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Ecological Speciation in Flowering Plants

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13.1 Introduction

Speciation refers to divergence of phenotype, and the evolution of reproductive barriers between populations that exhibit the different phenotypes. Either of these components of speciation may involve adaptation by natural selection. In this context several terms have been coined (see Chapter 1, in particular Box 1.1). Any speciation in which reproductive isolation follows directly or indirectly from adaptation to ecological conditions has been termed *ecological speciation* (e.g., Hatfield and Schluter 1999). In contrast, this volume defines the term *adaptive speciation* as lineage splitting in sympatry (or parapatry) through a process of frequency-dependent selection. This latter definition thus includes some, but not all, forms of ecological speciation and vice versa.

In this chapter we adopt the broader term ecological speciation, as we review the forms it may take in flowering plants, or angiosperms, and explore whether there is evidence for adaptive speciation more specifically. Most of the about 300 000 angiosperm species rely on animals for pollination, and the dramatic evolutionary radiation of the angiosperms over the past 100 million years is roughly contemporaneous with radiation in several animal taxa from which modern pollinators are drawn. As a result, pollination is thought by many investigators to be a critical ecological factor in speciation (Baker 1963; Crepet 1984; Eriksson and Bremer 1992; Grimaldi 1999; but see Ricklefs and Renner 2000). We outline several scenarios for pollinator-mediated speciation, paying special attention to the unusual role of pollinators as external agents of gene transfer. As both selection and gene flow depend on pollinator responses to floral traits, genetic changes in these traits may simultaneously generate phenotypic divergence and reproductive isolation. Alternatively, divergence of floral phenotypes may be decoupled from selection on traits that prevent successful crosses. A different likely mechanism of speciation is adaptation of plants to local environmental conditions. Finally, selection for floral and reproductive divergence may stem from pollination and other environmental sources combined. For each of these kinds of mechanism we present the available evidence and suggest avenues for future research.

Limited space restricts our attention here to divergence of two incipient species via adaptation to external biotic or abiotic conditions. This constitutes only part of angiosperm speciation. We do not discuss divergence caused by random genetic drift alone [see Levin (2000) for some putative examples]. Nor do we discuss speciation that results from the formation of stabilized diploid hybrids (Gallez and

Gottlieb 1982; Arnold *et al.* 1990; Rieseberg 1995, 1997) or changes in ploidy (Grant 1981; Soltis and Soltis 1993). Intra- and intergenomic conflicts may underlie other speciation events (see Haig and Westoby 1989; Gavrilets 2000b), but these foster adaptation and counteradaptation within the genic environment rather than an external environment.

13.2 Ecological Speciation Driven by Animal Pollinators

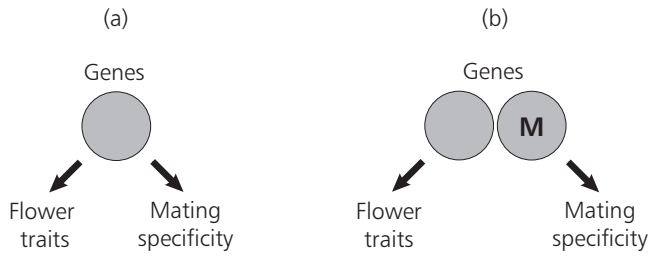
Mutualistic associations between plants and pollinators might drive ecological speciation in allopatry, parapatry, or sympatry. As we discuss below, similar mechanisms may generate reproductive isolation regardless of whether the incipient species are in ecological contact, based on the dual role of pollinators as agents of gene transfer and selection. Indeed, there is little direct evidence to date for pollinator-mediated speciation in the absence of spatial separation.

The basic scenario of *pollinator-mediated* divergent selection is especially transparent in the case of allopatry. Populations in geographic isolation encounter pollinators distinct from the ancestral ones (e.g., hummingbirds instead of large bees), and these pollinators select for different floral traits, and so produce phenotypic divergence. For example, the classic concept of pollination syndromes (Grant and Grant 1965; Faegri and van der Pijl 1979) predicts evolution of a deep, tube-shaped flower in a hummingbird-pollinated population (or "pollination race"), versus a broad bell-shaped flower in a bee-pollinated population. As gene flow between species also depends on pollinator behavior, a strong specialization for different pollinators also produces strong or complete reproductive isolation if the incipient species come into secondary contact (Grant 1949). The unusual dual role of pollinators (as agents of selection and movers of genes) thus suggests that speciation may sometimes proceed via a "single-variation" model (*sensu* Rice and Hostert 1993), with the same set of genes producing reproductive isolation as a pleiotropic by-product of phenotypic divergence (Box 13.1).

