CHAPTER 18

Floral biology of hybrid zones

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Outline

Hybridization between closely related species is relatively common in angiosperms and can create a natural hybrid zone. We review recent experimental studies of floral biology in pairs of hybridizing species, emphasizing comparisons of the floral morphology and nectar rewards of hybrid plants with that of their progenitors, and quantifying the influence of these floral traits on pollinator behaviour and pre-zygotic and post-zygotic reproductive isolation. Floral traits of hybrids can be intermediate or transgressive. Floral differences between species, which in one case are attributable to particular chromosomal regions, can have differing impacts on interspecific flights by pollinators. A simulation model of mating in a hybrid zone between *Ipomopsis aggregata* and *Ipomopsis tenuituba* shows that behavioural responses by hummingbird and hawk-moth pollinators affect pre-zygotic ethological isolation more strongly than mechanical isolation. We apply this model to compare contact sites between these species which differ greatly in the frequency of natural hybrids. Striking differences in hawk-moth behaviour between the sites generated large differences in the rate of interspecific pollen movement, potentially explaining the dissimilar frequencies of hybrids. Although floral traits influence both the formation and fitness of hybrids primarily through effects on pollinators, impacts on plant enemies also need consideration. Recent research has revealed much about how floral traits influence pollinator visitation, but mainly for systems with hummingbird versus insect pollinators. Such studies should be extended to other pollinators. Further research is also needed on how floral traits influence pollen dispersal and other post-visitation events that impact reproductive isolation, and their genetic basis in natural populations.

18.1 Introduction

Although no record of the word "hybrid" exists before the early seventeenth century (Oxford English Dictionary 1971), ancient Greek mythology was populated by mixed creatures, such as the chimaera, a composite of a lion, goat, and dragon, which were considered unnatural and monstrous. During the early twenty-first century, "hybrid" is more likely to connote an innovative and synergistic invention than a monstrosity. The evolutionary biologist's view of hybrids has changed similarly. Based largely on animal studies, natural hybrids were traditionally considered as either evolutionary dead-ends, occurring rarely and usually being sterile, or indicating a breakdown in reproductive isolation caused by disturbance, often attributed to human activity (Mayr 1963). However, evolutionary biologists now ascribe to hybrids a much wider range of evolutionary roles, including introgression of genes from another species, as stressed originally by Anderson (1949); persistence of stable hybrid zones (Barton and Hewitt 1985); introduction of novel genetic variation that can increase adaptation to a particular environment (Anderson and Stebbins 1954; Kim and Rieseberg 1999; Martinsen *et al.* 2001);

promotion of speciation through reinforcement (Servedio and Noor 2003); and production of a new hybrid species (Rieseberg *et al.* 1996; Rieseberg 1997).

Flowering plants hybridize commonly. Based on analysis of five floras, Ellstrand *et al.* (1996) estimated that hybrids comprise 9–22% of the total species in a particular region. More recently, Mallet (2005) reported that 25% of vascular plant species in the UK hybridize. Many of the observations or specimens undoubtedly come from hybrid zones, or areas of mixing between two related species that include some viable hybrids. Such a hybrid zone may be a result of secondary contact or ongoing speciation in sympatry or parapatry. Hybrid zones can form between native species, as for example between *Iris fulva*, *Iris brevicaulis*, and *Iris hexagona* in Louisiana (Arnold 1994), or between a recently introduced and a native species, as between two species of cordgrass (*Spartina*) in San Francisco Bay (Ayres *et al.* 1999).

Hybrid zones often present a striking profusion of flower morphologies. These floral traits can profoundly influence the degree of reproductive isolation between the species, by altering any of several steps in pre-zygotic and post-zygotic isolation (Fig. 18.1). Studies of *Phlox* provided an

early example: plants of *Phlox pilosa* with white corollas set a lower percentage of hybrid seed than did plants with pink corollas that were similar to the congener *Phlox glaberrima* (Levin and Schaal 1970). Perhaps the best-known mechanism for such pre-zygotic isolation depends on behaviour of animal pollinators; the floral morphology and rewards offered by each species may attract its own type of pollinator, which tends to move more often between flowers of the same species (ethological isolation, Fig. 18.1a). As outlined by Verne Grant (1949), seasonal and mechanical isolation (differences in floral structure restrict interspecific pollen movement: Fig. 18.1b) can also prevent interspecific mating, the deposition of heterospecific pollen on stigmas. Furthermore, recent studies have shown associations between certain floral traits and post-mating isolation that influence production of hybrid seed (Fig. 18.1c).

Floral traits may also influence post-zygotic reproductive isolation by altering several components of hybrid fitness (Fig. 18.1d–f). Divergent selection on alternative floral morphologies would coincide with low hybrid fitness, and could be important in driving floral diversification (Chapter 15). This impact on post-zygotic events has received less study than that of pre-zygotic events;
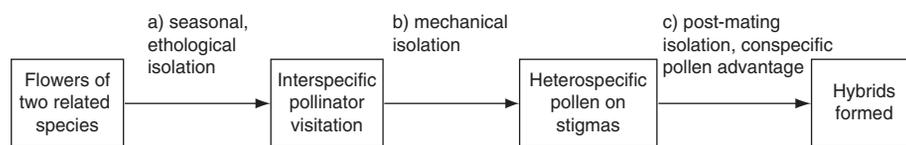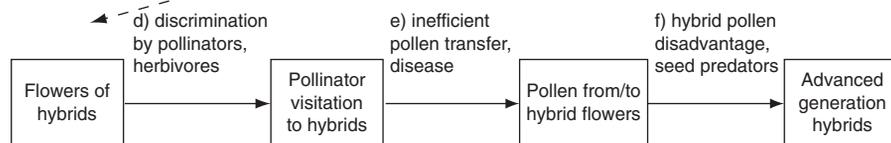

Figure 18.1 Steps in pre-zygotic and post-zygotic isolation that can be influenced by floral traits. The step from "hybrids formed" to "flowers of hybrids" represents survival and is shown by a dashed arrow as it is influenced indirectly via correlations with other traits.

however, in principle, floral traits could influence pollination success of hybrids directly, through both male and female functions (Chapter 2), and affect several components of fitness through interactions with floral herbivores, nectar thieves, and seed predators (Chapter 7).

The resulting fitness of hybrids relative to the parental species is critically important to evolution in hybrid zones and the origin and maintenance of species (Hatfield and Schluter 1999). Natural selection against intrinsically unfit hybrids balanced by some gene flow between the species (and hence incomplete pre-zygotic reproductive isolation) can maintain a spatially stable hybrid zone (Barton and Hewitt 1985). In this case, distinct floral morphologies and species could be maintained at opposite ends of a cline. Certain types of environmentally dependent selection on hybrids can also lead to a stable zone (reviewed in Arnold 1997). Alternatively, a hybrid zone might expand due to neutral mixing of genes following secondary contact between two species. If genes for floral traits are neutral, distinct floral morphologies would not persist. Finally a hybrid zone can shrink or disappear if reinforcement of reproductive isolation by selection against inter-specific mating decreases hybridization. Hybridization between invasive exotic and native species is of special concern as a cause of extinction and genetic assimilation of endangered plant species (Levin *et al.* 1996).

In this chapter, we explore the influences of floral traits on reproductive isolation in hybrid zones. We begin by examining the morphology of hybrid flowers, asking whether flowers of hybrids are intermediate or transgressive in phenotype, and how this trait distribution depends on the genetic architecture of floral morphology. We then examine effects of floral traits on pre-mating reproductive isolation (steps a and b in Fig. 18.1), especially that resulting from plant–pollinator interactions, and on post-mating reproductive isolation (step c). We find that despite early recognition of mechanisms of pre-zygotic isolation, surprisingly few studies have quantified the relative importance of various steps in the process. In addition to identifying specific forms of reproductive isolation that require more study, we

derive a model of pollinator behaviour to explain mating patterns and quantify impacts of ethological, mechanical, and post-mating isolation in hybrid zones between *Ipomopsis aggregata* and *Ipomopsis tenuituba*. We then examine more briefly recent studies exploring how floral traits influence hybrid fitness and thus post-zygotic isolation (steps d–f in Fig. 18.1). We conclude with recommendations for future study of reproductive isolation and hybridization.

