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Assortative Mating and Speciation as Pleiotropic Effects of Ecological Adaptation: Examples in Moths and Butterflies

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1. Introduction

Where divergent ecological adaptation also leads to assortative mating between populations, speciation is facilitated. We have extensively studied two examples from among the Lepidoptera, in which ecologically selected traits have pleiotropic effects on the mating system. Based on our understanding of mating behaviour and pleiotropy derived from these empirical examples, we here review pleiotropic effects in speciation and propose a classification depending on effects on the mating system. This classification helps us to interpret the way in which pleiotropy has led to assortative mating in nature, but will also, we hope, be of interest to theoreticians. Certain models of mating behaviour rarely considered in models of sympatric speciation seem to be those that are most empirically justifiable and also likely to be involved in speciation in the real world. We conclude that pleiotropy might be a rather more general route to speciation than previously supposed.

1.1 Definition

Pleiotropy is the phenomenon whereby a single gene affects several different aspects of the phenotype. In evolution, pleiotropy is commonly used to refer to phenotypic effects of a gene other than those originally favoured by natural selection. These pleiotropic effects are also, and perhaps more correctly, 'side-effects' of evolution at genes under selection. In the context of speciation we are particularly interested in how genes favoured by ecological selection might cause reproductive isolation as a side-effect.

Here we concentrate on the effects on pre-mating isolation (mate choice), which can be readily interpreted in the light of pleiotropy. Instead, most recent

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commentary has been focused on pleiotropic effects on post-mating isolation (hybrid inviability and sterility), particularly on the genetic bases of Dobzhansky–Müller incompatibilities (Turelli and Orr, 1995, 2000; Orr and Turelli, 2001). A great deal is known about the genetics of post-mating isolation, but we have little idea of the original roles of genes causing hybrid breakdown (but see Barbash *et al*., 2003; Presgraves *et al*., 2003). Sterility and inviability must be secondary pleiotropic effects, because low fitness cannot be favoured within populations. In contrast, traits affecting mating behaviour are more visible, and their original role in ecological adaptation might be more easily deduced. In this chapter we explore the pleiotropic effects acting on mate choice, and show how it is likely to be common for ecologically selected genes to have incidental effects on pre-mating reproductive isolation.

1.2 Pleiotropic effects in sympatric and allopatric speciation

Speciation is one example of the process by which one population splits into two genetically distinguishable daughter populations. Under almost any species definition, an important characteristic of a species is the ability to coexist with others without fusion into a single gene pool. Stable coexistence is possible only if closely related sexual species are somewhat reproductively isolated and also ecologically distinct. Perhaps the major challenge in understanding speciation is therefore to explain how genes for ecological distinctness become associated with genes that prevent gene flow.

In allopatric speciation, associations between genes are caused in part by geography, such that ecological traits, mating traits and mating preferences will often diverge in concert between two populations. The speed of speciation in allopatry will be enhanced if genes involved in ecological divergence also cause reproductive isolation. As recognized by Dobzhansky (1937), the common occurrence of 'ecological and seasonal isolation' implies that genes for habitat choice often result in reproductive isolation. Hence, a pleiotropic role of ecological adaptation in causing reproductive isolation is implied, even if not explicitly stated in the 'modern synthesis' view of allopatric speciation.

In sympatric speciation, divergent selection on its own must lead to reproductive isolation. It has long been realized that recombination rapidly breaks down associations between genes under selection and genes for mate choice, making sympatric speciation difficult (Felsenstein, 1981). Unsurprisingly therefore, pleiotropy, which ensures an inherent association between assortative mating genes and ecological traits, could play an important role in sympatric and parapatric speciation (Bush, 1975; Moore, 1981; Doebeli, 1996). In summary, therefore, both sympatric and allopatric speciation may involve pleiotropic effects of genes under natural selection as a cause of reproductive isolation. Their importance is supported by laboratory experiments showing that the evolution of reproductive isolation as a by-product of adaptation to distinct environments is likely (Rice and Hostert, 1993). Although in neither case are such effects necessary, speciation will be easier and more rapid when ecological selection contributes to reproductive isolation.

1.3 A classification of pleiotropic effects

Although pleiotropy is sometimes invoked in theoretical models of speciation, there has been little attempt to distinguish the ways in which pleiotropic effects might act. Based on our contemplation of empirical examples, we here propose a classification of pleiotropic effects according to their effects on the mating system. The aim of this classification is twofold. First, we believe that it will help in understanding empirical examples. Second, we hope to stimulate a greater realism in theoretical models of speciation. As we discuss below, some of the most common forms of pleiotropy in nature are rarely considered in theoretical models of sympatric speciation.