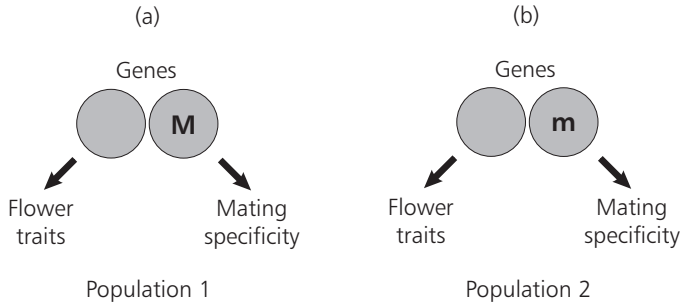
Initial differences in floral phenotype might often arise by mutations of large rather than small phenotypic effect (Gottlieb 1984; Bradshaw *et al.* 1995), and thus accelerate a "niche shift" to the use of a new pollinator resource. If this process occurs in local isolates, as argued by Levin (2000), then pollination races and ultimately the new species that result may be polyphyletic until lineage sorting (see Box 15.3) and extinction are complete (Levin 1993, 2000; Rieseberg and Brouillet 1994). When the incipient species come into secondary contact, it is possible for reproductive isolation imposed by pollinators to be reinforced by *postpollination* events, if negative assortative mating lowers fitness by generating phenotypes poorly adapted to either pollinator. For example, heterospecific pollen may fail to reach ovules or be outcompeted by conspecific pollen (e.g., Kölreuter 1763; Darwin 1876; Darlington and Mather 1949, p. 253; Paterniani 1969; Rieseberg 1995; although see Mather 1947; Alarcón and Campbell 2000). Reinforcement by a one-allele substitution is essentially automatic if appropriate genetic variation arises (e.g., Crosby 1970; Waser 1993; Waser and Price 1993), and even reinforcement by a two-allele substitution is likely, if reproductive isolation is strong already (Box 13.1; Felsenstein 1981; Dieckmann and Doebeli 1999; Chapter 5).

Box 13.1 Pleiotropy and linkage disequilibrium in ecological speciation

Phenotypic differentiation and reproductive isolation evolve in concert if they are pleiotropic effects of the same genes [figure below, panel (a)]. This "single-variation" model does not necessarily mean only one gene is involved, but rather that the same suite of genes produces phenotypic differentiation and reproductive isolation. For example, imagine a change in floral morphology that corresponds to a shift in major pollinators, with pollinator fidelity strong enough to eliminate pollen exchange between the derivative and parent species. A similar situation arises if separate genes code for phenotype and for mating specificity, so long as positive assortative mating is enhanced in all populations by substitution of the same allele [M in panel (b)]:



This is the "one-allele" substitution of Felsenstein (1981). In contrast, assortative mating may require substitution of alternative alleles, M and m, in different populations:



This "two-allele" substitution (Chapters 3 and 5) requires a statistical association between alleles at mating loci and at loci that code for phenotype. Such a linkage disequilibrium will arise and be maintained only with strong selection against hybrids, and genetic drift (or another mechanism such as partial flower constancy of pollinators) sufficient to generate an initial association (Felsenstein 1981; Dieckmann and Doebeli 1999).

In spite of its logical simplicity, this scenario has enjoyed little direct support to date. Many studies describe strong pollinator-mediated selection on floral phenotypes (e.g., Campbell 1989; Johnston 1991; Andersson and Widén 1993; Galen 1996), which illustrates the potential for rapid evolutionary change. However, the usual focus is on selection *within* a species rather than divergence *between* incipient species, so we have only indirect support for the scenario. We now need to extend the studies of selection not only to related species, but also to larger

spatial scales within species that are appropriate for exploring divergence in allopatry or parapatry – efforts that will require unusual collaboration and resources to succeed. Examples of such an approach include Levin's and colleagues' (Levin and Kerster 1967; Levin and Schaal 1970) demonstration that *Phlox* species exhibit character displacement in flower color, and reduced hybridization by butterflies upon secondary contact; and preliminary evidence (Peter E. Scott, personal communication) that flower morphology and color, and flowering phenology, vary geographically in ocotillo (*Fouquieria splendens*) in response to changes in pollination environment (e.g., large bees versus resident hummingbirds versus transient migratory hummingbirds).

Another reason for the scanty support for this scenario may be that species interactions are not always as tidy as it tacitly assumes. Many pollination interactions are generalized and opportunistic (e.g., Herrera 1996; Waser *et al.* 1996; Memmott 1999). In such cases, one-on-one coevolution of plants and pollinators is unlikely, and instead new pollination races may adapt to *mixtures* of pollinator species that differ *quantitatively*, not qualitatively, from those of parents (Grant and Grant 1965), with weak-to-moderate reproductive isolation on secondary contact (Waser 1998, 2001).

The scenario of pollinator-mediated speciation in allopatry can be extended to situations in which ongoing gene exchange counteracts divergence (i.e., to parapatry and sympatry). If different pollinator types exhibit sufficiently narrow and invariant preferences, they might select disruptively on floral phenotype even within a single population, which would lead to phenotypic divergence and, pleiotropically, to reproductive isolation. The amount of isolation that ensues depends on how often pollinators fly between flowers of the same rather than different phenotype, and on how much pollen is transferred, two aspects historically labeled "ethological" and "mechanical" isolation, respectively (Grant 1949). If some isolation via pollinators is in place, it may again be reinforced by postpollination events.

