in traits and fitness across arrays and treatments are eliminated. Eliminating differences in mean fitness among pollinator treatments improves our ability to detect differences in selection among pollinator treatments using ANCOVA (below), as it equalizes the elevations and centers of the regression lines without changing their slopes. Note that it is these slopes that are the estimate of the strength of selection (i.e., the selection gradients), and these are not dependent on the average seed set or seeds sired in each treatment (i.e., the elevation or intercepts of the selection gradients). The only floral trait that differed across arrays or treatments before relativization was flower size in the cage experiment and the only difference in fitness among pollinator treatments before relativization was lower fitness in the sweat bee treatment compared to all other treatments (see Results).

TESTING FOR DIFFERENCES IN SELECTION AMONG POLLINATORS

We used analysis of covariance (ANCOVA) to test for differences in selection among pollinators and genders, with separate ANCOVA models for the cage experiment and the single taxon day study. Differences in linear selection gradients were tested by fitting a model including the floral traits, pollinator, and gender main effects (not including the combined pollinator treatment in the cage experiment), and all trait by gender and trait by pollinator interactions. A significant pollinator \times trait interaction is evidence that pollinators differ in their selection on that floral trait, whereas a significant gender \times trait interaction indicates selection differs between male and female fitness.

We tested for differences in nonlinear selection using separate ANCOVAs where both linear and quadratic floral trait terms and their interactions among pollinator treatments were included in the model (Lande and Arnold 1983). The squared term for flower number was not included in the model because we were only interested in it as a covariate and because sample sizes were moderate ($N = 24$ or 60 plants). Furthermore, the squared term for flower number was not significant for any pollinator in preliminary analyses.

All ANCOVAs were performed in SAS using PROC MIXED. A repeated subject term was included in all models because the

fitness of each plant is represented in the model multiple times (i.e., male and female fitness and fitness in different pollinator environments). For the single taxon ANCOVA, stamen dimorphism was not included in the model because no plants from the dimorphism artificial selection lines were used and sample sizes were low ($n = 24$).

ESTIMATING SELECTION GRADIENTS

We estimated linear and nonlinear (quadratic) selection gradients for each of the six pollinator treatments. Linear estimates of selection were estimated from a model that did not include the squared terms, and quadratic gradients presented are the doubled quadratic-regression coefficients (Lande and Arnold 1983). Due to the presence of several outliers in our datasets, 95% confidence intervals for selection gradients were obtained by bootstrapping the original data 10,000 times. Two-tailed P -values were determined as the proportion of bootstrapped selection gradients above or below zero (for negative and positive selection gradients, respectively) multiplied by two (Crowley 1992; Legendre and Legendre 1998). Regression analyses were done using PROC REG in SAS (9.1) and bootstrapping was done using the SAS MACRO language.

TEST FOR NONADDITIVE SELECTION

To test whether multiple pollinators interacted in the cage experiment to alter selection by each pollinator individually, we compared the observed selection gradients when honey bees, bumblebees, and cabbage butterflies were all together with the gradients expected if selection by each pollinator taxon was purely additive in the cage environment. The additive expectation was generated by averaging the three selection gradients from the three individual pollinator treatments for each trait; this average was weighted by the pollinator visitation rates for each taxon in the combined treatment (see above) because honey bees were so dominant (making 55% of all visits) when all three pollinators were combined due to their greater number of foraging individuals in the cage. Weighting the average selection gradient ensures that any differences between the expected and actual gradients in the combined treatment are due to nonadditive selection and not due to the experimental artifact of differences in numbers of the different pollinator taxa in the combined treatment. This additive expected selection for each trait was compared to the actual selection gradient for the same traits in the combined treatment using ANCOVA, in which a significant trait \times treatment interaction is evidence for nonadditive selection among pollinators.

AVERAGE SELECTION THROUGH MALE VERSUS FEMALE FITNESS

We tested for overall differences in the magnitude of selection through male and female fitness by performing a paired

t-test on the absolute values of selection gradients estimated through both fitness components for each trait (both linear and quadratic) in each pollinator environment ($N = 38$). Although traits are correlated, and thus to some extent nonindependent, selection gradients are already corrected for correlations among traits because they are calculated using a multiple regression, and correlations among traits were generally low (mean = 0.28; range = 0.08–0.45).

Results

An ANOVA revealed that the average number of seeds produced per flower per day differed across pollinator treatments ($P < 0.0001$, $F = 9.85$, $df = 5$), where sweat bees produced significantly fewer seeds than all other pollinators. This was likely due to the much lower visitation rates during the day they were pollinating (Table 1). However, any differences in mean fitness across pollinator treatments were removed from the selection analyses by relativizing fitness within arrays within each pollinator treatment (see Methods). Thus, differences in visitation rates or seed production across pollinator treatments have no effect on our tests for differences in selection among pollinators.