This is intended to be complementary to a recent classification of speciation models (Kirkpatrick and Ravigné, 2002) that did not directly consider 'pleiotropy' in our sense. The distinct classes listed below are an extension of the subcategories of prezygotic isolating mechanisms, or 'Element II' of Kirkpatrick and Ravigné. Our classification distinguishes between mating cues and mating preferences. Perhaps most commonly these will be male traits and female preferences, although it need not be that way around – in the *Heliconius* example below, male preference is more important than female preference.

1. ***Pleiotropic assortment traits.*** A single trait under divergent ecological selection also causes assortative mating, essentially by affecting both male and female components of behaviour. For example, habitat choice or the time of adult emergence can affect temporal or spatial location of both sexes. This is typified by host races, where adaptation to a new host leads directly to assortative mating.
2. ***Pleiotropic mating cues.*** Traits under divergent selection are also used as cues in mating. Here, genes under divergent ecological selection are not directly involved with assortative mating, but instead form part of a suite of traits chosen during mate selection. Speciation therefore requires evolution of mate preference subsequent to the initial divergence of mating cues caused by disruptive natural selection. An example would be divergence in butterfly wing patterns between two populations. If such patterns are also used in mate recognition, then mate preferences may also diverge between populations, and so generate assortative mating.
3. ***Pleiotropic mating preferences.*** Genes under divergent selection also affect mate preference. Female preferences may diverge under natural selection, perhaps due to adaptation of the sensory system to a novel habitat. Subsequently, male traits coevolve to exploit the novel female sensory system. For example, the spectral sensitivity of a visual predator may be locally adapted to divergent light environments, leading to females in different populations becoming responsive to different signals. Subsequent sexual selection of male signals in response to changes in female sensitivity might lead to assortative mating between divergent populations.

Any combination of pleiotropy types 1–3 is of course possible. Alternatively, we may have:

4. ***No pleiotropy.*** Neither preference nor the traits chosen during mating are under direct ecological selection.

We now discuss each category in more detail.

1.3.1 Pleiotropic assortment traits – where traits affect both male and female behaviour

In some circumstances niche choice may equate to mate choice. The classic case in which pleiotropic effects cause reproductive isolation in this manner is during host shifts among phytophagous insects (Bush, 1969). Clearly, given the great diversity of host-plant relationships found among the insects, and the phylogenies of the insects and of their host plants, host-plant use is a trait that is evolutionarily labile (Mitter and Brooks, 1983). Furthermore, some insects use their host plants as a cue in mating behaviour, so that individuals choosing to exploit a novel host will only encounter mates who have made a similar host choice. The classic example is *Rhagoletis pomonella*, in which males patrol host fruits and wait for and court ovipositing females. Release experiments have shown that apple and hawthorn-associated host races show a strong preference for alighting on their native host. Since mating occurs on hosts, this leads to a reduction in cross-mating of over 90% between host races (Feder *et al*., 1994). However, *Rhagoletis* is perhaps an extreme example, where mate-finding is based almost exclusively on habitat cues. In other cases mates are guided by a mixture of habitat cues and habitat-independent signals, such as sex pheromones, a good example being the host races of *Zeiraphera* moths described below.

> Even where mating does not occur directly on the host plant, host phenology may induce assortment between ecotypes. Host plants may differ in nutritional suitability through the year, leading to differences in emergence time between races found on different hosts. If mating receptivity is sufficiently short-lived then this can lead to assortative mating, as in *Enchenopa* treehoppers (Wood and Keese, 1990). Phenology switching may even drive speciation in the absence of host specialization or host choice (Butlin, 1990). Similarly, adaptation to latitudinal differences in climate can lead to different numbers of generations per year, which can also lead to genetic isolation due to a lack of seasonal overlap in parapatric populations, as in *Pieris* and *Aricia* butterflies (Held and Speith, 1999; I.R. Wynne, R.J. Wilson, A.S. Burke, F. Simpson, A.S. Pullin, J. Mallet and C.D. Thomas, unpublished). Hence genes initially selected for adaptation to host phenology produce assortative mating between ecotypes.

