**MINI REVIEW**

# Ecological speciation in phytophagous insects

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**Abstract**

Divergent natural selection has been shown to promote speciation in a wide range of taxa. For example, adaptation to different ecological environments, via divergent selection, can result in the evolution of reproductive incompatibility between populations. Phytophagous insects have been at the forefront of these investigations of 'ecological speciation' and it is clear that adaptation to different host plants can promote insect speciation. However, much remains unknown. For example, there is abundant variability in the extent to which divergent selection promotes speciation, the sources of divergent selection, the types of reproductive barriers involved, and the genetic basis of divergent adaptation. We review these factors here. Several findings emerge, including the observation that although numerous different sources of divergent selection and reproductive isolation can be involved in insect speciation, their order of evolution and relative importance are poorly understood. Another finding is that the genetic basis of host preference and performance can involve loci of major effect and opposing dominance, factors which might facilitate speciation in the face of gene flow. In addition, we raise a number of other recent issues relating to phytophagous insect speciation, such as alternatives to ecological speciation, the geography of speciation, and the molecular signatures of speciation. Throughout, we aim to both synthesize what is known, as well as highlight areas where future work is especially needed.

The idea that adaptation to different ecological environments can promote speciation dates back at least to Darwin (1859), and work on the adaptation of phytophagous insects to their host plants has featured prominently in the development of this idea. For example, soon after the publication of the Origin of Species, Walsh (1867) proposed that insect speciation could be driven by shifting and adapting to new host plants. Subsequent work on host plant feeding preference and host-specific mating played an important role in establishing the possibility of speciation in the face of gene flow (Mayr, 1947, 1963; Maynard Smith, 1966; Bush, 1969), a major controversy of the modern synthesis which persists today (Coyne & Orr, 2004; Mallet, 2008). More recent work has examined

in great detail the manner and extent to which adaptation to different host plants might reduce gene flow between phytophagous insect populations (Funk, 1998; Berlocher & Feder, 2002; Drés & Mallet, 2002; Jiggins, 2008). Thus, research on phytophagous insects has contributed strongly to the development of a general hypothesis which is now referred to as 'ecological speciation' (Funk, 1998; Schluter, 2000, 2001, 2009; Rundle & Nosil, 2005).

We review here ecological speciation as it pertains to phytophagous insects. Consistent with past work, we define ecological speciation as the process by which barriers to gene flow evolve between populations as a result of ecologically-based divergent selection (Schluter, 2000, 2009). Selection is ecological when it arises as a consequence of the interaction of individuals with their environment during resource acquisition or from the interaction of individuals with other organisms in their attempt to achieve resources (e.g., competition). Selection is divergent when it acts in contrasting directions in the two popu-

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lations (this includes the case in which selection favors opposite phenotypes within a single population, normally termed disruptive selection).

We consider here the role of divergent selection in driving the evolution of barriers to gene flow (i.e., reproductive incompatibility). Thus, in some sense we adopt the biological species concept. However, we stress that the arguments made here apply to other species concepts as well. For example, in addition to considering the effects of divergent selection on the strength of barriers to gene exchange (i.e., reproductive isolation), one might focus instead on the effects of selection on the degree of morphological distinctiveness, the extent of genotypic clustering or lineage sorting, and the sharpness of geographic clines in gene frequencies (Mallet et al., 2007; Nosil et al., 2009a,b). Our focus here on reproductive incompatibility is useful, as it allows us to explicitly consider the role of selection in generating specific forms of barriers to genetic exchange, as well as on the genetic mechanisms linking selection to these specific barriers to gene flow (Coyne & Orr, 2004; Gavrilets, 2004).

Ecological speciation differs from models of speciation that involve processes other than ecologically-based divergent selection. These other models include those in which chance events play a key role, such as speciation by genetic drift and founder-events/population bottlenecks (reviewed in Coyne & Orr, 2004). Non-ecological speciation also includes models in which selection is involved, but is non-ecological or is not divergent between environments. Examples include certain models of speciation by sexual selection (Lande, 1981) and sexual conflict (Chapman et al., 2003) as well as scenarios involving the fixation of different, incompatible alleles in populations experiencing similar selection regimes (termed 'mutation-order speciation', Schluter, 2009). An alternative definition of ecological speciation would restrict it to situations in which barriers to gene flow are ecological in nature. However, when the goal is to understand mechanisms of speciation (as here), it is of interest when both ecological and non-ecological forms of reproductive isolation evolve ultimately due to a specific process (i.e., divergent selection). Another critical point is that ecological speciation can occur under any geographic arrangement of populations (allopatry, parapatry, or sympatry), so long as divergent selection is the evolutionary process driving population divergence (Funk, 1998; Schluter, 1998, 2001; Coyne & Orr, 2004; Rundle & Nosil, 2005).

The process of ecological speciation makes some clear predictions, for example that ecologically-divergent pairs of populations will exhibit greater reproductive isolation than ecologically-similar population pairs (Funk, 1998; Funk et al., 2002). Another prediction is that traits under

divergent selection will affect reproductive compatibility (Schluter, 1998; Jiggins et al., 2001). These predictions have been supported in laboratory experimental evolution studies (reviewed in Rice & Hostert, 1993) and in nature (reviewed in Rundle & Nosil, 2005). However, a number of questions remain unanswered, and we use the framework of Rundle & Nosil (2005) to review these questions in relation to phytophagous insect speciation. Specifically, Rundle & Nosil (2005) focused on all organisms and considered three components of ecological speciation: a source of divergent selection, a form of reproductive isolation, and a genetic mechanism to link the two (Figure 1). In phytophagous insects, we note that performance traits (those affecting growth and survival on different plants) can generally be thought of as traits under selection, whereas preference traits (those involved in alighting, feeding, and oviposition preference) might generally be thought of as reproductive isolation (Table 1). In addition to the three components mentioned above, we consider several other topics that are particularly relevant to phytophagous insect speciation: the individual genetic bases of host preference and performance, alternatives to ecological speciation, the geography of speciation, and the molecular signature of ecological speciation. We do not consider hybrid speciation, as it has been well-reviewed elsewhere (Schwarz et al., 2005; Gompert et al., 2006; Mallet, 2007).

Although we follow the framework of Rundle & Nosil (2005), we stress that the current review expands upon and differs from previous reviews of ecological speciation in several important ways. First, some topics in the current review were not covered in past reviews. Examples are the individual genetic basis of host plant preference and performance (Tables 2 and 3, respectively), the consideration of alternatives to ecological speciation in phytophagous insects, and the discussion of the molecular signatures of speciation. In other sections, there is some topical overlap with past reviews, but the current review expands on past studies by either providing a more systematic appraisal (i.e., the review of reproductive barriers in Table 1 and the consideration of different hypotheses for the causes of host-preference evolution) or, at the very least, by providing updated references to recent studies and emerging new trends in the literature. Indeed, our focus here on phytophagous insects, rather than all organisms, necessitated a focus on many examples and topics not covered by past reviews. Thus, although some material covered here will be familiar (cf. Rundle & Nosil, 2005 and Schluter, 2009), there are numerous novel aspects to the current review.

Our aim is to describe and integrate the many factors affecting ecological speciation, thus providing a broad overview of this process in herbivorous insects. At the same time, we focus more closely on less explored areas,
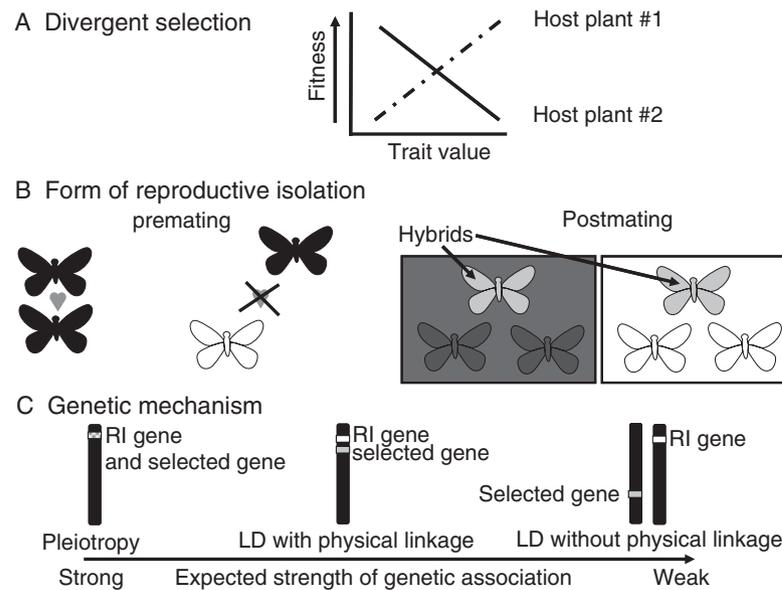
**Figure 1** The three components of ecological speciation (cf. Rundle & Nosil, 2005). (A) A source of divergent selection. The commonly recognized sources are differences between environments, interactions between populations, and sexual selection. (B) A form of reproductive isolation, which might act before or after mating (premating and postmating isolation, respectively). The example of premating isolation here concerns divergent mating preferences such that there is assortative mating based upon color (=sexual isolation). The example of postmating isolation concerns environment-dependent selection against hybrids such that the intermediate phenotype of hybrids renders them more vulnerable to visual predation (=less fit) in each parental environment (=extrinsic hybrid inviability). (C) A genetic mechanism linking selection to reproductive isolation. The genes under selection and conferring reproductive isolation may be one and the same (pleiotropy) or physically different (but statistically associated via linkage disequilibrium, LD). Modified from Nosil & Rundle (2009) and reprinted with permission of Princeton University Press.

such as the genetics and genomics of ecological speciation. For detailed reviews of how to test for ecological speciation, and the conceptual origin of ideas regarding divergent host adaptation and insect speciation, we refer readers to past reviews (Funk, 1998; Berlocher & Feder, 2002; Drés & Mallet, 2002; Funk et al., 2002, 2006; Funk & Nosil, 2008; Jiggins, 2008).
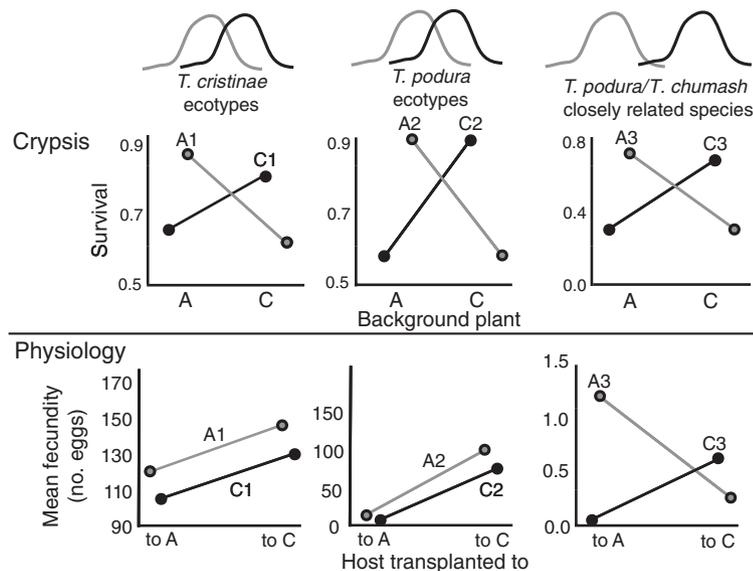
## A source of divergent selection

Three main forms of divergent selection are commonly recognized: differences between environments, interactions between populations (e.g., competition, predation), and sexual selection. Here we consider all three, but focus primarily on differences between environments (i.e., between host plants) because they have received by far the most attention. The term 'selection' is often used hereafter as shorthand for 'divergent selection' (which itself is defined by crossing fitness functions; Figure 1A).