As a consequence of the dual role of pollinators, sympatric speciation, like allopatric speciation, can proceed via a single-variation model (see Box 13.1). In this case, the usual difficulty imposed by sympatric speciation of generating linkage disequilibrium between mating loci and a fitness locus is circumvented. Whether sympatric speciation of this form really does occur, however, depends critically on the *strength* of disruptive selection and reproductive isolation produced by preferences of different pollinators for alternative phenotypes.

Once again, there are few telling studies. Mather (1947) inferred (without direct observation) that pollinator behavior explained the rarity of F1 hybrids between the snapdragons *Antirrhinum majus*, *A. glutinosa*, and *A. orontium* placed in mixtures. More recent studies have observed pollinator visitation directly in mixed-species arrays. For example, Fulton and Hodges (1999) reported strong fidelity of hummingbirds to the columbine *Aquilegia formosa*, and of hawkmoths to *Aq. pubescens*. However, unless preferences are absolute (and they rarely are, at least in temperate systems; Wilson and Thomson 1996), it cannot be assumed that

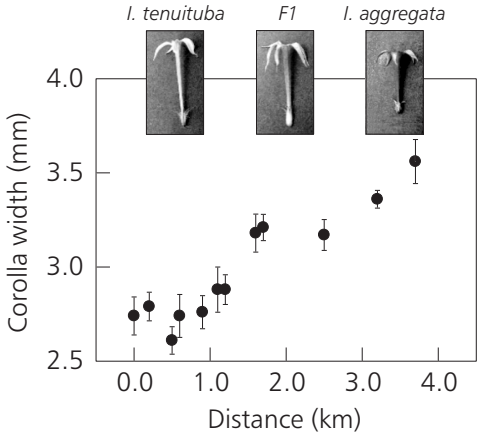


Figure 13.1 Several floral traits show clinal variation across a 4 km wide hybrid zone of *Ipomopsis*. Plants of *I. aggregata* have wider corolla tubes than those of *I. tenuituba*, and populations along the hybrid zone show a gradual transition in this trait. F1 hybrids are intermediate in corolla width, length, and flower color. Bars indicate standard errors.

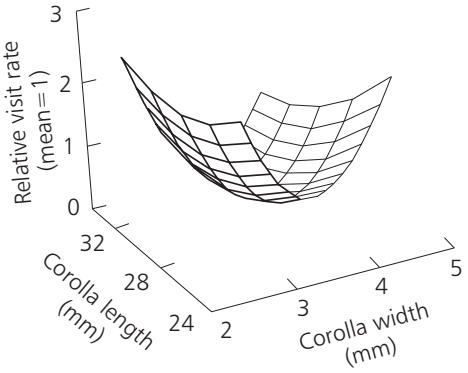
divergent preferences will always result in the lowest overall visitation to intermediate phenotypes. With the inclusion of natural or experimentally produced hybrids, or selected mutants, it is possible to generate a wider range of floral phenotypes, including intermediates, and to estimate selection more precisely than when only parental species are presented to pollinators. For example, when arrays of hybrid monkeyflowers (*Mimulus*) that express a wide range of floral traits are exposed to hummingbirds and bumblebees, selection favors elements of the phenotypes of the parental species (Sutherland and Vickery 1993; Vickery 1995; Schemske and Bradshaw 1999), but intermediate phenotypes also received substantial visits (Vickery 1992). Similarly, Ippolito and Holtsford (1999) documented disruptive selection by the combined visitation of hawkmoths and hummingbirds to arrays of F2 and F3 hybrids between two species of Brazilian *Nicotiana*. In contrast, Wesslingh and Arnold (2000) found that F1 *Iris* hybrids were visited more frequently than the two parental species. In none of these cases is it yet clear that selection is strong and consistent enough to drive complete divergence of the phenotype, much less reproductive isolation. Inconsistent selection is not a trivial issue. For example, Campbell *et al.* (1997) also documented disruptive selection on flower width in hybrid populations of *Ipomopsis* (Figure 13.1 and Box 13.2), but such selection occurred only in rare years when hawkmoths were present along with the more reliable hummingbirds (Plate 6).

More study certainly is needed of the strength and consistency of disruptive selection mediated by pollinators. This should be followed by investigations of whether differences in the selected traits also confer some reproductive isolation, and whether other traits (including postpollination traits noted earlier) also contribute. Few of the studies described above reported the frequency of interspecific pollinator flights in addition to the overall rate of visitation; those that did found weak-to-moderate, rather than strong-to-absolute, ethological isolation (Campbell *et al.* 1998, 2002; Wesslingh and Arnold 2000).

