

## REVIEW

## Ecological speciation

Howard D. Rundle<sup>1\*</sup> and Patrik Nosil<sup>2</sup>

<sup>1</sup>Department of Zoology and Entomology, University of Queensland, Brisbane, Queensland 4072, Australia

<sup>2</sup>Department of Biological Sciences, Simon Fraser University, Burnaby, BC V5A 1S6, Canada

\*Correspondence: E-mail: h.rundle@uq.edu.au

**Abstract**

Ecological processes are central to the formation of new species when barriers to gene flow (reproductive isolation) evolve between populations as a result of ecologically-based divergent selection. Although laboratory and field studies provide evidence that ‘ecological speciation’ can occur, our understanding of the details of the process is incomplete. Here we review ecological speciation by considering its constituent components: an ecological source of divergent selection, a form of reproductive isolation, and a genetic mechanism linking the two. Sources of divergent selection include differences in environment or niche, certain forms of sexual selection, and the ecological interaction of populations. We explore the evidence for the contribution of each to ecological speciation. Forms of reproductive isolation are diverse and we discuss the likelihood that each may be involved in ecological speciation. Divergent selection on genes affecting ecological traits can be transmitted directly (via pleiotropy) or indirectly (via linkage disequilibrium) to genes causing reproductive isolation and we explore the consequences of both. Along with these components, we also discuss the geography and the genetic basis of ecological speciation. Throughout, we provide examples from nature, critically evaluate their quality, and highlight areas where more work is required.

**Keywords**

Divergent selection, natural selection, reinforcement, reproductive isolation, sexual selection.

*Ecology Letters* (2005) 8: 336–352

**INTRODUCTION**

The past decade has seen a revival of the idea that the macroevolutionary phenomenon of speciation is the result of the microevolutionary process of ecologically-based divergent selection (Funk 1998; Schluter 2000, 2001). While the idea dates back at least to the modern evolutionary synthesis (e.g. Mayr 1942, 1947; Dobzhansky 1951), renewed interest in it has gone hand-in-hand with a reclassification of speciation models from a scheme of geography (i.e. sympatric vs. allopatric) to one that focuses on mechanisms for the evolution of reproductive isolation (Schluter 2000, 2001; Via 2001).

Although ecology may contribute to many mechanisms of speciation, our focus here is on the ecological model in which its contribution is fundamental. Consistent with its recent usage (Schluter 2000, 2001), we define ‘ecological speciation’ as the process by which barriers to gene flow evolve between populations as a result of ecologically-based divergent selection. Selection is ecological when it arises as a consequence of the interaction of individuals with their environment during resource acquisition. Ecologically-based

selection can thus arise, for example, from an individual’s quest to obtain food and other nutrients, attract pollinators, or avoid predators. It can also arise from their interaction with other organisms in their attempt to achieve these goals (e.g. competition). Selection is divergent when it acts in contrasting directions in the two populations and we include here the special case in which selection favours opposite, usually extreme, phenotypes within a single population (termed disruptive selection), as occurs during sympatric speciation.

Ecological speciation is distinguished from other models of speciation in which the evolution of reproductive isolation involves key processes other than ecologically-based divergent selection. These include models in which chance events play a central role, including speciation by polyploidization, hybridization, 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 and/or is not divergent between environments. Examples include certain models of speciation by sexual selection (e.g. Fisher’s runaway, Lande 1981; sexual conflict, Chapman *et al.* 2003)

in which selection arises from the interaction of the sexes and is not divergent between environments, and models involving the fixation of incompatible alleles in allopatric populations experiencing similar selection (Schluter 2001).

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. ecologically-based divergent selection). Distinguishing ecological from non-ecological mechanisms, however, does not imply that the processes involved in the latter are not important to speciation, nor that they may not influence the likelihood and outcome of ecological speciation. Indeed, both possibilities are important topics and a comprehensive understanding of the role of ecologically-based divergent selection in speciation will require careful consideration of numerous non-ecological factors (e.g. see Gavrillets 2004).

Laboratory experiments have shown that ecological speciation can occur; reproductive isolation has evolved as a by-product of adaptation to different environments in manipulative experiments (reviewed in Rice & Hostert 1993). There is also convincing evidence for its operation in nature (reviewed in Schluter 2001; Coyne & Orr 2004). For example, ecological speciation is directly implicated when traits causing reproductive isolation are under ecologically-based divergent selection (e.g. Macnair & Christie 1983; Filchak *et al.* 2000; Via *et al.* 2000; Jiggins *et al.* 2001). It is also implicated when reproductive isolation is shown to have evolved among replicate, independent populations in correlation with environment (i.e. parallel speciation; e.g. Funk 1998; Rundle *et al.* 2000; Nosil *et al.* 2002; McKinnon *et al.* 2004).

Here we review the process of ecological speciation. Because the above research demonstrates that it occurs, we focus on understanding the details of the process. To do this, we separate ecological speciation into three necessary components: an ecological source of divergent selection, a form of reproductive isolation, and a genetic mechanism to link them. This approach is based on a similar classification of theoretical models of speciation used by Kirkpatrick & Ravigné (2002). It is useful because the effects of these components can be studied, to a certain extent, in isolation of one another, and because it highlights areas that have received less attention. As we will see, our understanding of some components is good, whereas critical tests of others are lacking. We also consider two additional topics that have received less attention in previous reviews: the geography and genetic basis of ecological speciation. The literature on ecological speciation is rapidly growing and our review is by no means exhaustive. Rather, we present a selection of

studies that illustrate certain points or address understudied topics.

## ECOLOGICAL CAUSES OF DIVERGENT SELECTION

The first component required for ecological speciation is a source of divergent selection. Three ecological causes have been recognized (Schluter 2000, 2001; Kirkpatrick & Ravigné 2002). Although they are not fully independent and distinguishing between them may not be easy, their separate treatment is useful because it highlights the diversity of ways in which ecology may be involved and their consequences for speciation may vary. This is because the efficacy with which divergent selection is transmitted into reproductive isolation, as well as the forms of reproductive isolation that evolve, will depend on the traits under selection and how they are related genetically to those causing reproductive isolation. In this section, we outline the ecological causes and consider the evidence for the contribution of each to ecological speciation. Although all three ecological causes can, in theory, generate almost any form of reproductive isolation (ecological or not), in the next section we discuss the likelihood that particular forms will evolve via specific ecological causes.

### Environmental differences

Divergent selection can arise because of differences between populations in their environments, including, for example, habitat structure, climate, resources, and the suite of predators or competitors present (Schluter 2000). Divergent selection between environments is consistent with the classic model of allopatric speciation (e.g. Mayr 1942, 1947), although geographic separation is not a pre-requisite. Divergent selection may also arise between sympatric populations occupying separate niches within a single geographic area.

The contribution of environmental differences to ecological speciation is reasonably well-understood, in part because the majority of research has focused on this mechanism. Replicated laboratory experiments have directly shown that adaptation to different environments can generate some reproductive isolation, both in sympatry (Rice & Salt 1990) and allopatry (Rice & Hostert 1993). Environmental differences also appear to be frequent sources of divergent selection in nature (reviewed in Schluter 2000). For example, reciprocal transplant experiments, the classic ecological technique for studying local adaptation of divergent forms, have shown that tradeoffs are common such that traits enhancing fitness in one environment reduce it in the other, implying divergent selection between environments. Environmental differences have also been implicated in the evolution of reproductive

isolation in a few well studied cases of ecological speciation in nature (e.g. Macnair & Christie 1983; Nagel & Schluter 1998; Via *et al.* 2000; Jiggins *et al.* 2001; Linn *et al.* 2003; see Schluter 2001).

Nevertheless, our understanding of the role of environmental differences in ecological speciation is incomplete. Most laboratory experiments, for example, have addressed the evolution of one form of reproductive isolation (sexual isolation); data on the role of environmental differences in the evolution of other forms is limited or non-existent. In addition, reproductive isolation failed to evolve in a number of these experiments (e.g. Rundle 2003; see Rice & Hostert 1993), but we have little understanding as to why. Even more remarkable, for cases in which reproductive isolation did evolve, the traits responsible were generally not even identified. Future experiments that explore how divergent selection between environments affects specific phenotypic traits causing reproductive isolation may be especially useful in addressing these gaps in our knowledge.

The prevalence in nature of divergent selection between environments is also unclear. Although reciprocal transplant experiments suggest it is common, insufficient attention has been given to the possibility of intermediate environments and, when they exist, the fitness of intermediate forms inhabiting them (Schluter 2000). If intermediate environments exist and intermediate phenotypes do well in them, then in theory it is possible for populations adapted to different environments to have diverged from one another by genetic drift alone (Schluter 2000; Gavrillets 2004). Although the end product is the same (e.g. populations that exhibit fitness tradeoffs when reciprocally transplanted), divergent selection need not have been involved in their divergence.

Environmental differences have been implicated in a number of speciation events in nature, but additional cases are needed in other systems. Of particular importance will be those that consider agents of divergent selection that have received less attention. For example, predation is ubiquitous in natural populations and adaptation to it may have important consequences for reproductive isolation. However, predator-generated divergent selection has been implicated in the evolution of reproductive isolation in only a handful of cases (e.g. Jiggins *et al.* 2001; Vamosi & Schluter 2002; Nosil 2004). Additional tests of the role of predation, and other enemies (e.g. parasites, pathogens), are badly needed.

Finally, we note that one of the strongest tests of the role of environmental differences has yet to be performed. If speciation is caused by adaptation to different environments, for some taxa at least we should be able to recreate the initial stages of this process in a controlled laboratory setting. In one such experiment, suggested by Schluter (2000), hybrids between divergent taxa are placed into

separate environments that differ only in the aspects hypothesized to have caused their speciation. Reproductive isolation should then evolve in correlation with environment, building between populations in different environments and being absent between laboratory and natural populations from similar environments. Depending on the natural history of the taxa, a similar experiment could involve individuals from an ancestral species (e.g. the mainland ancestor of an island endemic) placed into a novel environment characteristic of a descendant. Other variants are also possible, but the key to such experiments is that they permit the ecological cause of selection, as well as the traits on which it acts, to be isolated and tested in a replicate manner.