SELECTION ON FLORAL TRAITS

When we measured selection only on those days that *Dialictus* or *Toxomerus* were the most abundant visitor, there was no significant difference in selection through male and female fitness for any floral trait. We found a significant difference between these pollinators in their nonlinear selection on flower size (Table 2)—*Dialictus* exerted stabilizing selection on flower size (only significant through female fitness), and *Toxomerus* did not exert any significant selection on this trait (Appendix 3, Fig. 2C,D). There was also a significant flower number \times pollinator interaction, where *Toxomerus* selected for more flowers through female fitness but *Dialictus* exerted no selection on this trait (Appendix 2, Fig. 2A). Averaging over male and female fitness and both pollinators, there was marginally significant stabilizing selection on both flower size ($P = 0.07$) and anther exertion ($P = 0.09$; trait main effects in Table 2). Examination of individual gradients for anther exertion shows that *Dialictus* exerted strong significant stabilizing selection on anther exertion through both male and female fitness whereas *Toxomerus* did not exert significant selection on this trait (Fig. 2E,F, Appendix 4). In sum, there was no evidence for conflicting selection by these two pollinators or across genders, but three traits seem to be under selection by only one of the pollinators (trait specialization).

For the cage experiment, the only significant difference in selection on floral traits between the three pollinator taxa was for stamen dimorphism—both the linear and the quadratic dimor-

Table 2. ANCOVA results testing for differences in selection between pollinators (*Toxomerus* and *Dialictus*) and between genders (male vs. female fitness) during the single-taxon days study. Trait main effects represent selection averaged over pollinators and genders and interactions among traits and pollinators or genders test for differences in selection among these categorical variables. Plant, pollinator, and gender main effects were also included in the model but are not shown for simplicity. Linear and quadratic terms are from separate ANCOVAs (Lande and Arnold 1983). Values in bold are significant at $P \leq 0.05$.

Source	β or γ	df	F-ratio	P-value
Flower number	0.11	1	2.20	0.15
Flower size	0.11	1	1.59	0.21
Flower size ²	-0.24	1	3.57	0.07
Anther Exsertion	0.01	1	0.02	0.89
Anther exertion ²	-0.20	1	3.07	0.09
Flower number \times pollinator		1	7.65	0.009
Flower size \times pollinator		1	0.00	0.96
Flower size² \times pollinator		1	4.52	0.04
Anther exertion \times pollinator		1	1.84	0.18
Anther exertion ² \times pollinator		1	1.61	0.21
Flower number \times gender		1	1.87	0.18
Flower size \times gender		1	0.01	0.94
Flower size ² \times gender		1	0.57	0.45
Anther exertion \times gender		1	0.40	0.53
Anther exertion ² \times gender		1	0.00	0.98

phism \times pollinator interaction terms were significant (Table 3). However, the individual selection gradients for each pollinator taxon revealed that this significant interaction term did not signify a trade-off; rather, stamen dimorphism was only under selection by one pollinator taxon, honey bees, which selected for less stamen dimorphism (total selection, $\beta = -0.11$; $\gamma = 0.20$, Appendix 5). The negative beta term and positive gamma term for the honey bee fitness function indicates a rapid fitness decline as stamen dimorphism increases from the smallest values, but a fairly constant lower fitness at larger values (Fig. 3G,H). The pattern of selection on stamen dimorphism by honey bees was similar through both male and female fitness.

Directional selection on flower number and anther exertion differed between genders averaged over the three pollinators in the cage experiment (trait \times gender interactions in Table 3). There was selection for more exerted anthers through male fitness (significant only for bumblebees, marginally significant for honey bees, Fig. 3F, Appendix 4) and for increased flower number through female fitness (Fig. 3A, Appendix 2), but little selection on these traits through the other gender.

In general, traits showed a similarity in selection between male and female fitness, as evidenced by the significant correlations between selection gradients estimated through male and

