

CHAPTER 14

Ecological genetics of floral evolution

Jeffrey K. Conner

*Kellogg Biological Station and
Department of Plant Biology, Michigan State University, East Lansing, MI, USA*

Outline

The diversity of floral forms in nature can be explained largely as adaptations to the diversity of biotic and abiotic selective agents with which different plant species interact. Ecological genetics is the study of the process of adaptation, and therefore is an ideal approach to understanding floral adaptations. Here I review work on selection and genetic variance and covariance of floral traits, as these are the principal determinants of adaptive evolution. Early work focused on simple floral polymorphisms, because they were amenable to study, but more recent work has used their simplicity to understand the genetic mechanisms underlying adaptation in unprecedented detail. Because most floral adaptations are not simple polymorphisms, I also review studies that have measured selection and the **G** matrix (additive genetic variances and covariances among traits) for quantitative floral traits. I present new results from my research group on highly correlated traits in wild radish flowers, showing how these traits can evolve independently despite the constraint caused by the genetic correlation. A study of the same traits across the Brassicaceae suggests that macroevolution may be guided by the **G** matrix, but that independent evolution of highly correlated traits can also occur during these longer periods. I close by reviewing some topics for future study that have been opened up by recent technical advances, and which have the potential to expand our understanding of the mechanisms of floral adaptation greatly.

14.1 Introduction

Natural selection acts on phenotypic variation, and if some of this phenotypic variation is due to underlying genetic variation, then adaptation through genetic change can occur in the population. However, the rate of adaptive evolution can be constrained by either a lack of genetic variance for the adaptive trait or genetic correlations between the adaptive trait and other traits under selection. Ecological genetics focuses on the process of adaptive evolution, especially the direction and strength of natural selection and the nature of genetic variation and covariation underlying adaptive phenotypic traits (Conner and Hartl 2004).

Ecological genetics provides an ideal approach to understanding key factors underlying floral

evolution. Flowers affect fitness directly through their role as the organs of mating and sexual reproduction in angiosperms. The primary selective agent on floral traits is clearly the pollen vector, usually animals or wind, although secondary selective agents, such as herbivores or abiotic factors, also influence floral evolution (Galen 1999; Chapter 7). Different floral organs (e.g., petals, stamens, pistil) may be under selection to work together as a functional unit (functional integration), which may alter correlations among floral parts (Armbruster 1991; Conner and Via 1993; see below), or conversely genetic correlations may constrain independent evolution of different floral parts (see below; Chapter 7).

In this chapter, I review current understanding of floral adaptations and suggest some lines of

inquiry that will be particularly fruitful in the future. I first discuss floral colour polymorphisms, because simple polymorphisms were the first traits studied by ecological geneticists and their simplicity enabled recent detailed genetic analysis. However, most floral adaptations involve quantitative floral traits, so I next consider the genetic variance and covariance of these more complex, continuous traits. I then briefly review studies of natural selection on quantitative traits and identify continuing gaps in our knowledge. Next I present an overview of my current research, which illustrates the twin roles of covariance in integrating traits and constraining independent trait evolution. Finally, I discuss some directions for future analysis of the ecological genetics of floral traits that hold considerable promise for new insights into the process of floral adaptation.

14.2 Simple polymorphisms: floral colour

Early studies of ecological genetics focused on simple polymorphisms: traits that involve a few discrete types. These traits are more amenable to study than continuous traits, because the discrete variation arises from allelic differences at only one or at most a few gene loci, and the traits are not strongly affected by the environment. Classic examples of this research include industrial polymorphism in peppered moths and other insects, banding patterns in snails, and heterostyly in primrose and other plants (Ford 1975; Chapter 13). Here I will briefly discuss studies of another simple polymorphism, flower colour, both for historical context and because this work has progressed recently in important new directions. Because the genetics of polymorphic traits are simple, past work has focused on selection by pollinators and the maintenance of polymorphism, as selection favouring one form over another tends to eliminate variation. These twin themes of pollinator-mediated selection and genetic variation recur throughout this chapter. More recent work on floral polymorphisms has exploited the genetic simplicity of these traits to reveal the genetic mechanisms of adaptation in unprecedented detail; the genetic mechanisms underlying more

complex adaptations are an important area for future research (Section 14.6).

Most, but not all, studies of selection on floral colour have found clear evidence for selection by pollinator preferences, but the form of these preferences varies. Both bumble bees and hummingbirds prefer the common blue flowers over rare white forms in *Delphinium nelsonii*, probably because the white flowers lack nectar guides, which increases the pollinators' handling time per flower (Waser and Price 1981). This pollinator preference probably explains the greater female fitness (seed production) of blue-flowered plants. Cabbage butterflies also prefer pigmented yellow flowers over white in wild radish (*Raphanus raphanistrum*), but this preference does not cause differences in female fitness (Fig. 14.1a and b; Stanton *et al.* 1986). This result suggests that fruit production is limited by resources other than pollen import (e.g., light, water, or soil nutrients). However, yellow-flowered plants sired more seeds than their white counterparts, showing that increased pollinator visitation enhanced male fitness (Fig. 14.1c). This was one of the first studies of floral evolution to measure seed siring success, which is crucial, but often neglected, in studies of floral evolution (see Section 14.4).

As in wild radish and *Delphinium*, pollinators of morning glories (*Ipomoea purpurea*) prefer pigmented flowers and discriminate against white flowers; however, in this species the selection is frequency dependent. Specifically, bumble bees prefer blue and pink flowers when they are common (greater than 75% combined), but exhibit no preference between white and pigmented flowers when they are roughly equally abundant (Epperson and Clegg 1987). In contrast to the previous examples, pollinators do not seem to discriminate between white and blue flowers in *Linanthus parryae* in the Mojave Desert (Schemske and Bierzychudek 2001). Nevertheless these morphs experience selection, and this selection fluctuated between years at one site: white-flowered plants produced more seeds during years with high rainfall, whereas blue-flowered plants produced more seeds during drier years. The cause of this selection remains unclear, although it is probably related to availability of essential cations in the soil

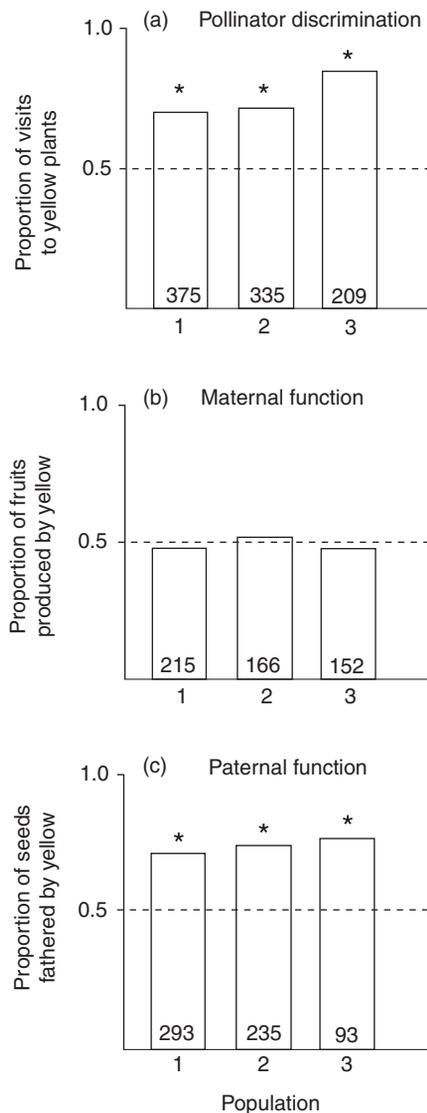


Figure 14.1 Pollinator preference for yellow-flowered plants over white-flowered plants in three populations of *Raphanus raphanistrum* (a) has no effect on female fitness (fruit production, b), but does enhance male fitness (siring success, c). An asterisk indicates a significant deviation from equal performance by the two colour morphs. Reprinted, with permission, from Stanton *et al.* 1986.

(D. W. Schemske personal communication). Thus, in this species selection on flower colour does not involve direct selection based on pollinator preference, but rather is indirect, caused by pleiotropic effects of the pigment gene on other plant

functions. Floral pigments serve various functions in plants, such as protection from UV radiation and from herbivores (Chapter 7).