### Sexual selection

The second ecological source of divergent selection involves sexual selection. Because it acts on traits directly involved in mate recognition, sexual selection may be a powerful force in the evolution of reproductive isolation (Panhuis *et al.* 2001). Speciation models involving sexual selection can be classified into two types depending on whether or not differences in mate preferences evolve ultimately 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). Examples that do not involve divergent selection between environments, and are hence not components of ecological speciation, are models in which sexual selection arises from the interaction of the sexes. This includes Fisher's runaway (Lande 1981) and sexual conflict (Chapman *et al.* 2003). Sexual selection can thus be involved in both ecological and non-ecological speciation (Schluter 2000, 2001).

The evidence for sexual selection in ecological speciation is weaker. Although comparative studies suggest that sexual selection is associated with speciation in nature in some taxa, these tests cannot discriminate among its various causes (reviewed in Panhuis *et al.* 2001; Coyne & Orr 2004), most notably ecological vs. non-ecological. Direct tests of ecologically-based sexual selection in speciation in nature are beginning to accumulate (see Boughman 2002). For example, allopatric populations of *Anolis cristatellus* lizards from two environments (mesic and xeric) occupy distinct habitats with respect to light intensity and spectral quality, and the design of their dewlaps (a trait important in social communication, including mating) has diverged between populations in a way that increases signal detectability in each habitat (Leal & Fleishman 2004). Likewise, in freshwater limnetic and benthic threespine stickleback

(*Gasterosteus aculeatus* spp.) fish, male nuptial colour and female perceptual sensitivity both vary among lakes in correlation with light environments, resulting in environment-specific signal preferences (Boughman 2001). In both cases, adaptive signal divergence appears to contribute to reproductive isolation. More studies on diverse taxa are needed, however, to provide general insights.

In contrast to accumulating evidence from natural systems, ecological models of speciation by sexual selection have never been evaluated in manipulative laboratory experiments. This is a conspicuous oversight. It is difficult to implicate any one model of sexual selection in a speciation event in nature, in part because the various models depend on parameters that are difficult to measure (Turelli *et al.* 2001). Specific predictions for some ecological models have been identified (e.g. Boughman 2002), although an alternative interpretation exists (see Coyne & Orr 2004) for even the strongest case (Boughman 2001). Laboratory experiments may be crucial in addressing such issues, allowing the feasibility of various models to be tested and providing insight into how signal traits and preferences, and hence reproductive isolation, evolve under different scenarios. Ultimately, it may be possible in some taxa to recreate speciation by ecologically-based sexual selection in the laboratory, thus gaining some of the strongest evidence possible.

### Ecological interactions

Divergent selection may also arise between populations as a result of their ecological interaction with one another. Ecological interactions are distinguished from other sources of divergent selection because they occur in sympatry, although exceptions could entail allopatric populations interacting indirectly via a separate, mobile species. In addition, divergent selection arising from ecological interactions is frequency dependent because individual fitnesses depend on the frequency of the various phenotypes (Taper & Case 1992; Schluter 2000). Frequency dependent ecological interactions among individuals within a population may also generate disruptive selection that can, in theory, cause sympatric speciation (reviewed in Turelli *et al.* 2001).

At least one form of ecological interaction, interspecific competition, appears common in nature. Observational studies implicate it as the predominant source of divergent selection during ecological character displacement and, although direct tests have just begun to accumulate, they support this conclusion (Taper & Case 1992; Schluter 2000). Nevertheless, despite the apparent prevalence of character displacement, as far as we are aware there are no direct tests, from nature or the laboratory, linking the evolution of reproductive isolation to interspecific competition. Although divergent selection can also arise from other

types of interactions (e.g. mutualism, facilitation, apparent competition; Abrams 2000; Doebeli & Dieckmann 2000; Day & Young 2004), their prevalence in nature and role in ecological speciation are also relatively unexplored. Interactions via shared predators have been shown to alter competitive interactions and affect divergent selection in a pond experiment in sticklebacks (Rundle *et al.* 2003), but the consequences for speciation are not known.

The role of ecological interactions in generating disruptive selection and causing sympatric speciation is similarly unknown. Laboratory experiments have shown that frequency-dependent competition is responsible for the sympatric, ecological diversification of single strains of asexual taxa (e.g. Friesen *et al.* 2004). Implications for ecological speciation are limited, however, because reproductive isolation does not apply. In sexual taxa, competitive interactions have also been shown to generate disruptive selection within a single population of sticklebacks in the wild (Bolnick 2004), although in this case there is no evidence that any reproductive isolation has evolved.

The absence of direct tests of the role of ecological interactions in speciation may be explained, in part, because research has focused on the consequences of a different interaction: heterospecific matings. If heterospecific matings reduce the fitness of the individuals involved, or their hybrid offspring, selection will favour individuals that mate within their own population. This will strengthen pre-zygotic isolation in a process known, in the broad sense, as reinforcement (Servedio & Noor 2003). Although it features prominently in many models of speciation, 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 originated from ecological causes (e.g. Kirkpatrick 2001), then it is tempting to consider reinforcement as a component of ecological speciation. The situation is unclear even in this case, however, because reinforcing selection need not be divergent. In classic 'one-allele' models, a single allele, causing individuals to mate with other, phenotypically similar individuals, is favoured by selection in both populations (Felsenstein 1981; Servedio & Noor 2003). Therefore, whether reinforcement is a component of ecological speciation depends upon the specific circumstances.

Under this broad definition, reinforcement may not be a rare occurrence (Servedio & Noor 2003), although just how common and how often it has an ecological basis is not well understood. Ecological causes have been implicated in some cases (e.g. Rundle & Schluter 1998; Nosil *et al.* 2003; Albert & Schluter 2004). Understanding the contribution of reinforcement to ecological speciation will require careful consideration of all costs to heterospecific matings and the mechanisms (ecological or not) by which they evolved.

Finally, separating the effects on ecological speciation of reinforcement and character displacement may be difficult. Both occur in sympatry from the interaction of populations and can produce the same evolutionary outcome: stronger pre-zygotic isolation between sympatric than allopatric populations (Servedio & Noor 2003). The extent of this problem will not be known until we determine how frequently pre-zygotic isolation is strengthened as a by-product of character displacement. Studies of reinforcement are beginning to consider the possibility: results of one suggest character displacement was not involved (Nosil *et al.* 2003) and two others attempted to control for its contribution (Rundle & Schluter 1998; Albert & Schluter 2004). Studies that estimate the independent and combined roles of both are badly needed. The control facilitated by laboratory experiments may be especially useful in distinguishing these processes and exploring their interaction. For example, by exposing allopatric populations to experimental sympatry in the laboratory, reinforcement was directly implicated in the strengthening of pre-zygotic isolation between the Australia fruit flies *Drosophila serrata* and *D. birchii* (Higgie *et al.* 2000). In future experiments, the opportunity for reinforcing selection could be directly manipulated by housing populations sympatrically or allopatrically during mating (reinforcing selection present or absent, respectively); the opportunity for competition and other ecological interactions could also be manipulated by raising the populations sympatrically or allopatrically during the rest of their life cycles (interactions permitted or prevented, respectively).

## FORMS OF REPRODUCTIVE ISOLATION

Many forms of reproductive isolation exist that can block gene flow between populations in different ways (Coyne & Orr 2004). Below we describe seven forms: four of pre-zygotic isolation and three of post-zygotic isolation. One is the unique product of ecologically-based divergent selection and its existence implies ecological speciation, whereas some others can be produced by any mechanism of speciation. A key question for each thus concerns the role of ecologically-based divergent selection in its evolution. We evaluate the evidence for this and highlight further types of data required. Examples from nature of each are given in Table 1.

### Habitat and temporal isolation

Pre-zygotic isolation can arise when populations are separated in space (habitat) or time (Dres & Mallet 2002; Funk *et al.* 2002). Habitat isolation occurs when populations exhibit genetically-based preferences for separate habitats, reducing the likelihood of heterospecific encounters (Rice &

Salt 1990; Johnson *et al.* 1996). Divergent habitat preferences are most likely to cause pre-zygotic isolation when mating occurs in or near the preferred habitat (Johnson *et al.* 1996; Funk *et al.* 2002). For example, divergent host-plant preferences cause partial reproductive isolation between herbivorous insect populations that mate on the plant on which they feed (Table 1). Temporal isolation occurs when populations exhibit divergent developmental schedules such that mating occurs at different times in the populations. Importantly, both habitat and temporal isolation may be common during ecological speciation because adaptation to different environments or resources will generate selection for divergent habitat preferences or developmental schedules (e.g. individuals preferring the habitat to which they are best adapted will have higher fitness).

Although habitat and temporal isolation appear common (Table 1), little attention has been given to their mechanisms of evolution. Non-ecological processes, such as genetic drift, are unlikely if trait differences can be shown to be adaptive in each habitat, or if they evolve in parallel multiple times (Schluter & Nagel 1995). Different forms of ecologically-based divergent selection could be involved, however, and their relative importance is unknown. Habitat and temporal isolation may both evolve as by-products of adaptation to different environments. However, as noted above, both may also be favoured by selection if traits enhancing fitness in one environment (or when exploiting one resource) decrease it in the other. Alternatively, habitat and temporal isolation could also be favoured by selection if they altered ecological interactions between populations (e.g. reduced competition) or decreased the likelihood of heterospecific matings (i.e. by reinforcement).