18.2   Genetic architecture of species differences; what do hybrid flowers look like?

The distribution of floral morphologies in hybrid zones depends on two major factors. First, because of the underlying genetic architecture, hybrids need not be intermediate in floral morphology or other traits between the parental species, but instead can be transgressive, or more extreme than either parent (Rieseberg and Ellstrand 1993). Second, the distribution may reflect past selection for hybrids with particular phenotypes.

Hybrids necessarily have intermediate phenotypes only if they are F_1 individuals, as in a *Rhododendron* hybrid zone (Milne *et al.* 2003), and the traits measured vary solely due to additive effects of genes. In contrast to this very restrictive situation, many hybrid zones are dominated by hybrids from later generations, as in the Louisiana irises for which no F_1 individuals have been found in the wild (Hodges *et al.* 1996). Even among F_1 individuals, variation due to dominance causes the phenotypic mean to deviate from that of the parents. Such effects are well known in agriculture, as many crop-development programmes take advantage of heterosis shown by F_1 individuals, whose vigour exceeds that of the parents. Furthermore, second generation (F_2) hybrids formed by crossing two F_1 individuals often exhibit transgressive traits (reviewed by Rieseberg *et al.* 1999a).

Transgressive traits can arise from epistasis, in which trait values deviate from the summed effects at two loci. Such epistasis can produce hybrid breakdown due to Dobzhansky–Muller incompatibilities (Rhode and Cruzan 2005), as

found in monkey flowers (*Mimulus guttatus* and *Mimulus nasutus*; Fishman and Willis 2001). Although rarely documented, epistasis could also generate transgressive floral traits.

Second, transgression can arise from complementary effects of additive alleles at multiple loci. If one species has an overall higher phenotypic value than the species with which it hybridizes, because the positive effects of alleles at some loci outweigh the negative effects of alleles at other loci, a recombinant F_2 individual might by chance inherit all positive alleles and be transgressive with a yet higher phenotypic value. Recent studies using QTL mapping have suggested that complementary genes are the major cause of transgression in plants (Rieseberg *et al*. 1999a).

Note that transgressive phenotypes are not an exception, but occur frequently in crosses between plant species. For example, 44% of traits reviewed by Rieseberg *et al*. (1999a: 58% if intraspecific crosses are included) exhibited transgression, although floral traits were not identified specifically. An example of transgressive floral traits comes from QTL mapping of species differences between the monkey flowers *Mimulus cardinalis* and *Mimulus lewisii* (Bradshaw *et al*. 1998). Several floral traits in the F_2 generation exhibited transgression, notably the size-related characters of corolla width and petal width, and nectar volume. Characters related to flower colour showed little transgressive segregation, which might reflect the influence of only a few major QTLs. In contrast, F_2 progeny of the columbines *Aquilegia formosa* and *Aquilegia pubescens* were intermediate for all five floral traits measured (Hodges *et al*. 2002). The possibility of transgressive segregation calls into question the use of morphological indices by themselves to assess the frequency of plant hybrids, but such indices can be valuable in combination with controlled crosses that characterize the trait values of F_1 and F_2 individuals to determine which traits are transgressive (Section 18.4.1).

18.3 Floral traits and the frequency of mating between species and hybrids

Production of hybrids is the converse of reproductive isolation. Therefore, studying the causes of hybrid production leads to an examination of forms of potential reproductive isolation and their roles in speciation. We begin with pre-mating isolation (Fig. 18.1a and b), which can occur through seasonal, ethological, or mechanical mechanisms (Grant 1949, 1994). Seasonal isolation refers to phenological differences in flowering that prevent mating (Husband and Schemske 2000; Chapter 8). Ethological isolation occurs when behavioural preferences of pollinators for particular floral traits restrict their movement between flowers of different species. Complete ethological isolation results if pollinators of different types (e.g., bumble bees and hummingbirds) have narrow and non-overlapping preferences. Such strong preferences are envisioned in the textbook concept of "pollinator syndromes" in which suites of floral traits lead to visitation by a particular type of pollinator (Faegri and van der Pijl 1966; Baker and Hurd 1968); for example, "hummingbird flowers" are red with broad corolla tubes. Alternatively, mechanical isolation results when differences in floral structures restrict interspecific pollen movement. For example, the positions of stigma and anthers may cause pollen grains from two species to be carried on different parts of a pollinator's body. Morphological adaptation to a specific pollinator could evolve in response to selection exerted by pollinators that visit a species most frequently and effectively ("the most effective pollinator": Stebbins 1970).

18.3.1 Ethological isolation

Although some degree of ethological isolation can be inferred from observations in natural hybrid zones, quantifying the process requires observations at experimental arrays of plants that provide simultaneous choice to pollinators. Observations of plants growing *in situ* are usually not sufficient, as biased visitation to one species can result instead from spatial separation of the species. Randomization of the locations of plants in an array also allows assessment of the relative importance of pollinator preference for floral traits of one plant species versus constancy, whereby individual pollinators visit both species but make long bouts of visits to one species before switching to the

other (Waser 1986). Table 18.1 summarizes recent experimental array studies of pollinator visitation between potentially hybridizing species, using either randomized or alternating positions of species (the latter can underestimate ethological isolation). Some arrays used just the two parental species, illustrating what might happen during initial contact between two species, whereas others also included hybrids, illustrating the potential for interspecific pollinator movement in established hybrid zones. For each case, we calculated reproductive isolation due to ethological isolation from the relative frequency of pollinator movement between plants of different species (Ramsey et al. 2003):   RI = 1 − (heterospecific transitions/conspecific transitions). Not all of the studies reported the necessary information, instead focusing on the demonstration of pollinator preference. However, estimates of ethological RI varied across the entire range from 0 (random visitation) to 1 (complete pollinator specialization; Table 18.1).

Many of these studies focused on situations with one hummingbird and one insect-pollinated species. In all such cases, the two major types of pollinators showed contrasting preferences, although they varied so much in strength that RI still ranged from 0 to 1, suggesting that the variation is not explained simply by pollinator type. In arrays of red-flowered *I. fulva* and blue-flowered *I. brevicaulis*, along with F_1 and backcross hybrids, hummingbirds preferred *I. fulva* flowers and bumble bees preferred F_1s (Wesselingh and Arnold 2000). However, both pollinator types visited nearest neighbouring plants nearly 80% of the time, generating zero or weak ethological isolation. In a similar experiment involving blue-flowered *I. hexagona* instead of *I. brevicaulis*, bumble bees preferred *I. hexagona* and hummingbirds preferred *I. fulva*, but both moved frequently between a parental species and F_1 individuals (Emms and Arnold 2000). These backcross movements were more common than heterospecific movements,

Table 18.1 Experimental array studies of ethological isolation between closely related species due to pollinator visit transitions.