The strong selection often reported in these studies raises the question of how genetic variation is maintained, because selection will tend to eliminate the polymorphisms. The authors of these studies postulate different mechanisms in each case, encompassing most of the major mechanisms that are thought to maintain genetic variation in general. Waser and Price explained the maintenance of the polymorphism in *Delphinium* as a balance between new mutations for white flowers and selection against the white morph. In wild radish, differences in colour preference among pollinators may maintain the polymorphism, as a series of studies of wild radish in England found that butterflies, syrphid flies, and honey bees preferred yellow flowers, whereas most bumble bees preferred white flowers (Kay 1978). Similarly, the fluctuating selection between wet and dry years in *Linanthus* could maintain the colour polymorphism in that species (Turelli *et al.* 2001). Finally, the negative frequency-dependent selection in morning glories would automatically maintain polymorphism.

Recent molecular studies of floral pigments in morning glory have revealed important new insights into the molecular mechanisms generating phenotypic variation and the biochemical and genetic basis of adaptation. One set of studies revealed that the mutations underlying the genetic variation in flower colour were caused mostly by transposable elements (Clegg and Durbin 2000), rather than by simple DNA base-pair substitutions. Other work examined different *Ipomoea* species in which red flowers evolved as part of an evolutionary switch from bee to hummingbird pollination. Zufall and Rausher (2004) determined that this switch involved inactivation of an enzyme in the pathway to blue pigments, which then shunts substrates along a different metabolic pathway, resulting in the synthesis of red pigments. Rarely is the genesis of an adaptive trait understood in this detail; knowledge of the molecular genetics of adaptation will go a long way towards improving our limited understanding of how adaptation occurs (Phillips 2005).

14.3 Ecological genetics of quantitative traits

Simple floral polymorphisms have provided and will continue to provide important insights into floral adaptation; however, most floral traits are not simple polymorphisms, but rather vary continuously. Examples include the sizes and shapes of petals and corolla tubes, the lengths of the stamens and pistils and the resulting placements of the anthers and stigmas, volume of nectar produced, and the sizes and numbers of ovules and pollen grains. Such continuous phenotypic distributions result from the action of more gene loci and/or stronger environmental effects compared with simple polymorphisms. Although both aspects of quantitative traits complicate their analysis, understanding floral adaptation has progressed significantly during the past 25 years, in part because of the availability of new theoretical, statistical, and molecular techniques, with the promise of even greater advances during the next decade (see also Chapter 2).

Two closely related equations encapsulate the key factors determining the adaptive evolution of quantitative traits:

$$R = h^2 S, \quad (1)$$

$$\Delta \bar{z} = \mathbf{G}\beta. \quad (2)$$

Both R and $\Delta \bar{z}$ represent the change in the mean phenotype from one generation to the next, that is, short-term adaptive evolution (Falconer and Mackay 1996; Conner and Hartl 2004). The magnitude of phenotypic evolution depends on the product of the amount of genetic variation for the trait, represented by heritability, h^2 , or the genetic variance/covariance matrix, \mathbf{G} , and the strength of phenotypic selection, represented by the selection differential, S , or the selection gradient, β . Therefore, directional selection can cause extensive phenotypic evolution (a change in mean) between generations if selection is strong (large S or β), which occurs when the environment changes (including changes caused by human activity). The magnitude of this evolution also depends on the standing genetic variation for the trait under selection (h^2 or \mathbf{G}). Note that these equations focus

on the change in the mean of a trait due to directional selection only, and do not model changes in trait variance caused by stabilizing and disruptive selection (cf. Chapter 13).

These equations differ in scope: the first considers evolution in one trait only, whereas the second is multivariate, considering multiple traits simultaneously. The multivariate approach offers two crucial advantages. First, the univariate selection differential estimates the combined selection on the trait, including both direct, adaptive selection and indirect, non-adaptive selection due to phenotypic correlations among traits. In contrast, the selection gradient estimates direct, adaptive selection, because it is based on multiple regression, so the effects of correlations among measured traits are removed. In fact, a well-conducted study estimating selection gradients is one of the best ways to determine what traits are adaptive in an undisturbed natural population (Conner and Hartl 2004).

The second advantage of the multivariate approach is that \mathbf{G} includes both the genetic variance (as does the heritability) and genetic covariances among traits. The rate and short-term direction of adaptive evolution depend not only on selection and genetic variance, but also on genetic covariances. Genetic correlations (the standardized version of genetic covariances) result from pleiotropy (one gene locus affects variation in multiple traits) or gametic-phase disequilibrium (non-random association between alleles at distinct loci, each of which affect variation in one trait of interest). Such correlations have two important consequences for adaptive evolution. First, selection on one trait causes evolution in all other traits with which it is correlated genetically. This correlated response to selection can either accentuate or slow adaptive evolution. In the latter case, a genetic correlation acts as an evolutionary constraint, because correlated responses are not necessarily adaptive. For example, in scarlet gilia (*Ipomopsis aggregata*) direct selection favours increases in both corolla length and the proportion of time in the pistillate phase (when the stigma is receptive), but a negative genetic correlation between these traits slows the expected increases (Campbell 1996). Second, genetic correlations can themselves be adaptations, resulting from past

selection for functional integration among traits that act together. For example, stabilizing selection on anther exsertion (the projection of the anthers beyond the opening of the corolla tube) through differences in male fitness in wild radish (Fig. 14.2; Morgan and Conner 2001) increases the correlation and integration between the filaments and corolla tube. Specifically, pollinators remove more pollen from flowers with anthers of intermediate height relative to the opening of the corolla tube than from flowers with lower or higher anthers. Thus, filament and corolla tube lengths are functionally integrated, because their relative lengths determine anther exsertion, which in turn affects successful pollination and subsequent male fitness.

Three approaches are used to estimate heritabilities, genetic correlations, and their unstandardized counterparts, genetic variances and covariances (the **G** matrix). The most common method involves the mating designs that plant and animal breeders have used for decades, such as offspring–parent regression, sibling analysis, and diallel mating crosses (Falconer and Mackay 1996; Conner and Hartl 2004). These techniques use controlled crosses to create sets of individuals of known genetic relationship, and then regression or analysis of variance to estimate genetic variances and covariances. For example, Campbell (1996) used both offspring–father regression and half-sibling analysis to demonstrate significant heritability for corolla length, width, and the positions of anthers and stigma of scarlet gilia, as well as genetic correlations among most traits. Many studies have used mating designs to estimate genetic variances and covariances for floral traits (e.g., Shore and Barrett 1990; Mazer and Schick 1991; O’Neil and Schmitt 1993; Conner *et al.* 2003a; Caruso 2004). These studies have found that significant genetic variance for, and covariance among, floral traits is very common in natural populations of a wide variety of plant species.

A newer approach uses molecular markers to estimate the relatedness of individuals in a population, instead of controlled crosses that create known relatedness (Ritland 2000; Thomas *et al.* 2000). This procedure allows estimation of quantitative genetic parameters for undisturbed natural populations and species for which controlled

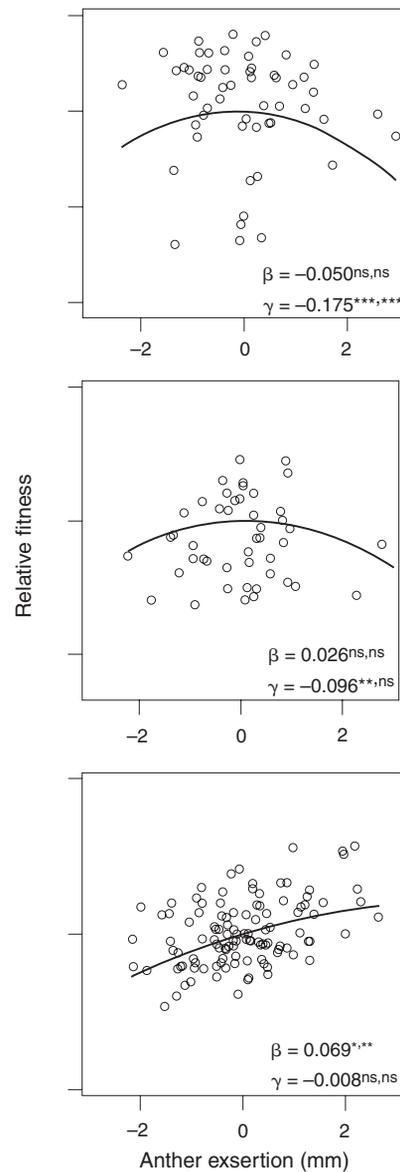


Figure 14.2 Relations of lifetime male fitness (seed siring success) to anther exsertion in wild radish during three field seasons. β and γ are the linear and quadratic selection gradients, respectively, and asterisks represent the outcomes of chi-square and simulation tests ($*P < 0.05$, $**P < 0.01$, $***P < 0.005$). Adapted from Morgan and Conner 2001.

crosses are difficult, although it has not been developed and tested as thoroughly as standard crossing methods. Using a marker-based method, Ritland and Ritland (1996) reported significant

heritability for both flower size and number in two natural populations of yellow monkeyflower, *Mimulus guttatus*. In contrast, Ritland and Ritland detected significant heritability only for flower size with more traditional offspring–parent regression methods, and all heritability estimates were lower. This contrast suggests that controlled crosses underestimate true heritability under natural conditions and/or that the marker-based method overestimates heritability. Clearly, the marker-based method requires more testing (but see Thomas *et al.* 2000).