In fact, any system where there is divergent habitat choice and where mating occurs in the habitat chosen provides a *pleiotropic assortment trait*. Mosaic hybrid zones provide a good example, where habitat choice leads to assortative mating on a regional scale (Howard and Waring, 1991; MacCallum *et al*., 1998). Nonetheless, few habitats are as clearly defined in space as the host plants of herbivorous insects, which perhaps explains why studies of sympatric speciation have concentrated on these organisms (Drès and Mallet, 2002). In conclusion, there are many excellent examples of pleiotropic assortative mating traits, whereby adaptation to a novel niche leads directly to appreciable pre-mating isolation. Indeed, it seems likely to be much more common than hitherto believed.

Despite many good examples of *pleiotropic assortment traits* in nature, they are rarely considered in theoretical models. In one of the earliest sympatric speciation models, Maynard Smith specifically dealt with the case 'habitat selection' in which mating occurred within a chosen habitat (Maynard Smith, 1966: 643). In contrast, subsequent arguments about sympatric speciation were based on models that ignored this scenario (e.g. Felsenstein, 1981). These models led to a general belief that the major difficulty for sympatric speciation is that associations between mating and ecological traits are broken down by recombination. However, in mating systems that involve *pleiotropic assortment traits*, mating and ecological adaptation are affected by the same genes, so this argument does not apply. The applicability of sympatric speciation theory therefore depends heavily on the specifics of the mating system.

One reason why *pleiotropic assortment traits* have been ignored in sympatric models of speciation is that adaptation to particular habitats can be regarded as a form of partial allopatry or parapatry. To reverse the argument of Kirkpatrick and Ravigné (2002), genes for *pleiotropic assortment traits* are identical in their effects on gene flow to an allopatric distribution. Models of sympatric speciation have typically focused on 'pure sympatry', where individuals are as likely to meet and can potentially mate with the other incipient species as they are with their own type, whatever their genetic constitution (Kondrashov and Mina, 1986). In nature, habitat choice may cause a restriction of gene flow and therefore, arguably, parapatric speciation is more generally applicable (Gavrilets, 2003). In our view, treating *pleiotropic assortment traits* as a form of allopatry would, however, confuse cases of reduced gene flow due to genetic constitution of the individuals involved (*pleiotropic assortment*) with cases where reduced gene flow is due entirely to environmental factors beyond genetic control (true allopatry), and it is this distinction that is at the crux of the argument about allopatric versus sympatric speciation.

1.3.2 Pleiotropic mating cues – where traits chosen during mating are under ecological selection

Pleiotropic mating cues may be more difficult to detect, as it is necessary to study both traits under divergent selection and mating preferences. Perhaps for this reason there are fewer well-studied examples. Butterfly wing patterns are used for hiding from or signalling to predators, as well as signalling to conspecifics (Vane-Wright, 1979; Nijhout, 1991; Vane-Wright and Boppré, 1993; Brunton and Majerus, 1995). Hence, divergence in wing pattern initiated by selection for better defensive capability may lead to divergent selection on mate preferences (Vane-Wright and Boppré, 1993; Jiggins *et al.*, 2001b). Below, we review our work in *Heliconius* butterflies demonstrating the coevolution of mate preferences with mimetic colour patterns and habitat preferences, leading to ecological and reproductive isolation between populations with distinct mimetic patterns.

In cactophilic *Drosophila*, larvae raised on different hosts emerge as adults with distinct cuticular hydrocarbon compositions. These hydrocarbons influence pre-mating isolation between populations (Etges, 1992; Brazner and Etges, 1993; Etges and Ahrens, 2001). It seems plausible, therefore, that speciation in this system might occur through shifts in host-plant use leading to divergent mating signals, followed by the evolution of preferences involving the novel hydrocarbon signals. Nonetheless, a significant proportion of the hydrocarbon differences between populations are unrelated to host use (Etges and Ahrens, 2001) and there is no clear evidence as yet for adaptation of mate preferences to locally occurring hydrocarbons. Hence the evidence in this case remains equivocal. Another example is 'Darwin's finches', in which beak size is adapted to exploiting particular foods, but divergence in beak size leads to a correlated change in song produced by males (Podos, 2001). Presumably there may be subsequent adaptation of female preferences to these novel songs, although learnt mate choice by female nestlings based on their father's song can preclude the need for genetic evolution of mating preferences and lead more directly to assortment between individuals with divergent beak sizes (Grant, 1986).