Species	Hybrids included?	Pollinators	Floral traits selected	Pre-zygotic ethological RI	Source
Iris brevicaulis, I. fulva	Yes	Bumble bees, hummingbirds		0–weak	Wesselingh and Arnold (2000)
Iris hexagona, I. fulva	Yes	Bumble bees, hummingbirds		0–0.69[a]	Emms and Arnold (2000)
Mimulus lewisii, M. cardinalis	Yes	Bees, hummingbirds	Colour (bees), nectar (birds)	Strong, 0.976[b]	Schemske and Bradshaw (1999)
Aquilegia pubescens, A. formosa	No	Hawk moths, hummingbirds	Orientation (moths)	0.85–1.00[c]	Fulton and Hodges (1999)
Nicotiana alata, N. forgetiana	Some arrays	Hawk moths, hummingbirds		0.59	Ippolito et al. (2004)
Ipomopsis tenuituba, I. aggregata	Some arrays	Hawk moths, hummingbirds	Colour (birds), width (both)	0.50, 0.30–0.96[d]	Campbell et al. (2002), Aldridge and Campbell, unpublished and this study
Baptisia leucophaea, B. sphaerocarpa	Some arrays	Bumble bees		0.48–0.84	Leebens-Mack and Milligan (1998)
Asclepias spp.	No	Generalist insects		0.39–1.00[e]	Kephart and Theiss (2003)

Ethological RI was calculated as 1 − (heterospecific plant transitions/conspecific transitions), unless otherwise noted.

[a] Value depends on site.

[b] The estimate of 0.976 is based on frequencies of foraging bouts that included one versus both species in an area of natural sympatry (Ramsey et al. 2003).

[c] Estimates based on frequencies of foraging bouts.

[d] Value depends on site.

[e] Value depends on pollinator type.

suggesting some ethological isolation between the species, but little pollinator discrimination against hybrids (step d in Fig. 18.1)

Only a few studies have used phenotypic or genetic manipulation to demonstrate effects of particular floral traits on pollinator visitation within and between species (Table 18.1). One case involved two *Mimulus* species (Schemske and Bradshaw 1999) that show strong ethological isolation in nature (Table 18.1). The pink-flowered *M. lewisii* has a wide corolla, produces little nectar, and is predominantly bee pollinated, whereas the red-flowered *M. cardinalis* has a narrower corolla, secretes more nectar, and is hummingbird pollinated. Including artificially produced hybrids in the array allowed investigation of how major QTLs for floral traits generate ethological isolation. The QTL marker genotype for petal carotenoid concentration dramatically affected visitation by bees, but not by hummingbirds. Conversely, the QTL for nectar production affected only hummingbird visitation.

In columbines (*Aquilegia*), flower orientation contributes significantly to ethological isolation (Fulton and Hodges 1999). *Aquilegia formosa* has pendent, red and yellow flowers with short nectar spurs, whereas *A. pubescens* has upright, pale flowers with long spurs. The two species produce hybrid zones in the southern Sierra Nevada Mountains of California. In an array of *A. pubescens* in which half of the flowers were tied to make the flowers pendent and thus given the trait of the other parental species, hawk moths visited upright flowers more than 10 times as often as pendent flowers. Manipulation of this one trait reproduced the strong preference for *A. pubescens* exhibited by hawk moths at arrays of the two parental species (Fulton and Hodges 1999).

Many pollinators may respond to suites of floral characters. An example of interacting effects of floral traits comes from hybrid zones between *Ipomopsis aggregata* and *I. tenuituba*. Hummingbirds prefer to visit the red-flowered *I. aggregata* when given simultaneous choice in arrays, visiting them 3–4 times as often as *I. tenuituba* (Campbell *et al.* 1997). However, other floral traits are also involved, as hummingbirds preferentially visit flowers with wide corolla tubes (Campbell *et al.* 2002) and can rapidly learn to associate white, instead of red, with high nectar reward. Manipulation of flower colour

in arrays showed that the combination of traits found naturally in *I. aggregata* induces higher hummingbird preference than occurs when flowers differ only in colour, suggesting that multiple characters contribute to the rate of hybridization (Melendez-Ackerman and Campbell 1998).

The presence of hybrids could lead to high interspecific pollen transfer, even though pollinators seldom move between species when hybrids are absent. Experiments that contrast responses to arrays of only the parental species and those including hybrids can evaluate the importance of this "hybrid bridge" (Leebens-Mack and Milligan 1998). Studies of *Nicotiana alata*, *Nicotiana forgetiana*, and their F_1 hybrids in Brazil provide a good example (Ippolito *et al.* 2004). In experimental plots with only plants of the two species, hummingbirds visited *N. forgetiana* exclusively, and hawk moths strongly preferred *N. alata*, in agreement with their pollinator syndromes. However, when plots contained the two species and F_1 individuals, hummingbirds visited both species, so that their movements could have produced interspecific gene flow, and backcrossing was also possible. This finding suggests that even if F_1 hybrids arise rarely due to pre-zygotic ethological isolation, their presence can accelerate further gene flow between species.

18.3.2   Mechanical isolation

Mechanical isolation is often suggested if pollen of two species is carried on different parts of pollinators' bodies, if a pollinator fails to contact reproductive parts of one species due to poor fit to the flower, or if hybrid production differs from interspecific pollinator movement (Macior 1965; Kephart and Theiss 2003; Ippolito *et al.* 2004). However, quantifying mechanical isolation requires tracking the movement of pollen grains within and between flower species. These patterns of pollen movement depend on the number of flowers displayed on a plant and on pollen carryover, the pattern of flower-to-flower pollen transfer (Chapter 5).

Few studies of pollen carryover have included related plant species (Levin and Berube 1972; Stucky 1985). One exception based on pollen dispersal within a species showed an intriguing

asymmetry in pollen transfer by hummingbirds and bumble bees visiting *Penstemon* (Castellanos *et al.* 2003). Because they carried pollen to a longer sequence of recipient flowers, birds could pollinate the bee-syndrome *Penstemon strictus* flowers nearly as well as bees, whereas bees pollinated the bird-syndrome *Penstemon barbatus* poorly, suggesting that mechanical isolation would be asymmetric. However, as far as we are aware, the measurements of pollen dispersal between anthers and stigmas of two related flower species by individual pollinators that are necessary to quantify mechanical isolation are available only from studies of *Ipomopsis*. On a per-visit basis, hummingbirds transfer little pollen from the highly exserted anthers of *I. aggregata* subsp. *formosissima* to the inserted stigma of *Ipomopsis arizonica*, but much pollen in the opposite direction, demonstrating asymmetric mechanical isolation between these species visited by the same hummingbird pollinators (Wolf *et al.* 2001). Similarly, hummingbirds transfer about 35% as much pollen from a donor flower of *I. tenuituba* to a recipient flower of *I. aggregata* as they do to another conspecific flower [mechanical $RI = 1 - (\text{transfer to heterospecific/transfer to conspecific}) = 0.65$], with no demonstrable mechanical isolation in the opposite direction (Campbell *et al.* 1998).

18.4 The relative importance of ethological and mechanical isolation

Studies of *I. aggregata*, *I. tenuituba*, and their hybrids allow the opportunity to quantify mechanical isolation and compare it with the strength of ethological isolation. This system offers the further advantage that hybridization varies across the geographical range (Grant and Wilken 1988). Comparison of ethological isolation at contact sites with and without large numbers of hybrids, while controlling for other features of the species, provides a powerful assessment of its role in determining the frequency of hybrid formation.

18.4.1 Floral traits in *Ipomopsis aggregata* and *I. tenuituba* and pollinator behaviour

To set the stage for these comparisons, we first describe in more detail differences in flowers between the two species and hybrids. *I. aggregata* subsp. *aggregata* has red flowers with relatively short, wide corolla tubes, high nectar production, and slightly inserted to exserted reproductive organs. *Ipomopsis tenuituba* has white to pale pink flowers with a long, narrow corolla tube, low nectar production, and strongly inserted reproductive organs (Plate 8). Flowers of both species are protandrous, and the plants are self-incompatible. The most common pollinators are hummingbirds and hawk moths. Hybrid zones form frequently, but not always, where the species come into contact in the western mountains of the USA (Grant and Wilken 1988).