The third general approach for estimating genetic variances and correlations involves artificial selection, which has been practiced by humans for millennia, resulting in all our domesticated plants and animals. To perform artificial selection, the investigator measures the phenotypic trait of interest on a controlled population, and selects individuals with the most extreme trait (in both directions, e.g., large and small) to breed the next generation. This process is typically continued for several generations. A significant difference between the lines selected in different directions provides good evidence for genetic variance for the trait in the original population. In addition, significant differences in any other traits that were not subject to selection demonstrates their genetic correlation with the selected trait. In essence, the investigator applies a known strength of selection (S) and measures the phenotypic change or response to this selection (R or $\Delta\bar{z}$) to infer the magnitude of heritability and parts of the \mathbf{G} matrix (see eqs 1 and 2). Artificial selection is most advantageous as a direct test of whether a given trait can evolve in response to a known strength of selection. However, artificial selection can be time and labour intensive, it provides the genetic variances of only the selected trait(s) (usually only one) and covariances with that trait, rather than the entire \mathbf{G} matrix, and the species used must be easily maintained and crossed (see Conner 2003 for further discussion).

To date, relatively few papers have reported the use of artificial selection to understand ecological genetics of floral traits. One of the first was by Huether (1968), who both increased and decreased petal number of *Linanthus androsaceus* during five

generations of selection, even though five petals are diagnostic for the Polemoniaceae. Similarly, Karoly and Conner (2000) decreased the height difference between the short and long stamens of *Brassica rapa* during only three generations; this trait is diagnostic of another large family, the Brassicaceae. Finally, Stanton and Young (1994) changed the ratio of petal size and pollen production in *Raphanus sativus* with a single generation of selection, even though these two traits are subject to a strong positive genetic correlation, which should oppose such a response to selection. Taken together, these studies suggest weak evolutionary constraints on adaptive floral evolution, at least in the short term.

A few studies have used artificial selection to test for a trade-off between flower size and number that is expected if flower production draws from a limited resource pool. Worley and Barrett (2000) selected for larger and smaller flowers, as well as increased flower production per inflorescence, in *Eichhornia paniculata*. Interestingly, the predicted negative correlated responses occurred only in lines selected for smaller flowers, resulting in a correlated increase in flower number. Delph *et al.* (2004) also used artificial selection to study flower number–size trade-offs, but with the additional goal of understanding genetic correlations between the sexes in a dioecious plant, *Silene latifolia*. In this species, males produce more, smaller flowers than females. To reduce this sexual dimorphism, Delph *et al.* selected for larger flowers in males or smaller flowers in females and observed correlated responses between the sexes. Female flower size increased in the lines in which males were selected, and male flower size decreased in lines in which the females were selected. In addition, flower number exhibited negative correlated responses, decreasing under selection for larger flowers and vice versa.

Additional evidence for negative genetic correlations and possible constraints is evident from responses to selection on male versus female function within hermaphroditic flowers. Selection for production of more ovules and anthers in separate lines of *Spergularia marina* caused direct responses by each trait and negative correlated responses in the other trait, indicating a negative

genetic correlation (Mazer *et al.* 1999). In contrast, Sarkissian and Harder (2001) reported significant direct responses to three generations of selection for large and small pollen grains in *Brassica rapa*, and a *positive* correlated response for ovule number. However, pollen number exhibited a negative response to selection on pollen size, indicating a negative genetic correlation and possible trade-off between pollen size and number in this species.

To summarize, studies of floral traits using different methods have commonly found significant genetic variance for floral traits, so that they can respond to selection. Genetic correlations among floral traits are also very common, which should constrain adaptive evolution, although these constraints might not be particularly strong (also see below). Consequently, additional measurements of genetic variation and covariance by themselves are no longer very useful, although such studies remain useful in the context of more comprehensive studies of adaptation and constraint.

14.4 Natural selection on floral traits

Selection has been measured for diverse floral traits in a wide variety of plant species (see Table 15.2 and associated electronic materials). Many authors have measured phenotypic selection on continuous floral traits (e.g., Schemske and Horvitz 1989; Johnston 1991; Caruso 2000; Maad and Alexandersson 2004; Chapter 15), and a few have combined such studies with estimates of genetic variances in the field for the same traits (Campbell 1996; Galen 1996). These studies often, but not always, detect strong directional selection on floral traits caused by pollinators, which demonstrates that quantitative floral traits are commonly adaptations for successful pollination. Stabilizing and disruptive selection seem to be less common, but this may be due to the greater statistical power needed to detect these forms of selection. These general results are mirrored in studies of natural selection in general (Kingsolver *et al.* 2001)

Despite many studies, important aspects of selection on floral traits remain poorly understood. Importantly, most studies focus exclusively on measures of female fitness, such as fruit and seed production, ignoring or estimating male fitness

inadequately. The importance of male fitness for floral evolution has been recognized for more than 25 years (Willson and Price 1977), based on Bateman's principle (Bateman 1948), which predicts stronger selection on floral traits through differences in male fitness than through differences in female fitness. This prediction applies when the availability of resources other than pollen, such as light, water, or soil nutrients, limit female fertility, whereas male fitness depends on success in pollen export and ovule fertilization. Stanton *et al.*'s (1986) analysis of selection on flower colour in wild radish, discussed above, supported this prediction. How commonly Bateman's principle applies to plants has been subject to debate, as it depends largely on how commonly female fitness is pollen limited (Wilson *et al.* 1994; Larson and Barrett 2000; Ashman *et al.* 2004; Chapter 4). However, this debate does not diminish the relevance of male fitness in floral adaptation, because half of all genes are transmitted through pollen to the next generation.

Early attempts to measure male fitness focused on pollen removal from flowers, first in milkweeds and orchids, which package pollen in pollinia (e.g., Willson and Price 1977; Queller 1983; Nilsson 1988), and later in species with granular pollen, particularly after automated particle counters made such measurements more practical and accurate (see Galen and Stanton 1989; Harder and Barrett 1993). However, Harder and Thomson (1989), using a combination of theoretical and empirical approaches, showed that many floral traits may promote male siring success by *reducing* the number of pollen grains removed by each pollinator and instead placing pollen on more pollinators (Thomson and Thomson 1992; see also Stanton 1994). Therefore, pollen removal may often be a misleading proxy for male fitness.

For example, consider my research group's study of the effects of differences in anther height on both pollen removal and siring success in wild radish (Conner *et al.* 2003b). Like most members of the Brassicaceae, wild radish has four long and two short stamens, producing two anther heights within each flower. Using both experimental manipulation and natural variation, we found maximal single-visit pollen removal from flowers

with the least difference in anther height. However, molecular genetic paternity analysis (see below) revealed stabilizing selection around the prevailing population mean difference in anther height during one year, perhaps reflecting a balance between the conflicting effects of increased pollen removal by individual pollinators and increased numbers of pollinators transporting pollen. In contrast, the same study found non-significant directional selection for *increased* differences during two other years. Thus, pollen removal was not a good guide to the pattern of selection based on siring success.

Surprisingly, few studies have measured selection on floral traits through seed siring success, even though the importance of male fitness in floral function and evolution has been long appreciated and the variety of genetic techniques, including AFLP, that enable measurement of siring success continues to increase. Stanton *et al.*'s (1986) work on floral colour polymorphism, discussed above, was among the first to do this. Their work was facilitated by the dual role of the floral colour trait as the trait of interest and as a genetic marker (inherited in a simple Mendelian fashion), so that the seed siring success of the yellow-flowered morphs could be measured directly by simply counting yellow-flowered offspring. In studies of quantitative traits, which do not exhibit simple Mendelian inheritance, molecular markers that are independent for the traits of interest are needed. Most studies of selection on floral traits through seed siring success have used allozymes; however, newer DNA-based markers, such as AFLP and microsatellites (see Conner and Hartl 2004 for an overview), reveal more variation than allozymes (increasing the power of inference) and can be used on virtually any species, with little development time in the case of AFLP.