### Natural selection against immigrants (immigrant inviability)

Pre-zygotic isolation can arise when migrants between populations suffer reduced survival because they are poorly adapted to their non-native habitat. Although not normally considered 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 review). By reducing interbreeding between populations, natural selection against immigrants constitutes a legitimate reproductive barrier, although it is the direct consequence of ecologically-based divergent selection. Despite being opposite sides of the same coin, the separate consideration of divergent selection and immigrant inviability is useful because the presence of the former does not guarantee that the latter was an important source of reproductive isolation during

**Table 1** Forms of reproductive isolation with examples from nature. Also indicated is whether a particular form commonly evolves by ecologically-based divergent selection (ecological selection) and the ecological causes of divergent selection that could contribute

Form of reproductive isolation	Ecological selection?	Ecological causes of selection	Examples
Pre-zygotic			
Habitat	Probably	DE, EI, RS	Leaf beetles (Funk 1998); pea aphids (Via 1999); ladybird beetles (Katakura <i>et al.</i> 1989); leaf-mining flies (Tavormina 1982); <i>Rhagoletis</i> fruit flies (Feder <i>et al.</i> 1994; Linn <i>et al.</i> 2003); <i>Eurosta</i> galling fly (Craig <i>et al.</i> 1993)
Temporal	Probably	DE, EI, RS	<i>Enchenopa</i> leafhoppers (Wood & Keese 1990), <i>Rhagoletis</i> fruit flies (Feder <i>et al.</i> 1994; Filchak <i>et al.</i> 2000); <i>Banksia</i> plants (Lamont <i>et al.</i> 2003)
Selection against migrants	Yes	DE, EI, RS	Leaf beetles (Funk 1998); <i>Littorina</i> snails (Rolan-Alvarez <i>et al.</i> 1997); <i>Bombina</i> toads (Kruuk & Gilchrist 1997); <i>Heliconius</i> butterflies (Mallet & Barton 1989; Mallet 1989); pea aphids (Via <i>et al.</i> 2000), <i>Timema</i> walking-sticks (Nosil 2004); <i>Artemisia</i> sagebrush (Wang <i>et al.</i> 1997); <i>Gilia</i> plants (Nagy 1997)
Sexual	Unknown (probably)	All	Intertidal snails (Cruz <i>et al.</i> 2004); leaf beetles (Funk 1998); freshwater stickleback (Nagel & Schluter 1998; Rundle <i>et al.</i> 2000; Boughman 2001); <i>Timema</i> walking-sticks (Nosil <i>et al.</i> 2002, 2003); <i>Heliconius</i> butterflies (Jiggins <i>et al.</i> 2001); marine/freshwater stickleback (McKinnon <i>et al.</i> 2004)
Post-mating, pre-zygotic	Unknown	All	<i>Drosophila</i> (Price <i>et al.</i> 2001); ground crickets (Howard <i>et al.</i> 1998); <i>Helianthus</i> plants (Rieseberg <i>et al.</i> 1995)
Post-zygotic			
Intrinsic	Unknown	All	<i>Drosophila</i> spp. (Ting <i>et al.</i> 1998; Presgraves <i>et al.</i> 2003; Barbash <i>et al.</i> 2004; Wu & Ting 2004)
Ecologically- dependent	Yes (unique*)	DE, EI	Freshwater stickleback (Hatfield & Schluter 1999; Rundle 2002); pea aphids (Via <i>et al.</i> 2000); <i>Eurosta</i> galling fly (Craig <i>et al.</i> 1997); water lily leaf beetle (Pappers <i>et al.</i> 2002)
Sexual selection against hybrids	Unknown	All	Freshwater sticklebacks (Vamosi & Schluter 1999); <i>Heliconius</i> butterflies (Naisbit <i>et al.</i> 2001)

DE, divergent environments; EI, ecological interactions; SS, sexual selection; RS, reinforcing selection.

\*This can only evolve by ecologically-based divergent selection.

the speciation process. When speciation is allopatric, for example, 'parental' individuals may never migrate between environments and ecological speciation may occur entirely via the evolution of other forms of reproductive isolation.

Demonstrating natural selection against immigrants is consistent with ecological speciation, although as noted earlier, in theory it is possible for genetic drift to produce divergent populations that exhibit fitness tradeoffs when reciprocally transplanted (Schluter 2000; Gavrilets 2004). This alternate drift-based possibility can be ruled out if the fitness of intermediate forms (i.e. hybrids) is also reduced by ecological mechanisms (see 'ecologically-dependent post-zygotic isolation' below). Quantification of the individual components of reproductive isolation in diverse taxa reveals that natural selection against migrants tends to be strong and that its relative contribution to total isolation may often be greater than that of more commonly considered forms (e.g. sexual isolation, hybrid

inviability; Nosil *et al.* 2005). Our understanding of the divergent selection involved is limited, however, because data addressing the sources and phenotypic targets of selection are few (Schluter 2000). A more detailed understanding will require experiments that directly manipulate agents of selection and identify the traits involved (e.g. Nosil 2004).

### Sexual isolation (pollinator isolation)

Pre-zygotic isolation can arise because individuals from different populations are less attracted to, or do not recognize, one another as potential mates. Such sexual isolation is one of the most commonly recognized forms of pre-zygotic isolation, but its ecological basis is unfortunately also one of the most difficult to determine. This is because sexual isolation usually involves the interaction of signal traits in one sex with preferences in the other. Differences

among populations in both of these will generally arise as a by-product of mate choice evolution within populations, a process that necessarily involves sexual selection and may involve natural selection and genetic drift as well (Kirkpatrick & Ryan 1991; Coyne & Orr 2004). An ecological basis is expected whenever sexual selection has an ecological component. As outlined earlier, this can occur when ecologically-important characters also influence mate choice, or when environmental differences generate divergent selection on mating or communication systems. Sexual isolation can also evolve by reinforcing selection within an ecological context (i.e. if the cost to heterospecific matings originated by ecological mechanisms).

Sexual isolation has received much attention in nature and a number of lines of evidence implicate ecologically-based divergent selection in its evolution. For example, pairs of populations independently adapted to different environments exhibit stronger sexual isolation than those independently adapted to similar environments (Funk 1998; Rundle *et al.* 2000; Nosil *et al.* 2002; McKinnon *et al.* 2004). In addition, traits under divergent natural selection have been shown to influence mate choice in a number of systems (e.g. Nagel & Schluter 1998; Jiggins *et al.* 2001; see Schluter 2001). Divergent selection on mating systems has also been implicated in a few cases (e.g. Boughman 2001, 2002; Leal & Fleishman 2004), and there is evidence consistent with ecologically-based reinforcement (Rundle & Schluter 1998; Nosil *et al.* 2003; Albert & Schluter 2004).

In plants, populations in different environments can be exposed to selection to adapt to different pollinators. The subsequent divergence in pollinator-related traits will generate pollinator isolation. Such pollinator isolation has been strongly implicated in monkeyflowers (Schemske & Bradshaw 1999; Bradshaw & Schemske 2003; Ramsey *et al.* 2003) and may be common in other plants (see Coyne & Orr 2004).

### Post-mating, pre-zygotic isolation

Post-mating, pre-zygotic isolation exists when barriers, acting after copulation is initiated, either reduce or prevent the fertilization of eggs with heterotypic sperm. 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 and it is not immediately apparent what their potential role is in ecological speciation. Although reproductive proteins involved in gametic interactions often evolve rapidly via selection (Swanson & Vacquier 2002), the source of this selection is generally not known and a role for ecological causes is not required (Vacquier *et al.* 1997). Examples exist

that are consistent with both ecological and non-ecological selection (see Coyne & Orr 2004). Distinguishing among the various mechanisms for the evolution of this type of barrier may require detailed knowledge of individual cases.

### Intrinsic post-zygotic isolation

Post-zygotic isolation can result from genetic incompatibilities between genomes that are expressed when they are brought together in hybrids (Rice & Hostert 1993; Rundle & Whitlock 2001; Coyne & Orr 2004). These incompatibilities reduce the fitness of hybrids and, although their effects may be environment-dependent (e.g. greater consequences in a more harsh environment; see Rundle & Whitlock 2001), they do not depend on an ecological interaction between phenotype and environment. Intrinsic post-zygotic isolation has received much attention in the literature, although work has focused primarily on understanding the genetic basis of two extreme forms (hybrid sterility and inviability; Wu & Ting 2004) and on exploring theoretical models for its evolution (reviewed in Coyne & Orr 2004). Its role in ecological speciation has been generally overlooked (but see Lu & Bernatchez 1998), likely in part because it can be produced by any mechanism of speciation. It is possible, however, that genetic incompatibilities evolve more rapidly under divergent selection and that they are thus an important cause of ecological speciation. Consistent with this, in all three cases where a gene causing intrinsic post-zygotic isolation has been identified, there is evidence that it has evolved via positive selection (Hmr, Barbash *et al.* 2004; Nup96, Presgraves *et al.* 2003; OdsH, Ting *et al.* 1998; Wu & Ting 2004 for review). However, causes of selection (e.g. ecological or not) cannot be determined from these data alone. Sister group comparisons, similar to those used to test for a role of sexual selection in speciation (see Panhuis *et al.* 2001) may be useful in asking whether intrinsic incompatibilities evolve sooner or more frequently when divergent selection is stronger.

### Ecologically-dependent post-zygotic isolation

Post-zygotic isolation can also arise when hybrid fitness is reduced because of an ecological mismatch between hybrid phenotype and their environment (Rice & Hostert 1993; Rundle & Whitlock 2001; Coyne & Orr 2004). Basically, hybrids are not well adapted to either parental environment, and in effect, fall between niches. Ecologically-dependent post-mating isolation is analogous to immigrant inviability above except that divergent selection is acting against hybrids instead of parental individuals. As with immigrant inviability, ecologically-dependent post-zygotic isolation and divergent selection between environments can be considered two sides of the same coin (Coyne & Orr 2004). In

contrast to intrinsic post-zygotic isolation, ecologically-dependent (or extrinsic) post-zygotic isolation has received less attention. This is despite the fact that this form of isolation is a unique prediction of ecological speciation. To the extent that hybrid phenotypes are intermediate, ecologically-dependent post-zygotic isolation is a necessary consequence of divergent selection between environments.

There are at least three techniques for demonstrating ecologically-dependent post-zygotic isolation. In the first, the fitness of hybrids in the wild is compared with that in a benign environment (e.g. Hatfield & Schluter 1999). The benign environment is assumed to remove the ecological factors that reduce hybrid fitness, thus permitting an estimate of any intrinsic genetic isolation. Comparison of hybrid fitness in the wild to that in the benign environment yields an estimate of ecologically-dependent isolation. Caution is warranted, however, because non-ecological reductions in hybrid fitness may differ between environments, complicating this method (see Hatfield & Schluter 1999). In the second, backcrosses of F1 hybrids to both parental forms are used in reciprocal transplants between environments (e.g. 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). In the third technique, which has never been attempted to our knowledge, parental individuals are phenotypically modified to resemble hybrids. Given proper controls for this manipulation, the fitness of these individuals in the parental environments estimates ecologically-dependent isolation alone. Such modifications may be straightforward to apply in many plants (e.g. Hodges *et al.* 2002).