### Differences between environments

Different host plant species might differ in their chemistry and morphological structure. Thus, selection might act in contrasting directions between populations of insects feeding on different host species, favoring opposing trait values on different hosts. Although this concept and the pattern predicted by it is simple (Figure 1A), the actual detection of divergent selection is complicated by the fact that not all traits need be subject to divergent selection between hosts. Thus, if divergent selection is not detected, this might represent a true lack of any selection, insufficient statistical power to detect selection on traits that were measured, selection acting on only a small subset of loci affecting a trait (such that data pooled across all loci do not result in a strong signal of selection), or selection that acts only on traits that were not measured. We refer readers to past reviews for more detailed treatment of these issues (Fry, 1996, 2003), and here focus on the types of divergent selection between hosts that have been detected using reciprocal transplant experiments.

The use of different host plants might impose divergent selection on digestive and physiological traits related to processing different plant chemicals. We refer to this hereafter using the general term 'selection on physiology', which has been measured in many groups of insects (Schluter, 2000; Funk et al., 2002; Nosil et al., 2005). In

**Figure 2** An empirical example of divergent selection arising from differences between environments (in this case, differences between host plant species). The graphs represent fitness functions such that crossing fitness functions are indicative of divergent selection. Shown here is the nature of selection on crypsis and physiology for three taxon pairs of *Timema*. A1 and C1 refer to ecotypes of *Timema cristinae* (A, *Adenostoma* and C, *Ceanothus* hereafter). A2 and C2 refer to ecotypes of *Timema podura*. A3 and C3 refer to the species pair *Timema podura* and *Timema chumash*, respectively. The ecotype pairs exhibit weaker divergence in morphology, host preference, and mitochondrial DNA than the species pair [denoted here by greater overlap of trait distributions for the ecotype pairs; see Nosil & Sandoval (2008) for details], and are also subject to divergent selection on fewer traits (i.e., crypsis alone, rather than both crypsis and physiology). Modified from Nosil & Sandoval (2008) and reprinted with permission of the Public Library of Science.

some cases, divergent selection on physiology was not detected (Wiklund, 1975; Courtney & Forsberg, 1988; Kibota & Courtney, 1991; Gratton & Welter, 1998). In such cases, future work could focus on the explanations for a lack of selection (as described above). In other cases, divergent selection on physiology was detected such that each insect form grew and survived better on its native host plant (Rausher, 1984; Via, 1984, 1991; Mackenzie, 1996; Funk, 1998; Nosil & Sandoval, 2008). When such selection is detected, future work could examine why poor growth and survival on non-native hosts occurred. For example, did low fitness arise because of low levels of ingestion, indigestibility of particular plant compounds that were ingested, or inhibition of digestive enzymes by secondary compounds?

As noted above, a lack of selection on physiology does not preclude the existence of selection on other traits. For example, Bernays & Graham (1988) argued that predation might be a major source of divergent selection. Studies of *Timema* species walking-stick insects support this claim (Sandoval & Nosil, 2005; Nosil & Crespi, 2006a,b; Nosil & Sandoval, 2008). More specifically, these studies showed that divergent selection between hosts on cryptic morphology (i.e.,

camouflage), exerted from visual predators, occurred for all three taxon pairs tested. In contrast, divergent selection on physiology (in the absence of predation) did not occur between ecotypes using different host plants within species, but did occur for a distinct species pair that uses different host species. Thus, although divergent selection on both cryptic morphology and physiology occurs in *Timema* species, the former appears to be more widespread and to arise earlier in the speciation process (Figure 2). Further studies that estimate selection on different types of traits are needed. In particular, traits other than physiology and cryptic morphology warrant examination. For example, selection might act on parasite resistance (Feder, 1995) or on traits related to biomechanics/maneuvering on different types of plant substrates (Moran, 1986; Bernays, 1991; Soto et al., 2008).

A few additional points warrant mention. Although reciprocal transplant experiments are the main method used to test for divergent selection, a powerful alternative is multi-generation selection experiments (Agrawal, 2000; Fry, 2003). Such selection experiments might be particularly feasible in phytophagous insects with short generation times. Another point is that if fitness trade-offs

involve co-adapted gene complexes, they will be detectable only after hybridization [i.e., they will not be detectable via measuring the fitness of parental types (de Jong & Nielsen, 2002)]. A final point is that there is essentially no data linking divergent selection between hosts directly to the evolution of other forms of reproductive isolation (such as divergent mate or host preference).

### Interactions between populations (e.g., competition, predation, and reinforcement)

Divergent selection may also arise between populations as a result of their ecological interaction with one another. Ecological interactions differ from other sources of divergent selection because they occur in sympatry (or parapatry) and often involve frequency-dependent disruptive selection. Interspecific competition in insects has been documented, but its role in generating divergent selection between hosts has been less studied (Denno et al., 1995, 2002, 2003). Thus, although interspecific competition may be a common source of divergent selection in vertebrates (Schluter, 2000; Bolnick & Lau, 2008), its importance in phytophagous insects needs further study.

Another interaction involves selection against heterospecific mating. If heterospecific mating reduces the fitness of the individuals involved, or their hybrid offspring, selection will favor individuals that mate within their own population. This can strengthen prezygotic isolation in a process known, in the broad sense, as reinforcement (Servedio & Noor, 2003). Reinforcement is difficult to categorize because it can complete a speciation process initiated by any mechanism, ecological or not (Schluter, 2001; Rundle & Schluter, 2004). If the cost to heterospecific mating originates from ecological causes (e.g., Kirkpatrick, 2001), then it is reasonable to consider reinforcement as a component of ecological speciation, as documented in a phytophagous insect system (Nosil et al., 2003). Other cases of reinforcement in insects have been documented, but the role of divergent natural selection is unclear (e.g., Phelan & Baker, 1987). Understanding the contribution of reinforcement to ecological speciation will require consideration of the costs to heterospecific mating and the mechanisms (ecological or not) by which they evolved.

Finally, we note that empirically separating the effects on ecological speciation of competition vs. reinforcement may be difficult. Both occur in sympatry from the interaction of populations and can produce the same evolutionary outcome: stronger premating isolation between sympatric than allopatric populations (Servedio & Noor, 2003). Future speciation studies should focus on distinguishing the effects of competition vs. reinforcement.

### Sexual selection

Divergent sexual selection can potentially arise from ecological sources. Specifically, speciation models involving sexual selection can be classified into two types, depending on whether or not differences in mate preferences evolve because of divergent selection between environments (Schluter, 2000, 2001; Boughman, 2002). Models involving divergent selection between environments include spatial variation in natural selection on secondary sexual traits (Lande, 1982) and on mating or communication systems (Ryan & Rand, 1993; Boughman, 2002). For example, if different habitats differ in their signal transmission properties, different trait values for signal and mating preference traits might accrue high fitness in different habitats, resulting in habitat-specific and divergent selection on sensory systems and mating preferences (i.e., 'sensory drive' – see Endler, 1992; Boughman, 2002 for reviews). In contrast, examples of sexual selection that do not involve divergent selection between environments, and are thus not components of ecological speciation, are models in which sexual selection arises from the interaction of the sexes [e.g., Fisher's runaway (Lande, 1981); sexual conflict (Chapman et al., 2003)]. Sexual selection can thus be involved in both ecological and non-ecological speciation (Schluter, 2000, 2001; Nosil et al., 2007).

The evidence for ecologically-based divergent sexual selection in insect speciation is limited, mostly because tests are lacking. An excellent example of how insect communication signals can be shaped by the ecological environment, in this case differences in host plant use, stems from work on vibrational communication in *Enchenopa* spp. (Rodríguez et al., 2004, 2007; McNett & Cocroft, 2008). In this system, the predictions of the sensory drive hypothesis were supported. First, the insect taxon pair examined differs in both host plant use and male mating signals (e.g., signal frequency). Second, the different host species used by the insects differ in their signal transmission properties. Third, male signal frequencies on each host match the peak of optimal transmission, thereby maximizing signal detection in each environment (i.e., on each host). Finally, signal divergence is genetically based and generates premating isolation. In two other systems in which similar tests have been conducted, one reports matching of insect signals to transmission properties of common hosts (Miklas et al., 2001; Cokl et al., 2005), whereas the other reports no such matching (Henry & Wells, 2004). McNett & Cocroft (2008) suggest that these differences might be explained by the degree of host specificity, with signal divergence being most likely when each insect form feeds on only a single host plant. Further work is required to determine how often differences between host plants cause divergent sexual selection.

## A form of reproductive isolation

During ecological speciation, divergent selection causes the evolution of barriers to genetic exchange (i.e., reproductive isolation). Reproductive isolation itself can occur before or after mating (premating and postmating isolation, respectively). Within these two classes, various types of isolating barriers are possible. Here we review examples of four premating barriers, one postmating-prezygotic barrier, and three postzygotic barriers (Table 1). We consider which isolating barriers are particularly likely to be involved in ecological speciation, the relative contribution of different types of barriers to total reproductive isolation, and at which point in the speciation process different barriers evolve.