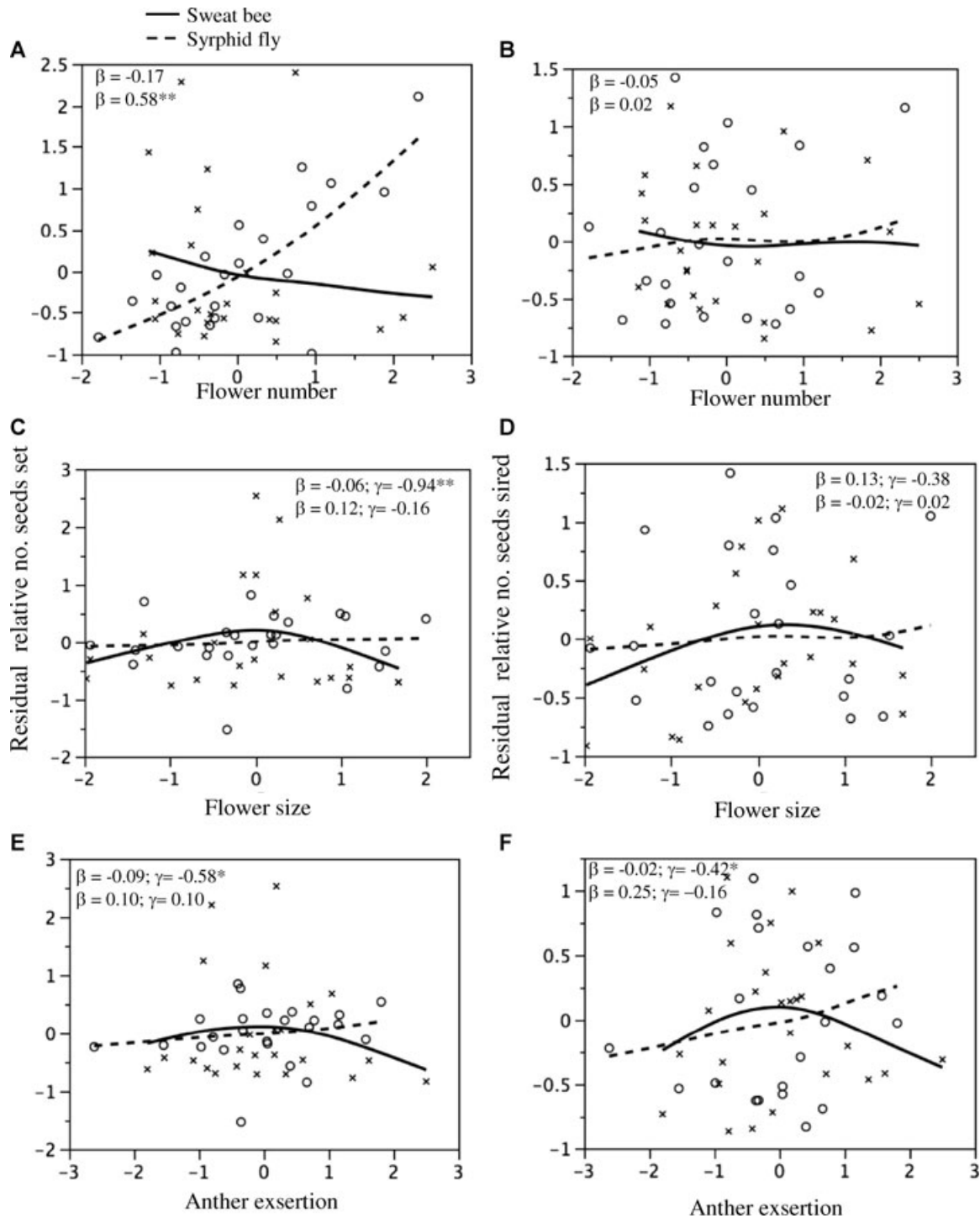


Figure 2. Fitness functions for each floral trait in the single taxon days experiment. Sweat bee (*Dialictus*) gradients are solid lines with "x" markers and syrphid fly (*Toxomerus*) gradients are dashed lines with "o" markers; selection through female fitness is plotted in the panels on the left, male fitness on the right. The curves are cubic spline fits with a smoothness (lambda) of 5. Values on the x-axis are traits values standardized to a mean = 0 and variance = 1. * $P \leq 0.05$; ** $P \leq 0.01$.

female fitness ($R = 0.54$, $P = 0.0005$). Selection through female fitness was significantly stronger than through male fitness (paired $t = -2.17$, $P = 0.04$; $N = 38$), although this difference was largely due to strong selection on flower number through female fitness

and no selection on flower number through male fitness. If flower number is removed from the analysis, the magnitude of selection is no longer different through male versus female fitness (paired $t = -1.11$, $P = 0.28$; $N = 32$).

Table 3. ANCOVA results testing for differences in selection on floral traits between pollinators (honey bees, bumblebees, and cabbage butterflies) and genders in the cage experiment. Trait main effects represent total selection averaged over all treatments and both genders, and interactions between traits and pollinators or gender test for differences in selection among these categorical variables (the combined pollinator treatment was not included). Plant, pollinator, and gender main effects were also included in the model but are not shown for simplicity. Linear and quadratic estimates are from separate ANCOVAs (Lande and Arnold 1983). Values in bold are significant at $P \leq 0.05$.

Source	β or γ	df	F-ratio	P value
Flower number	0.13	1	21.53	<0.0001
Flower size	-0.009	1	0.08	0.78
Flower size ²	-0.06	1	1.04	0.31
Anther exertion	0.05	1	1.62	0.20
Anther exertion ²	0.04	1	0.55	0.46
Stamen dimorphism	-0.04	1	0.90	0.34
Stamen dimorphism ²	0.06	1	1.91	0.17
Flower number \times pollinator		2	1.95	0.14
Flower size \times pollinator		2	1.45	0.24
Flower size ² \times pollinator		2	0.97	0.38
Anther exertion \times pollinator		2	2.01	0.14
Anther exertion ² \times pollinator		2	0.77	0.47
Stamen dimorphism \times pollinator		2	3.15	0.04
Stamen dimorphism² \times pollinator		2	3.13	0.05
Flower number \times gender		1	17.80	<0.0001
Flower size \times gender		1	2.31	0.13
Flower size ² \times gender		1	2.45	0.12
Anther exertion \times gender		1	8.30	0.004
Anther exertion ² \times gender		1	0.01	0.93
Stamen dimorphism \times gender		1	0.39	0.53
Stamen dimorphism ² \times gender		1	2.73	0.10

TEST FOR NONADDITIVE SELECTION

In the ANCOVA comparing the observed and expected selection gradients when all three pollinators were combined, there were no significant treatment \times trait interactions, indicating no evidence for nonadditive selection among pollinators (Table 4). When all three pollinators were combined there was significant stabilizing selection on flower size through both genders (Appendix 3) and significant disruptive selection on stamen dimorphism through female fitness (Appendix 5), as also detected in the significant ANCOVA main effects for these two traits (Table 4).