New methods of analysis also increase the power to detect selection through differences in male fitness. For example, in a study of selection through male function on floral traits of *R. raphanistrum*, I used molecular genetic markers to genotype all possible fathers, mothers, and a sample of offspring to estimate seed siring success for each potential father. Initially, I used these data to estimate paternal fitness in a selection gradient

analysis (Conner *et al.* 1996b); however, both steps in this two-step estimation process (first siring success and then the selection gradients) are subject to error, which can reduce the statistical power for detecting selection. Indeed, this indirect approach to measure selection found little evidence for selection on floral traits (Conner *et al.* 1996b). In contrast, reanalysis of the same data by direct estimation of the selection gradients from the molecular marker data and the measurements of the phenotypic traits of interest (Morgan and Conner 2001; Chapter 2) detected significant selection on all three measured traits during several years, although the form of selection varied among years for flower size and anther exertion (Fig. 14.2). The stabilizing selection on anther exertion during 1991 and 1992 suggests that an intermediate anther position was most effective in placing pollen on the pollinators, but it is unclear why selection favoured the most exerted anthers during 1993. The estimates of the selection gradients from both analyses were very similar, but the direct method produced smaller standard errors, facilitating rejection of a hypothesis of no selection.

All studies to date that have measured selection through both female and male fitness have found contrasting patterns of selection (Conner *et al.* 1996a; Kobayashi *et al.* 1999; Elle and Meagher 2000; Morgan and Conner 2001; van Kleunen and Ritland 2004; Wright and Meagher 2004). For example, in our work on wild radish, flower size experienced positive directional selection through female fitness, but stabilizing selection through male fitness during 1992 (Conner *et al.* 1996a; Morgan and Conner 2001). The differences in selection through male versus female fitness are not always as predicted; for example, van Kleunen and Ritland (2004) found selection for increased anther length through female, but not male, fitness. Therefore, measurements of selection through female fitness alone are likely to be misleading concerning both selection through male function and the total selection acting on floral traits.

In addition to the need for more estimates of selection based on male fitness, two aspects of natural selection on floral traits are obvious candidates for more thorough study. First, selection is

rarely, if ever, measured during the entire life cycle. This problem is most acute in perennials, for which a heavy investment in reproduction in one season might lead to a high fitness contribution during that season at the expense of a reduced lifespan (also see Chapter 3). Thus selection favouring one trait during one year may be opposed by selection favouring a different trait during subsequent years, so that measurement of selection during a single season may mislead. Even in annuals, selection may be complicated by the “invisible fraction” (Grafen 1988), whereby differential germination success and/or mortality before flowering can cause non-adaptive evolution of floral traits if they are genetically correlated with traits that promote survival to flowering. Selection due to the invisible fraction is very difficult to measure, because it occurs before the floral traits of interest are expressed, so they cannot be measured. Therefore, the invisible fraction continues to be little studied (but see Bennington and McGraw 1995) and it remains a difficult problem for estimating natural selection in the field.

The second poorly understood aspect of selection on floral traits involves the spatial and temporal variation in selection on a given trait (also see Chapter 15). Unless selection is measured during multiple years at multiple sites, an assessment of the strength and nature of selection may be incomplete. In the context of floral evolution, variation in selection is particularly likely for species served by diverse pollinators, as the composition of the pollinator fauna may vary spatially and temporally. For example, Schemske and Horvitz (1989) measured selection on corolla length in *Calathea ovandensis* during three years, but found significant selection for decreased corolla length during only the year when one of the most effective pollinator species was present in appreciable numbers. Similarly, scarlet gilia (*Ipomopsis aggregata*) experienced significantly stronger selection for increased corolla length in the presence of another flowering species that competes with it for pollinators than when the competitor was absent (Caruso 2000). Such spatial and temporal variation in selection is probably ubiquitous, but the frequency with which selection acts in opposite directions on the same trait from year to year or site to site remains poorly studied. Regardless of how often selection changes direction, spatial and

temporal variation will cause single-year studies conducted at single sites to misrepresent the strength of selection experienced by a trait.

14.5 Independent evolution of correlated traits in radish

As noted in Section 14.3 genetic correlation is often invoked as a likely evolutionary constraint, but this hypothesis is rarely tested directly. As a detailed illustration of the quantitative genetics of floral traits, I now describe a test of this hypothesis for wild radish flowers conducted by my research group. We have focused particularly on the lengths of the filaments and corolla tube, because together they determine the positions of the anthers relative to the opening of the corolla tube (anther exertion), which affects the contact between anthers and pollinators. The phenotypic and genetic correlations between these traits are very strong (typically between 0.8 and 0.9), and significantly exceeds the average correlation between other pairs of floral traits in wild radish and some other closely related species in the Brassicaceae (Conner and Sterling 1995; J.K. Conner, K. Karoly, C. Stewart, V. Koelling, A.K. Monfils, L.A. Prather and H. Sahli unpublished manuscript). In wild radish this correlation is stable among populations and environments (Conner and Sterling 1995; J.K. Conner, K. Karoly, C. Stewart, V. Koelling, A.K. Monfils, L.A. Prather and H. Sahli unpublished manuscript), perhaps because it results from pleiotropy or extremely tight linkage (Conner 2002). A strong, stable genetic correlation caused by pleiotropy should constrain the independent evolution of filaments and corolla tubes. Below I describe both microevolutionary and macroevolutionary tests of this hypothesis.

14.5.1 Microevolution

To test whether the filament–corolla tube correlation constrains the independent evolution of these traits in wild radish over the short term, the research groups of Jeffrey Conner and Keith Karoly selected for increased and decreased anther exertion (two lines per direction) for five or six generations, while maintaining two randomly mated control lines. Exsertion was defined as

filament length minus corolla tube length (Plate 5). Our goal was to select in the direction that should be least responsive to change, that is, perpendicular to the major axis of the correlation between filament and corolla tube (Fig. 14.3). The major axis of the correlation is the direction of greatest genetic variance in two dimensions, so selection in this direction should produce the most rapid evolutionary response. This has been called the genetic line of least resistance (Schluter 1996). Perpendicular to the major axis is the direction of least genetic variance, so selection should be most constrained in this direction. Note that Stanton and Young's (1994) experiment discussed above (Section 14.3) selected perpendicular to the major axis of the correlation between petal size and pollen production.

Figure 14.4 illustrates the vectors of selection that we applied and the evolutionary responses to this selection. These vectors are the bivariate selection differentials, which depict the strength and direction of selection for both traits simultaneously in

two dimensions. Although we sought to select perpendicular to the major axis of genetic variation, we were not entirely successful, because the direction of the selection vector depends on the phenotypic variance in each trait during each generation and their relative means (recall that selection was applied to the difference between filament and corolla tube, rather than to the individual traits themselves). These phenotypic means and variances depend strongly on the environment, as illustrated by the randomly mated controls (Fig. 14.4). For example, selection seems to have increased flower size overall during the experiment and flower size fluctuated from generation to generation. However, similar changes in the control lines indicated that these patterns resulted from environmental differences in a glasshouse among generations, rather than genetic changes in response to selection. Regardless, selection for increased anther exertion achieved this outcome relative to selection for reduced exertion in both replicates

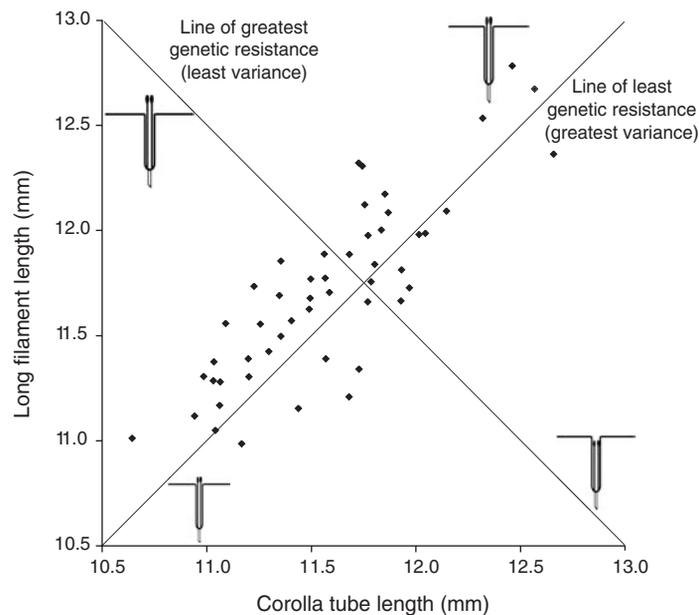


Figure 14.3 Genetic lines of least and greatest resistance. The points are means for filament and corolla tube length for half-sibling families of wild radish, *Raphanus raphanistrum* (data from Conner and Via 1993), so together they depict the additive genetic correlation between the two traits. The lines are the major and minor axes of this correlation, which correspond to the directions of greatest and least additive genetic variation in two dimensions. Selection along the major axis will produce the fastest evolutionary response, whereas selection along the minor axis will produce the slowest response; thus, they are known as the lines of least and greatest genetic resistance, respectively (Schluter 1996). The diagrams depict the relative sizes of the two traits at the four corners.