### Habitat and temporal isolation

When populations use different host plant species, the evolution of preference for the populations 'native host' (the one commonly utilized in the local environment) can result in population divergence in host preference, which can in turn generate premating isolation (Futuyma & Moreno, 1988; Jaenike, 1990; Egan & Funk, 2006; Table 1). Specifically, the tendency of adult insects to remain on or return to the larval or nymphal host plant (='host fidelity' Bush, 1969; Feder et al., 1994; Funk et al., 2002), coupled with the occurrence of mating on the host plant, can directly generate reproductive isolation, by reducing encounters and interbreeding between populations adapted to different hosts (i.e., habitat isolation).

Most studies of divergent host preferences have been conducted in the laboratory (Funk et al., 2002). In some cases, divergent host preferences have been observed in the wild, but even amongst these examples there are few cases where divergent preferences have actually been shown to result in reproductive isolation (i.e., assortative mating or reductions in gene flow). A few examples exist from field surveys and field cage experiments. Clover and alfalfa races of the pea aphids, *Acyrthosiphon pisum* (Harris), prefer the natal host plant both in laboratory choice experiments (Via, 1999) and in field surveys (Via et al., 2000). Host fidelity for the natal host plant most likely causes strong habitat isolation between the two races. The ability of host fidelity to generate assortative mating between apple and hawthorn host races of *Rhagoletis pomonella* (Walsh) flies was shown using mark-recapture experiments in the wild (Feder et al., 1994). Finally, assortative mating driven by host fidelity was directly observed in a host-associated species pair of *Henosepilachna* ladybird beetles, *H. niponica* (Lewis) and *H. yasutomii* Katakura (which use the host plants thistle and blue cohosh, respectively). The two species exhibited strong assortative mating in field cages in which only the two native host plants of the species were planted. However, these species exhibited weaker assortative mating in cages which included the two native host species plus an additional acceptable host plant, the Japanese nightshade, presumably because the nightshade acted as a bridge to gene flow between the species (Hirai et al., 2006). Divergent host preferences are likely to generate habitat isolation in other group of insects as well (Wood, 1980; Craig et al., 1993, 2001; Feder et al., 1994; Funk, 1998; Wood et al., 1999; Emelianov et al., 2001, 2003; Forister, 2004, 2005). Thus, it appears that habitat isolation may be a common form of reproductive isolation in phytophagous insects. Although habitat isolation is the most commonly reported form of reproductive isolation in herbivorous insects (Table 1), we note that this pattern is likely influenced by a strong bias towards studying this form of isolation [see Bakker et al. (2008) for an example of a lack of host fidelity].

Two main hypotheses exist to explain the evolution of divergent host preferences (Funk et al., 2002 and Tilmon, 2008 for thorough reviews). The first is the 'fitness trade-offs hypothesis,' in which host preferences diverge via selection against individuals that switch between hosts. Under this scenario, preference for the native host is favored because individuals are maladapted to non-native hosts such that individuals choosing a non-native host exhibit lower fitness than individuals that choose the native host to which they are well adapted. In this hypothesis, selection only acts in populations in which there is the opportunity for switching between utilized hosts (i.e., when more than one utilized host is available in the environment). One possible outcome is greater preference divergence in geographic regions where multiple hosts are utilized (in sympatry or parapatry) than between geographically-isolated populations that use a single, yet different, host (allopatry). This pattern can be thought of as 'character displacement' of host preference, with host preferences evolving in a reinforcement-like process (Nosil & Yukilevich, 2008). Tests for such character displacement of host preference are few, and at least one study testing for such displacement did not detect it (Nosil et al., 2006b).

In a second 'information processing hypotheses', there is no selection against switching between utilized hosts, because only one host is utilized in the local environment. Under this scenario, search and efficiency costs can favor increased preference for the single, utilized host because individuals without strong preferences accrue lower fitness, but for reasons other than switching to an alternate host (Bernays & Wcislo, 1994; Funk & Bernays, 2001; Egan & Funk, 2006). For example, due to constraints associated with information processing, generalized individuals without strong preferences might take longer to locate or to

**Table 1** Forms of reproductive isolation (RI) between phytophagous insect taxa

| Insect systems | Habitat isolation | Immigrant inviability | Temporal isolation | Sexual isolation | Gametic incompatibility | CSP* | Intrinsic $F_1$ inviability | Extrinsic $F_1$ inviability | Sexual selection against hybrids | References |
|---|---|---|---|---|---|---|---|---|---|---|
| Evolution caused by divergent selection? | Probably | Yes | Probably | Possibly | Possibly | Possibly | Possibly | Yes | Possibly | |
| Phasmida | | | | | | | | | | |
| *Timema cristinae* (walking stick) | | | | | | | | | | |
| *Adenostoma* ecotype × *Ceanothus* ecotype | + | + | ✓ | + | ± | ? | − | + | ? | 1–8 |
| Hemiptera | | | | | | | | | | |
| *Acyrthosiphon pisum* (pea aphid) | | | | | | | | | | |
| alfalfa race × clover race | + | + | − | − | ? | ? | − | + | ? | 9–13 |
| *Enchenopa binotata* (treehoppers) | | | | | | | | | | |
| among host-associated species complex | + | + | + | ✓ | ? | ? | ✓ | ? | ? | 14–20 |
| Coleoptera | | | | | | | | | | |
| *Chrysolina angusticollis* (leaf beetle) | | | | | | | | | | |
| form A × form B | − | − | − | + | + | ? | − | ? | ? | 21–24 |
| *Galerucella nymphaeae* (leaf beetle) | | | | | | | | | | |
| *Rumex* race × *Polygonum* race | + | + | ? | − | − | ? | − | + | ? | 25, 26 |
| *Henosepilachna* (ladybird beetle) | | | | | | | | | | |
| *Henosepilachna pustulosa* × | | | | | | | | | | |
| *Henosepilachna vigintioctomaculata* | + | + | ✓ | + | + | + | − | − | − | 27–31 |
| *Henosepilachna yasutomii* × | | | | | | | | | | |
| *Henosepilachna niponica* | + | + | ✓ | − | − | ? | − | ± | ? | 27, 32–34 |
| *Neochlamisus bebbianae* (leaf beetle) | | | | | | | | | | |
| maple race × willow race | + | + | − | + | ? | ? | ? | ? | ? | 35, 36 |
| Diptera | | | | | | | | | | |
| *Eurosta solidaginis* (goldenrod gall maker) | | | | | | | | | | |
| *Solidago gigantea* race × *Solidago altissima* race | + | + | + | + | ? | ? | ✓ | ± | ? | 37–43 |
| *Rhagoletis pomonella* (apple maggot fly) | | | | | | | | | | |
| apple race × hawthorn race | + | + | + | − | − | ? | − | + | ? | 44–49 |
| Lepidoptera | | | | | | | | | | |
| *Acrocercops transecta* (leaf mining moth) | | | | | | | | | | |
| *Juglans* race × *Lyonia* race | + | + | − | − | − | ? | − | + | − | 50, 51 |
| *Heliconius* (mimetic butterfly) | | | | | | | | | | |
| *Heliconius melpomene* × *Heliconius cydno* | + | ? | ? | + | ? | ? | + | ? | + | 52–54 |
| *Ostrinia nubilalis* (European corn borer) | | | | | | | | | | |
| maize race × mugwort race | + | + | + | + | ? | ? | ? | − | ? | 55–58 |
| *Zeiraphera diniana* (larch bud moth) | | | | | | | | | | |
| larch race × pine race | + | + | + | + | ? | ? | ? | ? | ? | 59–62 |
| Total number of tested taxa | 14 | 13 | 12 | 14 | 7 | 1 | 12 | 9 | 3 | |
| The number of taxa exhibiting positive isolation | 13 | 12 | 8 | 9 | 2 | 1 | 3 | 5 | 1 | |

**Table 1** Continued

| Insect systems | Habitat isolation | Immigrant inviability | Temporal isolation | Sexual isolation | Gametic incompatibility | CSP* | Intrinsic $F_1$ inviability | Extrinsic $F_1$ inviability | Sexual selection against hybrids |
|---|---|---|---|---|---|---|---|---|---|
| Evolution caused by divergent selection? | Probably | Yes | Probably | Possibly | Possibly | Possibly | Possibly | Yes | Possibly |
| The number of taxa exhibiting no isolation | 1 | 1 | 4 | 5 | 4 | 0 | 9 | 4 | 2 |

The evidence in each example is summarized as follows: −, no RI detected; +, positive RI detected; ±, mixed evidence; <, RI present but very weak; ?, not measured; *CSP indicates conspecific sperm precedence.

1 = Nosil, 2004; 2 = Nosil, 2007; 3 = P Nosil, unpubl; 4 = Nosil & Crespi, 2004; 5 = Nosil & Crespi, 2006a; 6 = Nosil et al., 2002; 7 = Sandoval, 1994a; 8 = Sandoval, 1994b; 9 = Caillaud & Via, 2000; 10 = Hawthorne & Via, 2001; 11 = Via, 1991; 12 = Via, 1999; 13 = Via et al., 2000; 14 = Rodríguez et al., 2004; 15 = Tilmon et al., 1998; 16 = Wood, 1980; 17 = Wood & Guttman, 1982; 18 = Wood & Guttman, 1983; 19 = Wood & Keese, 1990; 20 = Wood et al., 1999; 21 = Saitoh & Katakura, 1991; 22 = Saitoh et al., 2008; 23 = Katakura et al., 1996; 24 = Arita, unpubl.; 25 = Pappers et al., 2002a; 26 = Pappers et al., 2002b; 27 = Katakura, 1997; 28 = Katakura & Nakano, 1979; 29 = Matsubayashi & Katakura, 2007; 30 = Matsubayashi & Katakura, 2009; 31 = Nakano, 1985; 32 = Katakura et al., 1989; 33 = Katakura & Hosogai, 1997; 34 = Kuwajima, unpubl; 35 = Funk, 1998; 36 = Funk et al., 2002; 37 = Brown et al., 1995; 38 = Craig et al., 1993; 39 = Craig et al., 1997; 40 = Craig et al., 2000; 41 = Craig et al., 2001; 42 = Horner et al., 1999; 43 = Itami et al., 1998; 44 = Feder et al., 1997; 45 = Filchak et al., 2000; 46 = Prokopy et al., 1982; 47 = Prokopy et al., 1988; 48 = Reissig & Smith, 1978; 49 = Berlocher & Feder, 2002; 50 = Ohshima, 2008; 51 = I Ohshima, unpubl.; 52 = Jiggins et al., 2001; 53 = Naisbit et al., 2001; 54 = Naisbit et al., 2002; 55 = Bethenod et al., 2005; 56 = Calcagno et al., 2007; 57 = Malausa et al., 2005; 58 = Thomas et al., 2003; 59 = Emelianov et al., 1995; 60 = Emelianov et al., 2001; 61 = Emelianov et al., 2003; 62 = Day, 1984.

decide whether to feed on the utilized host, thereby wasting time and energy while increasing predation risk. Alternatively, such individuals may suffer low fitness because they attempt to use a 'non-utilizable' host. When preference evolution is driven by such selection, populations in habitats where only a single host is utilized still evolve preference for that host. Thus, unlike the host-switching scenario above, the information processing hypothesis can drive preference evolution between allopatric populations that use a single (but different) host each. This hypothesis could thus explain allopatric insect speciation (and it does not predict character displacement).