RESPONSE TO SELECTION AFTER ONE GENERATION

The only trait that had diverged significantly among the pollination treatments in the cage experiment was stamen dimorphism

($F = 3.3$, $P = 0.02$), which was also the only trait for which selection differed significantly among pollinators (Table 3). A Tukey's HSD test revealed significantly less stamen dimorphism in the honey bee treatment than in the bumblebee treatment (Fig. 4) after one generation of natural selection. The cabbage butterfly treatment mean was similar to the bumblebee mean, whereas the combined treatment mean was similar to the honey bee mean. This is consistent with the negative directional selection on stamen dimorphism by honey bees, the lack of significant selection by the other pollinators, and the combined treatment being dominated by honey bee visitation (Appendix 5).

Discussion

SUPPORT FOR CONFLICTING SELECTION LEADING TO TRADE-OFFS

Trade-offs, which can result from conflicting selection, are traditionally thought to constrain the evolution of increased generalization because a change in a trait may increase adaptation to one resource but decrease adaptation to another. However, we found little evidence for trade-offs in adaptation to different pollinators through a single component of fitness. In the two cases with a significant trait \times pollinator interaction, only one pollinator exerted significant selection on the trait. This provides more support for the trait specialization hypothesis, where a trait is under selection by a subset of the pollinators.

However, there was some support for the trade-off hypothesis when we examined selection on anther exertion through male fitness. Both bumblebees and honey bees selected for increased anther exertion, whereas the small sweat bee, *Dialictus*, selected for an intermediate optimum anther exertion. Thus, optimal adaptation of anther exertion for pollination by sweat bees would cause a maladaptation for both of the large bees. In this case, size seems to be a factor influencing the optimal anther exertion for pollination by different pollinators. It is not surprising that larger pollinators selected for more exerted anthers, as the anthers may need to be more exerted to come in contact with a honey bee or bumblebee's head, whereas a sweat bee may not contact highly exerted anthers as effectively because they are positioned too far above the petal where the bee lands. Therefore, trade-offs caused by conflicting selection do seem to occur for adaptation to these different sized bees, at least through male fitness.

SUPPORT FOR COMMON ADAPTIVE PEAKS VERSUS TRAIT SPECIALIZATIONS

Although results from this study do suggest a possible trade-off in anther exertion, we also found evidence for both the common adaptive peaks hypothesis and the trait specialization hypothesis. In the cage experiment, although neither pollinator alone