Box 13.2 Pollinator-mediated disruptive selection

Several quantitative traits of the flowers of *I. aggregata* are under phenotypic selection during the pollination phase of the life cycle (Campbell 1989), and heritabilities and genetic correlations suggest that this could cause rapid evolution (Campbell 1996). In some cases the mechanism of selection is known to involve pollinators. For example, experiments show that hummingbirds select for wider floral tubes (fused corollas), which they probe deeply, thereby removing more pollen per visit (Campbell *et al.* 1991, 1996).

In some parts of the western USA, *I. aggregata* grows in contact with the congener *I. tenuituba* and the two form hybrids with intermediate floral phenotypes. In one such hybrid zone, Campbell *et al.* (1997) used multivariate selection analysis to detect disruptive selection on corolla width within single populations. Here, the component of fitness is rate of visitation to flowers by hummingbirds [broad-tails (*Selasphorus platycercus*) and rufous (*S. rufus*)], and hawkmoths (*Hyles lineata*), which prefer broad and narrow corollas, respectively (Plate 6). The quadratic relationship for visitation (relative to the mean) as a function of corolla width and length, at a value of flower color equal to the average for natural hybrids, is:



This selection gradient occurs only in rare years when hawkmoths appear in appreciable numbers; in most years pollination is predominantly by hummingbirds. However, this example illustrates how pollinator-mediated disruptive selection may operate in some systems, and so lead to speciation.

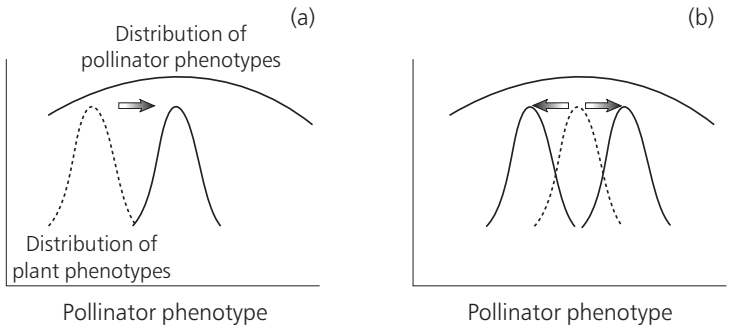
Another promising experimental approach is the genetic or phenotypic manipulation of a trait already shown to differ between closely-related species, to see whether elimination of the difference increases interspecific pollination. As is true in some animals (Chapter 16), color patterns often distinguish flowers of different species, and are amenable to manipulation (Waser and Price 1985b). When Meléndez-Ackerman and Campbell (1998) painted flowers of *I. tenuituba* and hybrids red, to match flowers of the congener *I. aggregata*, more of the resultant seeds were hybrid than in a control population with the species differences intact, which suggests that color differences confer some reproductive isolation. A similar approach could be taken with other traits, using phenotypic manipulation or plants known to differ at major loci for floral traits.

Box 13.3 Adaptive dynamics of pollinator-mediated speciation

Disruptive or divergent pollinator-mediated selection on floral phenotypes seems to require, at first glance, two distinct modes of pollinator phenotype (e.g., two pollinators that differ markedly in the lengths of their tongues, proboscises, or beaks). Adaptive dynamics models, however, highlight the existence of an alternative mechanism (Metz *et al.* 1996; Dieckmann and Doebeli 1999). In this mechanism, which also has a unimodal and continuous distribution of pollinator phenotypes (e.g., describing the relative frequencies of pollinators as a function of their tongue length), disruptive selection can result from frequency-dependent competition for pollination among plants.

In this scenario each plant phenotype corresponds to a pollinator phenotype to which it is best adapted. The pollination resources available to a particular plant phenotype are therefore proportional to the frequency of the corresponding pollinator. Models for adaptive dynamics driven by frequency-dependent competition for a unimodal resource (in the present case represented by the pollinators) are then directly applicable (Chapter 5).

These models predict the following. First, the mean phenotype in the parental plant species evolves to occupy the peak of the pollinator distribution, as illustrated by the arrow in panel (a) below:



Once the mean phenotype in the plant population reaches this point, rare phenotypes on either side of the mean phenotype attract visits by pollinators from more marginal ranges of the pollinator distribution and thereby experience less competition for visits by pollinators. For disruptive selection to occur, this advantage to rare phenotypes must outweigh the disadvantage that results from the smaller abundance of pollinators away from the peak of their distribution and the potential disadvantage of finding other plants to mate with. If these disadvantages are overcome, evolutionary branching in the plant population can bring about two daughter species, as illustrated in panel (b) above.

The pollinator distribution in this scenario corresponds to the resource distribution used in the general models of evolutionary branching driven by intraspecific competition. Also, note the congruence with MacArthur's (1969) "Q-minimization" model, which predicts an optimal match of resource utilization and resource functions for a set of species.