Few studies have applied these above techniques and the extent of ecologically-dependent post-zygotic isolation in nature is unknown. When conducting such studies, it is important to consider the possibility of intermediate environment (Schluter 2000). Average hybrid fitness may not be reduced if such an environment is accessible and hybrids perform well in it (e.g. Wang *et al.* 1997). Finally, although demonstrating ecologically-dependent isolation is an important first step, its ecological causes are also of interest. If hybrids are used, experiments designed to measure divergent selection between environments can provide important information about the ecological mechanisms of reduced hybrid fitness, such as the traits involved (e.g. Nagy 1997).

### Sexual selection against hybrids

Finally, post-zygotic isolation can also arise if hybrids, despite surviving to sexual maturity, are less likely to secure a mate. Sexual selection against hybrids, however, may or may not contain an ecological component (Schluter 2000).

For example, hybrid attractiveness could be reduced as a consequence of genetic incompatibilities that accumulated from non-ecological processes. Thus, although sexual selection against hybrids appears common (Schluter 2000; Coyne & Orr 2004), the key for ecological speciation lies in understanding its origin. An ecological component is clear if hybrid sexual displays are maladapted to their environment (e.g. intermediate displays are less visible). An ecological component is also implicated if sexual display traits are condition-dependent, as theory suggests they should often be (Rowe & Houle 1996), and hybrid condition is reduced as a result of ecological mechanisms (P. Edelaar *et al.*, unpubl. manuscript). Finally, ecology is also implicated if mate preferences diverge between parental species as a consequence of ecological mechanisms and this renders hybrids unattractive because of their intermediate phenotypes. The above possibilities have received little attention, although the latter situation appears to be involved in the reduced mating success of hybrids between species of *Heliconius* butterflies. Colour-patterns of these butterflies, which diverged as adaptations to mimic different model taxa, are also important traits in mate choice. Hybrid colour-patterns are intermediate and fall largely outside of the range of parental mate preferences (Naisbit *et al.* 2001). Pollinator-based discrimination against hybrid plants possessing intermediate floral traits may also be a common example of the latter scenario (e.g. Schemske & Bradshaw 1999; Emms & Arnold 2000). Additional tests of all possibilities are required.

### Importance for ecological speciation

As we have seen above, many forms of reproductive isolation exist and they vary in the potential role of ecological processes in their evolution. Although examples exist of all types in nature (Table 1), the extent and relative strength of these barriers is poorly understood. This is because there are only a handful of cases in which the relative contribution of multiple barriers has been addressed in a single system (Coyne & Orr 2004). Doing so may provide important insights into the roles of ecological and non-ecological processes in speciation. For example, Ramsey *et al.* (2003) conclude that, despite multiple and substantive forms of pre- and post-zygotic isolation between the two species of monkeyflower discussed earlier, ecological factors stemming for their adaptation to different environments played the central role. In whitefish ecotypes, both ecological and intrinsic genetic barriers exist, although it is not known how the latter evolved so the role of ecological selection remains unclear (Lu & Bernatchez 1998). Finally, in host-associated *Timema* walking-stick insects, natural selection against immigrants and sexual isolation contribute similarly to total pre-zygotic isolation

and both appear to have evolved by ecological mechanisms (Nosil *et al.* 2002, 2003; Nosil 2004).

Being specific predictions of the ecological model, many studies of ecological speciation consider those forms of reproductive isolation that are likely to have been produced by ecologically-based divergent natural selection. The relative contribution of divergent selection to the evolution of those forms commonly attributed to non-ecological processes has been largely overlooked. As we noted for intrinsic post-zygotic isolation, although genetic incompatibilities can evolve by drift and uniform selection, ecologically-based divergent selection may speed their accumulation. The contribution of divergent selection to the evolution of all forms of reproductive isolation requires investigation.

The barriers to gene flow important to speciation are those that evolve before reproductive isolation is yet complete. Thus when multiple barriers exist between taxa, the temporal order of their evolution is key and may shed light on the mechanism of speciation. The relative importance of current barriers, however, may not be indicative of their historical importance (Coyne & Orr 2004). Little is known about the relative rates of evolution of various forms of reproductive isolation. Data from phytophagous insects suggest that ecological forms can evolve prior to others that may involve non-ecological process (Funk *et al.* 2002 for review). Likewise, comparative studies indicate that sexual isolation can evolve before intrinsic post-zygotic isolation (Coyne & Orr 1997; Mendelson 2003). In Coyne & Orr's (1997) study of various *Drosophila* species, this result was entirely the product of sexual isolation evolving faster between sympatric than allopatric species pairs. This suggests that post-zygotic isolation may often be the engine that drives the evolution of pre-zygotic isolation via reinforcement, although ecological interactions could also be involved. Clearly much work is needed to produce a comprehensive understanding of the temporal order of the evolution of reproductive isolation. The forms that exist between partially isolated taxa in nature are thus of great interest.

### LINKING DIVERGENT SELECTION AND REPRODUCTIVE ISOLATION

The final component of ecological speciation is the genetic mechanism by which selection on ecological traits is transmitted to the genes causing reproductive isolation. There are two ways this can occur, distinguished by the relationship between the genes under divergent selection (i.e. those affecting ecological traits) and those causing reproductive isolation (Kirkpatrick & Ravigné 2002). In the first, these genes are one in the same. In this case, reproductive isolation evolves by direct selection because it is the pleiotropic effect of the genes under selection (Kirkpatrick & Barton 1997; termed 'single-variation'

models by Rice & Hostert 1993). In the second, genes under divergent selection are physically different from those causing reproductive isolation. In this case, reproductive isolation evolves by indirect selection arising from the non-random association (linkage disequilibrium) of the genes for reproductive isolation and those for ecological traits (Kirkpatrick & Barton 1997; termed 'double-variation' models by Rice & Hostert 1993). Note that the relationship of direct and indirect selection with pleiotropy and linkage disequilibrium differs when considering selection at the genetic (as here) or phenotypic (e.g. Lynch 1985) level.

The nature of these genetic relationships is important for two reasons. First, pleiotropy and linkage disequilibrium will affect the strength of selection transmitted to the genes affecting reproductive isolation and, depending on the nature of the relationship, may facilitate or hinder speciation. Second, the genes involved will determine the form of reproductive isolation that evolves. If, for example, pleiotropy is more common between certain ecological traits and particular forms of reproductive isolation, such traits should feature prominently in ecological speciation.

### Direct selection and pleiotropy

Speciation is facilitated when genes under divergent selection cause reproductive isolation pleiotropically. There are numerous ways this can occur. For example, habitat isolation will evolve as a direct consequence of selection on habitat preference genes if individuals mate in their preferred habitat. This is the route by which sympatric speciation is thought to be most likely (Johnson *et al.* 1996) and has been demonstrated in a laboratory experiment (Rice & Salt 1990). Sexual isolation can evolve because of changes in mate preferences that arise as a pleiotropic consequence of the adaptive divergence of mating or communication systems (Ryan & Rand 1993; Boughman 2002). Such changes in mate preferences may also cause sexual selection against hybrids as a direct consequence (Liou & Price 1994). In plants, pollinator isolation is a direct consequence of adaptation to different pollinators (e.g. Schemske & Bradshaw 1999) and temporal isolation, caused by differences in flowering time, may arise as the pleiotropic effect of adaptation to different environments (e.g., Macnair & Gardner 1998). Intrinsic post-zygotic isolation can arise pleiotropically if alleles favoured by selection within each population contribute to incompatibilities between them. Finally, ecologically-based reductions in parental (i.e. immigrant inviability) and hybrid (i.e. ecologically-dependent post-zygotic isolation) fitness are facilitated when genes favoured by selection in one environment directly reduce fitness in the other (Via & Hawthorne 2002).

### Indirect selection and linkage disequilibrium

Indirect selection is thought to be less effective than direct selection in the evolution of reproductive isolation (Kirkpatrick & Ryan 1991; Kirkpatrick & Barton 1997). This is because the genetic association between the genes under selection and those causing reproductive isolation (i.e. linkage disequilibrium) is not perfect, thus weakening selection on the latter (Kirkpatrick & Ravigné 2002). The amount of linkage disequilibrium that exists is affected by three factors. The first is the genetic basis of reproductive isolation. As pointed out by Felsenstein (1981), there are two distinct possibilities, termed one- and two-allele mechanisms. In a one-allele mechanism, reproductive isolation is caused by the same allele fixing in both populations (e.g. an allele causing individuals to prefer mates phenotypically similar to themselves). In a two-allele mechanism, different alleles fix in each population (e.g. a preference allele for large individuals in one population and small individuals in the other). This distinction is important when considering the effects of recombination. Recombination in a two-allele mechanism breaks down linkage disequilibrium, randomizing the association between genes under divergent selection and those causing reproductive isolation (Felsenstein 1981). In contrast, recombination creates no such problem for a one-allele mechanism and it is therefore a more powerful mechanism of speciation than a two-allele.

The second is physical linkage. The maintenance of linkage disequilibrium is greatly facilitated by the physical linkage of genes on a chromosome because the likelihood of a recombination event declines with decreasing genetic map distance (Lynch & Walsh 1998). Chromosomal inversions may play a similar role in suppressing recombination and, by protecting large regions of the genome, may foster speciation by maintaining barriers to gene flow between hybridizing species (Ortiz-Barrientos *et al.* 2002). Reduced recombination, however, may decrease the chance of favourable gene combinations being brought together, interfering with the initial build up of linkage disequilibrium (Kirkpatrick *et al.* 2002). Therefore, whether reduced recombination promotes or impedes ecological speciation will depend on the relative importance of building up vs. maintaining appropriate forms of linkage disequilibrium.