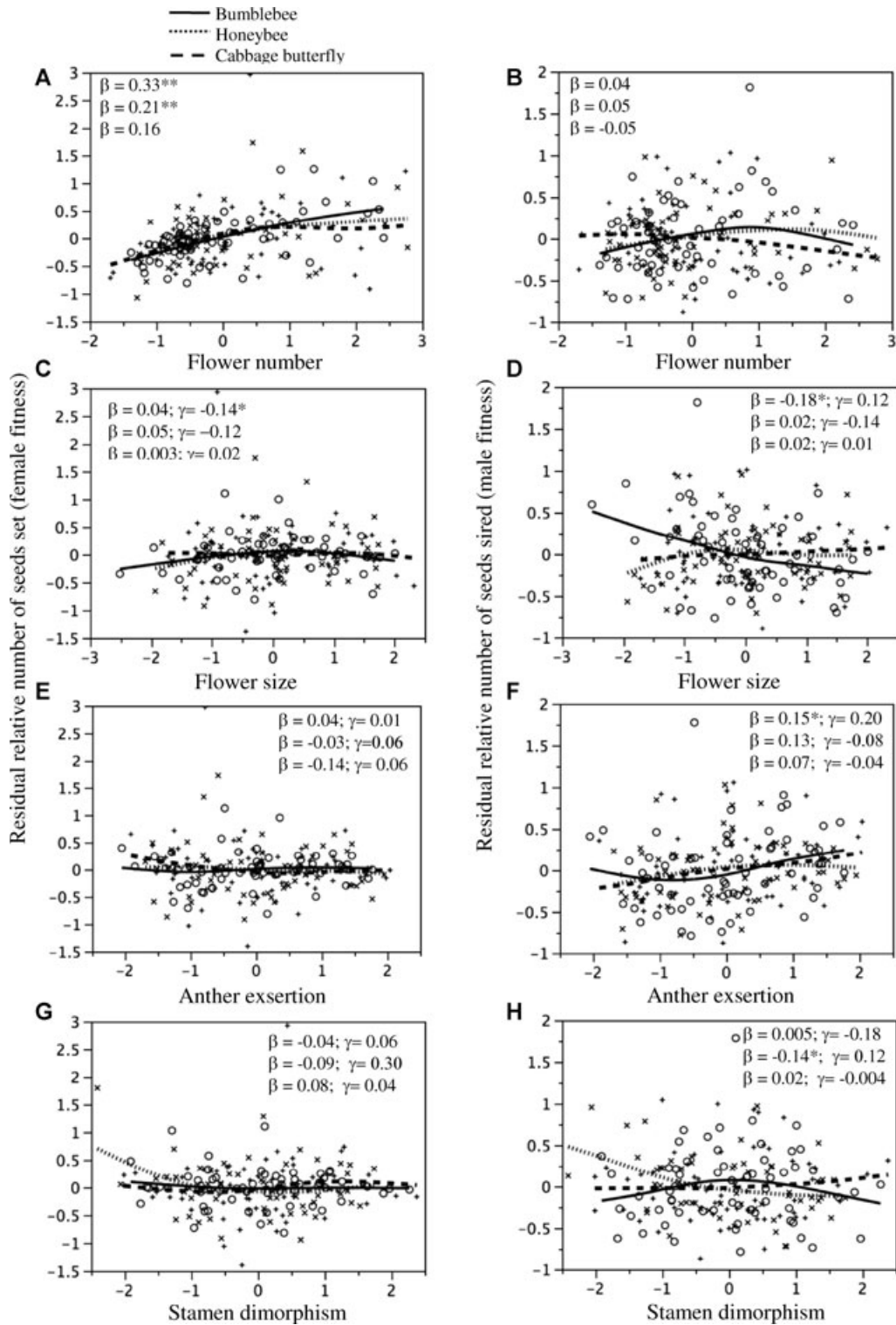


Figure 3. Fitness functions for each floral trait in each pollination environment for the cage experiment. Bumblebees gradients are solid lines with "o" markers, honey bees are dotted lines with "x" markers, and cabbage butterflies are dashed lines with "+" markers; selection through female fitness is plotted in the panels on the left, male fitness on the right. The curves are cubic spline fits with a smoothness (lambda) of 5. Values on the x-axis are traits values standardized to a mean = 0 and variance = 1. * $P \leq 0.05$; ** $P \leq 0.01$.

Table 4. ANCOVA testing for differences in selection between the combined pollinator treatment versus the weighted average selection based on selection by each pollinator taxon individually. A trait \times treatment interaction would indicate that the expected selection based on each pollinator's individual selection gradient is significantly different from the observed selection gradient when all three pollinators co-occur. Treatment and gender main effects were also included in the model, but are not shown for simplicity; interactions with gender were not included (see Methods). Linear and quadratic estimates are from separate ANCOVAs. Values in bold are significant at $P \leq 0.05$.

Source	β or γ	df	F-ratio	P value
Flower number	0.04	1	6.33	0.01
Flower size	-0.03	1	0.34	0.56
Flower size²	-0.08	1	7.68	0.006
Anther exertion	0.02	1	0.47	0.49
Anther exertion ²	0.04	1	0.18	0.67
Stamen dimorphism	-0.07	1	0.82	0.36
Stamen dimorphism²	0.16	1	4.53	0.03
Flower number treatment		1	1.41	0.24
Flower size \times treatment		1	2.7	0.10
Flower size ² \times treatment		1	0.66	0.41
Anther exertion \times treatment		1	1.76	0.19
Anther exertion ² \times treatment		1	0.00	0.95
Stamen dimorphism \times treatment		1	1.82	0.18
Stamen dimorphism ² \times treatment		1	1.48	0.22

exerted significant selection on flower size, there was significant stabilizing selection on flower size when all pollinators were combined. We also found strong stabilizing selection on flower size by *Dialictus* through female fitness. Taken together, these findings suggest that selection on flower size fits the multiple

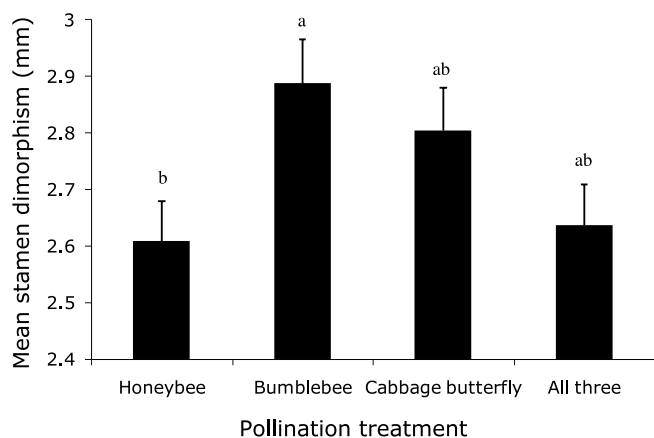


Figure 4. Mean stamen dimorphism (\pm SE) in each pollination treatment in the cage experiment after one generation of natural selection. Treatments that do not share a letter in common are significantly different ($P < 0.05$) using a Tukey's HSD.

adaptive peaks hypothesis, where several pollinators exert similar stabilizing selection on flower size. This can result in a flower size that is optimal for attraction or effectiveness of multiple pollinator taxa.