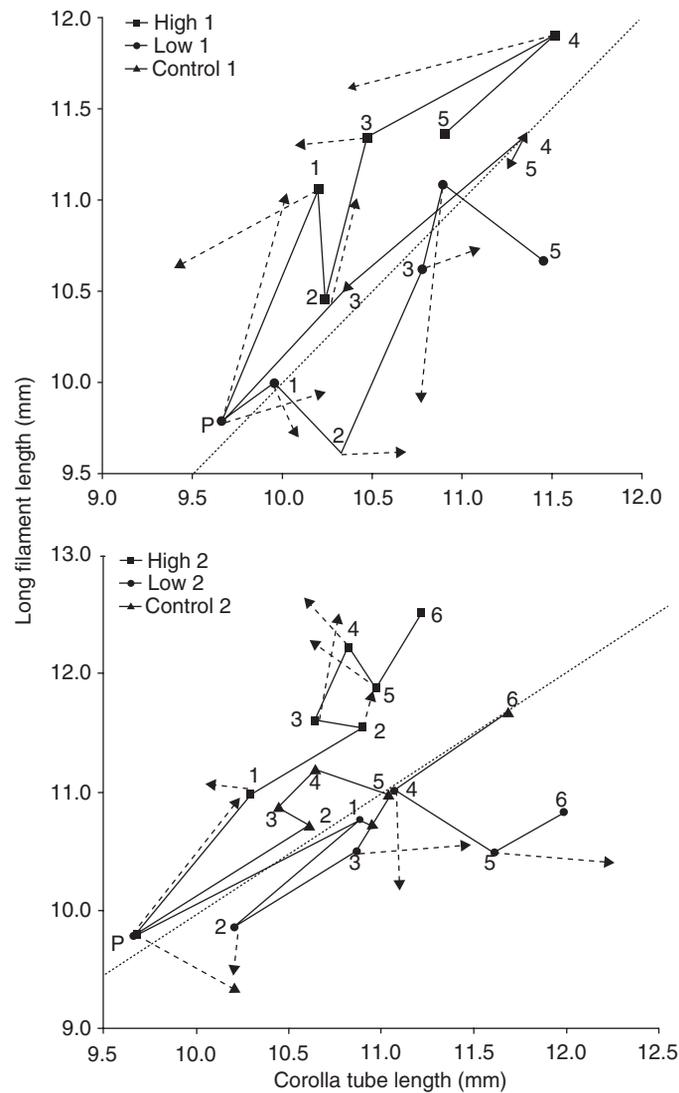


Figure 14.4 Bivariate selection differentials (dashed vectors) and overall changes in mean corolla tube and filament lengths (solid lines) of *Raphanus raphanistrum* during five and six generations of artificial selection in two replicate experiments. Each symbol represents the bivariate mean during the generation indicated by the adjacent number (P = parental), with squares indicating lines selected for increased exertion, circles depicting lines selected for reduced exertion, and triangles representing control lines. Control plants were not measured during the first two generations for replicate 1 and the first generation for replicate 2.

(Fig. 14.4), so that by the end of the experiment the lines had diverged perpendicularly to the major axis of genetic variation (see Plate 5 for examples).

Similarly, environmental differences among generations often affected the mean phenotype, despite the well-controlled glasshouse, which complicated assessment of the response to selection, especially for

traits subject to genetic correlations. The genetic responses can be isolated partially from the influence of among-generation environmental differences by examining the phenotypic differences between the lines selected for increased and reduced anther exertion (Fig. 14.5), which were raised together simultaneously in the same glasshouse.

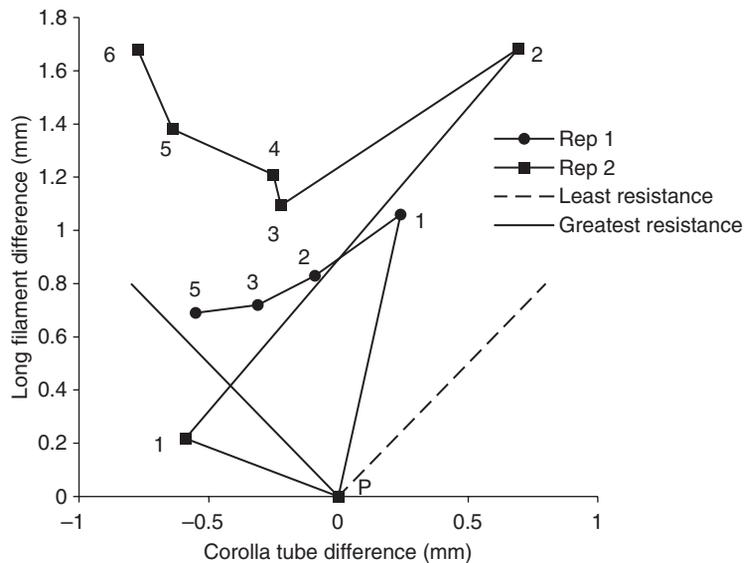


Figure 14.5 Mean differences in filament and corolla tube lengths between lines of *Raphanus raphanistrum* selected for high and low anther exertion in two replicate experiments during five and six generations, respectively. Numbers beside each point indicate the respective generation (P = parental). Lines of greatest and least genetic resistance to selection, based on the genetic correlation between the traits, are plotted for reference. Results for generation 4 of replicate 1 are not plotted, because the selection lines were not grown simultaneously.

In replicate 1, anther exertion changed considerably during the first generation, evolving along a trajectory roughly 30° from the line of least genetic resistance (represented by equal change for the two traits). This change in exertion resulted from a large difference in filament length between lines and a much smaller difference in corolla tube length. During the next generation of selection the trajectory of evolution turned sharply, and further divergence between the lines selected for increased and reduced anther exertion involved mainly evolution of tube length, with less change in filament length. Thus, replicate 1 never evolved along lines of maximum genetic resistance, that is, perpendicular to the major axis of variation, but rather at an angle to it.

Replicate 2 followed a somewhat different evolutionary trajectory. During the first generation of selection this replicate evolved roughly along the line of greatest genetic resistance (and perpendicular to the line of least resistance), as a result of both an increase in filament length and a decrease in tube length in the high lines relative to the low lines (Fig. 14.5). During the second generation, this replicate evolved parallel to the line of least

resistance, resulting in a bivariate mean difference between lines similar to that in replicate 1 after one generation, with a large divergence in filament length and a smaller divergence in tube length between lines. As in replicate 1, the trajectory then turned sharply during the next generation and evolved in almost the opposite direction to that during generation two, again roughly parallel to the line of least resistance. Finally, the trajectory of divergence turned again and evolved along lines of greatest resistance during the final three generations.

The preceding results indicate that both traits evolved independently in both replicates, ultimately resulting in longer filaments and shorter corolla tubes in the lines selected for increased anther exertion than in those selected for reduced exertion. In the aggregate, anther exertion evolved very nearly along the line of greatest resistance (especially in replicate 2), even though many individual segments of the trajectory deviated from this course. This outcome demonstrates that genetic correlations are not a strong constraint to independent evolution, at least over a few generations, and that net evolution can occur quite

rapidly and predictably in the direction of greatest genetic resistance (least genetic variance) if selection is in this direction. However, the precise trajectories of evolution during each generation are less predictable.