The third is the strength of selection. In a two-allele mechanism, linkage disequilibrium between genes affecting ecological traits and genes conferring reproductive isolation can be generated and maintained by strong selection, but only if selection acts directly on both loci to favour specific combinations of alleles (i.e. correlational selection; Diehl & Bush 1989). Although not relevant to ecological speciation in allopatry (because reproductive isolation is a neutral trait), such conditions may exist if speciation occurs by disruptive

selection in sympatry. Finally, there is one situation in which linkage disequilibrium can be high and indirect selection therefore strong. It exists when matings occur between divergent populations, as happens during reinforcement after secondary contact (Kirkpatrick & Ravigné 2002). Thus, although reinforcement relies on linkage disequilibrium between the genes that reduce fitness during heterospecific encounters and those that strengthen pre-zygotic isolation, it occurs under conditions that are most conducive for indirect selection (Kirkpatrick & Ravigné 2002).

### Examples from nature

There is little evidence examining the relationships between genes under divergent selection and those causing reproductive isolation. In practice, separating pleiotropy from indirect selection facilitated by close physical linkage will be a difficult task. Linkage disequilibrium caused by tight physical linkage, however, may represent a 'fundamental' relationship similar in effect to pleiotropy (Via & Hawthorne 2002). An important question is how common pleiotropy and tight physical linkage are, and how often they are of the form that would facilitate ecological speciation.

Data are sparse. Quantitative trait locus (QTL) mapping in pea aphids identified loci with opposite effects on fecundity on the two hosts, suggesting alleles with pleiotropic effects or tight physical linkage. Such a fundamental genetic trade-off in fecundity on the two hosts could contribute to two forms of reproductive isolation: ecologically-based reductions in parental (i.e. immigrant inviability) and hybrid (i.e. ecologically-dependent post-zygotic isolation) fitness during ecological speciation. A number of loci affecting performance and habitat preference also appeared to reside in similar regions of the genome, again suggesting pleiotropy or tight physical linkage (Hawthorne & Via 2001; Via & Hawthorne 2002). Such genetic correlations were also of the form that would facilitate ecological speciation. For the latter case, however, there is some doubt as to whether the experimental design actually measured two traits (Coyne & Orr 2004). Performance and host preference appear unlinked in other systems (Ortiz-Barrientos *et al.* 2002).

In monkeyflowers, hybrid sterility is either a pleiotropic effect of an allele for resistance to copper contaminated soils, or is caused by something tightly linked to it, again facilitating speciation (Macnair & Christie 1983). In two other species of monkeyflower, flower colour, an important trait contributing to pollinator isolation, is controlled in large part by a single locus (*YUP*). In the predominately bumblebee pollinated *Mimulus lewisii*, substitution of the *YUP* allele from the hummingbird pollinated *M. cardinalis* increased its attractiveness to hummingbirds and pleiotropically decreased its attraction

to bumblebees, facilitating the evolution of pollinator isolation. In contrast, introgression of the *M. lewisi* YUP allele into *M. cardinalis* increased its attractiveness to bumblebees, but had little effect on its attractiveness to hummingbirds. Similarly, the genotype at a QTL locus for nectar volume significantly affected hummingbird but not bumblebee visitation (Schemske & Bradshaw 1999; Bradshaw & Schemske 2003). Accumulating evidence for reinforcement also implies that indirect selection is important. Whether reinforcement commonly involves one- or two-allele mechanism is not known (but see Servedio & Noor 2003).

Finally, in the columbines *Aquilegia formosa* and *A. pubescens*, pleiotropy or close physical linkage appears to integrate a number of floral traits that contribute to pollinator isolation (Hodges *et al.* 2002). Although pleiotropy and physical linkage of genes affecting multiple selected traits is not required for ecological speciation, it may affect its likelihood. This is because these relationships, depending on their nature, may either enhance or inhibit the response to selection of the traits involved (Barton 1995; Orr 2000; Otto 2004).

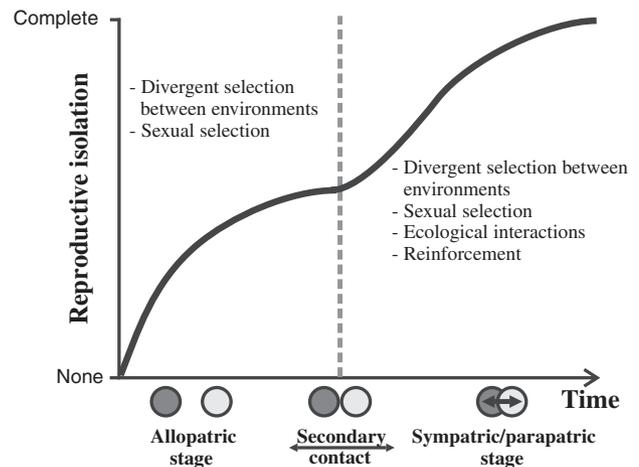
The increasing sophistication of mapping studies offers promise in exploring the genetic architecture of ecological traits and reproductive isolation. Other approaches may also be informative. Laboratory experiments, for example, could play an important role in furthering our understanding of direct and indirect selection and one- and two-allele mechanisms in ecological speciation. For example, the only laboratory test of sympatric speciation involving direct selection was successful, whereas only three of 24 involving indirect selection succeeded (Kirkpatrick & Ravigné 2002). As noted earlier, when experiments fail the reasons why may be particularly informative and more attention is needed exploring the contribution of genetic causes of such failures.

## GEOGRAPHY OF ECOLOGICAL SPECIATION

Although ecological speciation can occur under any geographic context, geography is still important because it affects the ecological sources of divergent selection that can act, as well as the possibility of gene flow between the populations. We address both issues below.

### The two stages of ecological speciation

A number of studies suggest that the traditional models of allopatric and sympatric speciation represent opposite extremes of the geography of speciation and may be overly simplistic (Grant & Grant 1997; Schluter 2001; Rundle & Schluter 2004). Rather, speciation in nature may often occur between these extremes and involve an allopatric and a



**Figure 1** A general scenario for speciation under any geographic context. Reproductive isolation between two populations is absent at the beginning of the speciation process (at the left) and evolves to completion (at the right). Populations are initially allopatric, but secondary contact can occur at any time (dashed vertical line), commencing the second stage of the speciation process. The ecological causes of divergent selection by which reproductive isolation may evolve are listed within the panel for each stage. Depicted is an intermediate scenario in which partial reproductive isolation evolves in allopatry, but speciation is completed in sympatry.

sympatric (or parapatric) stage (Fig. 1). The idea is that speciation begins when populations are allopatric, with reproductive isolation accumulating as a by-product of divergent selection between their environments. The second stage is initiated upon secondary contact. Ecological interactions between the populations are added as a potential source of divergent selection and, if reproductive isolation is not yet complete, heterospecific matings may occur, adding the potential for gene flow and reinforcement as well. The amount of reproductive isolation that evolves during each stage indicates the geographic context of speciation: if reproductive isolation is complete prior to secondary contact, speciation was allopatric, whereas if little reproductive isolation existed at the time of secondary contact, speciation was essentially sympatric. The latter scenario includes in cases in which reproductive isolation evolves within a single, continuous population; it also includes the situation of parapatric speciation in which gene flow is reduced through isolation-by-distance, but is not eliminated. Key questions thus concern how often one or the other stages are absent, and when both are present, the relative importance of each to the evolution of reproductive isolation.

This two stage scenario arose, in part, from recent work on present-day sympatric limnetic and benthic threespine sticklebacks. Their speciation appears to have involved an

initial allopatric and subsequent sympatric stage, with some reproductive isolation evolving during each (Albert & Schluter 2004; Rundle & Schluter 2004). Recent sequence data from the apple and hawthorn host-races of the apple maggot fly *Rhagoletis pomonella*, a classic case put forward in support of sympatric speciation, also suggest a more complex geographic scenario (Feder *et al.* 2003). Inversion polymorphisms, containing genetic variation affecting ecologically important diapause traits that differ between the host-races, trace their origins to allopatric populations in Mexico. Gene flow from the Mexican populations likely introduced this variation into the North American populations. It is unlikely that this introgression was responsible for any immediate reproductive isolation between populations, although it may have provided the genetic variation necessary to facilitate the subsequent host shift (Feder *et al.* 2003). Key traits that generate some pre-zygotic isolation between the host races, such as olfactory preferences for their respective fruits, appear to have evolved recently and in sympatry (Linn *et al.* 2003). The relative roles of divergence in allopatry and sympatry are not yet fully understood in either of these examples.

Inferring the geography of past speciation events is difficult and recent attention has focused on phylogenetic comparative methods for its reconstruction. However, the ability of these methods to test alternative hypotheses concerning the geography of speciation appears limited. This is because the key assumption of these models, that historical distributions at the time of speciation can be inferred from present-day species ranges, is generally not met (Losos & Glor 2003). Alternate population genetic and coalescent approaches hold some promise, but require simplifying assumptions of their own and their utility remains to be determined (Losos & Glor 2003). The study of ongoing speciation events, for which the geographic context can be more directly observed, is thus an important task.

### Effects of secondary contact on speciation

Secondary contact occurs when individuals from separate populations encounter one another through migration or dispersal, or when range shifts or expansions bring formerly allopatric populations into sympatry. Gene flow between populations is possible once secondary contact is established and its occurrence is generally thought to erode their differences, hampering speciation (Servedio & Kirkpatrick 1997; Servedio & Noor 2003). However, secondary contact also permits additional sources of divergent selection, such as ecological interactions between the populations, and it allows for the possibility of reinforcement (Fig. 1). Thus secondary contact can exert dual and opposing effects on the likelihood of speciation.

Consider the example of reinforcement; increased heterospecific encounter rates increases the opportunity for both reinforcement and gene flow. In theory, the magnitude of pre-zygotic isolation that evolves is expected to reflect a balance between these opposing forces (Kirkpatrick 2000; Servedio & Noor 2003 for review). A study of walking-stick insects demonstrates that pre-zygotic isolation is strongest between similar sized populations, supporting this prediction (Nosil *et al.* 2003). Furthermore, sexual isolation was found to be strongest when both divergent selection between environments and reinforcement operated. Further empirical and theoretical studies are needed that explore the interaction of gene flow with reinforcing selection and various forms of ecologically-based divergent selection (e.g. Kirkpatrick 2001).