Additionally, although selection on anther exertion by sweat bees seems to oppose selection by larger bees, we did find that bumblebees and honey bees may exert similar selection pressure on anther exertion. Based on pollen removal studies, honey bees and cabbage butterflies were expected to select for increased anther exertion as they removed more pollen from anthers that were more exerted (Conner et al. 1995). For honey bees, it seems that this increased pollen removal from more exerted anthers translates into higher siring success, although it is difficult to determine if the finding of marginally significant selection by honey bees is due to low power to detect selection or due to no actual selection on this trait.

Finally, we found that one trait, stamen dimorphism, was only under significant selection by honey bees, and this selection to decrease dimorphism resulted in decreased stamen dimorphism, relative to bumblebees, after just one generation. The reasons behind this selection for decreased dimorphism are currently unknown. Conner et al. (2003) found that flowers with monomorphic anthers (i.e., dimorphism = 0) had more pollen removed from them than flowers with stamens of two different heights. Thus, if pollen removal is correlated with seed-siring success, this would mean selection to decrease dimorphism and could explain why honey bees select for decreased dimorphism. However, selection for more dimorphic anthers, which may be a mechanism to limit pollen removal during a single visit (Conner et al. 2003), may occur in environments where pollinators are reliably present and where pollinators differ in their effectiveness (Thomson and Thomson 1992).

Both our finding of selection on dimorphism by only one pollinator, and similar selection on flower size by multiple pollinators, reveal that a generalist species can evolve to be well adapted to multiple pollinators. Our results for anther exertion, however, suggest conflicting selection by small versus large bees. Thus far, two studies examining the effects of floral traits on visitation rate or pollen-transfer efficiency of different pollinators in more specialized plant species have found evidence for conflicting selection (Schemske and Bradshaw 1999; Muchhala 2007) but work by Galen (1996) is supportive of the trait specialization hypothesis, and work by Aigner (2004) is suggestive of either the trait specialization or common adaptive peaks hypothesis. There have still been too few studies measuring selection by different pollinators on floral traits to determine the relative importance of each of our three proposed hypotheses for adaptation in generalist organisms, but results from our study and previous studies suggest that all three scenarios may be important.

THE IMPACT OF POLLINATOR IMPORTANCE ON TOTAL SELECTION

It is important to keep in mind that although pollinators may differ in their selection on a trait, differences in pollinator frequency can alter the total selection on a trait by weighting selection by one pollinator more heavily than selection by another pollinator. The large variance among pollinators of *R. raphanistrum* in their importance to plant reproduction (Sahli and Conner 2007) and differences among pollinators in the selection they exert indicates that total selection on a trait is dependent on the relative visitation frequencies of each taxon. Although multiple pollinators may co-occur in plant populations and differ in their selection on traits, if one pollinator dominates and selects on floral traits, the population may adapt to the most common pollinator (Stebbins 1970). Conner et al. (2003) found stabilizing selection on both anther exertion and flower size in 1992 when they estimated selection on arrays under natural pollination conditions. During the 1992 study, small bees made 40% of all visits, similar to our findings in 2002. Results from our study have now shown that *Dialictus* does, indeed, exert stabilizing selection on both anther exertion and flower size. Thus, the stabilizing selection on anther exertion detected in nature when multiple pollinators are present, and the little variation in anther exertion in natural populations, may be due to stabilizing selection exerted by just one pollinator, *Dialictus*. The stabilizing selection on anther exertion is equivalent to correlational selection on filament and tube length, and thus may be responsible for the very strong correlations between these traits (Conner and Via 1993; Conner 2002).

SELECTION THROUGH MALE VERSUS FEMALE FITNESS

We found significant differences between genders in selection on two traits, anther exertion and flower number. Differences in selection between genders is predicted by theory (Morgan and Schoen 1997) but has had little previous empirical support (but see Morgan 1992). However, we did not find overall differences in the “strength” of selection through male versus female fitness except for flower number, which affected female fitness much more than male fitness. A recent review of seven studies measuring selection on attraction traits through both male and female fitness (Ashman and Morgan 2004) also found little difference between the magnitude of selection through male and female fitness. Our results emphasize the fact that selection through male fitness is just as important as through female fitness, and thus should be measured, but do not support the view that selection is stronger through male than through female fitness (Bateman’s rule). However, seed production in this study was likely not as resource-limited as in nature because our plants were grown in pots in the greenhouse with ample fertilizer and water and produced fewer seeds than field plants (4 vs. 12.7 seeds/per day alive,

Conner et al. 1996) because they were not pollinated every day. Increasing resource availability can increase pollen limitation of seed production, which in turn increases the opportunity for selection through female fitness (Totland 2001; Ashman and Morgan 2004; Maad and Alexandersson 2004; Caruso et al. 2005). Thus, our findings of strong selection on floral traits through female fitness may be overestimates of those in nature. Clearly more studies measuring selection through both male and female fitness on more plant species are needed, as this is just one of a handful of studies to date that has measured selection through male seed-siring success.