To determine whether floral traits of hybrids are intermediate or transgressive to those of the parental species, we analysed seven traits of screen-house-grown F_2 hybrids between *I. aggregata* subsp. *aggregata* and *I. tenuituba* subsp. *tenuituba* collected near a hybrid zone at Poverty Gulch, CO (PG). The mean phenotype was intermediate or similar to one parent for corolla size, nectar volume, and flower colour (assessed by optical density), and significantly exceeded that of both parents (positively transgressive) only for style length (Fig. 18.2). Next we constructed a composite index of floral morphology by finding the canonical discriminant function (CDF) that maximized the difference between the two species ($P < 0.001$). The CDF correlated strongly and positively with corolla width, nectar volume, and colour ($r = 0.87$, 0.97, and 0.74, respectively), and negatively with corolla length ($r = -0.69$). Based on this CDF, the mean phenotype for F_2 individuals (6.8) was intermediate between those for the parental species (11.5 and 0.9), suggesting that a composite index can be useful as a hybrid index, even when an individual trait may not be intermediate.

As described in Section 18.3.1, some of these floral traits influence pollinator behaviour in a way that impacts ethological isolation. That ethological isolation is evident in a reduction in hybrid formation. Painting all flowers red to eliminate the difference in flower colour between the plant species reduced the percentage of seeds formed that were conspecific, as shown by multilocus paternity analysis (Campbell 2004). This trait manipulation alone caused RI, based on the relative formation of
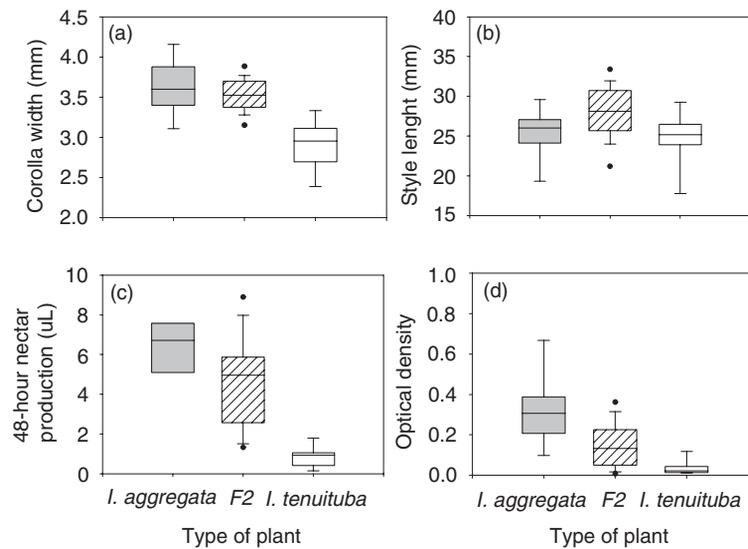
Figure 18.2 Representative floral traits for *Ipomopsis aggregata*, *I. tenuituba* and F_2 hybrids. Each box plot shows the 10th, 25th, 50th, 75th, and 90th percentiles (and 5th and 95th where the number of plants permitted). Traits were measured using methods described in Campbell *et al.* (2002), with two to five flowers measured per plant and averaged prior to analysis. For corolla width (a) the F_2 differs from *I. tenuituba* (*t*-test, $P < 0.0001$). For traits shown in (b), (c), and (d), both parental species differed from the F_2 (all $P < 0.05$), but only style length (b) is transgressive. Corolla length, and maximum and minimum stamen lengths (not shown) were also included in the canonical discriminant analysis.

hybrid and conspecific seed, to drop from 0.81 to 0.60 in arrays visited by hummingbirds. Whereas hummingbirds preferentially visit flowers with traits characteristic of *I. aggregata* (Campbell *et al.* 2002), hawk moths (usually rare at Poverty Gulch) slightly prefer plants with narrow corolla tubes, typical of *I. tenuituba* (Campbell *et al.* 1997).

To quantify the effects of the ethological isolation on pollen transfer, Campbell *et al.* (2002) set up arrays with equal numbers of *I. aggregata*, *I. tenuituba*, and hybrids (F_1 or F_2). The pollen in dehiscing anthers was marked with different colours of dye, so that it was possible to estimate interspecific pollen movement to stigmas simultaneously with interspecific flights by pollinators. Dye placed on anthers of one of the parental species reached heterospecific stigmas 7% as often as conspecific ones (Fig. 18.3a). This difference resulted partly from ethological isolation, as hummingbirds starting at one of the parental species flew to a heterospecific plant only 50% as often as they flew to a conspecific (Fig. 18.3b). However, ethological isolation was far from complete, and the rate of backcrossing exceeded

interspecific pollen movement, as estimated by dye dispersal (Fig. 18.3a).

18.4.2   Simulation model of ethological and mechanical isolation

To determine the relative importance of ethological versus mechanical isolation in reducing interspecific pollen movement, Campbell *et al.* (2002) constructed a simulation model in which these parameters could be varied realistically. We review that study of a single hybrid zone and then apply a modification of the model to a new investigation of how geographical variation in pollinator behaviour influences pre-zygotic reproductive isolation.

The model simulated the movement of pollen grains carried by pollinators in an array of plants consisting of equal numbers of the two parental species and hybrids. Ethological isolation was determined by the sequence of plants visited by a pollinator, the usual method of measurement (Table 18.1). Visit sequences sampled with replacement from foraging bouts observed at
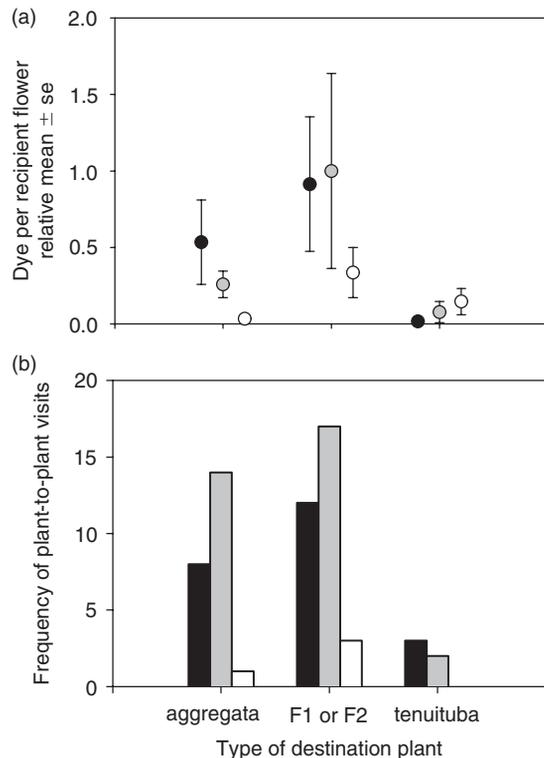
Figure 18.3 Relative amounts of (a) pollen transfer as estimated by dye particles and (b) frequency of visits by hummingbirds in experimental arrays with equal numbers of *Ipomopsis aggregata*, *I. tenuituba* and F_1 or F_2 hybrids. Responses are shown for the nine combinations of transfer or visit transition, with the type of the source plant indicated by shading (*I. aggregata*, closed symbol or bar; *I. tenuituba*, open symbol or bar; F_1 or F_2 hybrids, grey symbol or bar) and the type of the destination plant identified on the abscissa. Adapted from Campbell *et al.* (2002). © 2002 by The University of Chicago.

arrays in the field were used to simulate natural levels of ethological isolation, whereas random movements between plants simulated no ethological isolation. Patterns of pollen movement cannot be obtained simply from combining such measurements of ethological isolation with mechanical isolation, as they also depend on the floral display. Thus pollen export and import by a plant depended also on field measurements of the number of flowers displayed, the proportion of flowers in female or male phase, the probability that a pollinator visits a given flower once at a plant, the amount of pollen it picks up at a male-phase

flower, and the probability of pollen deposition on a flower.