Finally, separate from the above considerations, gene flow between species involves hybridization that can, under certain circumstances, foster speciation. For example, by recombining divergent parental genomes and generating new gene combinations, hybrid species of *Helianthus* sunflowers have undergone large and rapid adaptive transitions (Rieseberg *et al.* 2003). Although ecological divergence appears critical to the survival of the hybrid species, this does not appear to be a mechanism of ecological speciation because initial reproductive isolation appears to be the product, at least in part, of non-ecological fertility selection (Rieseberg 2000).

### GENETIC BASIS OF ECOLOGICAL SPECIATION

Earlier we considered how pleiotropy and linkage disequilibrium transmit divergent selection into reproductive isolation. Here we are concerned with other aspects of the genetic architecture of ecological speciation including the number of genes involved, their location in the genome, the distribution of their effect sizes, and the nature of the interactions within (dominance) and among (epistasis) them. Such topics have received much attention in the study of speciation and species differences (reviewed respectively in Coyne & Orr 2004 and Orr 2001). However, as we discuss below, their study in ecological speciation is hampered in two ways. First, empirical data specific to ecological speciation are limited. Second, the implications of such data for our understanding of how ecological speciation occurs are not clear.

What is known specifically about the genetic basis of ecological speciation? Empirical studies have shown that traits evolving via ecological selection, and that confer reproductive isolation, can be affected by few or many genes, of small or large effect, that vary in their dominance and epistatic interactions (e.g. Hatfield 1997; Schemske & Bradshaw 1999; Peichel *et al.* 2001; Bradshaw & Schemske 2003; Naisbit *et al.* 2003). Ecological speciation can proceed via divergence in just

a few key genomic regions (e.g. Campbell & Bernatchez 2004; Emelianov *et al.* 2004) and can involve a small number of traits (e.g. Bradshaw & Schemske 2003; McKinnon *et al.* 2004). Little is known regarding the contribution of mutation vs. standing variation. The genetic basis of parallel evolution can determine whether independently evolved ecological traits that confer reproductive isolation involve the same or different genetic architectures, but has also received limited attention (Schluter *et al.* 2004). Different genetic architectures imply few genetic constraints on ecological speciation (e.g. Naisbit *et al.* 2003), but also suggest the possibility of non-ecological speciation of parallel evolving populations due to the fixation of incompatible alleles. Finally, ecologically-dependent reductions in hybrid fitness require phenotypes that are intermediate between parental forms. Dominance and epistasis, however, can cause departures from this. Although not specific to ecological speciation, data on the genetics of ordinary phenotypic differences between species tend to show roughly additive effects (Orr 2001).

What are the consequences for ecological speciation of such data? The hallmark of ecological speciation is adaptation to different environments, so it is tempting to use what is known about the population genetics of adaptation as a guide. For example, effect size and dominance may affect ecological speciation because they influence the probability that new mutations are fixed and thus the rate of adaptation (Turner 1981; Orr 2000). However, we lack quantitative genetic models that specifically examine the effects on ecological speciation of these aspects of genetic architecture. Such models are required because ecological speciation is concerned with the evolution of reproductive isolation, a complication absent during adaptation. Reproductive isolation is the property of pairs of populations and the genetic basis of certain forms may differ profoundly from that of ordinary traits (Orr 2001; Coyne & Orr 2004). Until such models are considered, the genetic architecture of ecological speciation will remain a descriptive endeavour.

### Genes causing ecologically-based reproductive isolation

The identification of individual genes conferring reproductive isolation warrants special attention because it can potentially provide unique insight into ecological speciation. For example, once such genes are identified, tests for selection at the molecular level are possible. A number of such tests have been conducted and selection has been strongly implicated in the evolution of reproductive isolation (Swanson & Vacquier 2002; Presgraves *et al.* 2003; Barbash *et al.* 2004; Coyne & Orr 2004; Wu & Ting 2004). Such tests, however, tell us little about the form of selection responsible (e.g. ecological vs. non-ecological; Vacquier *et al.* 1997). For example, although positive selection on a gene in two

lineages is consistent with divergent selection, it could also be produced by uniform selection with different advantageous mutations fixing in each. Insight into the form of selection may still be possible, however, by determining the normal function of the gene in the parental populations and how it causes reproductive isolation (e.g. Sun *et al.* 2004).

### CONCLUSIONS

The study of ecological speciation has come a long way in recent years. Mechanisms have been clarified, specific predictions have been recognized, and much data has been collected. Most importantly, ecologically-based divergent selection has been implicated in the evolution of reproductive isolation in a number of cases. Nevertheless, a detailed understanding of the process still eludes us, even in the best studied model systems. The reason is that the ecological speciation is complex and can encompass many different scenarios. Divergent selection can have various ecological causes, numerous forms of reproductive isolation can result, and there are different genetic mechanisms than can link them. Reinforcement may also strengthen reproductive isolation in sympatry and may itself be ecological or not. And all of this can occur under different geographic contexts. It will be no small task to evaluate all of these possibilities to develop a general understanding of how speciation proceeds from beginning to end.

Like many rapidly growing fields, much of the evidence is indirect, relying on observational and comparative studies. Even direct tests of specific predictions of the best understood components, such as the role of environmental differences, are in some respects qualitative. For example, sexual isolation has been shown to be stronger between populations inhabiting different, as opposed to similar, environments (e.g. Funk 1998; Rundle *et al.* 2000; Nosil *et al.* 2002, 2003). However, quantitative links between the strength of divergent selection and the magnitude of reproductive isolation are lacking. In addition, in some taxa a detailed understanding of ecological speciation should permit at least the early stages of the process to be recreated in replicate populations under controlled laboratory conditions, providing some of the strongest evidence possible.

For many topics, it is the classic ecological processes that have received the least attention. For example, we know only a little about the role of competitors and predators in the evolution of reproductive isolation, and even less concerning other possibilities such as parasites, mutualists, or facilitators. Similarly, there are few tests for ecologically-dependent post-zygotic isolation in nature, although a number of techniques exist to do so. Finally, the influence of other factors on ecological speciation has yet to be considered. For example, population structure is common in nature and is known to affect many evolutionary processes.

However, its effect on ecological speciation has received little attention. In addition, although the colonization of novel habitats may often involve reductions in population size, the interaction between drift and divergent selection during ecological speciation has generally been overlooked (but see Rundle 2003). The influence of shared ancestry is also not known. Closely related populations may share biases in their standing genetic variation and in their production of new variation (Schluter *et al.* 2004). How such biases affect adaptive divergence and the evolution of reproductive isolation has not been considered. Understanding the influence of these above factors will require ecological studies that integrate molecular, population and quantitative genetics, and that consider the phylogenetic history of the system (e.g. Bernatchez *et al.* 1999).

Nevertheless, we close by noting that our most general conclusion is promising. Much progress has been made in recent years and where gaps in our knowledge exist, it is often clear what needs to be done and the tools are generally available.

## ACKNOWLEDGEMENTS

We thank A. Betancourt, M. Blows, J. Boughman, F. Breden, S. Chenoweth, B. Crespi, E. Elle, S. Gavrillets, M. Higgie, M. Kawata, A.O. Mooers, D. Ortíz-Barrientos, D. Presgraves, J. McKinnon, the FAB-laboratory at Simon Fraser University, and two anonymous referees for comments on earlier versions. Our work is funded by the Australian Research Council (H.D.R.) and the Natural Sciences and Engineering Research Council of Canada (P.N.).

## REFERENCES

- Abrams, P.A. (2000). Character shifts of prey species that share predators. *Am. Nat.*, 156, S45–S61.
- Albert, A. & Schluter, D. (2004). Reproductive character displacement of male stickleback mate preference: reinforcement or direct selection? *Evolution*, 58, 1099–1107.
- Barbash, D.A., Awadalla, P. & Tarone, A.M. (2004). Functional divergence caused by ancient positive selection of a *Drosophila* hybrid incompatibility locus. *PLoS Biol.*, 2, 839–848.
- Barton, N.H. (1995). Linkage and the limits to natural selection. *Genetics*, 140, 821–841.
- Bernatchez, L., Chouinard, A. & Lu, G. (1999). Integrating molecular genetics and ecology in studies of adaptive radiation: whitefish, *Coregonus* sp., as a case study. *Biol. J. Linn. Soc.*, 68, 173–194.
- Bolnick, D. (2004). Intraspecific competition generates disruptive selection in natural populations of three-spined stickleback. *Evolution*, 58, 608–618.
- Boughman, J.W. (2001). Divergent sexual selection enhances reproductive isolation in sticklebacks. *Nature*, 411, 944–948.
- Boughman, J.W. (2002). How sensory drive can promote speciation. *Trends Ecol. Evol.*, 17, 571–577.
- Bradshaw, H.D. & Schemske, D.W. (2003). Allele substitution at a flower colour locus produces a pollinator shift in monkey-flowers. *Nature*, 426, 176–178.
- Campbell, D. & Bernatchez, L. (2004). Genomic scan using AFLP markers as a means to assess the role of directional selection in the divergence of sympatric whitefish ecotypes. *Mol. Biol. Evol.*, 21, 945–946.
- Chapman, T., Arnqvist, G., Bangham, J. & Rowe, L. (2003). Sexual conflict. *Trends Ecol. Evol.*, 18, 41–47.
- Coyne, J.A. & Orr, H.A. (1997). “Patterns of speciation in *Drosophila*” revisited. *Evolution*, 51, 295–303.
- Coyne, J.A. & Orr, H.A. (2004). *Speciation*. Sinauer Associates, Inc., Sunderland, MA.
- Craig, T.P., Itami, J.K., Abrahamson, W.G. & Horner, J.D. (1993). Behavioral evidence for host-race formation in *Eurosta solidaginis*. *Evolution*, 47, 1696–1710.
- Craig, T.P., Horner, J.D. & Itami, J.K. (1997). Hybridization studies on the host races of *Eurosta solidaginis*: implications for sympatric speciation. *Evolution*, 51, 1552–1560.
- Cruz, R., Carballo, M., Conde-Padin, P. & Rolan-Alvarez, E. (2004). Testing alternative models for sexual isolation in natural populations of *Littorina saxatilis*: indirect support for by-product ecological speciation? *J. Evol. Biol.*, 17, 288–293.
- Day, T. & Young, K.A. (2004). Competitive and facilitative evolutionary diversification. *BioScience*, 54, 1101–1109.
- Diehl, S.R. & Bush, G.L. (1989). The role of habitat preference in adaptation and speciation. In: *Speciation and Its Consequences* (eds. Otte, D. & Endler, J.). Sinauer Assoc., Sunderland, MA. pp. 345–365.
- Dobzhansky, T. (1951) *Genetics and the Origin of Species*, 3rd edn. Columbia University Press, New York.
- Doebeli, M. & Dieckmann, U. (2000). Evolutionary branching and sympatric speciation caused by different types of ecological interactions. *Am. Nat.*, 156, S77–S101.
- Dres, M. & Mallet, J. (2002). Host races in plant-feeding insects and their importance in sympatric speciation. *Phil. Trans. R. Soc. Lond. B*, 357, 471–492.
- Emelianov, I., Marec, F. & Mallet, J. (2004). Genomic evidence for divergence with gene flow in host races of the larch budmoth. *Proc. R. Soc. Lond. B*, 271, 97–105.
- Emms, S.K. & Arnold, M.L. (2000). Site-to-site differences in pollinator visitation patterns in a Louisiana Iris hybrid zone. *Oikos*, 91, 568–578.
- Feder, J.L., Opp, S.B., Wlazlo, B., Reynolds, K., Go, W. & Spsisak, S. (1994). Host fidelity is an effective premating barrier between sympatric races of the apple maggot fly. *Proc. Natl. Acad. Sci. U S A*, 91, 7990–7994.
- Feder, J.L., Berlocher, S.H., Roethele, J.B., Dambroski, H., Smith, J.J., Perry, W.L. *et al.* (2003). Allopatric genetic origins for sympatric host-plant shifts and race formation in *Rhagoletis*. *Proc. Natl. Acad. Sci. U S A*, 100, 10314–10319.
- Felsenstein, J. (1981). Skepticism towards Santa Rosalia, or why are there so few kinds of animals? *Evolution*, 35, 124–138.
- Filchak, K.E., Roethele, J.B. & Feder, J.L. 2000. Natural selection and sympatric divergence in the apple maggot *Rhagoletis pomonella*. *Nature*, 407, 739–742.
- Friesen, M.L., Saxer, G., Travisano, M. & Doebeli, M. (2004). Experimental evidence for sympatric ecological diversification