The adaptive dynamics approach may thus offer a resolution to the apparent paradox, observed by Ollerton (1996), that pollinator-mediated plant speciation seems to require a multimodal "pollination space", whereas such a setting seems

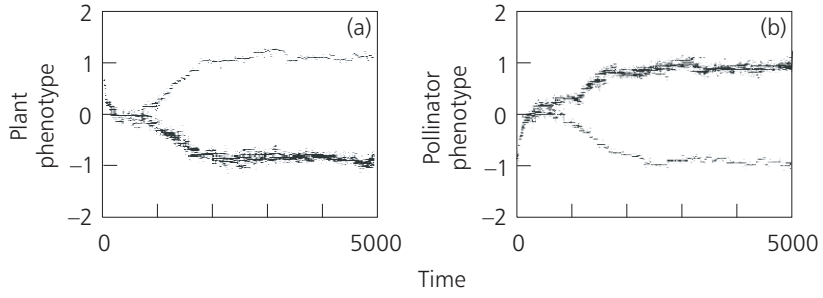
continued

Box 13.3 *continued*

unlikely to exist in nature given the generalization and opportunism in plant-pollinator interactions (see Herrera 1996; Waser *et al.* 1996). The scenario described above assumes that, on the time scale of plant speciation, little or no evolutionary change occurs in the distribution of pollinator phenotypes. This assumption may apply to plants that rely on many pollinator species (see Memmott 1999).

In addition, adaptive dynamics models can be constructed for more complex scenarios that lead to the cospeciation of tightly associated plants and pollinators – a process that may have happened, for instance, in some figs and fig wasps (Herre *et al.* 1996).

An example for such a model was provided by Doebeli and Dieckmann (2000). Based on a Lotka–Volterra system for two mutualistically interacting species, individuals in each species are characterized by a quantitative trait that determines the distribution of mutualistic benefit given to individuals of the other species. In this coevolutionary scenario, plants are viewed as resources for the pollinators, and vice versa. The allocation of this benefit depends on the phenotypes of the interacting individuals in such a way that the level of support increases with phenotypic similarity. In addition to determining the strength of the mutualistic interaction, the quantitative trait also determines the amount of external resources available to each individual. This imposes a stabilizing effect on the quantitative traits, with a different optimal trait value in each of the two species. Thus, while the mutualistic interaction is frequency dependent and selects for phenotypic similarity, the stabilizing selection imposed by the distribution of external resources selects for specific trait values that are different in the two species.



If the conflict between these different sources of selection is large enough, coevolution can result in concurrent evolutionary branching in both species [cospeciation; see panels (a) and (b) above]. These results suggest that evolutionary diversification can also arise when plants and pollinators are specialized to each other, as long as additional external resources are critical and provide the necessary selection for distinct quantitative phenotypes.

The "adaptive dynamics" framework (Chapters 4, 5, and 7) suggests a variant on pollinator-mediated ecological speciation in sympatry or parapatry (Box 13.3). According to this model, distinct types of pollinators, or quantitatively distinct mixtures of the same pollinator types, are not required for the initial pollinator-mediated divergence in floral phenotype. Even with a distribution of pollinator

phenotype that is unimodal rather than bi- or multimodal, rapid evolution of floral phenotype and splitting into multiple phenotypic classes might be observed. However, to date no evidence indicates this mechanism of adaptive speciation in angiosperms.

13.3 Adaptation and Speciation in Different Environments

Another potential route to speciation involves adaptation to different environments (other than animal pollinators), with reproductive isolation following as a pleiotropic by-product. Environment differences may be abiotic, such as in soil moisture, chemistry, or solar insolation (e.g., McNeilly and Antonovics 1968; Davies and Snaydon 1976; Lovett Doust 1981; Galen and Stanton 1991), or biotic, involving interactions with soil fungi, herbivores, competitors, and diseases (e.g., Turkington and Harper 1979; Parker 1985). A special case arises when abiotic conditions lead to a paucity of animal pollinators, which may select for traits such as predominant self-pollination or greatly modified pollen, flowers, or inflorescences to achieve wind pollination, any of which will enhance reproductive isolation between the original species and its derivative (e.g., Berry and Calvo 1989; Weller *et al.* 1998).

A famous example of genetic divergence that involves soil chemistry and competition is the adaptation of plant populations to soils with high concentrations of salts of heavy metals. On tailings (spoils) from British lead and zinc mines (dating in some cases to Roman times), plants are tolerant to the metals, whereas genotypes from adjacent pasture are unable to grow. Conversely, tolerant genotypes are overshadowed and excluded from the pasture by faster-growing intolerant genotypes (McNeilly and Antonovics 1968; Hickey and McNeilly 1975). The boundary between tailings and pasture is abrupt, as is that between genotypic classes. In some species, such as the grasses *Agrostis tenuis* and *Anthoxanthum odoratum*, tolerant and intolerant races differ in seasonal time of flowering, and so are partially isolated reproductively at a prepollination stage. Flowering phenology can evolve rapidly under selection (e.g., Stanford *et al.* 1962; Carey 1983), so this difference might represent adaptive reinforcement of postzygotic reproductive isolation (hybrid inviability). However, more direct causes of different flowering phenology cannot be ruled out (Antonovics 1968; McNeilly and Antonovics 1968), such as a difference in the timing of appropriate moisture conditions of the soil in pasture versus tailings. In a similar case that involves *Mimulus* species endemic to serpentine soils, Macnair and Gardner (1998) proposed that nutrient-poor, dry soils select for early flowering and self-fertilization. This has the effect of reducing gene flow between species, but it does not constitute adaptive reinforcement.