Mechanical isolation was incorporated by varying the probability of pollen deposition on a stigma as a function of the type of pollen donor and flower recipient. The simulation incorporated estimates of the natural incidence of mechanical isolation from measurements of pollen transfer between hand-held flowers during visitation by captive hummingbirds (Campbell *et al.* 1998). Birds removed pollen from one of three types of donor flowers (*I. aggregata*, *I. tenuituba*, or hybrid) and then visited a long series of emasculated recipient flowers including all three types. The amounts of pollen deposited on the series of recipients fitted well to a model of pollen carryover in which each pollen grain has the same probability of being deposited on a flower's stigma ($P_{deposit}$), resulting in exponential decay in the amount deposited on subsequently visited stigmas (see Chapter 5). When a hummingbird removed pollen from an *I. tenuituba* donor, $P_{deposit}$ was higher for subsequent visits to conspecific flowers than for visits to *I. aggregata* (0.182 versus 0.064; Campbell *et al.* 1998). For all nine combinations of pollen donor and recipient, this probability of pollen deposition varied from 0.064 to 0.226 (for full parameter set see Campbell *et al.* 2002). A constant $P_{deposit}$ for all combinations of donor and recipient simulated no mechanical isolation.

To assess the impacts of ethological and mechanical isolation, Campbell *et al.* (2002) ran four types of simulations: presence or absence of each type of isolation in a crossed design. Pollinators moved either randomly between plants (no ethological isolation) or by sampling from observed visitation sequences. The probability of pollen deposition ($P_{deposit}$) was either constant (no mechanical isolation) or depended on the combination of donor and recipient flower. With both ethological and mechanical isolation, the simulation predicted relative amounts of pollen dispersal between various combinations of flower types well (compare Fig. 18.3a with Fig. 18.4d), and the fit improved further when the simulation included the few visitation sequences by bees (Campbell *et al.* 2002). Note that the distribution of predicted pollen transfer (Fig. 18.4d) differed from that of
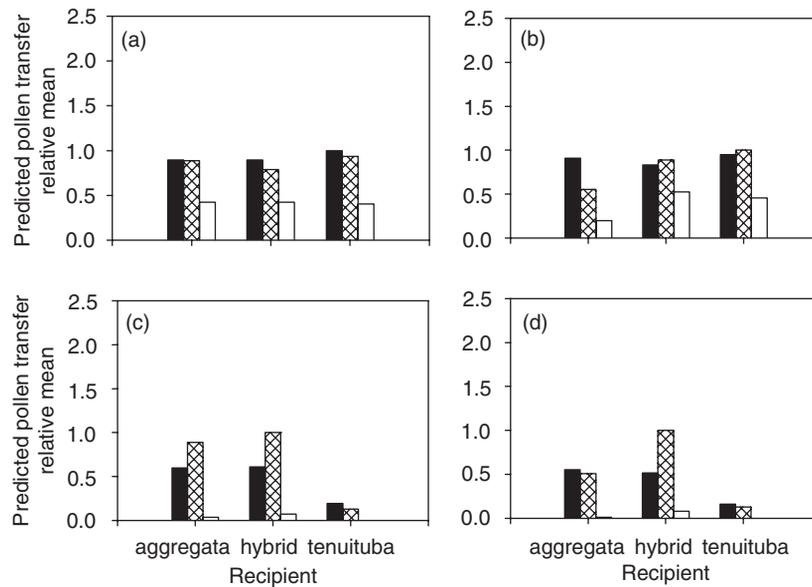
Figure 18.4 Effects of ethological and mechanical isolation on predicted pollen export per flower from *Ipomopsis aggregata* (filled bars), *I. tenuituba* (open bars) and F_1 or F_2 hybrids (hatched bars) in a simulation model of a community with the three classes of plants at equal frequencies. The four cases illustrate the effects of: (a) random visitation sequences (no ethological isolation) and a constant probability ($P_{deposit}$) of 0.135 that a pollen grain on a pollinator is deposited on a flower during an individual flower visit (no mechanical isolation); (b) random visitation sequences (no ethological isolation) and $P_{deposit}$ determined by the particular combination of pollen donor and recipient (mechanical isolation); (c) observed bird visitation sequences (ethological isolation) and constant $P_{deposit}$ (no mechanical isolation); and (d) observed bird visitation sequences (ethological isolation) and variable $P_{deposit}$ (mechanical isolation). Each panel is based on 200 replicates. Adapted from Campbell *et al.* (2002). © 2002 by The University of Chicago.

pollinator visits (Fig. 18.3b). For example, 52% of all pollinator movements, but only 42% of predicted pollen transfer, were of a backcross type. In the null model with no ethological or no mechanical isolation (Fig. 18.4a), interspecific pollen transfer equalled conspecific pollination. Introducing ethological isolation reduced the relative interspecific pollen transfer (Fig. 18.4c) to 38% that of conspecific transfer (RI = 0.62). In contrast, inclusion of variable pollen carryover, and hence the influence of mechanical isolation (Fig. 18.4b), reduced interspecific pollination to only 84% of conspecific pollination (RI = 0.16). Thus ethological isolation appears to be more effective than mechanical isolation in this system.

18.4.3 Geographical variation in pre-mating isolation

To identify the reproductive isolating mechanisms that best explain geographical variation in hybrid

formation, we compared the subalpine site described above (PG) with a sage-oak site where natural hybrids between the same *Ipomopsis* species are mostly absent (Grizzly Ridge, CO [GR]; Aldridge 2005). Both sites have the same pollinators, but hummingbirds at GR visit only *I. aggregata*, and hawk moths visit only *I. tenuituba* in natural populations, suggesting complete ethological isolation, rather than the partial isolation seen at PG (G. Aldridge and D.R. Campbell unpublished manuscript). This difference could reflect dissimilar floral morphology, as the species are more divergent for corolla width at GR (mean = 2.8 versus 4.3 mm for *I. tenuituba* and *I. aggregata*) than at PG (2.9 versus 3.6 mm), or it could result from contrasting pollinator behaviour between sites. To test these hypotheses, we set up experimental arrays of potted *I. aggregata* and *I. tenuituba* plants in the four combinations of site of origin and site of observation (PG or GR; G. Aldridge and D.R. Campbell unpublished manuscript). Hummingbirds preferred *I. aggregata* in

all situations, although their preference was lower for plants from PG. In contrast, hawk moths exhibited both strong preference for *I. tenuituba* (one-sample *t*-test, $P < 0.001$) and constancy (*G*-test, $P < 0.001$) only for plants from GR and observed at GR, with 95% of plant-to-plant movements being conspecific. In contrast, the proportion of conspecific movements by hawk moths foraging on GR plants translocated to PG fell below the random expectation of 50%. Based on the combined visits from birds and moths, the intensity of ethological isolation depended on both plant traits (compare Fig. 18.5a with Fig.