Finally, selection by honey bees, bumblebees, and cabbage butterflies acted in an additive manner in our experiments. To our knowledge, this is the first study to test for interactions among pollinators in their selection on floral traits. Total selection in the field may be more complicated given the presence of additional important pollinators and additional flowering plant species. It is important to note that our test for nonadditive selection would only detect nonadditive effects due to interactions among pollinators affecting the pollinator’s behavior once it lands on a flower or due to interactions altering which flowers a pollinator chooses to visit. Our method would not detect nonadditive selection caused by overall differences in visitation rates because we took visitation rates into account when determining the expected selection. However, this is a first step to studying nonadditive selection among pollinator taxa.

Conclusions

In sum, this study revealed that traits in generalist species may evolve to be adaptations to a subset of resources, that is, pollinators in this case. This work also supports the idea that the strength and direction of net selection on floral traits may be strongly dependent on the local abundance of each pollinator taxon in the community. When populations contain both large bees and small bees, adaptation of anther exertion may be hindered due to a trade-off in selection by these different pollinators. However, in populations where *Dialictus* are abundant pollinators, our results predict evolution toward an intermediate optimum anther exertion, and in populations where honey bees or bumblebees are abundant, we would expect to see the evolution of more exerted anthers. Flower size should be under stabilizing selection regardless of the composition of the visiting pollinator taxa. This study also emphasizes the importance of measuring total selection through both male and female fitness, because selection differed significantly between genders for two of the four traits studied. The total selection exerted on traits may strongly depend on whether plants are more resource or pollen limited in their female reproduction, which could alter the detection of a fitness trade-off.

ACKNOWLEDGMENTS

We thank M. Morgan for estimating fractional male fertilities, S. Emery, H. Emery, D. Powless, C. Stewart, and T. Thompson for help with fieldwork, KBS volunteers for help with building the cages, N. Consolatti and C. Wei for their help with beekeeping, and Z. Huang for supplying the honey bees. R. Lenski, A. Prather, and D. Schemske, were helpful in designing the experiment and we thank them and T. L. Ashman, C. Gross, M. Geber, R. LaRosa, A. Royer, E. Stacy, and anonymous reviewers for their comments on the manuscript. This research was supported by a Lauff Research Scholarship, by the National Science Foundation under grants DEB 9903880, DEB 0108354, and DDIG DEB-0408055, and by the Cooperative State Research, Education, and Extension Service, US Department of Agriculture, under Agreement No. 2002*35320-11538. This is KBS contribution no. 1582.

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Associate Editor: T. Juenger

Appendix 1. Eigenvectors and the total variance explained by the first principal component of the six morphological traits in each of the four pollinator treatments.

Trait	Eigenvectors					
	Sweat bees	Syrphid flies	Bumble bees	Honey bees	Cabbage butterflies	All three cage species combined
Corolla tube length	0.35	0.35	0.41	0.35	0.37	0.36
Long filament length	0.51	0.50	0.46	0.46	0.45	0.47
Short filament length	0.52	0.48	0.46	0.48	0.48	0.49
Pistil length	0.29	0.34	0.46	0.44	0.36	0.45
Petal width	0.33	0.30	0.29	0.30	0.36	0.33
Petal length	0.40	0.44	0.34	0.39	0.41	0.31
Total variance explained	45%	55%	55%	54%	55%	52%

Appendix 2. Standardized linear (β) selection gradients for flower number by different pollinators (95% confidence intervals). Values in bold are significant at $P \leq 0.10$.

Pollinator	Female fitness β	Male fitness β	Total Selection β
Sweat bees	-0.17 (-0.64, 0.28)	-0.05 (-0.33, 0.26)	-0.11 (-0.44, 0.22)
Syrphid flies	0.58** (0.20, 0.84)	0.02 (-0.40, 0.39)	0.30* (0.04, 0.50)
Bumblebees	0.33** (0.22, 0.44)	0.04 (-0.09, 0.19)	0.15** (0.05, 0.28)
Honey bees	0.21** (0.05, 0.36)	0.05 (-0.06, 0.17)	0.13** (0.03, 0.24)
Cabbage butterflies	0.16 (-0.03, 0.34)	-0.05 (-0.15, 0.06)	0.05 (-0.06, 0.16)
All three cage species combined	0.17* (0.03, 0.29)	0.07 (-0.04, 0.19)	0.12* (0.03, 0.20)

* $P \leq 0.05$; ** $P \leq 0.01$.

Appendix 3. Standardized linear (β) and quadratic (γ) selection gradients for flower size by different pollinators (95% CIs). β estimates represent directional selection, and γ terms represent quadratic selection, estimating curvature of the fitness function. Values in bold are significant at $P \leq 0.10$.