In addition to habitat isolation, the dependency of phytophagous insects on their host plants may impose selection to match the phenology of the host plant. If populations adapt to different host species with divergent phenologies, then discordance between insect populations in their developmental time, mating season, and ovipositing season can generate 'temporal isolation'. For example, apple and hawthorn plants differ in their fruiting time and apple vs. hawthorn host races of *R. pomonella* exhibit divergent phenologies, which are each synchronized to the fruiting time of their natal host, thereby generating partial reproductive isolation between the races (Feder et al., 1997; Feder, 1998; Filchak et al., 2000). Evidence for temporal isolation also exists for other host races and closely-related insect species (Wood & Keese, 1990; Itami et al., 1998; Groman & Pellmyr, 2000; Thomas et al., 2003).

In theory, habitat isolation and temporal isolation could be produced by any diversifying evolutionary process. However when such reproductive isolation results from traits associated with adaptation to different hosts, divergent selection is the most likely explanation. Which form of divergent selection contributes most to the evolution of habitat and temporal isolation is often unclear (Berlocher & Feder, 2002; Drés & Mallet, 2002). Thus, future studies could focus on distinguishing the role of different forms of selection in the evolution of habitat and temporal isolation, for example by contrasting allopatric population pairs to non-allopatric ones (Nosil et al., 2006a; Nosil, 2007). Finally, an outstanding question is the extent to which host choice involves preference for the native host, avoidance of alternative hosts, or both (Forbes et al., 2005; Feder & Forbes, 2008).

**Immigrant inviability**

Premating isolation can arise when migrants between populations suffer reduced survival because they are poorly adapted to their non-native habitat. Although not normally considered as a form of reproductive isolation, such 'immigrant inviability' can directly reduce gene flow between populations by lowering the rate of heterospecific

mating encounters (Funk, 1998; Via et al., 2000; Nosil, 2004; Nosil et al., 2005 for a review). By reducing inter-breeding between populations, natural selection against immigrants constitutes a legitimate reproductive barrier, even though it is the direct consequence of ecologically-based divergent selection. Immigrant inviability also imposes selection for the evolution of host preference, contributing to the evolution of habitat isolation via the aforementioned 'fitness trade-offs hypothesis'. Immigrant inviability should be a common form of reproductive isolation in phytophagous insects because it will act whenever populations are divergently adapted to different host plants.

### Sexual isolation

Divergent mating cues and preferences can reduce the probability of between-population mating, generating sexual isolation, as observed in some groups of phytophagous insects (Table 1; Funk, 1998; Funk et al., 2002). For example, sexual isolation has been documented between host forms of *Eurosta solidaginis* (Fitch) (Craig et al., 1993, 1997), *Neochlamisus bebbianae* (Brown) (Funk, 1998), *Zeiraphera diniana* (Guenée) (Emelianov et al., 2001), *Timema cristinae* (Vickery) (Nosil et al., 2002), and between the species pair *Henosepilachna vigintioctomaculata* (Motschulsky) and *Henosepilachna pustulosa* (Kôno) (Matsubayashi & Katakura, 2007). In some cases, the evolution of sexual isolation was clearly promoted by divergent host-plant adaptation, because population pairs on different hosts exhibited greater sexual isolation than similarly-aged population pairs on the same host (Funk, 1998; Nosil et al., 2002). Additional studies that examine both different-host and same-host pairs, to control for divergence that is not host-associated, are required.

Sexual isolation might arise because differentiation in host chemical composition affects cuticular hydrocarbon composition, which in turn affects mating preferences (Stennett & Etges, 1997; Etges, 1998; Etges & Ahrens, 2001; Etges et al., 2009). Other types of traits, such as morphological traits related to host plant use, might also generate sexual isolation. The actual traits contributing to sexual isolation between populations adapted to different hosts are usually unknown and finding such traits represents a major avenue for further research (Jiggins et al., 2001; Fordyce et al., 2002; Nosil et al., 2007). Additionally, the role of genetic factors vs. plasticity in generating sexual isolation requires more work, as only genetically-based sexual isolation constitutes a true reproductive barrier (Coyne & Orr, 2004).

An interesting point is that some phytophagous insects adapted to different hosts do not exhibit sexual isolation. Examples of an apparent lack of sexual isolation include host forms of *R. pomonella* (Reissig & Smith, 1978) and *A. pisum* (Via, 1999), as well as the species pair *H. niponica* and *H. yasutomii* (Katakura et al., 1989). One explanation for this variability is that if strong host preferences evolve early in the speciation process, hybridization can be reduced to the extent that reinforcing selection on mating preferences to avoid hybridization becomes weak or absent (Nosil & Yukilevich, 2008). Another explanation is that divergent host adaptation only sometimes involves traits that incidentally also affect mating preference.

### Postmating-prezygotic isolation

Postmating, prezygotic isolation exists when there is a reduction in the fertilization success of between-population matings, or a reduction in female fitness following between-population copulation. Examples include poor transfer or storage of sperm (Price et al., 2001), failure of fertilization when gametes come into contact (Vacquier et al., 1997; Palumbi, 1998), and conspecific sperm or pollen preference (Rieseberg et al., 1995; Howard et al., 1998). Such barriers can evolve via numerous processes, such that their role in ecological speciation is unclear.

In phytophagous insects, postmating-prezygotic isolation might arise when host-related differentiation affects external or internal genitalia, or the physical condition of gametes during spermatogenesis and oogenesis (which in turn might affect the physical matching of sperm and egg). Although the extreme diversity of insect genitalic structures suggests that postmating-prezygotic isolation could be common in insects (Shapiro & Porter, 1989; Sota & Kubota, 1998; Hosken & Stockley, 2004), there is very little data linking divergent selection to the evolution of this form of reproductive isolation. A clear example stems from ecotypes of *T. cristinae*, in which female fecundity is greatly reduced following between-population mating (relative to fecundity following within-population mating), but only when mating is between populations using different host plants (i.e., fecundity is not reduced following mating between different populations using the same host) (Nosil & Crespi, 2006a). Although the mechanism reducing female fitness in this example is unknown, the observation of postmating-prezygotic isolation only between populations adapted to different hosts shows that divergent selection can drive the evolution of this form of reproductive isolation. More attention to the association between divergent host use and these types of barriers is warranted, especially given the diversity of male/female interactions in insects (Choe & Crespi, 1997).

### Intrinsic hybrid inviability

Postmating isolation can result from genetic incompatibilities between divergent genomes, caused by negative

interactions between genes that differ between populations, when these genes are brought together in hybrids (Rice & Hostert, 1993; Rundle & Whitlock, 2001; Coyne & Orr, 2004). These incompatibilities reduce the fitness of hybrids and they do not depend on an ecological interaction between phenotype and environment. However, it is still possible that such incompatibilities evolve as a by-product of ecologically-based divergent natural selection, for example if the different alleles favored by selection within each population are incompatible with one another when brought together in the genome of a hybrid. Intrinsic hybrid inviability is well known in insects, for example having been documented in many crosses between species or subspecies of *Drosophila* (Coyne & Orr, 2004). However, the role of divergent selection in driving the evolution of such incompatibilities is generally unknown. Evidence that divergent selection can promote the evolution of intrinsic hybrid inviability comes from studies demonstrating a positive relationship between the degree of divergence in host plant use and the magnitude of intrinsic hybrid inviability between taxon pairs of Lepidoptera and *Drosophila* (Funk et al., 2006; Funk & Nosil, 2008).

### Extrinsic hybrid inviability

Postmating isolation can also arise when hybrid fitness is reduced because of an ecological mismatch between intermediate hybrid phenotypes and the environment. Such extrinsic hybrid inviability (or ecologically-dependent postmating isolation) is closely tied to divergent host adaptation, with $F_1$ hybrids being intermediate between parental forms in adaptive characters and thus exhibiting lower fitness on each host than the native parental form. However, a difficulty when testing for extrinsic hybrid inviability is distinguishing low $F_1$ fitness that arises due to a true mismatch between phenotype and environment from low $F_1$ fitness that is due to genetic incompatibilities in the hybrids. One direct test for extrinsic postmating isolation involves backcrosses of $F_1$ hybrids to both parental forms in reciprocal transplants between environments (e.g., Rundle & Whitlock, 2001; Rundle, 2002). A comparison of the fitness of the two types of backcrosses estimates a component of ecologically-dependent isolation while controlling for any genetic incompatibilities (Rundle & Whitlock, 2001; see Emelianov et al., 2001; Linn et al., 2004 for other types of tests). Although it is intuitively acceptable that hybrids between forms adapted to different host plants will exhibit extrinsic hybrid inviability, we are aware of only a single direct test that explicitly controlled for genetic incompatibility using the method of Rundle & Whitlock (2001). This study detected clear evidence for ecologi-

cally-dependent postmating isolation (Egan & Funk, 2009). Nonetheless, indirect evidence for extrinsic hybrid inviability stems from numerous studies showing low performance of hybrids on the hosts used by the parental species (e.g., Craig et al., 1997; Feder, 1998; Via et al., 2000; Forister, 2005; Ohshima, 2008).