18.5b) and differences in pollinator behaviour between sites (compare closed and open bars). The difference between sites may relate partly to warmer temperatures at GR, which allow some nocturnal foraging, in contrast to the largely diurnal foraging at the high-elevation PG site (Campbell *et al.* 1997). Under low light, the difference in flower colour may be more visible, causing hawk moths to restrict their visits to the white-flowered species, in keeping with the hawk-moth pollination syndrome. These site-to-site differences underscore the importance of considering the "pollinator geographical mosaic"
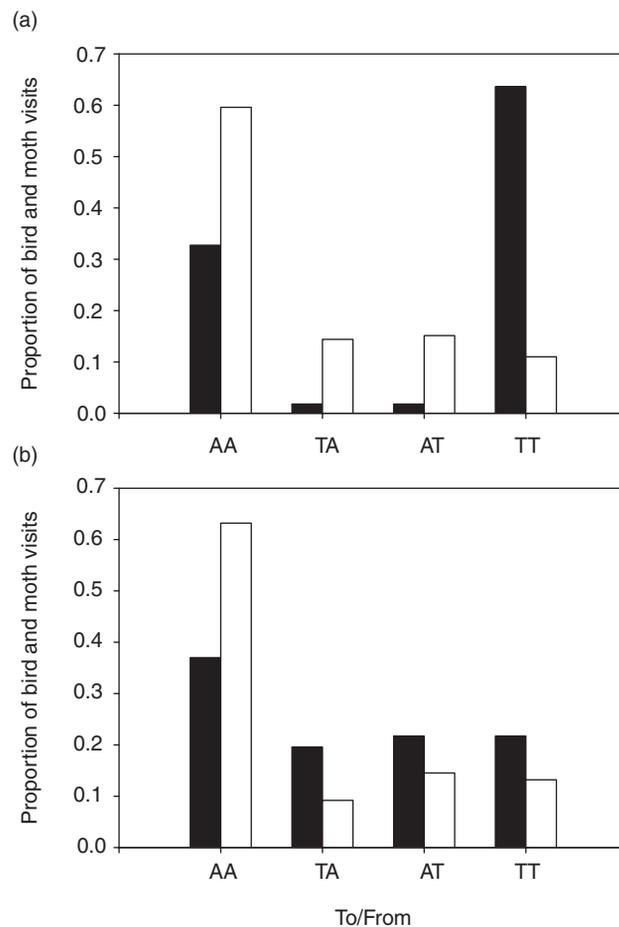

Figure 18.5 Effects of sites of observation and origin of *Ipomosis aggregata* and *Ipomosis tenuituba* plants on the proportions of pollinator movements in experimental arrays at Grizzly Ridge (GR, filled bars) and Poverty Gulch (PG, open bars). AA, movements to *I. aggregata* from *I. aggregata*, AT to *I. aggregata* from *I. tenuituba*, TA to *I. tenuituba* from *I. aggregata*, and TT to *I. tenuituba* from *I. tenuituba*. Comparisons of panels (a) plants originating from Grizzly Ridge and (b) plants originating from Poverty Gulch illustrate the effect of site of origin, whereas comparisons within a panel illustrate the effect of site of observation. Based on 686 plant-to-plant movements by hummingbirds and hawk moths.

(Chapter 15) and show that geographical differences in pollinator behaviour can appear even in the absence of spatial variation in the pollinator community.

We now explore the importance of this variation in ethological isolation to the production of hybrids by altering the simulation model to reflect the differences in plant-to-plant movements among the four array types. To incorporate these new data, we modified Campbell *et al.*'s (2002) model to allow changes in positions of plants within the arrays between foraging bouts. The number of flowers per plant and the probability of visiting a particular flower were altered to reflect our new field observations. The latter parameter varied significantly among the four array types; both plant origin and site of study influenced the proportion of flowers visited on an inflorescence (main effects in two-way ANOVA, both $P < 0.001$), so we used separate values for each situation. Visitation sequences were obtained by sampling with replacement from the observed sequences by hawk moths and hummingbirds. No data are available on dispersal of pollen by hawk-moths, let alone how it changes between conspecific and heterospecific visits, but Hodges' (1995) study of *Mirabilis* suggested extensive carryover, with $P_{deposit} = 0.05$. We ran the model with four scenarios for $P_{deposit}$: (1) fixed at 0.135 (the overall estimate for hummingbirds in Campbell *et al.* 1998); (2) fixed at 0.05; (3) 0.135 for hummingbird sequences, but 0.05 for hawk-moth sequences; and (4) variable pollen deposition for hummingbird sequences, reflecting natural mechanical isolation, but 0.05 for hawk-moth sequences.

With $P_{deposit}$ fixed at 0.135, the model predicted that just 4% of pollen transfer would be heterospecific at GR with GR plants presented in a mixture of the two species, compared with 29% at PG with PG plants (Fig. 18.6). Thus both plant differences and site differences acting in combination could account for a seven-fold difference in interspecific pollen movement between these two natural situations. These figures are similar to the percentage of interspecific plant-to-plant flights (4% and 24%, respectively, in Fig. 18.5), indicating that ethological isolation alone primarily explains the lack of hybrids at GR; although note that predicted pollen transfer at PG is higher than the observed interspecific visitation.

Predicted heterospecific transfer was greatest for PG plants at GR (40%), suggesting that overall differences in ethological isolation could produce a 10-fold difference in the rate of hybridization.

Variation in pollen carryover within limits indicated by the available data had relatively little impact on heterospecific pollen transfer. A reduction of $P_{deposit}$ from 0.135 to 0.05, thus increasing carryover, increased the relative frequency of heterospecific transfer slightly (Fig. 18.7). This change in $P_{deposit}$ also reduced total pollen transfer, as more pollen remained on a pollinator's body when it left the simulated array. A 10-fold increase in $P_{deposit}$ to 0.5 lowered the frequency of heterospecific transfer from 32% to 27% for PG plants at PG (results not shown), but in all cases heterospecific transfer remained higher than the percentage of heterospecific visit transitions (24%). Addition of the measured mechanical isolation for hummingbirds had little impact on the percentage of heterospecific pollen transfer (compare grey and hatched bars in Fig. 18.7). In sum, the model illustrates some differences between heterospecific pollinator movements and heterospecific pollen transfer. However, ethological isolation due to the visitation behaviour of hawk moths at GR would remain a powerful reproductive isolating mechanism over a wide range of pollen carryover and probably explains most of the reduction in hybrids at that site.

18.5 Post-mating isolation

Even if pollen is transferred between species, formation of hybrids may be reduced if heterospecific pollen germinates poorly on stigmas or few pollen tubes grow down styles (step c in Fig. 18.1). In at least one case, this post-mating isolation acts in combination with mechanical isolation to prevent hybridization in nature; *I. arizonica* pollen performs poorly on *I. aggregata* stigmas (Wolf *et al.* 2001) and hummingbirds move little pollen in the reverse direction (Section 18.3.2). Heterospecific pollen that fertilizes when present by itself on a stigma may perform poorly when competing with conspecific pollen (Darwin 1859; Arnold 1997). Examples of such a conspecific pollen advantage include the Louisiana irises (Carney *et al.* 1996),
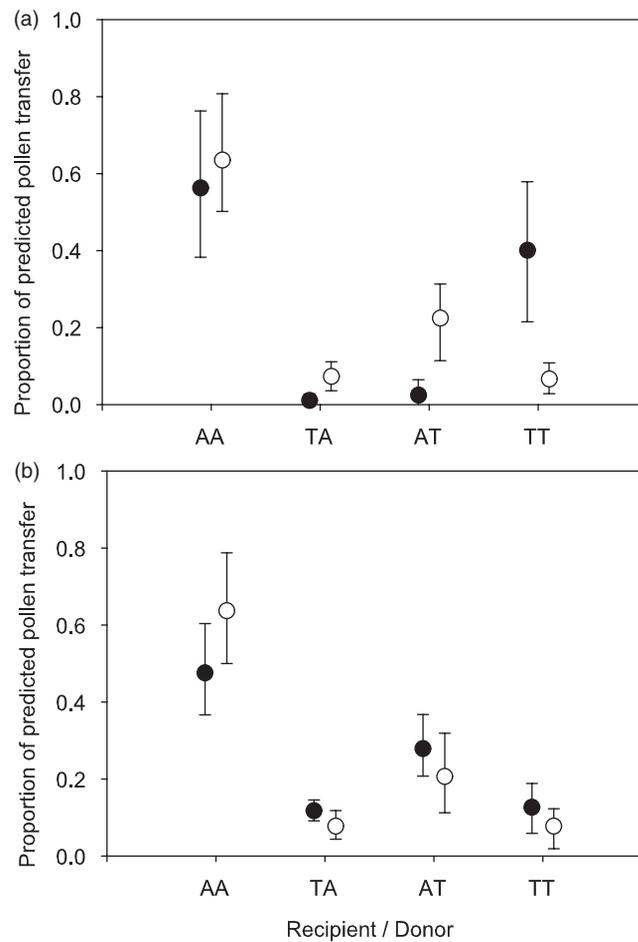
Figure 18.6 Mean (±95% confidence interval) proportion of pollen transfer per flower predicted for visitation sequences observed for *Ipomopsis aggregata and I. tenuituba* plants originating from (a) Grizzly Ridge (GR, filled symbols) and (b) Poverty Gulch (PG, open symbols: see Fig. 18.5) in 100 replicates of a simulation model. Comparisons within a panel illustrate the effect of site of observation. $P_{deposit} = 0.135$. Number of flowers displayed = 13 for *I. aggregata* and 12 for *I. tenuituba*. Probability of visiting a flower once at a plant = 0.31 and 0.35 for plants at GR and PG in (a), and 0.34 and 0.50 in (b). Proportion of flowers in female phase = 0.44 for *I. aggregata* and 0.23 for *I. tenuituba* (based on Campbell *et al.* 2002). Pollen removal per visit to a male-phase flower = 1713 grains for *I. aggregata* and 482 grains for *I. tenuituba*.