14.5.2 Macroevolution

To test whether the type of independent evolution of filament and corolla tube observed in the preceding experiment is reflected in species divergence, Alan Prather, Anna Wiese, and I, along with members of my research group, measured floral traits in 23 species drawn broadly from the Brassicaceae, plus *Cleome spinosa*, representing an outgroup (Capparaceae). For these species, the mean lengths of the long filaments and corolla tubes have clearly evolved along lines of least genetic resistance (Fig. 14.6): most species cluster near the line of equality. Thus, most of the evolution in filament and corolla tube length in the Brassicaceae

has resulted from changes in flower size, without much change in these traits relative to each other. Whether this isometry represents the influence of a genetic constraint is less clear. Schluter (1996) noted that isometry could occur if the line of least resistance also includes adaptive trait combinations. Accordingly, the observed isometry could reflect correlational selection for equal-length filament and corolla tubes within the Brassicaceae. We have evidence for this correlational selection in wild radish (Fig. 14.2; Morgan and Conner 2001), although whether this selection has occurred throughout such a large (>3000 species) and diverse family is unknown. The constraint is clearly not absolute, as very exerted anthers (filaments much longer than the corolla tube) have evolved in *Aethionema* and *Stanleya*, and highly inserted anthers (filaments shorter than corolla tube) have evolved in *Hesperis* and *Matthiola* (see Plate 5). These genera mirror the results of our artificial selection for increased and reduced

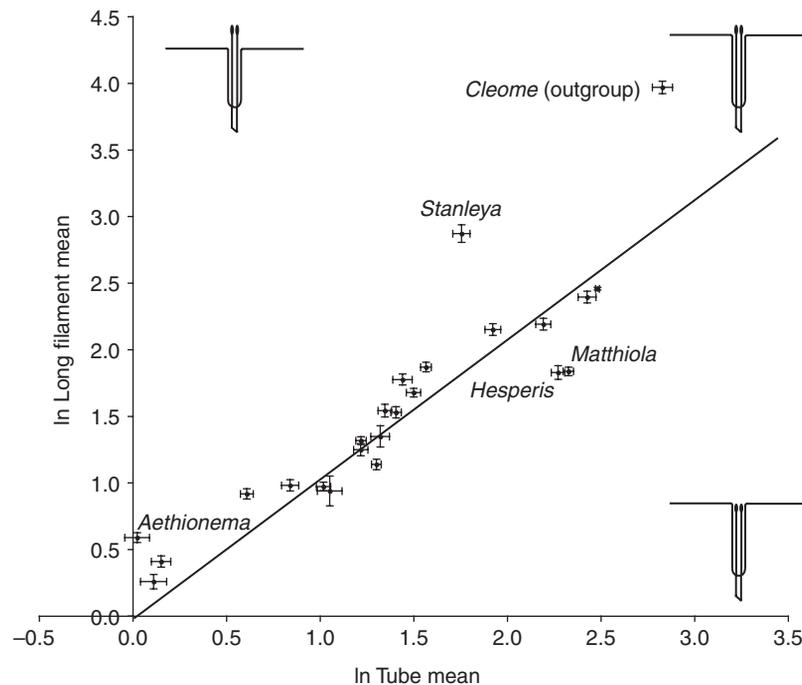


Figure 14.6 Macroevolution (mostly) along lines of least genetic resistance. Shown are ln-transformed mean ($\pm 95\%$ confidence intervals) filament and corolla tube lengths of 23 species of Brassicaceae plus *Cleome* (Capparaceae). Some species were measured in the field, whereas others were measured in a glasshouse, so environmental differences are included. Schematic floral cross-sections depicting anther exertion in the different regions of the graph are also shown.

anther exertion, respectively. Overall, these comparative results suggest that genetic correlations among traits can guide the direction of interspecific phenotypic differentiation, but that these constraints can be overcome by selection.

14.6 Future directions

What directions might studies of the genetics of floral traits take in the near future? Here I highlight four areas:

Selection on floral traits through male fitness: More studies of selection on floral traits that incorporate lifetime male fitness, estimated through molecular genetic paternity analysis, are clearly needed. Because selection often acts differently through male than through female fitness (see Section 14.4 above), this information is critical to identify what floral trait values are adaptive and in what ways selection through male and female fitness conflicts. Although selection on floral traits through male fitness and functional gender have been major topics in plant evolutionary ecology for 25 years (Lloyd 1980; Lloyd 1984; Chapter 1), data that address these topics directly with reliable estimates of male fitness have been sorely lacking.

The genetic architecture of floral adaptations: The quantitative genetic techniques described in this chapter treat the genome as a black box, using the statistical abstractions of variance and covariance. This approach will continue to be extremely fruitful, especially to predict evolutionary change quantitatively using eqs 1 and 2 (Section 14.3), but it does not provide information on the details of genetic variances and covariances at the level of individual loci and alleles at those loci. The genetic architecture of a trait includes several interesting and fundamental issues. For example, is variation in a given trait governed by a few loci with large influence on phenotypic variance, many loci of small effects, or a mixture of both? The latter possibility seems most likely and is supported by many of the studies of quantitative trait loci (QTL) discussed below. If this result is confirmed, it implies that the initial stages of adaptation may be quite rapid, involving fixation of the large-effect loci, but the latter stages will be slower, relying instead on the greater number of loci with smaller

phenotypic effect. Another key aspect of genetic architecture is whether genetic correlations between traits result from pleiotropy or gametic-phase disequilibrium (Conner 2002). Correlations caused by gametic-phase disequilibrium are unlikely to cause long-term evolutionary constraint; conversely, a finding of gametic-phase disequilibrium suggests that selection or some other evolutionary process is operating to maintain the disequilibrium. Finally, how important is epistasis i.e., interactions among gene loci, in adaptive evolution? Gene interactions are ubiquitous and they seem to be important in experimental evolution of microbes (Elena and Lenski 2001); however, their importance in adaptive evolution of more complex organisms and traits, such as floral traits, is largely unknown.

Mapping of QTL takes a first step towards characterizing genetic architecture. QTL mapping seeks to associate variance in complex phenotypic traits with molecular markers scattered throughout the genome. For floral traits this technique has been used mainly to map genes causing interspecific differences (e.g., Lin and Ritland 1997; Bradshaw *et al.* 1998). An exception was provided by Juenger *et al.* (2000), who mapped eight floral traits in the well-studied Landsberg *erecta* X Columbia recombinant inbred lines of *Arabidopsis thaliana*. They found 18 QTL that affected at least one trait, with 11 affecting more than one trait, indicating pleiotropy or fairly tight linkage. QTL mapping is only a first step in determining genetic architecture of complex traits, because the QTL identified are fairly large sections of chromosomes that can contain dozens or perhaps hundreds of genes. Because the *A. thaliana* genome has been sequenced, Juenger *et al.* could identify candidate genes (genes whose function has been identified in other studies) for some of these QTL. This is one approach that can facilitate the identification of the gene loci affecting a trait, and thus understanding of the genetic architecture of adaptive floral traits at the level of individual loci.

The relative roles of regulatory versus structural genes: A third fundamental question considers the degree to which variation in complex traits results from variation in structural genes (those coding for proteins) versus regulatory genes (those affecting

the expression of other genes). Changes in regulatory genes can create dramatic and coordinated phenotypic change, as the effects of one regulatory change can cascade through a large metabolic pathway or regulatory network. Regulatory genes underlie major morphological adaptations in vertebrates (Abzhanov *et al.* 2004; Shapiro *et al.* 2004) and the evolution of regulatory elements has been proposed to be the main source of novel morphologies (Doebley and Lukens 1998). Although changes in regulatory genes may be particularly important in morphological evolution, changes in structural genes may be more important in simpler traits involving single molecules, such as resistance to insecticides and toxins (Geffeney *et al.* 2002; Ffrench-Constant *et al.* 2004) and pigmentation (Hoekstra and Nachman 2003). For example, recall that red flowers evolved in *Ipomoea* through changes in an enzyme-coding structural gene (Section 14.2; Zufall and Rausher 2004). Nevertheless, a substantial body of work demonstrates that regulatory genes control floral-organ identity (reviewed in Ng and Yanofsky 2001). Whether the same loci vary within natural populations, and are responsible for genetic variance in adaptive floral traits, is mostly unknown. The increasing availability of whole-genome microarrays and quantitative PCR are improving the ability to measure gene expression, so these questions are now more tractable.

Combined approaches: The largest advances in understanding the ecological genetics of flowers may result from studies that combine different approaches, such as QTL mapping and microarray analysis (Wayne and McIntyre 2002), artificial selection and QTL mapping (reviewed in Conner 2003), and quantitative genetics within species with phylogenetic studies (Schluter 1996; Baker and Wilkinson 2003; Fig. 14.6). These combinations will probably provide the most rapid progress towards some long-term goals in the ecological genetics of flowers, such as understanding complex floral adaptations at the level of allele frequencies at individual gene loci, and understanding the interplay between genetics and selection in creating floral diversification among species and even higher taxonomic groups. To date, these combined approaches have seldom

been applied to floral traits, but advanced molecular genetic tools are currently under development for several plant species in addition to *A. thaliana*, including *Mimulus* (<http://www.biology.duke.edu/mimulus/>) and *Aquilegia* (S. A. Hodges personal communication), so that understanding of the genetic influences on floral adaptation is certain to continue evolving.