### Sexual selection against hybrids

Reduced mating success of hybrids can also result in postmating isolation, although examples linking reduced mating success of hybrids to divergence in host plant use are lacking. An example that does have an ecological context concerns the *Heliconius* butterflies, *H. cydno* Doubleday and *H. melpomene* L. $F_1$ hybrids between these species mate readily with one another, but hybrid color-patterns are intermediate and fall largely outside of the range of parental mate preferences, so that hybrids exhibit low mating success with the parental forms (Naisbit et al., 2001).

### Multiple components of isolation and the timing of their evolution

The sections above and the data in Table 1 make it clear that there is at least some evidence for selection driving the evolution of each type of barrier considered (although as noted, some are inherently more prone to evolving via divergent selection than others). Given that selection can drive the evolution of diverse types of barriers, future work could fruitfully focus on two topics: 1) for a given stage or time period in the speciation process, determining the relative importance of each type of reproductive isolation for total reproductive isolation, and 2) among different stages of the speciation process, the timing of the evolution of different forms of reproductive isolation.

On the first topic, although reproductive isolation between closely related taxa can involve multiple components of isolation (Table 1), the nature of multiple components of isolation is poorly understood. Consider two examples of closely related taxa in which the strength of multiple isolating barriers is well documented. In the case of *T. cristinae*, two ecotypes occurring on different host plants in parapatry exhibit at least four different forms of reproductive isolation (Figure 3A), yet their total reproductive isolation is far from complete. These ecotypes have evolved reproductive isolation via a combination of divergent selection and ecologically-based reinforcing selection (Nosil et al., 2002, 2003; Nosil, 2007). The second example concerns closely related species of the phytophagous ladybird beetles *H. vigintioctomaculata* and *H. pustulosa*, which exhibit six isolating barriers (Figure 3B). Interestingly, although each individual barrier is incomplete, the joint action of all the different barriers results in almost complete reproductive isolation (Matsubayashi & Katakura,

**A** *Timema cristinae* ecotypes

**B** Closely related *Henosepilachna* ladybird beetles
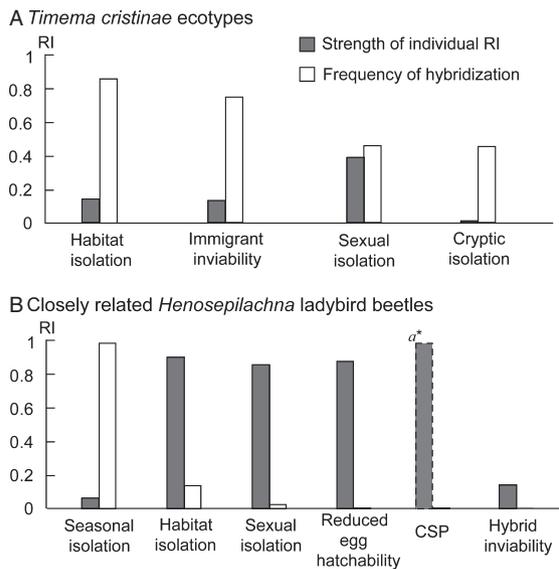
**Figure 3** Empirical examples of multiple components of reproductive isolation. (A) Host ecotypes of *Timema cristinae* from parapatric populations using different hosts (data from Nosil, 2007). (B) Closely related species of *Henosepilachna* ladybird beetles from sympatric populations utilizing different hosts (data from Matsubayashi & Katakura, 2009). Gray bars represent mean individual strength of reproductive isolation (RI), which is estimated for each reproductive barrier using the methods in Ramsey et al. (2003) for *Timema* and the related methods of Martin & Willis (2007) for *Henosepilachna*. Blank bars denote the estimated frequency of hybridization after the sequential action of the isolating barriers, which is estimated as: 1−cumulative strength of total RI (following Ramsey et al., 2003). Conspecific sperm precedence (CSP) in the ladybird beetle example varied in strength from zero to complete, depending on the frequency of multiple mating of females (Matsubayashi & Katakura, 2009). Thus, the maximum case ($a^*$) is represented here. Interbreeding/hybridization between the *Timema* ecotypes is moderately reduced after the action of all measured isolating barriers, whereas hybridization between the *Henosepilachna* species is reduced to nearly zero before postmating isolation (reduced egg hatchability) even acts.

2009). The example shows the ability of multiple, yet incomplete, components of isolation to almost completely prevent gene flow. Focused studies on multiple components of isolation are still rare, and further accumulation of such studies is needed to understand which barriers contribute to speciation and how different barriers might interact (Coyne & Orr, 2004).

A related question concerns the timing of evolution of reproductive barriers. The barriers to gene flow important to speciation are those that evolve before reproductive isolation is yet complete. Thus, the temporal order of the evolution of different barriers is important for understanding speciation, particularly because the relative importance of

current barriers is not necessarily indicative of their historical importance (Coyne & Orr, 2004). Little is known about the relative rates of evolution of various forms of reproductive isolation, although data from phytophagous insects suggests that ecological forms of reproductive isolation sometimes evolve prior to forms that are non-ecological (Funk et al., 2002 for a review; Table 1). Much more work is needed to produce a comprehensive understanding of the temporal order of the evolution of reproductive isolation.

## A genetic mechanism linking selection to reproductive isolation

Ecological speciation requires a genetic mechanism by which selection on genes conferring local adaptation is transferred to genes conferring reproductive isolation. Two main mechanisms are recognized, distinguished by whether these genes are (1) one and the same (i.e., pleiotropy – a gene affecting performance on a host plant also affects reproductive isolation) or (2) physically different from one another (in which case evolution proceeds due to linkage disequilibrium between selected and reproductive isolation genes) (Kirkpatrick & Ravigné, 2002; Coyne & Orr, 2004; Rundle & Nosil, 2005). In the case of pleiotropy, reproductive isolation evolves by direct selection on a gene affecting adaptation to a host plant. Direct selection is thought to be a powerful mechanism of speciation (Figure 4). In contrast, speciation is more difficult when genes responsible for host adaptation are physically different from those causing reproductive isolation. In this case, reproductive isolation evolves because the effects of selection on loci conferring adaptation spill over to other loci that confer reproductive isolation. However, the strength of selection on reproductive isolation loci is mitigated by the imperfect genetic association between selected and reproductive isolation loci (Felsenstein, 1981; Kirkpatrick & Ravigné, 2002) (Figure 4). In this scenario of linkage disequilibrium, reproductive isolation is said to evolve by indirect selection. We review here examples of reproductive isolation evolving due to pleiotropy vs. linkage disequilibrium, as well as the individual genetic bases of performance and preference traits.

### Genetic mechanisms linking selection and reproductive isolation

(A) *Direct selection and pleiotropy.* There are some empirical examples of pleiotropy playing a role in insect speciation. For performance traits, negative genetic correlations on different host plants (i.e., genetic trade-offs) results in immigrants from different populations using a different host suffering reduced performance on their non-native host. Thus, selection acts directly as a form of reproductive

isolation between populations on different hosts (i.e., immigrant inviability). There are some examples of such trade-offs in phytophagous insects (Via, 1991; Nosil, 2004), although as discussed above, the overall evidence is mixed (Scheirs et al., 2005). In host races of pea aphids, QTL mapping studies revealed that alleles conferring high fecundity on alfalfa confer low fecundity on clover and vice versa, creating a strong barrier to gene flow (Via & Hawthorne, 2002). Similarly, selection might act directly on genes involved in phenology, leading to temporal isolation (Craig et al., 1993; Feder, 1998), or on host preference genes, leading to habitat isolation. Further genetic studies on these types of traits are clearly required.

(B) *Indirect selection and linkage disequilibrium.* In this scenario, genes conferring reproductive isolation evolve due to being in linkage disequilibrium with genes under host-associated selection. The main issue with speciation under such a scenario is that linkage disequilibrium can be broken down by recombination (Figure 4) (Felsenstein, 1981). However, several factors can reduce the effect of recombination on breaking up associations between selected and reproductive isolation loci, thereby facilitating speciation.

First, the maintenance of linkage disequilibrium can be facilitated by physical linkage between selected and reproductive isolation genes. Hawthorne & Via (2001) discovered possible tight physical linkage between preference and performance genes in pea aphids (Figure 4 [ii]). They demonstrated that the two sets of genes are localized mainly on two chromosomes and thus most likely closely physically linked with each other. As mentioned above, differences in host preference result in premating isolation between host races such that this physical linkage helps to transmit selection to reproductive isolation genes. However, it is possible that only a single trait was measured; because the races have a strong preference for their own host, they may have exhibited low fitness due to starvation on the alternative host. Given this scenario, preference, and performance are really the same 'trait', thus the findings represent pleiotropy rather than physical linkage. Nonetheless, Hawthorne & Via (2001) point out that pleiotropy and tight physical linkage have similar consequences for speciation because both generate strong genetic correlations between traits.

In a second and related scenario, the maintenance of linkage disequilibrium is facilitated by chromosomal inversions. For example, the recently-derived apple host race of the apple maggot fly shows consistent differences in allele frequency from the hawthorn host race at six allozyme loci (Feder et al., 1990), with all these loci correlating with the timing of adult eclosion (Feder et al., 1997). Mapping analyses revealed that the six allozyme loci were located on three different chromosomes, with inversions subsuming each of the three genetic regions containing the loci (Feder et al., 2003a,b). To the extent that host adaptation and reproductive isolation genes reside in inversions, the recombination suppressing effects of inversions could greatly promote speciation by maintaining associations between genes. However, recent theory indicates that these recombination suppressing effects must be very strong for inversions to promote speciation (e.g., as in cases where the genes involved in speciation reside near chromosomal breakpoints), because even low levels of recombination are effective at breaking down associations between genes (Feder & Nosil, 2009).

Third, the fixation of the same assortative mating allele in each of two diverging populations (i.e., a one-allele assortative mating mechanism) (Felsenstein, 1981) overcomes the problem of recombination breaking up associations between selected and reproductive isolation loci is, but only one explicit test for such a scenario exists (Ortiz-Barrientos & Noor, 2005).