Adaptation of plants to local abiotic and biotic conditions is reported on various spatial scales across a range from close parapatry to allopatry (e.g., Bradshaw 1960; Jain and Bradshaw 1966; Davies and Snaydon 1976; Turkington and Harper 1979; Waser and Price 1985a; Bennington and McGraw 1995; Mehrhoff and Turkington 1996; Ronsheim 1997; for a historical review see Langlet 1971). Examples of ecological and phenotypic radiation on oceanic islands and habitat

islands provide especially compelling cases of apparent adaptive radiation in strict allopatry (Levin 2000; Chapter 17). Thus, it appears possible that ecological speciation via this route is common. What is poorly understood is how reproductive isolation evolves. There is evidence for partial pre- and postzygotic barriers, both for short distances [e.g., the mine tailings example and Waser *et al.* (2000)] and on larger spatial scales within species (e.g., Kruckeberg 1957; Grant and Grant 1960; Hughes and Vickery 1974; Vickery 1978). In some cases these barriers are known to be direct pleiotropic effects of the genes involved in local adaptation (Macnair and Christie 1983; Galen and Stanton 1991; Stanton *et al.* 1994).

13.4 Combined Speciation Mechanisms

Pollinators are assumed to be central to one mode of ecological speciation and, as outlined in Section 13.2, this mechanism of *pollinator-mediated divergent selection* is likely, although evidence is scanty. In addition, pollinators might play a more subtle, indirect role by facilitating the mechanism of adaptation to local environments described in Section 13.3 (Waser 1998, 2001) and thus speciation in local isolates (Levin 2000). The scenario for *pollinator-facilitated local adaptation* is as follows. By their area-restricted foraging, itself a result of natural selection for efficiency in food harvest, pollinators move most pollen relatively short distances (e.g., Levin and Kerster 1974; Campbell and Waser 1989; Karron *et al.* 1995). The resultant restriction of pollen dispersal, coupled with restricted seed dispersal (the situation for most plant species), allows adaptation of conspecific plants in different populations to different local environments. In other words, gene flow between populations is insufficient to counterbalance local adaptation. Thus, pollinator behavior helps to foster phenotypic and reproductive distinctiveness.

This scenario remains mostly unexplored. We do not know, for example, how closely the scale of local selection matches restrictions on gene flow. Part of the uncertainty arises from the difficulty in estimating the long-distance tail of gene flow. New methods of paternity exclusion (e.g., Devlin and Ellstrand 1990) reveal surprising amounts of pollen flow in excess of 1 km in some species (5–24% of paternity in examples cited by Levin 2000), more than anticipated from direct studies of pollinator behavior or pollen dispersal from a source (Campbell 1991a). It remains unclear, however, where exactly this long-distance pollen comes from, how fit offspring sired by such pollen are (but see Waser and Price 1994; Waser *et al.* 2000), and, most importantly, how such gene flow interacts with locally variable selection to determine the final patterns of genetic differentiation.

Other combinations of mechanisms also seem likely. In particular, divergent selection in parapatry or allopatry (or disruptive selection in sympatry) might be mediated by a combination of pollinators and other environmental features. For example, divergent floral phenotypes in *Polemonium viscosum* along elevational gradients may represent different equilibria between opposing selection on flower size related to water loss from petals, attraction of nectar-robbing ants, and interaction with pollinator faunas that comprise different mixtures of bumblebees and flies (Galen 1983, 1996; Galen *et al.* 1999; Galen and Cuba 2001). Brody (1997)

Table 13.1 Selection on floral phenotype by combinations of pollinators and other environmental factors. In some cases the evidence for selection as tabulated here is circumstantial; in others it is more firmly established. In no cases are the mechanisms of selection fully worked out, and neither is net selection shown to vary from site to site, which would lead to divergent selection in parapatry or allopatry. Nonetheless, examples such as these point toward possible cases of floral divergence under the influence of multiple factors.