Pollinator	Female fitness		Male fitness		Total fitness	
	β	γ	β	γ	β	γ
Sweat bees	-0.06 (-0.42, 0.30)	-0.94** (-1.86, -0.28)	0.13 (-0.13, 0.42)	-0.38 (-0.94, 0.16)	0.04 (-0.31, 0.38)	-0.66* (-1.2, -0.12)
Syrphid flies	0.12 (-0.22, 0.44)	-0.16 (-0.80, 0.48)	-0.02 (-0.49, 0.46)	0.02 (-1.02, 1.1)	0.05 (-0.28, 0.38)	-0.07 (-0.69, 0.55)
Bumblebees	0.04 (-0.06, 0.14)	-0.14* (-0.34, -0.002)	-0.18** (-0.28, -0.06)	0.12 (-0.06, 0.32)	-0.06 (-0.17, 0.04)	-0.08 (-0.26, 0.06)
Honey bees	0.05 (-0.09, 0.18)	-0.12 (-0.32, 0.10)	0.02 (-0.09, 0.12)	-0.14 (-0.30, 0.04)	0.03 (-0.07, 0.13)	-0.12 (-0.28, 0.04)
Cabbage butterflies	0.003 (-0.13, 0.13)	0.02 (-0.22, 0.36)	0.02 (-0.08, 0.11)	0.01 (-0.20, 0.22)	0.009 (-0.08, 0.09)	0.02 (-0.14, 0.22)
All three cage species combined	0.03 (-0.11, 0.18)	-0.18* (-0.38, -0.01)	0.10 (-0.03, 0.22)	-0.16* (-0.36, -0.02)	0.06 (-0.05, 0.18)	-0.16* (-0.34, -0.06)

* $P \leq 0.05$; ** $P \leq 0.01$.

Appendix 4. Standardized linear (β) and quadratic (γ) selection gradients for anther exertion by different pollinators (95% CIs). β estimates represent directional selection, and γ terms represent quadratic selection, estimating curvature of the fitness function. Values in bold are significant at $P \leq 0.10$.

Pollinator	Female fitness		Male fitness		Total fitness	
	β	γ	β	γ	β	γ
Sweat bees	-0.09 (-0.47, 0.32)	-0.58* (-1.28, -0.06)	-0.02 (-0.26, 0.22)	-0.42* (-0.92, -0.08)	-0.06 (-0.39, 0.28)	-0.50* (-0.94, -0.06)
Syrphid flies	0.10 (-0.14, 0.32)	0.10 (-0.30, 0.50)	0.25 (-0.18, 0.69)	-0.16 (-1.14, 0.76)	0.18 (-0.16, 0.51)	-0.03 (-0.51, 0.45)
Bumblebees	0.04 (-0.05, 0.14)	0.01 (-0.16, 0.18)	0.15* (0.02, 0.29)	0.20 (-0.04, 0.40)	0.06 (-0.03, 0.15)	0.10 (-0.14, 0.28)
Honey bees	-0.03 (-0.17, 0.11)	0.06 (-0.18, 0.32)	0.13 (-0.008, 0.25)	-0.08 (-0.26, 0.12)	0.05 (-0.06, 0.16)	-0.01 (-0.16, 0.16)
Cabbage butterflies	-0.14 (-0.32, 0.02)	0.06 (-0.20, 0.30)	0.07 (-0.07, 0.22)	-0.04 (-0.32, 0.16)	-0.03 (-0.17, 0.09)	0.01 (-0.20, 0.18)
All three cage species combined	-0.07 (-0.19, 0.0)	0.06 (-0.16, 0.30)	-0.06 (-0.19, 0.07)	-0.004 (-0.24, 0.24)	-0.06 (-0.16, 0.03)	0.04 (-0.14, 0.20)

* $P \leq 0.05$.

Appendix 5. Standardized linear (β) and quadratic (γ) selection gradients for stamen dimorphism by different pollinators (95% CIs). β estimates represent directional selection, and γ terms represent quadratic selection, estimating curvature of the fitness function. Values in bold are significant at $P \leq 0.10$.

Pollinator	Female fitness		Male fitness		Total fitness	
	β	γ	β	γ	β	γ
Bumblebees	-0.04 (-0.13, 0.06)	0.06 (-0.08, 0.20)	0.005 (-0.13, 0.16)	-0.18 (-0.40, 0.06)	0.001 (-0.09, 0.13)	-0.06 (-0.26, 0.12)
Honey bees	-0.09 (-0.29, 0.08)	0.30 (-0.06, 0.56)	-0.14* (-0.25, -0.01)	0.12 (-0.08, 0.36)	-0.11 (-0.22, 0.008)	0.20* (0.02, 0.32)
Cabbage butterflies	0.08 (-0.05, 0.25)	0.04 (-0.16, 0.28)	0.02 (-0.09, 0.12)	-0.004 (-0.16, 0.14)	0.05 (-0.05, 0.16)	0.02 (-0.12, 0.14)
All three cage species combined	-0.02 (-0.14, 0.11)	0.16* (0.006, 0.40)	0.05 (-0.08, 0.18)	-0.06 (-0.24, 0.10)	0.01 (-0.08, 0.11)	0.06 (-0.08, 0.20)

* $P \leq 0.05$.