Finally, some theory and data from phytophagous insects indicate that linkage disequilibrium could simply be maintained by very strong divergent selection (Charlesworth et al., 1997; Smadja et al., 2008; Via & West, 2008). This process of 'divergence hitchhiking' could generate clusters of genetic differentiation within the genome, with such clusters harboring genes under divergent selection and exhibiting reduced introgression relative to undifferentiated regions (Gavrilets, 2004; Via & West, 2008; Nosil et al., 2009a). However, as in the case of inversions above, it is unclear the extent to which this mechanism can overcome the powerful effects of recombination.

### Genetic basis of performance and preference

Independent of how performance (i.e., selected) and preference (i.e., reproductive isolation) loci are associated with each other, their individual genetic bases are also of interest for understanding speciation. For example, the number of loci affecting a particular trait can influence the rate and likelihood of population divergence, by affecting per locus selection coefficients (Gavrilets, 2004; Gavrilets & Vose, 2005). Likewise, the number of loci underlying a trait can affect the manner in which genetic divergence is distributed throughout the genome (i.e., how localized it is).

There are two extremes under which the genetic basis of host-plant adaptation may fall: (1) many loci with a small effect (polygenic control) as opposed to (2) one or a few loci with a large effect (simple genetic control). For a given total strength of divergent selection, the fewer the genes responsible for host adaptation and thus the higher the per locus selection coefficient. Divergence for a given locus is a function of its selection coefficient and rates of gene flow

**Table 2** Genetic basis of host preference

| Insect systems | Experimental design[1] | Estimated number of loci mainly determining preference | Chromosomal location[2] | Existence and direction of dominance[3] | Maternal or cytoplasmic effect | Larval experience affects preference | References |
|---|---|---|---|---|---|---|---|
| **Hemiptera** | | | | | | | |
| *Acyrthosiphon pisum* — Alfalfa-race, Clover-race | F2 mapping analysis | 3–5 | A | N/A | N/A | N/A | Hawthorne & Via (2001); Via & Hawthorne (2002) |
| *Nilaparvata lugens* — Rice-race, *Leersia*-race | F1, F2, BC | 1–3 | A | No | No | No | Sezer & Butlin (1998a) |
| **Coleoptera** | | | | | | | |
| *Altica* flea beetle — *A. viridicyanea* (associated with *Geranium wilfordii*), *A. fragariae* (associated with *Duchesnea indica*) | F1, F2, BC | A few | A | + *Geranium* | No | No | Xue et al. (2009) |
| **Diptera** | | | | | | | |
| *Drosophila melanogaster* species complex — *D. sechellia* (having preference for *Morinda*), *D. melanogaster* (displaying avoidance of *Morinda*) | F1, BC mapping analysis | 2 | A | ++ avoidance of *Morinda* | No | No | Higa & Fuyama (1993); Jones (2005); Matsuo et al. (2007) |
| *Eurosata solidaginis* — *Solidago altissima*-race, *S. gigantea*-race | F1, F2, BC | A few | A | + *gigantea* | No | No | Craig et al. (2001) |
| *Proccidochares* flies — *P. australis* (associated with *Heterotheca*), *P.* spec. A (associated with *Machaeranthera*) | F1, F2, BC | 1 | A | No | No | Yes (partially) | Huettel & Bush (1972) |
| *Rhagoletis* flies — Apple-race, Hawthorn-race | F1, F2, BC | A few | A | No | + (only in F2 & backcross generations) | No | Dambroski et al. (2005) |
| **Lepidoptera** | | | | | | | |
| *Acrocercops transecta* — Juglans-race, *Lyonia*-race | F1, F2, BC | 1 | A | ++ *Lyonia* | No | No | I Ohshima (unpubl.) |
| *Helicoverpa* moths — *H. armigera* (associated with cotton), *H. assulta* (associated with pepper) | F1, F2, BC | 1 | A | + cotton | No | No | Tang et al. (2006) |

**Table 2** *Continued*

| Insect systems | Experimental design[1] | Estimated number of loci mainly determining preference | Chromosomal location[2] | Existence and direction of dominance[3] | Maternal or cytoplasmic effect | Larval experience affects preference | References |
|---|---|---|---|---|---|---|---|
| *Heliothis* moths | F1 | N/A | A | + tobacco | No | N/A | Sheck & Gould (1995) |
| *H. virescens* (associated with tobacco, cotton, and soybean), *H. subflexa* (associated with *Physalis pubescens*) | | | | | | | |
| *Mitoura* butterflies | F1 | A few | A | ++ *Calocedrus* | No | No | Forister (2005) |
| *M. nelsoni* (associated with *Calocedrus*), *M. muiri* (associated with *Cupressus*) | | | | | | | |
| *Papilio* butterflies | F1, F2 | N/A | Z | No | No | No | Thompson (1988) |
| *P. oregonius* (associated with *Artemisia*), *P. zelicaon* (associated with *Lomatium* and *Cymopterus*) | | | | | | | |
| *Polygonia c-album* | F1 | N/A | Z | No | No | No | Nygren et al. (2006); Janz et al. (2009) |
| Sweden population (polyphagous), Spain population (associated with *Urtica dioica*) | | | | | | | |
| *Yponomeuta* moths | F1, BC | N/A | A | + *Euonymus* | No | Yes (partially) | Hora et al. (2005) |
| *Y. cagnagellus* (associated with *Euonymus*), *Y. malinellus* (associated with *Malus*), *Y. padellus* (associated with Rosaceae plants) | | | | | | | |

[1]Hybrid generations used in the inference of the genetic basis. For classical crossing experiments, the hybrid generations assessed are shown. 'Mapping analysis' indicates that linkage analyses were conducted.

[2]A, autosome; Z, Z-chromosome.

[3]Dominant phenotypes. ++, complete dominance; +, incomplete dominance.

N/A, the relevant data was not collected.

**Table 3** Genetic basis of host performance

| Insect systems | | Experimental design[1] | Estimated number of loci mainly determining performance | Chromosomal location[2] | Existence and the direction of dominance[3] | Maternal or cytoplasmic effect | References |
|---|---|---|---|---|---|---|---|
| **Hemiptera** | | | | | | | |
| *Acyrthosiphon pisum* | Alfalfa-race, Clover-race | F₂ mapping analysis | 3–5 | A | N/A | N/A | Hawthorne & Via (2001); Via & Hawthorne (2002) |
| *Nilaparvata lugens* | Rice-race, *Leersia*-race | F₁, F₂, BC | 1–2 | A | ++ rice | No | Sezer & Butlin (1998b) |
| **Coleoptera** | | | | | | | |
| *Leptinotarsa decemlineata* | Tomato-adapted population, Population poorly adapted to tomato | F₁, F₂, BC | Polygenic | A | ++ resistance to tomato | Yes (only in F2 & backcross generations) | Lu et al. (2001) |
| *Phyllotreta nemorum* | *Barbarea*-adapted population, Population poorly adapted to *Barbarea* | F₁, F₂, BC | 1 | A & X & Y | ++ resistance to *Barbarea* | No | de Jong & Nielsen (1999); de Jong et al. (2000) |
| **Diptera** | | | | | | | |
| *Drosophila melanogaster* species complex | *D. sechellia* (having resistance to *Morinda*'s toxin), *D. simulans* (lacking resistance to *Morinda*'s toxin) | BC mapping analysis | 5 | A | ++ resistance to *Morinda* | No | Jones (1998, 2005) |
| *Eurosta solidaginis* | *Solidago altissima*-race, *S. gigantea*-race | F₁, F₂, BC | N/A | A | No | No | Craig et al. (1997) |
| **Lepidoptera** | | | | | | | |
| *Acrocercops transecta* | *Juglans*-race, *Lyonia*-race | F₁, F₂, BC | 1 | A | ++ *Juglans* | No | Ohshima (2008), unpubl. |
| *Heliothis* moths | *H. virescens* (associated with tobacco, cotton, and soybean), *H. subflexa* (associated with *Physalis pubescens*) | F₁, BC | Polygenic | A | + cotton and tabacco, ++ *Physalis* | No | Sheck & Gould (1993); Sheck & Gould (1996) |
| *Mitoura* butterflies | *M. nelsoni* (associated with *Calocedrus*), *M. muiri* (associated with *Cupressus*) | F₁ | A few | A | ++ *Calocedrus* | No | Forister (2005) |

**Table 3** *Continued*

| Insect systems | Experimental design[1] | Estimated number of loci mainly determining performance | Chromosomal location[2] | Existence and the direction of dominance[3] | Maternal or cytoplasmic effect | References |
|---|---|---|---|---|---|---|
| *Polygonia c-album* | Sweden population (polyphagous), Spain population (associated with *Urtica dioica*) | F$_1$ | N/A | A | No | No | Nygren et al. (2006) |

[1]Hybrid generations used in the inference of the genetic basis. For classical crossing experiments, the hybrid generations assessed are shown. 'Mapping analysis' indicates that linkage analyses were conducted.

[2]A, autosome; Z, Z-chromosome.

[3]Dominant phenotypes. ++, complete dominance; +, incomplete dominance.

N/A, the relevant data was not collected.

(Gavrilets, 2004; Mallet, 2005). Thus, it may be easier for selection to overcome gene flow and maintain adaptive divergence in sympatry and parapatry when few loci are involved. However, if only few genes diverge, then it is unlikely that much reproductive isolation will incidentally evolve via the fortuitous effects of hitchhiking and pleoitropy (Rice & Hostert, 1993). In contrast, if divergent selection acts on many genomic regions, it can cause genomically-widespread divergence, leading to incidental divergence in the genes yielding additional isolation (e.g., genes responsible for mating preference or intrinsic hybrid sterility). In essence, simple genetic control of host adaptation makes divergence in the face of gene flow more likely, but polygenic control results in a greater correlated evolutionary response, and thus may be more likely to result in reproductive isolation between populations (Nosil et al., 2009b for a review). Therefore, the consequences of divergent selection for speciation depend on the genetic basis of host adaptation.

Here, we review the genetic basis of host preference and performance (Tables 2 and 3, respectively). Relevant studies were located using a Web of Science search using the terms 'genetic basis and host and preference', 'genetic basis and host and performance', and 'host race and insect', as well as from citations in the articles recovered by our Web of Science searches. In order to focus on the number, location, and mode of inheritance of preference and performance loci, we considered studies that included experimental crossing between populations or closely related species and assessed phenotypes in F$_1$, F$_2$, or backcross generations.