two *Brassica* species (Hauser *et al.* 1997), *Helianthus* (Rieseberg *et al.* 1995), *Piriqueta* (Wang and Cruzan 1998), and *Senecio chrysanthemifolius* (Chapman *et al.* 2005).

Whereas post-mating isolation is well documented, less is known about particular floral traits involved in natural contact sites. Pollen-tube growth rate has been associated with both the pollen source and characteristics of the recipient pistil (e.g., Kerwin and Smith-Huerta 2000). Asymmetries in the advantage of one species may reflect a tendency for species with longer styles to

have faster-growing pollen tubes (Arnold 1997). In addition, crosses between species with different mating systems often show unilateral interspecific incompatibility in which pollen from the self-incompatible species fertilizes the self-compatible species, but the reciprocal cross fails (e.g., Harder *et al.* 1993). Such asymmetries could produce an asymmetrical pattern of hybridization and help to strengthen reproductive isolation (Brandvain and Haig 2005).

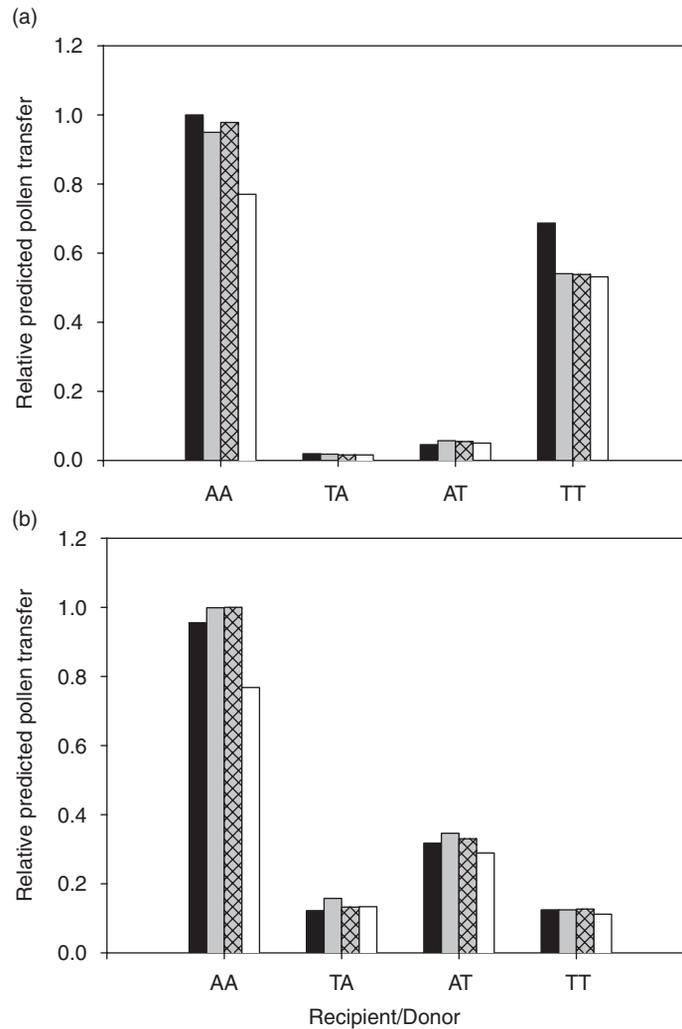Incorporation of pollen competition into the model described in Section 18.4.3 allowed

Figure 18.7 Effects of pollen carryover by hummingbirds and hawk moths on predicted pollen transfer per flower in mixed arrays of *Ipomopsis aggregata* (A) and *I. tenuituba* (T) for (a) visitation sequences for Grizzly Ridge (GR) plants observed at GR, and (b) Poverty Gulch (PG) plants observed at PG. Pollen transfer is expressed relative to the highest mean. Filled bars: $P_{deposit} = 0.135$. Shaded bars: $P_{deposit} = 0.135$ for hummingbird sequences and $P_{deposit} = 0.05$ for hawk-moth sequences. Hatched bars: $P_{deposit} = 0.133, 0.115, 0.064$, and 0.182 for A to A (*I. aggregata* to *I. aggregata*), A to T (*I. aggregata* to *I. tenuituba*), T to A, and T to T transitions in hummingbird sequences, and $P_{deposit} = 0.05$ for hawk-moth sequences. Open bars: $P_{deposit} = 0.05$.

quantitative evaluation of the impact of post-mating isolation. At the GR site, *I. aggregata* has a longer style than *I. tenuituba*. When equal amounts of pollen of the two species were placed together on stigmas, *I. aggregata* sired 70–80% of the seeds produced by recipient plants of either species (Aldridge and Campbell 2006). Thus, rather than a conspecific pollen advantage, there was a unilateral advantage to *I. aggregata*. Incorporating this threefold seed siring advantage in the simulation model has its greatest impact on the relative expected production of the reciprocal hybrids, altering the mix from 29% of F_1 individuals with a *tenuituba* mother and *aggregata* father (TA; GR plants at GR in Fig. 18.6a) to 56% in this direction of hybridization.

Whereas most studies of pre-zygotic isolation in angiosperms have focused on particular mechanisms, such as ethological isolation, an alternative approach uses genetic markers to determine the mating patterns in natural hybrid zones. Rieseberg *et al.* (1998) used a multilocus allozyme analysis of the progeny of open-pollinated maternal families and showed that hybrid plants were more likely to be fertilized by pollen from *Helianthus petiolaris* than from *H. annuus*. This approach may be particularly useful for wind-pollinated species. In principle, genetic markers could also be used to ascribe the likelihood of paternity to individual plants in natural populations (Smouse *et al.* 1999), and thereby examine how patterns of mating depend on floral traits. If floral traits are critical to reducing hybridization, such an analysis would show that mating occurs more often between plants of similar phenotype.

18.6 Floral traits and the fitness of hybrids

In addition to affecting hybrid formation, floral traits can influence hybrid fitness and thus the level of post-zygotic reproductive isolation (Fig. 18.1d–f). Floral traits play this role most directly by affecting pollen export and import by hybrids. Low pollination success of hybrids could cause either low female fitness (seed production) or low male fitness (seeds sired). David Lloyd (1980) first articulated in detail that the functional gender of individual hermaphroditic plants varies within populations, depending on their relative success as female and male parents. Male fertility is usually difficult to measure in natural plant populations, because of the large number of potential fathers for each seed (Meagher 1986), and this has rarely been attempted for natural hybrids (Melendez-Ackerman and Campbell 1998). However, continuing advances in molecular markers, such as microsatellites, and statistical analysis of male fertility (Smouse *et al.* 1999; Morgan and Conner 2001) make this increasingly feasible (Chapter 14).