- due to frequency-dependent competition in *Escherichia coli*. *Evolution*, 58, 245–260.
- Funk, D.J. (1998). Isolating a role for natural selection in speciation: host adaptation and sexual isolation in *Neochlamisus bebbianae* leaf beetles. *Evolution*, 52, 1744–1759.
- Funk, D.J., Filchak, K.E. & Feder, J.L. (2002). Herbivorous insects: model systems for the comparative study of speciation ecology. *Genetica*, 116, 251–267.
- Gavrilets, S. (2004). *Fitness Landscapes and the Origin of Species*. Princeton University Press, Princeton, NJ.
- Grant, P.R. & Grant, B.R. (1997). Genetics and the origin of bird species. *Proc. Natl. Acad. Sci. U S A*, 94, 7768–7775.
- Hatfield, T. (1997). Genetic divergence in adaptive characters between sympatric species of stickleback. *Am. Nat.*, 149, 1009–1029.
- Hatfield, T. & Schluter, D. (1999). Ecological speciation in sticklebacks: environment dependent hybrid fitness. *Evolution*, 53, 866–873.
- Hawthorne, D.J. & Via, S. (2001). Genetic linkage of ecological specialization and reproductive isolation in pea aphids. *Nature*, 412, 904–907.
- Higgie, M., Chenoweth, S. & Blows, M.W. (2000). Natural selection and the reinforcement of mate recognition. *Science*, 290, 519–520.
- Hodges, S.A., Whittall, J.B., Fulton, M. & Yang, J.Y. (2002). Genetics of floral traits influencing reproductive isolation between *Aquilegia formosa* and *Aquilegia pubescens*. *Am. Nat.*, 159, S51–S60.
- Howard, D.J., Gregory, P.G., Chu, J.M., Cain, M.L. (1998). Conspecific sperm precedence is an effective barrier to hybridization between closely related species. *Evolution*, 52, 511–516.
- Jiggins, C.D., Naisbit, R.E., Coe, R.L. & Mallet, J. (2001). Reproductive isolation caused by colour pattern mimicry. *Nature*, 411, 302–305.
- Johnson, P.A., Hoppensteadt, F.C., Smith, J.J. & Bush, G.L. (1996). Conditions for sympatric speciation: a diploid model incorporating habitat fidelity and non-habitat assortative mating. *Evol. Ecol.*, 10, 187–205.
- Katakura, H., Shioi, M. & Kira, Y. (1989). Reproductive isolation by host specificity in a pair of phytophagous ladybird beetles. *Evolution*, 43, 1045–1053.
- Kirkpatrick, M. (2000). Reinforcement and divergence under assortative mating. *Proc. R. Soc. Lond. B*, 267, 1649–1655.
- Kirkpatrick, M. (2001). Reinforcement during ecological speciation. *Proc. R. Soc. Lond. B*, 268, 1259–1263.
- Kirkpatrick, M. & Barton, N.H. (1997). The strength of indirect selection on female mating preferences. *Proc. Natl. Acad. Sci. U S A*, 94, 1282–1286.
- Kirkpatrick, M. & Ravigné, V. (2002). Speciation by natural and sexual selection: models and experiments. *Am. Nat.*, 159, S22–S35.
- Kirkpatrick, M. & Ryan, M.J. (1991). The evolution of mating preferences and the paradox of the lek. *Nature*, 350, 33–38.
- Kirkpatrick, M., Johnson, T. & Barton, N. (2002). General models of multilocus evolution. *Genetics*, 161, 1727–1750.
- Kruuk, L.E.B. & Gilchrist, J.S. (1997). Mechanisms maintaining species differentiation: predator mediated selection in a *Bombina* hybrid zone. *Proc. R. Soc. Lond. B*, 264, 105–110.
- Lamont, B.B., He, T., Enright, N.J., Krauss, S.L. & Miller, B.P. (2003). Anthropogenic disturbance promotes hybridization between *Banksia* species by altering their biology. *J. Evol. Biol.*, 16, 551–557.
- Lande, R. (1981). Models of speciation by sexual selection on polygenic traits. *Proc. Natl. Acad. Sci. U S A*, 78, 3721–3725.
- Lande, R. (1982). Rapid origin of sexual isolation and character divergence in a cline. *Evolution*, 36, 213–223.
- Leal, M. & Fleishman, L.J. (2004). Differences in visual signal design and detectability between allopatric populations of *Anolis* lizards. *Am. Nat.*, 163, 26–39.
- Linn, Jr C., Feder, J.L., Nojima, S., Dambroski, H.R., Berlocher, S.H. & Roelofs, W. (2003). Fruit odor discrimination and sympatric host race formation in *Rhagoletis*. *Proc. Natl. Acad. Sci. U S A*, 100, 11490–11493.
- Liou, L. & Price, T. (1994). Speciation by reinforcement of pre-mating isolation. *Evolution*, 48, 1451–1459.
- Losos, J.B. & Glor, R.E. (2003). Phylogenetic comparative methods and the geography of speciation. *Trends Ecol. Evol.*, 18, 220–227.
- Lu, G. & Bernatchez, L. (1998). Experimental evidence for reduced hybrid viability between dwarf and normal ecotypes of lake whitefish (*Coregonus clupeaformis* Mitchell). *Proc. R. Soc. Lond. B*, 265, 1025–1030.
- Lynch, M. (1985). Spontaneous mutations for life-history characters in an obligate parthenogen. *Evolution*, 39, 804–818.
- Lynch, M. & Walsh, B. (1998). *Genetics and Analysis of Quantitative Traits*. Sinauer Assoc., Sunderland, MA.
- Macnair, M.R. & Christie, P. (1983). Reproductive isolation as a pleiotropic effect of copper tolerance in *Mimulus guttatus*? *Heredity*, 50, 295–302.
- Macnair, M.R. & Gardner, M. (1998). The evolution of edaphic endemics. In: *Endless Forms: Species and Speciation* (eds. Howard, D.J. & Berlocher, S.H.). Oxford University Press, Oxford, pp. 157–171.
- Mallet, J. (1989). The genetics of warning colour in Peruvian hybrid zones of *Heliconius erato* and *H. melpomene*. *Proc. R. Soc. Lond. B*, 236, 163–185.
- Mallet, J. & Barton, N.H. (1989). Strong natural selection in a warning-color hybrid zone. *Evolution*, 43, 421–431.
- Mayr, E. (1942). *Systematics and the Origin of Species*. Columbia University Press, New York.
- Mayr, R. (1947). Ecological factors in speciation. *Evolution*, 1, 263–288.
- McKinnon, J.S., Mori, S., Blackman, B.K., David L., Kingsley, D.M., Jamieson, L. *et al.* (2004). Evidence for ecology's role in speciation. *Nature*, 429, 294–298.
- Mendelson, T.C. (2003). Sexual isolation evolves faster than hybrid inviability in a diverse and sexually dimorphic genus of fish (Percidae: *Etheostoma*). *Evolution*, 57, 317–327.
- Nagel, L. & Schluter, D. (1998). Body size, natural selection, and speciation in sticklebacks. *Evolution*, 52, 209–218.
- Nagy, E.S. (1997). Selection for native characters in hybrids between two locally adapted plant subspecies. *Evolution*, 51, 1469–1480.
- Naisbit, R.E., Jiggins, C.D. & Mallet, J. (2001). Disruptive sexual selection against hybrids contributes to speciation between *Helioconius cydno* and *Helioconius melpomene*. *Proc. R. Soc. Lond. B*, 268, 1849–1854.
- Naisbit, R.E., Jiggins, C.D. & Mallet, J. (2003). Mimicry: developmental genes that contribute to speciation. *Evol. Dev.*, 5, 269–280.