(A) *Genetic basis of host preference.* We found 14 relevant studies stemming from Hemiptera, Coleoptera, Diptera, and Lepidoptera (Table 2). Of these, 10 revealed that host preference is determined by a few (1–5) loci. While publication bias likely contributes to this pattern, what can be said is that host preference can involve only a few loci of large effect. One milestone study is Matsuo et al. (2007), who detected and sequenced two preference genes in the *Drosophila melanogaster* Meigen species complex. The two genes code odorant binding proteins and are very tightly linked with each other (<500 base pairs).

Another finding is that preference genes are often located on autosomes. However, two butterfly systems, *Papilio* butterflies (Thompson, 1988) and *Polygonia c-album* L. (Nygren et al., 2006), report Z-linked inheritance of preference, consistent with a past review of sex-linked traits in lepidoptera which reported that traits which distinguish closely related Lepidoptera exhibit a bias towards being Z-linked (Prowell, 1998). Z-linkage of preference traits in Lepidopterans may thus play an important role in host selection in hybrid generations
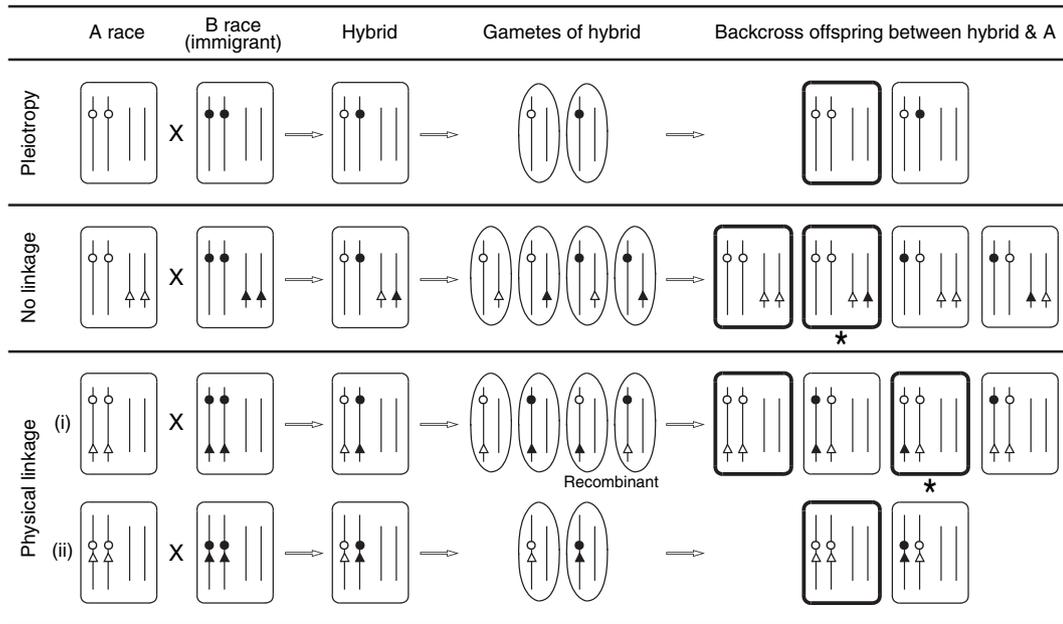
**Figure 4** The maintenance of linkage disequilibrium between a gene conferring host adaptation (e.g., performance) and a gene causing reproductive isolation (e.g., preference). The figure shows individual genotypes in hybridization scenarios between two host races (both races are 2n = 4). Two loci are shown, circles are performance loci and triangles are premating isolation loci. Open circles are alleles conferring high performance on the host of race A, and solid circles are those conferring high performance on the host of race B. Heterozygous genotypes suffer from reduced performance on each host (indicated by the thin line in the backcross generation) relative to homozygous genotypes (indicated by the thick line). Mating occurs more frequently between individuals possessing the same alleles at the premating isolation locus. In the case of pleiotropy, the performance gene and the isolation gene are the same such that individuals showing high performance on host A prefer to mate with race A, resulting in assortative mating between races. In contrast, when the performance and isolation genes are physically different and located on different chromosomes, backcrosses that show high performance on host A have low mating discrimination against the A race (marked with *). Even in the case of physical linkage, loose linkage can result in recombination between loci (i), breaking down linkage disequilibrium between them (marked with *). However, if physical linkage is tight enough, it can maintain linkage disequilibrium (ii).

(Prowell, 1998). Because females are the heterogametic sex in Lepidoptera, hybrid females inherit preference genes only from the paternal parent and exhibit only paternal host preference.

Eight of the studies reviewed here revealed that preference for one host was dominant over preference for the other. These dominance results, coupled with the above mentioned Z-linkage, indicate that hybrid generations often exhibit preference for only one of the parental hosts. Only one study, using *Rhagoletis* flies, detected cytoplasmic effects on preference. Females of $F_2$ and backcross generations between apple and hawthorn host races significantly preferred to orient to a maternal grandmothers' host (Dambroski et al., 2005). In two cases (*Procecidochares* flies and *Yponomeuta* moths), preference in hybrids was influenced partially by the host plant on which they fed in their larval stage, but a large role for genetic factors was nonetheless evident even in these specific examples, as well as more generally. Only one study focused on larval host-

plant preference of holometabolan insects and revealed that the preference was also under genetic control in *Helicoverpa* moths (Tang et al., 2006). Interplant movement in later instars of two *Helicoverpa* species was found to be extensive, thus differences in larval preference could result in habitat isolation. Also, in insects whose adults and larvae use the same host-plant tissue (e.g., Coleoptera and Hemiptera), adult feeding preference is one of the important factors causing habitat isolation. Only one study, using *Altica* flea beetles, assessed the genetic basis of adult feeding preference and showed that this trait was genetically determined (Xue et al., 2009). Studies focused on larval host preference or on adult feeding preference are very rare, and further studies are needed. Although we focus here on acceptance of hosts as the measure of 'preference', we note recent studies in *Rhagoletis* which uncovered an important role for host avoidance in host selection behavior (Forbes et al., 2005; Linn et al., 2005). Future genetic studies, particularly those including both preference and

avoidance data, are required before major generalizations can be made. However, what these results do indicate is that differences in host preference between closely related taxa can be under simple genetic control, although examples of polygenic control also exist (e.g., Nosil et al., 2006b).

(B) *Genetic basis of performance*. We identified 10 relevant studies involving Hemiptera, Coleoptera, Diptera, and Lepidoptera (Table 3). Six studies supported simple genetic control, but polygenic control was supported in the colorada potato beetle *Leptinotarsa decemlineata* Say (Lu et al., 2001) and in the *Heliothis* moths (Sheck & Gould, 1993, 1996). Specifically, *Heliothis virescens* (Fabricius) has a broad host range and is associated mainly with cotton, soybean, and tobacco whereas *Heliothis subflexa* (Guenée) has a narrow host range, feeding only on plants in the genus *Physalia*. Experimental crossing revealed that performance on these respective hosts was governed by independent loci and that these loci did not interact negatively. High performance on cotton, on tobacco, and on *Physalis* was inherited dominantly, and thus $F_1$ hybrids could grow as well on these plants as could the two pure species.

In all studies examined, performance loci were to some extent located on autosomes. In one case, both autosomal and sex chromosomes were involved (*Phyllotreta* beetles; de Jong & Nielsen, 1999; de Jong et al., 2000). Seven studies revealed complete dominance, indicating that $F_1$ hybrids would survive only on one of the parental hosts. Only one study provided evidence for maternal or cytoplasmic effects on performance (Lu et al., 2001). In summary, the studies examined exhibit a tendency towards simple genetic control, as reported for preference genes above. Again, there may be a publication bias and further studies are clearly warranted.

### Summary: genetics of speciation in phytophagous insects

Although the number of available studies is few, those reviewed here provide evidence that preference and performance can be under the control of few loci, potentially facilitating divergence in the face of gene flow and contributing to the propensity for phytophagous insects to undergo sympatric speciation (Berlocher & Feder, 2002; Drés & Mallet, 2002). Races or closely related species can show various types of host-independent reproductive isolation, such as differences in pheromone composition or mating behavior (e.g., Bethenod et al., 2005). If there is physical linkage between these host-independent isolation genes and those affecting preference or performance, genome-wide divergence may be facilitated. Indeed, a recent study combining QTL mapping and genome-scan methods revealed that differentiation between host races of pea

aphids comprises a large genomic region (20 centiMorgans) surrounding QTL for host preference and performance (Via & West, 2008). Future genetic analyses should include both host-dependent and host-independent isolation genes, to allow comparison of their genomic location and localization.

A second finding is that roughly half of the reviewed studies showed some evidence of dominance. Additionally, in the *D. melanogaster* species complex, *Acrocercops transecta* Meyriek, and *Mitoura* butterfly systems, the direction of dominance was opposite between preference and performance traits. This finding has implications for speciation because opposing dominance acts to reduce gene flow. Consider an example which illustrates this process. In *A. transecta*, performance on *Juglans* was completely dominant over that on *Lyonia*, but preference for *Lyonia* was completely dominant over that for *Juglans* (Ohshima, 2008; I Ohshima, unpubl.). Because of the dominant performance on *Juglans*, $F_1$ larvae can survive only on *Juglans*. This suggests that gene flow will occur (i.e., asymmetrically) from the *Lyonia* race to the *Juglans* race. However, eclosed $F_1$ females avoid ovipositing on *Juglans* due to the expression of the dominant *Lyonia* preference. This results in the genetic components that came from the *Lyonia* race being removed from the *Juglans* race. Thus, differences in the mode of dominance inheritance between preference and performance traits itself functions as a barrier to gene flow between populations adapted to different hosts (Forister, 2005; Ohshima, 2008; I Ohshima, unpubl.) (Figure 5). Differences in the chromosomal location of genes (autosomes vs. Z-chromosomes) could also contribute to this type of reproductive isolation (e.g., *P. c-album*, Nygren et al., 2006).