Species	Traits	Selection	References
Polemonium viscosum	Flower size, corolla flare	Preference by bumble bees, pollen transfer efficiency, water loss, damage from nectar robbers	Galen 1996; Galen *et al.* 1999; Galen and Cuba 2001
Phlox drummondii	Flower color	Pollinator preference, pleiotropic effect on survival and flower production	Levin 1972; Levin and Brack 1995
Delphinium nelsonii	Flower color, nectar guides	Pollinator preference, background color, environment	Waser and Price 1985b
Mimulus cardinalis and *M. lewisii*	Flower color and shape, leaf morphology	Pollinator preference, survival	Hiesey *et al.* 1971
Ipomoea purpurea	Flower color	Preference by bumble bees, pleiotropic effect on plant size and seed size via herbivore resistance	Rausher and Fry 1993; Fineblum and Rausher 1997
Clarkia gracilis	Petal color spotting	Preference by bees, differential survival	Jones 1996
Ipomopsis aggregata	Flower morphology, flowering phenology	Preference of pollinators and predispersal seed predators	Campbell 1991b; Brody 1997; Irwin and Brody 1999

and Irwin and Brody (1999) pointed out the potential for selection on floral phenotype jointly mediated by pollinators and by plant enemies, such as seed predators and nectar robbers. Flowering time is a trait that may be selected by pollinators, but also by other environmental conditions (e.g., Ehrendorfer 1953; McNeilly and Antonovics 1968; Waser 1978). Other possible cases of interaction between various mechanisms of selection and various targets of selection have been identified (Table 13.1), but usually not fully explored. Examples are likely to be common, but to detect them requires investigators not to focus too narrowly on only one aspect of plant biology, such as pollination or vegetative physiology, in isolation of the others.

One approach to study simultaneously pollinators and other potential sources of divergent or disruptive selection makes use of reciprocal transplants across species

boundaries. Reciprocal transplants have a long history in botany (Clausen *et al.* 1940; Langlet 1971), but have been applied only recently to mechanisms of speciation. They do not necessarily reveal the nature of selection that *generates* the original species differences, but can elucidate selection that *maintains* differences. The hope is that by observing species or subspecies that are closely related we can learn about the selection responsible for early stages of species differentiation (Harrison 1993). For example, consider an experimental reciprocal transplant across the zone of contact between two related plant species. If selection always favors the parental species, with hybrids less fit in both parental habitats, divergent selection in parapatry is implicated (ecological speciation model of Hatfield and Schluter 1999; Chapter 9). Whether such selection derives from pollinators, from other environmental factors, or from some mixture could then be distinguished by comparing selection at each site that arises from variation in pollination success with selection at other life-history stages. In sagebrush (*Artemisia*), each parental species survived better than the other parent or hybrids in its own home habitat, whereas hybrids survived better than either parent in a hybrid zone between the parental habitats (Wang *et al.* 1997). In contrast, F1 and F2 hybrids between two species of *Iris* survived as well as both parental species, regardless of habitat, once rhizomes had been established (Emms and Arnold 1997). In the *Iris* system, reproductive isolation appears to result from neither pollinator behavior (Wesslingh and Arnold 2000) nor physical features of the environment, but largely from postpollination events that limit the formation of F1 hybrids (Arnold *et al.* 1993; Carney *et al.* 1994).

To date, reciprocal transplant experiments generally have been conducted only over a small portion of the life history, but ultimately it will be necessary to extend them to allow direct comparison of pollination success of hybrids with other fitness components. In an *Ipomopsis* hybrid zone, differences in both pollination and vegetative survival contribute to fitness differences between the two parental species and F1 hybrids (Campbell and Waser 2001). *I. aggregata* appears to have higher fitness at low elevation, largely because its floral traits make it attractive to hummingbirds (Campbell *et al.* 1997), whereas *I. tenuituba* has higher fitness at high elevation largely because of a survival advantage. Reproductive isolation is not strongly developed, but F1 hybrids may suffer because pollinators move pollen more effectively from *I. aggregata* to *I. tenuituba* (Campbell *et al.* 1998, 2002), and hybrid seeds with the latter species as maternal parent survive poorly in parental habitats (Campbell and Waser 2001). Thus, a complex mixture of pollinator-mediated selection and other mechanisms is involved in the dynamics of this hybrid zone, and may have contributed to the original differentiation of the parental species.

In summary, a promising approach is to combine reciprocal transplant studies across multiple habitats with selection analysis (Nagy 1997), or with manipulation of specific traits, to determine if traits are selected divergently, and if so by pollinators, other features of the environment, or both.

Box 13.4 Hot research topics, old and new

The last major full treatment of angiosperm speciation is by Grant (1981). The major sections of his book consider the nature of plant species, divergence of species, secondary genetic fusion of species, derived genetic systems (such as polyploidy), and the evolution of hybrid (= species) complexes. Grant lists the following primary mechanisms of divergence of species:

- Geographical speciation;
- Quantum speciation;
- Sympatric speciation;
- Reproductive isolation as a by-product of divergence;
- Chromosomal repatterning.