Acknowledgements

I thank my collaborators, Keith Karoly, Alan Prather, Anna Wiese, and members of our research groups for helping to generate many of the ideas and data presented here. I am also grateful to my current research group, Meghan Duffy, Frances Knapczyk, Cindy Mills, Neil Patel, Angela Roles, and Heather Sahli, for comments on an earlier version of this chapter. My research has been supported by the National Science Foundation under grants DEB 9796183, DEB 9903880, DEB 0108354, and DBI 9605168 and by the Cooperative State Research, Education, and Extension Service, US Department of Agriculture, under Agreement No. 2002*35320-11538.

References

- Abzhanov A, Protas M, Grant BR, Grant PR, and Tabin CJ (2004). Bmp4 and morphological variation of beaks in Darwin's finches. *Science*, **305**, 1462-65.
- Armbruster WS (1991). Multilevel analysis of morphometric data from natural plant populations: insights into ontogenetic, genetic, and selective correlations in *Dalechampia scandens*. *Evolution*, **45**, 1229-44.
- Ashman TL, Knight TM, Steets JA, *et al.* (2004). Pollen limitation of plant reproduction: Ecological and evolutionary causes and consequences. *Ecology*, **85**, 2408-21.
- Baker RH and Wilkinson GS (2003). Phylogenetic analysis of correlation structure in stalk-eyed flies (*Diasemopsis*, Diopsidae). *Evolution*, **57**, 87-103.
- Bateman AJ (1948). Intra-sexual selection in *Drosophila*. *Heredity*, **2**, 349-68.
- Bennington CC and McGraw JB (1995). Phenotypic selection in an artificial population of *Impatiens pallida*: The importance of the invisible fraction. *Evolution*, **49**, 317-24.

- Bradshaw HD Jr, Otto KG, Frewen BE, McKay JK, and Schemske DW (1998). Quantitative trait loci affecting differences in floral morphology between two species of monkeyflower (*Mimulus*). *Genetics*, **149**, 367–82.
- Campbell DR (1996). Evolution of floral traits in a hermaphroditic plant: field measurements of heritabilities and genetic correlations. *Evolution*, **50**, 1442–53.
- Caruso CM (2000). Competition for pollination influences selection on floral traits of *Ipomopsis aggregata*. *Evolution*, **54**, 1546–57.
- Caruso CM (2004). The quantitative genetics of floral trait variation in *Lobelia*: potential constraints on adaptive evolution. *Evolution*, **58**, 732–40.
- Clegg MT and Durbin ML (2000). Flower color variation: A model for the experimental study of evolution. *Proceedings of the National Academy of Sciences of the United States of America*, **97**, 7016–23.
- Conner JK (2002). Genetic mechanisms of floral trait correlations in a natural population. *Nature*, **420**, 407–10.
- Conner JK (2003). Artificial selection: a powerful tool for ecologists. *Ecology*, **84**, 1650–60.
- Conner JK and Hartl DL (2004). *A primer of ecological genetics*. Sinauer Associates, Sunderland, MA.
- Conner JK and Sterling A (1995). Testing hypotheses of functional relationships: a comparative survey of correlation patterns among floral traits in five insect-pollinated plants. *American Journal of Botany*, **82**, 1399–406.
- Conner JK and Via S (1993). Patterns of phenotypic and genetic correlations among morphological and life history traits in wild radish, *Raphanus raphanistrum*. *Evolution*, **47**, 704–11.
- Conner JK, Rush S, and Jennetten P (1996a). Measurements of natural selection on floral traits in wild radish (*Raphanus raphanistrum*). I. Selection through lifetime female fitness. *Evolution*, **50**, 1127–36.
- Conner JK, Rush S, Kercher S, and Jennetten P (1996b). Measurements of natural selection on floral traits in wild radish (*Raphanus raphanistrum*). II. Selection through lifetime male and total fitness. *Evolution*, **50**, 1137–46.
- Conner JK, Franks R, and Stewart C (2003a). Expression of additive genetic variances and covariances for wild radish floral traits: comparison between field and greenhouse environments. *Evolution*, **57**, 487–95.
- Conner JK, Rice AM, Stewart C, and Morgan MT (2003b). Patterns and mechanisms of selection on a family-diagnostic trait: Evidence from experimental manipulation and lifetime fitness selection gradients. *Evolution*, **57**, 480–6.
- Delph LF, Gehring JL, Frey FM, Arntz AM, and Levri M (2004). Genetic constraints on floral evolution in a sexually dimorphic plant revealed by artificial selection. *Evolution*, **58**, 1936–46.
- Doebley J and Lukens L (1998). Transcriptional regulators and the evolution of plant form. *The Plant Cell*, **10**, 1075–82.
- Elena SF and Lenski RE (2001). Epistasis between new mutations and genetic background and a test of genetic canalization. *Evolution*, **55**, 1746–52.
- Elle E and Meagher TR (2000). Sex allocation and reproductive success in the andromonoecious perennial *Solanum carolinense* (Solanaceae). II. Paternity and functional gender. *American Naturalist*, **156**, 622–36.
- Epperson BK and Clegg MT (1987). Frequency-dependent variation for outcrossing rate among flower-color morphs of *Ipomoea purpurea*. *Evolution*, **41**, 1302–11.
- Falconer DS and Mackay TFC (1996). *Introduction to quantitative genetics*, 4th edition. Longman, Harlow, UK.
- Ffrench-Constant RH, Daborn PJ, and Le Goff G (2004). The genetics and genomics of insecticide resistance. *Trends in Genetics*, **20**, 163–70.
- Ford EB (1975). *Ecological genetics*. Chapman and Hall, London.
- Galen C (1996). Rates of floral evolution: adaptation to bumblebee pollination in an alpine wildflower, *Polemonium viscosum*. *Evolution*, **50**, 120–5.
- Galen C (1999). Why do flowers vary. *Bioscience*, **49**, 631–40.
- Galen C and Stanton ML (1989). Bumble bee pollination and floral morphology: Factors influencing pollen dispersal in the alpine sky pilot, *Polemonium viscosum* (Polemoniaceae). *American Journal of Botany*, **76**, 419–26.
- Geffeney S, Brodie ED, and Ruben PC (2002). Mechanisms of adaptation in a predator-prey arms race: TTX-resistant sodium channels. *Science*, **297**, 1336–9.
- Grafen A (1988). On the uses of data on lifetime reproductive success. In TH Clutton-Brock, ed. *Reproductive success*, pp. 454–71. University of Chicago Press, Chicago.
- Harder LD and Barrett SCH (1993). Pollen removal from tristylous *Pontederia cordata*: effects of anther position and pollinator specialization. *Ecology*, **74**, 1059–72.
- Harder LD and Thomson JD (1989). Evolutionary options for maximizing pollen dispersal of animal-pollinated plants. *American Naturalist*, **133**, 323–44.
- Hoekstra HE and Nachman MW (2003). Different genes underlie adaptive melanism in different populations of rock pocket mice. *Molecular Ecology*, **12**, 1185–94.
- Huether CA Jr (1968). Exposure of natural genetic variability underlying the pentamerous corolla constancy in *Linanthus androsaceus* ssp. *androsaceus*. *Genetics*, **60**, 123–46.
- Johnston MO (1991). Natural selection on floral traits in two species of *Lobelia* with different pollinators. *Evolution*, **45**, 1468–79.