Only a few studies present data on the pollination success of hybrid flowers and even fewer on how floral traits are involved. In the Louisiana irises, F_1 individuals received pollinator visits by bees and hummingbirds at a rate intermediate to that of the two parental species (Emms and Arnold 2000). Visitors to *Nicotiana* arrays exhibited less discrimination for or against hybrids than for the parental species (Ippolito *et al.* 2004). In experimental arrays of *Ipomopsis*, per-flower hummingbird visitation to hybrids ranged between years from intermediate between the two parental species *I. aggregata* and *I. tenuituba* to exceeding that of both parents (Campbell *et al.* 2002). In the latter case, pollen receipt as estimated by dyes followed the general pattern in pollinator visitation (Fig. 18.3). In a separate experiment, hybrids produced and sired intermediate numbers of seeds to those of the parental species (Melendez-Ackerman and Campbell 1998). These results do not follow the common expectation that hybrids have low pollination success, but they involve cases in which natural hybridization is relatively frequent.

In principle, if differences in floral traits impose strong ethological isolation, hybrids intermediate for those traits will receive few pollinator visits. This poor performance of hybrids constitutes disruptive or divergent selection on floral morphology, in which the low hybrid fitness represents a form of post-zygotic reproductive isolation. Thus, the same set of traits (and presumably genes) under divergent selection would also produce pre-zygotic reproductive isolation. In this "single-variation" model (Rice and Hostert 1993), sympatric or parapatric speciation occurs relatively easily (Rice 1987). A potential example involves corolla width in *Ipomopsis*, as plants with intermediate corolla widths receive less combined visitation by hummingbirds and hawk moths (Campbell *et al.* 1997). Whether the species difference in corolla width also enforces pre-zygotic isolation through assortative mating remains unknown.

The steps between pollinator visitation of hybrids and production of advanced generation hybrids (Fig. 18.1e and f) have rarely been studied in natural hybrid zones. In studies of efficiency of pollen transfer involving *I. aggregata* and *I. tenuituba*, hybrids between the two species actually received more pollen per visit than did the parental species (Campbell *et al.* 1998). This pattern reflected stabilizing selection on the position of the

stigma relative to the opening of the corolla tube, which is intermediate in hybrids, probably due to frequent misses of exserted stigmas by hummingbirds and inability of hummingbirds to insert their bill fully into narrow *I. tenuituba* flowers. Once pollen is deposited or exported from hybrids, its final success can depend on pollen interactions. Hybrid pollen between these two species of *Ipomopsis* is at a disadvantage in competing with pollen of the two species to fertilize ovules (Campbell *et al*. 2003).

Although floral traits are typically assumed to evolve in response to pollinators, they could also have other fitness consequences for hybrids. Selection by nectar thieves and by floral herbivores that damage petals or reproductive parts is well documented (Chapter 7) and provides a potential mechanism for reducing pollinator visitation to hybrids (step d in Fig. 18.1). Similarly, floral traits can influence fruit or seed predation, potentially reducing the production of viable seed by hybrids (step f). A more complex interaction occurs when pollinators are vectors for sexual disease, as the same floral trait can increase pollen transfer and spore transfer, causing sterility (Elmqvist *et al*. 1993). In some cases, the responses of pollinators and non-pollinators to the same floral trait lead to antagonistic selection on floral colour, shape, or size (Chapter 7). Despite being well documented in single-species populations, the influence of these effects on hybrid fitness is largely unknown and warrants more study. Hybrid zones between *I. aggregata* and *I. tenuituba* provide a potential example of antagonistic selection between hummingbird pollinators and seed predators. The anthomyiid fly *Hylemya* sp. (= *Delia*) lays eggs on the inside of the sepals, and the larvae consume seeds before pupating (Brody 1992). Oviposition rates correlated positively with corolla width in experimental arrays that included both plant species and hybrids (Campbell *et al*. 2002). This trait also increases hummingbird visitation, so damage by the seed predator and pollination can correspond closely, leading to opposing selection.

Floral traits can also be associated with aspects of survival, such as the ability to handle drought stress or resistance to vegetative herbivory. Usually a pleiotropic effect on a vegetative trait is involved, for example the multiple effects of the anthocyanin pathway on flower colour and vegetative traits (Chapter 7). In this case, indirect selection of the genetically correlated trait must be considered to predict the evolutionary fate of the floral trait (Lande and Arnold 1983, Chapter 14). In one example, the white allele that affects flower colour and pollinator visitation in the morning glory *Ipomoea purpurea* (Epperson and Clegg 1987) also controls stem colour (Schoen *et al*. 1984) and resistance to herbivory by tortoise beetles (Simms and Bucher 1996). Again, little is known about such indirect selection or genetic correlations between floral traits and vegetative traits in hybrid zones.

18.7 Conclusions and future directions

The studies reviewed above represent considerable progress during the past decade in understanding of the influences of floral traits on hybridization and reproductive isolation. However, further work is needed along several lines. First, even in the best-studied natural systems the influences of particular floral traits on reproductive isolation have seldom been quantified, largely because the data are restricted to a single step in the process. Most investigators have focused on pollinator visitation or post-mating isolation, and only rarely have all or most steps involved in reproductive isolation been examined (Campbell 2004), making it hard to assess their relative importance. Pollinator visitation is a topic with which to begin, because complete ethological isolation precludes opportunities for reproductive isolating mechanisms that act later. However, even when focusing on this initial step, pollinator transitions between species, rather than pollinator preference alone, must be measured.

Second, in systems with any breakdown of ethological isolation, later-acting processes that may influence pollen transfer, interspecific gene flow, or hybrid fitness should also be measured. For example, even if a pollinator visits a second species only occasionally during a long foraging bout, many plants could receive some heterospecific pollen if pollen carryover is extensive. Our simulation models illustrate that the details of

pollen carryover affect the correspondence between interspecific pollinator visitation and heterospecific pollen transfer. This link between pollinator visitation patterns and pollen movement warrants more study, especially for whole pollinator assemblages.

Third, the extent of interspecific gene flow can depend strongly on environmental characteristics of the contact site, and on the presence versus absence of hybrids, with pollinators showing stronger preference when the parental species only are present. In a study of *Ipomopsis* we found as much difference in ethological isolation between two contact sites visited by the same pollinators (0.30–0.96) as could be explained by pollinator type in the broader survey of studies (Table 18.1).

Fourth, molecular genetic approaches offer increasing promise for further understanding of how particular traits influence components of reproductive isolation. Initial studies of the genetic basis of reproductive isolation have proceeded by QTL analyses of floral traits in a glasshouse setting (Bradshaw *et al.* 1998), combined with separate field experiments to assess pollinator responses. In experimental arrays of *Mimulus*, in which the normal genetic associations between flower colour and nectar were disrupted, bee visitation depended on the presence/absence of an allele controlling carotenoid pigments in the petals (Schemske and Bradshaw 1999). Genetic mapping could also be used in natural hybrid zones to test more directly for QTLs that influence reproductive isolation. In one example, Rieseberg *et al.* (1999b) employed a large number of mapped RAPD markers to examine associations with reduced pollen fertility of natural sunflower hybrids. Use of such methods in natural hybrid zones will become easier as more species-diagnostic markers are developed. However, a simpler approach could be applied that involves planting a QTL mapping population of offspring of known parentage in the field and examining correlations of genetic markers specific to the original parents with floral traits and visitation by specific pollinators, or even patterns of pollen transfer (e.g., using dyes to mark pollen). Such field data could allow mapping of chromosomal blocks associated with particular floral traits and their influences in restricting pollination by a particular type of animal.

Finally, most of the more detailed studies of pre-mating isolation have focused on systems involving divergence of insect and hummingbird-pollinated species. The specific influences of floral traits on hybridization in such situations probably do not apply generally in other pollination systems. Therefore studies of other pollination systems, including those involving multiple types of insect pollinators, or animal versus wind-pollination, are essential to identify general principles of the floral biology of hybridization.

Acknowledgements

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