- Nosil, P. (2004). Reproductive isolation caused by visual predation against migrants between divergent environments. *Proc. R. Soc. Lond. B*, 271, 1521–1528.
- Nosil, P., Crespi, B.J. & Sandoval, C. (2002). Host-plant adaptation drives the parallel evolution of reproductive isolation. *Nature*, 417, 441–443.
- Nosil, P., Crespi, B.J. & Sandoval, C. (2003). Reproductive isolation driven by the combined effects of ecological adaptation and reinforcement. *Proc. R. Soc. Lond. B*, 270, 1911–1918.
- Nosil, P., Vines, T.H. & Funk, D.J. (2005). Perspective: reproductive isolation driven by natural selection against migrants between divergent environments. *Evolution*, 59, in press.
- Orr, H.A. (2000). Adaptation and the cost of complexity. *Evolution*, 54, 13–20.
- Orr, H.A. (2001). The genetics of species differences. *Trends Ecol. Evol.*, 16, 343–350.
- Ortiz-Barrientos, D., Reiland, J., Hey, J. & Noor, M.A.F. (2002). Recombination and the divergence of hybridizing species. *Genetica*, 116, 167–178.
- Otto, S.P. (2004). Two steps forward, one step back: the pleiotropic effects of favoured alleles. *Proc. R. Soc. Lond. B*, 271, 705–714.
- Palumbi, S.R. (1998). Species formation and the evolution of gamete recognition loci. In: *Endless Forms: Species and Speciation* (eds. Howard, D.J. & Berlocher, S.H.). Oxford University Press, New York. pp. 271–278.
- Panhuis, T.M., Butlin, R., Zuk, M. & Tregenza, T. (2001). Sexual selection and speciation. *Trends Ecol. Evol.*, 16, 364–371.
- Pappers, S.M., Van der Velde G., Ouborg, N.J. & Van Groenendael, J.M. (2002). Genetically based polymorphisms in morphology and life history associated with putative host races of the water lily leaf beetle, *Galerucella nymphalaeae*. *Evolution*, 56, 1610–1621.
- Peichel, C.L., Nereng, K.S., Ohgi, K.A., Cole, B.L.E., Colosimo, P.F., Buerkle, C.A. *et al.* (2001). The genetic architecture of divergence between threespine stickleback species. *Nature*, 414, 901–905.
- Presgraves, D.C., Balagopalan, L., Abmayr, S.M. & Orr, H.A. (2003). Adaptive evolution drives divergence of a hybrid inviability gene between two species of *Drosophila*. *Nature*, 423, 715–719.
- Price, C.S.C., Kim, C.H., Gronlund, C.J. & Coyne, J.A. (2001). Cryptic reproductive isolation in the *Drosophila simulans* species complex. *Evolution*, 55, 81–92.
- Ramsey, J., Bradshaw, H.D. Jr & Schemske, D.W. (2003). Components of reproductive isolation between the monkeyflowers *Mimulus lewisii* and *M. cardinalis* (Phrymaceae). *Evolution*, 57, 1520–1534.
- Rice, W.R. & Hostert, E.E. (1993). Laboratory experiments on speciation: what have we learned in 40 years. *Evolution*, 47, 1637–1653.
- Rice, W.R. & Salt, G.W. (1990). The evolution of reproductive isolation as a correlated character under sympatric conditions: experimental evidence. *Evolution*, 44, 1140–1152.
- Rieseberg, L.H. (2000). Crossing relationships among ancient and experimental sunflower hybrid lineages. *Evolution*, 54, 859–865.
- Rieseberg, L.H., Desrochers, A.M. & Youn, S.J. (1995). Interspecific pollen competition as a reproductive barrier between sympatric species of *Helianthus* (Asteraceae). *Am. J. Bot.*, 82, 515–519.
- Rieseberg, L.H., Raymond, O., Rosenthal, D.M., Lai, Z., Livingstone, K., Nakazato, T. *et al.* (2003). Major ecological transitions in wild sunflowers facilitated by hybridization. *Science*, 301, 1211–1216.
- Rolan-Alvarez, E., Johannesson, K. & Erlandsson, J. (1997). The maintenance of a cline in the marine snail *Littorina saxatilis*: the role of home site advantage and hybrid fitness. *Evolution*, 51, 1838–1847.
- Rowe, L. & Houle, D. (1996). The lex paradox and the capture of genetic variance by condition dependent traits. *Proc. R. Soc. Lond. B*, 263, 1415–1421.
- Rundle, H.D. (2002). A test of ecologically dependent post-mating isolation between sympatric sticklebacks. *Evolution*, 56, 322–329.
- Rundle, H.D. (2003). Divergent environments and population bottlenecks fail to generate pre-mating isolation in *Drosophila pseudoobscura*. *Evolution*, 57, 2557–2565.
- Rundle, H.D. & Schluter, D. (1998). Reinforcement of stickleback mating preferences: sympatry breeds contempt. *Evolution*, 52, 200–208.
- Rundle, H.D. & Schluter, D. (2004). Natural selection and ecological speciation in sticklebacks. In: *Adaptive Speciation* (eds. Dieckmann, U., Doebeli, M., Metz, J.A.J. & Tautz, D.). Cambridge University Press, Cambridge, pp. 192–209.
- Rundle, H.D. & Whitlock, M. (2001). A genetic interpretation of ecologically dependent isolation. *Evolution*, 55, 198–201.
- Rundle, H.D., Nagel, L., Boughman, J.W. & Schluter, D. (2000). Natural selection and parallel speciation in sympatric sticklebacks. *Science*, 287, 306–308.
- Rundle, H.D., Vamosi, S.M. & Schluter, D. (2003). Experimental test of predation's effect on divergent selection during character displacement in sticklebacks. *Proc. Natl. Acad. Sci. U S A* 100, 14943–14948.
- Ryan, M.J. & Rand, A.S. (1993). Species recognition and sexual selection as a unitary problem in animal communication. *Evolution* 47, 647–657
- Schemske, D.W. & Bradshaw, H.D. (1999). Pollinator preference and the evolution of floral traits in monkeyflowers (*Mimulus*). *Proc. Natl. Acad. Sci. U S A*, 96, 11910–11915.
- Schluter, D. (2000). *The Ecology of Adaptive Radiation*. Oxford University Press, Oxford.
- Schluter, D. (2001). Ecology and the origin of species. *Trends Ecol. Evol.*, 16, 372–380.
- Schluter, D. & Nagel, L.M. (1995). Parallel speciation by natural selection. *Am. Nat.*, 146, 292–301.
- Schluter, D., Clifford, E.A., Nemethy, M. & McKinnon, J.S. (2004). Parallel evolution and inheritance of quantitative traits. *Am. Nat.*, 163, 809–822.
- Servedio, M.R. & Kirkpatrick, M. (1997). The effects of gene flow on reinforcement. *Evolution*, 51, 1764–1772.
- Servedio, M.R. & Noor, M.A.F. (2003). The role of reinforcement in speciation: theory and data. *Annu. Rev. Ecol. Evol. Syst.*, 34, 339–364.
- Sun, S., Ting, C-T. & Wu, C.I. (2004). The normal function of a speciation gene, *Odysseus*, and its hybrid sterility effect. *Science*, 305, 81–83.
- Swanson, W.J. & Vacquier, V.D. (2002). The rapid evolution of reproductive proteins. *Nat. Rev. Genet.*, 3, 137–144.
- Taper, M. & Case, T.J. (1992). Coevolution among competitors. *Oxf. Surv. Evol. Biol.*, 8, 63–109.

- Tavormina, S.J. (1982). Sympatric genetic divergence in the leaf-mining insect *Liriomyza brassicae* (Diptera: Agromyzidae). *Evolution*, 36, 523–534.
- Ting, C.T., Tsaur, S.C., Wu, M.L. & Wu, C.I. (1998). A rapidly evolving homeobox at the site of a hybrid sterility gene. *Science*, 282, 1501–1504.
- Turelli, M., Barton, N.H. & Coyne, J.A. (2001). Theory and speciation. *Trends Ecol. Evol.*, 16, 330–343.
- Turner, J.R. (1981). Adaptation and evolution in *Heliconius*: a defense of NeoDarwinism. *Annu. Rev. Ecol. Syst.*, 12, 99–121.
- Vacquier, V.D., Swanson, W.J. & Lee, Y.H. (1997). Positive Darwinian selection on two homologous fertilization proteins: what is the selective pressure driving their divergence? *J. Mol. Evol.*, 44, S15–S22.
- Vamosi, S.M. & Schluter, D. (1999). Sexual selection against hybrids between sympatric stickleback species: evidence from a field experiment. *Evolution*, 53, 874–879.
- Vamosi, S.M. & Schluter, D. (2002). Impacts of trout predation on fitness of sympatric sticklebacks and their hybrids. *Proc. R. Soc. Lond. B*, 269, 923–930.
- Via, S. (1999). Reproductive isolation between sympatric races of pea aphids. I. Gene flow restriction and habitat choice. *Evolution*, 53, 1446–1457.
- Via, S. (2001). Sympatric speciation in animals: the ugly duckling grows up. *Trends Ecol. Evol.*, 16, 381–390.
- Via, S. & Hawthorne, D.J. 2002. The genetic architecture of ecological specialization: correlated gene effects on host use and habitat choice in pea aphids. *Am. Nat.*, 159, S76–S88.
- Via, S., Bouck, A.C. & Skillman, S. (2000). Reproductive isolation between divergent races of pea aphids on two hosts. II. Selection against migrants and hybrids in the parental environments. *Evolution*, 54, 1626–1637.
- Wang, H., McArthur, E.D., Sanderson, S.C., Graham, J.H. & Freeman, D.C. (1997). Narrow hybrid zone between two subspecies of big sagebrush (*Artemisia tridentata*: Asteraceae). IV. Reciprocal transplant experiments. *Evolution*, 51, 95–102.
- Wood, T.K. & Keese, M.C. (1990). Host-plant-induced assortative mating in *Enchenopa* treehoppers. *Evolution*, 44, 619–628.
- Wu, C.I. & Ting, C.T. (2004). Genes and speciation. *Nat. Rev. Genet.*, 5, 114–122.

Editor, Masakado Kawata

Manuscript received 17 August 2004

First decision made 30 September 2004

Manuscript accepted 22 November 2004