- Juenger T, Purugganan M, and Mackay TFC (2000). Quantitative trait loci for floral morphology in *Arabidopsis thaliana*. *Genetics*, **156**, 1379–92.
- Karoly K and Conner JK (2000). Heritable variation in a family-diagnostic trait. *Evolution*, **54**, 1433–8.
- Kay QON (1978). The role of preferential and assortative pollination in the maintenance of flower colour polymorphisms. In AJ Richards, ed. *The pollination of flowers by insects*, pp. 175–90. Academic Press, New York.
- Kingsolver JG, Hoekstra HE, Hoekstra JM, et al. (2001). The strength of phenotypic selection in natural populations. *American Naturalist*, **157**, 245–61.
- Kobayashi S, Inoue K, and Kato M (1999). Mechanism of selection favoring a wide tubular corolla in *Campanula punctata*. *Evolution*, **53**, 752–7.
- Larson BMH and Barrett SCH (2000). A comparative analysis of pollen limitation in flowering plants. *Biological Journal of the Linnean Society*, **69**, 503–20.
- Lin J-Z and Ritland K (1997). Quantitative trait loci differentiating the outbreeding *Mimulus guttatus* from the inbreeding *M. platycalyx*. *Genetics*, **146**, 1115–21.
- Lloyd DG (1980). Sexual strategies in plants III. A quantitative method for describing the gender of plants. *New Zealand Journal of Botany*, **18**, 103–8.
- Lloyd DG (1984). Gender allocations in outcrossing cosexual plants. In R Dirzo and J Sarukhán, eds. *Perspectives on plant population ecology*, pp. 277–300. Sinauer Associates, Sunderland, MA.
- Maad J and Alexandersson R (2004). Variable selection in *Platanthera bifolia* (Orchidaceae): phenotypic selection differed between sex functions in a drought year. *Journal of Evolutionary Biology*, **17**, 642–50.
- Mazer SJ and Schick CT (1991). Constancy of population parameters for life-history and floral traits in *Raphanus sativus* L. II. Effects of planting density on phenotype and heritability estimates. *Evolution*, **45**, 1888–907.
- Mazer SJ, Delesalle VA, and Neal PR (1999). Responses of floral traits to selection on primary sexual investment in *Spergularia marina*: The battle between the sexes. *Evolution*, **53**, 717–31.
- Morgan MT and Conner JK (2001). Using genetic markers to directly estimate male selection gradients. *Evolution*, **55**, 272–81.
- Ng M and Yanofsky MF (2001). Function and evolution of the plant MADS-box gene family. *Nature Reviews Genetics*, **2**, 186–95.
- Nilsson LA (1988). The evolution of flowers with deep corolla tubes. *Nature*, **334**, 147–9.
- O'Neil P and Schmitt J (1993). Genetic constraints on the independent evolution of male and female reproductive characters in the tristylous plant *Lythrum salicaria*. *Evolution*, **47**, 1457–71.
- Phillips PC (2005). Testing hypotheses regarding the genetics of adaptation. *Genetica*, **123**, 15–24.
- Queller DC (1983). Sexual selection in a hermaphroditic plant. *Nature*, **305**, 706–7.
- Ritland K (2000). Marker-inferred relatedness as a tool for detecting heritability in nature. *Molecular Ecology*, **9**, 1195–204.
- Ritland K and Ritland C (1996). Inferences about quantitative inheritance based on natural population structure in the yellow monkey flower, *Mimulus guttatus*. *Evolution*, **50**, 1074–82.
- Sarkissian TS and Harder LD (2001). Direct and indirect responses to selection on pollen size in *Brassica rapa* L. *Journal of Evolutionary Biology*, **14**, 456–68.
- Schemske DW and Bierzychudek P (2001). Perspective: evolution of flower color in the desert annual *Linanthus parryae*: Wright revisited. *Evolution*, **55**, 1269–82.
- Schemske DW and Horvitz CC (1989). Temporal variation in selection on a floral character. *Evolution*, **43**, 461–5.
- Schluter D (1996). Adaptive radiation along genetic lines of least resistance. *Evolution*, **50**, 1766–74.
- Shapiro MD, Marks ME, Peichel CL, et al. (2004). Genetic and developmental basis of evolutionary pelvic reduction in threespine sticklebacks. *Nature*, **428**, 717–23.
- Shore JS and Barrett SCH (1990). Quantitative genetics of floral characters in homostylous *Turnera ulmifolia* var. *angustifolia* Willd. (Turneraceae). *Heredity*, **64**, 1105–12.
- Stanton ML (1994). Male–male competition during pollination in plant populations. *American Naturalist*, **144**, S40–68.
- Stanton ML and Young HJ (1994). Selecting for floral character associations in wild radish, *Raphanus sativus* L. *Journal of Evolutionary Biology*, **7**, 271–85.
- Stanton ML, Snow AA, and Handel SN (1986). Floral evolution: attractiveness to pollinators increases male fitness. *Science*, **232**, 1625–7.
- Thomas SC, Pemberton JM, and Hill WG (2000). Estimating variance components in natural populations using inferred relationships. *Heredity*, **84**, 427–36.
- Thomson JD and Thomson BA (1992). Pollen presentation and viability schedules in animal-pollinated plants: consequences for reproductive success. In R Wyatt, ed. *Ecology and evolution of plant reproduction*, pp. 1–24. Chapman and Hall, New York.
- Turelli M, Schemske DW, and Bierzychudek P (2001). Stable two-allele polymorphisms maintained by fluctuating fitness and seed banks: Protecting the blues in *Linanthus parryae*. *Evolution*, **55**, 1283–98.
- van Kleunen M and Ritland K (2004). Predicting evolution of floral traits associated with mating system in a natural plant population. *Journal of Evolutionary Biology*, **17**, 1389–99.

- Waser NM and Price MV (1981). Pollinator choice and stabilizing selection for flower color in *Delphinium nelsonii*. *Evolution*, **35**, 376–90.
- Wayne ML and McIntyre LM (2002). Combining mapping and arraying: an approach to candidate gene identification. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 14903–6.
- Willson MF and Price PW (1977). The evolution of inflorescence size in *Asclepias* (Asclepiadaceae). *Evolution*, **31**, 495–511.
- Wilson P, Thomson JD, Stanton ML, and Rigney LP (1994). Beyond floral Batemanian: gender biases in selection for pollination success. *American Naturalist*, **143**, 283–96.
- Worley AC and Barrett SCH (2000). Evolution of floral display in *Eichhornia paniculata* (Pontederiaceae): Direct and correlated responses to selection on flower size and number. *Evolution*, **54**, 1533–45.
- Wright JW and Meagher TR (2004). Selection on floral characters in natural Spanish populations of *Silene latifolia*. *Journal of Evolutionary Biology*, **17**, 382–95.
- Zufall RA and Rausher MD (2004). Genetic changes associated with floral adaptation restrict future evolutionary potential. *Nature*, **428**, 847–50.

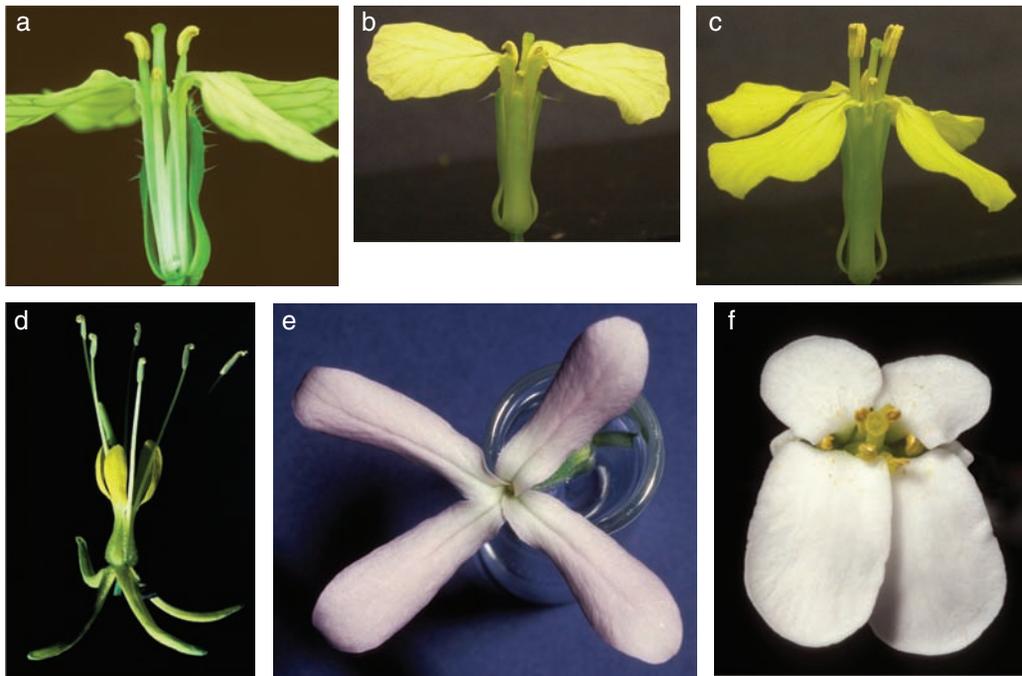


Plate 5 Flowers of selected species in the Brassicaceae: (a) wild radish *Raphanus raphanistrum* from a natural population, with one petal and sepal removed to show the filament and corolla tube lengths; (b) wild radish flower from a line subject to artificial selection for reduced anther exsertion (Section 14.5.1); (c) wild radish flower from a line selected for increased anther exsertion; (d) *Stanleya pinnata*, with naturally highly exserted anthers; (e) *Matthiola*, with highly inserted anthers; and (f) *Iberis*, with intermediate anther exsertion, typical of most species in the Brassicaceae (see Chapter 14). Photographs by Jeffrey K. Conner.