Finally, we note that the observed differences in dominance direction between performance and preference traits suggest that genes responsible for the two traits are likely physically different from one another. This means that genetic covariance between the two traits cannot be caused by pleiotropy, and thus that host-associated divergence involves multilocus evolution (e.g., Via, 1986; Singer et al., 1988; but see Fox, 1993). There are at least two mechanisms that can generate genetic covariance between traits controlled by different genes (Armbruster & Schwaegerle, 1996). One is physical linkage between the loci governing performance and preference (e.g., Hawthorne & Via, 2001). The other is migration between populations, which can generate linkage disequilibrium between unlinked loci, so long as those loci exhibit differences between populations (Nei & Li, 1973; Kirkpatrick et al., 2002). To our knowledge, only one study tested for this latter scenario and reports that genetic covariance between preference
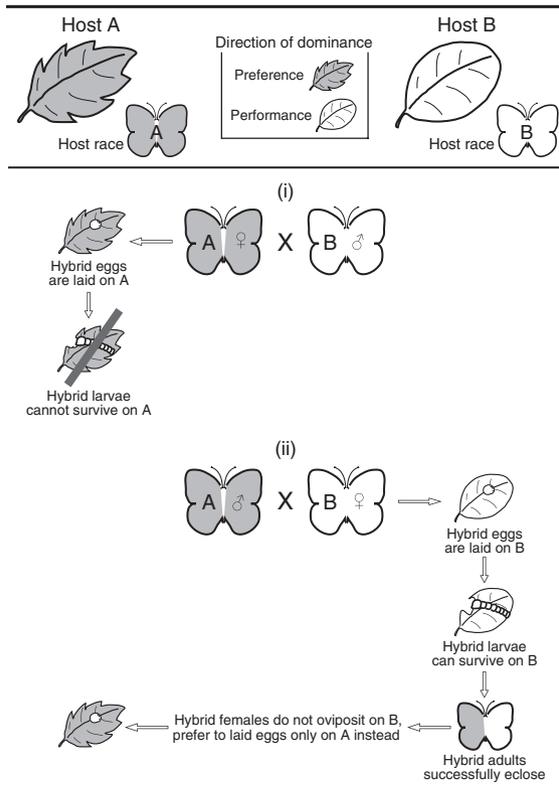
**Figure 5** The effects on reproductive isolation of contrasting modes of inheritance of preference and performance (see also Forister, 2005). The figure shows two butterfly host races associated with host plants A and B. In this host-race system, preference for plant A is dominant over preference for B. In contrast, for adaptation, high performance on B is dominant over high performance on A. Such inheritance patterns were documented in our empirical review of the genetic basis of preference and performance (Tables 2 and 3). The two host races can mate with each other in both crossing directions: (i) and (ii). (i) When hybridization occurs between A-race females and B-race males, $F_1$ hybrid eggs are laid exclusively on plant A due to strict preference of A-race females for that plant. However, hatched hybrid larvae lack high performance on plant A (because of dominance of high performance on plant B). Thus, gene flow in this direction of crossing is completely blocked. (ii) When hybridization occurs between A-race males and B-race females, $F_1$ eggs are laid only on plant B due to the strict preference of B-race females. $F_1$ larvae can survive on plant B as well as can individuals of the pure B race (due to the dominance of high performance on plant B). However, eclosed adult $F_1$ females do not oviposit on plant B, but rather oviposit on A because preference for A is dominant. If hybrid males will mate with B-race females, female offspring of this backcross mating also prefer to oviposit on plant A. Thus, the dominance of preference for plant A causes hybrids to not oviposit on plant B, thereby preventing the hybrids from crossing into the pure B race, and resulting in partial reproductive isolation between the two races.

and performance loci is generated, at least in part, by migration between populations adapted to different hosts (Nosil et al., 2006a). We suggest that future studies should consider the mechanisms establishing and maintaining the covariance between preference and performance, as well as their individual genetic bases (Jaenike & Holt, 1991; Gassmann et al., 2006; Matsuo et al., 2007; Ohshima, 2008; Agosta & Klemens, 2009).

## Outstanding topics

We have strived throughout to highlight areas where further work is required. We focus in this section on aspects that did not arise in the topics reviewed above, but appear to be particularly important directions for future research. Our treatment is brief, as little is known about each. Thus, the aim is to introduce topics that warrant further investigation, rather than systematically review them per se.

### Alternatives to ecological speciation

As described in the introductory text, various non-ecological factors can promote speciation. A number of characteristics of herbivorous insects make them good candidates for 'non-ecological speciation'. For example, patchy distributions, small local population sizes, and population bottlenecks caused by host-plant shifts all increase the likelihood of speciation via random genetic drift (Knowles et al., 1999). Likewise, the diverse array of mating systems and sexual interactions in insects makes speciation via non-ecological forms of sexual selection and sexual conflict a serious possibility (Choe & Crespi, 1997; Arnqvist, 1998). Future studies could focus not only on these non-ecological possibilities, but also on how they interact with the role of divergent selection. For example, how do population sizes affect the response to divergent selection, and how might sexual and natural selection interact to drive divergence?

### Geography of phytophagous insect speciation

Traditionally, speciation has been classified by the geographic context under which it occurs, rather than the mechanisms responsible (e.g., selection or drift). These geographic contexts include allopaty, parapatry, and sympatry, with each representing the occurrence of increasing geographic contact/overlap and gene flow during divergence. As described in the introductory text, ecological speciation might occur under any geographical arrangement of populations, so long as divergent selection is the process driving divergence. Insects have long been the focus of debates concerning the geographic mode of speciation, particularly the likelihood of the controversial mode of sympatric speciation. Proponents have argued

sympatric speciation in insects is likely, because many of them mate on their host plants and divergent selection between hosts can be strong (Walsh, 1867; Maynard Smith, 1966; Bush, 1969; Berlocher & Feder, 2002; Drés & Mallet, 2002). Opponents argued that sympatric speciation is theoretically difficult (Mayr, 1947, 1963; Coyne & Orr, 2004) and that the patchy distribution of insects makes allopatric speciation more likely (Futuyma, 2008). For more detailed treatment we refer readers to past reviews which focused on the geography of speciation (e.g., Via, 2001; Berlocher & Feder, 2002). We do note, however, that further studies of the geographic mode of insect speciation are warranted. In particular, studies of parapatric divergence are few. By 'parapatry' we mean situations in which there is geographic contact between adjacent patches of alternative host plants, but the patches are large relative to the dispersal ability of the insects. Thus, during parapatric divergence there is some spatial restriction to the opportunity for gene flow such that gene flow between populations on different hosts may be high right at the border between host patches, but relatively low elsewhere throughout the patches (Mallet et al., 2009).

Another point is that the classification of speciation into distinct geographic contexts may be overly simplistic. Ecological speciation may often occur in stages that involve different geographic contexts. For example, speciation might begin when populations are allopatric, with reproductive isolation accumulating as a by-product of adaptation to their different environments. A second stage is initiated upon secondary contact (parapatry or sympatry), with genetic exchange becoming possible at this point. Indeed, recent work on phytophagous insects has shown that the often extended process of speciation can have multiple modes, involving both an allopatric and a sympatric phase (Feder et al., 2003a; Xie et al., 2007).

### Molecular signature of ecological speciation

A final topic considers the molecular signatures of ecological speciation. Divergent selection during ecological speciation can cause levels of genetic differentiation between populations to be highly variable across the genome (Via, 2001, 2009; Wu, 2001), a pattern that can be referred to as 'heterogeneous genomic divergence' (Nosil et al., 2009a for a review). For example, loci under divergent selection and those tightly physically-linked to them may exhibit stronger differentiation than neutral regions with weak or no linkage to such loci (Charlesworth et al., 1997; Via, 2009). Divergent selection can also increase genome-wide neutral differentiation by reducing gene flow (e.g., by causing ecological speciation), thus promoting genome-wide divergence via the stochastic effects of genetic drift. These consequences of divergent selection

are being reported in recently accumulating studies that (1) document 'outlier loci' with higher levels of population differentiation than expected under neutrality, and (2) positive associations between the degree of adaptive phenotypic divergence and levels of molecular genetic differentiation across population pairs [isolation-by-adaptation (IBA)], even at neutral loci putatively unlinked to those under selection. For example, 'outlier' loci have been reported in several phytophagous insect systems (Emelianov et al., 2004; Scheffer & Hawthorne, 2007; Egan et al., 2008). In one case, both outlier loci and the pattern of IBA were reported in the same system (Nosil et al., 2008). These outlier and IBA studies can be combined with QTL mapping to determine the size and genomic location of regions of strong differentiation, as conducted for host races of pea aphids (Via & West, 2008). Further such studies will shed light into how host adaptation during speciation affects patterns of genomic differentiation. In particular, many questions remain about the number, size, and distribution of regions of differentiation in the genome during speciation.

### Conclusions

We reviewed here three components of ecological speciation in phytophagous insects. The most studied source of divergent selection is differences between environments (hosts), but even here there is much variability in whether divergent selection was detected and in which traits it acted upon. Thus, further studies examining the sources and agents of divergent selection are needed. Many different forms of reproductive isolation have been documented between closely-related insect taxa. Future studies should thus focus on examining multiple barriers within a single system, to ascertain their relative contribution to total reproductive isolation. A particularly pressing future direction concerns determining the rate at which different barriers evolve, because those barriers tending to evolve before speciation is complete are most causally related to the speciation process itself. Genetic studies of ecological speciation are accumulating, but further work is needed to understand how genes under selection and genes conferring reproductive isolation become and remain associated with one another. Additionally, further work might explore the extent to which simple genetic control and opposing dominance for host preference vs. performance facilitates speciation in the face of gene flow.

Finally, we note that there is much variability in the extent to which phytophagous insect speciation proceeds to completion, a topic that has long fascinated entomologists (Berlocher & Feder, 2002; Drés & Mallet, 2002 for reviews). In some instances only weak population diver-

gence occurs, in other cases host race formation, and in others complete speciation. A major outstanding question is the factors determining the extent to which speciation proceeds to completion. Studies examining a wide range of evolutionary divergence could yield insight into this question, thereby yielding a comprehensive understanding of how speciation unfolds, from beginning to end (Funk, 1998; Drés & Mallet, 2002; Funk et al., 2002; Funk & Nosil, 2007; Mallet et al., 2007; Nosil et al., 2009b; Peccoud et al., 2009).

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