We imagine that a modern update would include these same topics in some form. However, our ideal update would also stress some new issues, including:

- Studies of pollinator-mediated disruptive selection on various scales, and its exact mechanisms, including explicit attention focused on the behavior of the animals in choosing flowers and its root causes, such as cognitive constraint (e.g., see Chittka *et al.* 1999). These studies should be quantitative and coupled with investigations of reproductive isolation to determine whether the same floral traits under disruptive or divergent selection also confer reproductive isolation, as implied in the classic scenario.
- Assuming that the mechanisms of reproductive isolation are clearly determined, the investigation of their genetic basis [e.g., via quantitative trait locus (QTL) mapping; Bradshaw *et al.* 1995], with the proviso that the basis of species' differences may not fully reflect the basis of isolation mechanisms that constituted the actual speciation event (Harrison 1991). New molecular methods for the construction of isogenic lines should increase the range of characters that can be manipulated to identify the effects on reproductive isolation.
- The continued marriage of molecular systematics and ecology with the mapping of interactions, such as pollination and herbivory, onto family trees for the plants to infer evolutionary history and lability (see Armbruster 1996; Weller and Sakai 1999).
- Renewed focus on the plant phenotype, with emphasis on the integration of physiology and external morphology, and on the study of pleiotropic linkages between vegetative and reproductive traits (see Fineblum and Rausher 1997). Transplant studies with hybrids could be used to explore the relative importance of selection on vegetative versus floral traits. In addition, new morphometric approaches can be used to characterize the floral phenotype and to explore phenotypic selection (Herrera 1993).
- Study of the sizes of phenotypic changes that appear spontaneously by mutation in plants, and of the role of these in phenotypic divergence under natural selection. As Darwin stressed, *Natura non facit saltum*; but she does take steps of various sizes, and there is renewed discussion about the importance of "macromutations" (e.g., Gottlieb 1984; Doebley 1993).

continued

Box 13.4 *continued*

- Exploration of how reproductive isolation is manifest on a hierarchy of scales of spatial or genetic separation, with the goal of reconstructing the temporal sequence of evolution of isolating mechanisms (Coyne and Orr 1997). The spatial context could be incorporated by performing hybrid crosses over a wide range of spatial scales (Edmands 1999), and by combining phylogenetic information with biogeographic distributions (Schaal *et al.* 1998) to reveal the history of genetic exchange.
- A discussion of various forms of *intra- and intergenotypic conflict*, and how these might result in reinforcement of reproductive isolation between populations that diverge in parapatry or allopatry (e.g., Haig and Westoby 1989; Martienssen 1996; Grossniklaus *et al.* 1998; Rice 1998; Gavrilets 2000b). In particular, might such conflicts, and the selection "arms race" that they engender, turn out to be a major source of genetic incompatibilities between individuals from different populations, and if so on what spatial scales and under what circumstances?

13.5 Concluding Comments

In this chapter we are limited to a précis of ecological speciation. An updated treatment of all of angiosperm speciation, including ecological speciation, is overdue. However, a good argument can be made that this update must wait for the results of a renewed empirical attack. There is no lack of research topics at hand (Box 13.4), and new techniques promise answers to old and hitherto intractable questions.

In spite of empirical gaps, our conclusion is that ecological speciation in angiosperms is likely, via several mechanisms, but that no direct evidence is at hand for adaptive speciation as defined in this volume (Chapter 1). Given large values of phenotypic selection coefficients measured under natural conditions, and high heritabilities of phenotypic traits of flowers (Jain and Bradshaw 1966; Antonovics 1971; Bradshaw 1972; Davies and Snaydon 1976; Lovett Doust 1981; Campbell 1989, 1996; Armbruster 1990; Johnston 1991; Conner *et al.* 1996), speciation may also be quite rapid. Finally, divergence in sympatry is not ruled out. The task for the coming decades is to establish the relative importance of different mechanisms and spatial contexts.

In the study of speciation, flowering plants are worthy subjects in their own right. They are also of value as model systems. For example, individual plants "stay put to be counted" (Harper 1977) and so they have proved to be ideal subjects for transplant studies, which are impossible with most animals (Futuyma and Shapiro 1995), and this advantage remains to be fully exploited. While *exact* mechanisms of speciation in plants can no more be extended to other taxa than the reverse (e.g., Mitchell *et al.* 1993), the lessons learned from angiosperms promise to shed much light, however oblique, on ecological speciation in general.



Plate 6 Disruptive selection due to different pollinators (Chapter 13).
Hawkmoths (*Hyles lineata* – top) and hummingbirds (*Selasphorus* spp. – bottom) pollinating the red flowers of *Ipomopsis aggregata* (left) and the pale pink flowers of *I. tenuituba* (right). Both pollinators visit flowers of both species in a hybrid zone in Colorado, USA. However, hawkmoths prefer narrow flowers, while hummingbirds prefer wide, red flowers. As a result, disruptive selection on floral phenotype can occur in this hybrid zone, as postulated in some models of plant speciation. Because pollinator preferences are not absolute, however, they would not impose strong reproductive isolation between *I. aggregata* and *I. tenuituba* in sympatry.

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