When pleiotropy is considered in theoretical treatments of speciation, it is often as a *pleiotropic mating cue*. A particular trait is imagined to diverge under ecological selection. This trait is involved in the mating system such that female preferences subsequently diverge, leading to assortative mating. For example Maynard Smith considered a scenario that involved '*modifier genes'* in which a novel allele *B* at a modifier locus causes assortative mating depending on the ecologically selected locus *A* (Maynard Smith, 1966). Therefore *b* has no effect on mating, but once *B* is fixed, *A* always mates with *A*, and *a* with *a*. The fixation of *B* can be favoured in a process analogous to reinforcement because of selection against *Aa* heterozygotes, which tend to be found in an inappropriate environment due to hybridization. This is an example of what Felsenstein called a 'one-allele' model, in which a single allele increases assortativeness of mating based on the trait locus (Felsenstein, 1981; Kirkpatrick and Ravigné, 2002). However, divergence of mate preferences could also be modelled in a two-allele scenario in which allele *b* might represent preference for *a* and allele *B* preference for *A*. In general, two-allele models seem far more likely. In butterflies, for example, it is easier to imagine one phenotype being a preference for red males and the alternative phenotype being a preference for blue males. The corresponding, and rather improbable, one-allele scenario would be a single 'choosiness' phenotype that caused red to mate with red and blue with blue. However, two-allele models make speciation much harder, as alternative alleles must go to fixation in each of the divergent ecotypes, and any hybridization may cause recombination to act against speciation (Felsenstein, 1981). Hence, there are various ways in which speciation might occur subsequent to divergence in a *pleiotropic mating cue*.

1.3.3 Pleiotropic mating preferences – where mate preferences are under ecological selection

Pleiotropic mating preferences can arise where the environment chosen by an organism constrains or otherwise influences intraspecific communication. This is implied in the 'sensory drive' hypothesis (Endler, 1992; Endler and

Basolo, 1998; Boughman, 2002; see also West-Eberhard, 1983). Boughman described three related processes that affect signal evolution.

1. *Signal transmission*. Signals must pass through the local environment before being received and are thus subject to alteration by properties of that environment.
2. *Perceptual tuning*. Individuals receiving signals will have their receptor mechanisms adapted to the local environment for foraging, predator detection etc., as well as sexual selection, and thus are likely to be more receptive to certain kinds of signals. This adaptation of the sensory system to the local environment is likely to lead to changes in the kinds of signals most readily detected by females. Hence genes for adaptation of the sensory system to a local habitat are likely to have pleiotropic effects on mate recognition.
3. *Signal matching*. The signals themselves will become adapted to maximize 'visibility' to the particular receptor system of the local population. This third stage refers to selection on the mating cue itself, driven by prior changes in female preferences, and therefore represents a form of sexual selection that occurs subsequent to pleiotropic divergence of female preferences.

One of the best-studied examples involves stickleback ecotypes that are adapted to habitats with differing degrees of water clarity (Boughman, 2001). Males in clear limnetic habitat have more red coloration, those living in murkier waters are blacker, these colours being the most contrasting signals in the respective habitats. Females have corresponding differences in their detection thresholds for red light. One possible sequence of events is that female visual perceptivity changed to maximize foraging ability in the distinct habitats, and was followed by divergence in the male trait to enhance conspicuousness to the perceptual systems of locally occurring females (Boughman, 2001). As far as we are aware there are no well-studied examples of sensory drive in insects, but this may be because their sensory systems are poorly understood.

2. Empirical examples

Here, we discuss examples studied extensively by us which provide evidence that pleiotropic assortment traits and pleiotropic mating cues are generally important in natural systems.

2.1 Speciation in the larch budmoth

Phytophagous insects that use long-distance pheromones to attract mates are seemingly the last kinds of insects one might expect to show pleiotropy between host use and mating behaviour. In many night-flying Lepidoptera, females 'call' for males by producing pheromones. Calling females can attract males from distances far greater than from within a single host individual. Hence, unlike the situation in *Rhagoletis* (see above), host choice does *not* equate to mate choice. For example, larch- and pine-adapted host races of the larch budmoth *Zeiraphera diniana* occur sympatrically and are genetically differentiated, implying significant reproductive isolation (Emelianov *et al*., 1995). Assortative mating is primarily due to major blend differences in the pheromones between races, which are unrelated to host use. Hence we did not expect to find pleiotropy between host use and mate choice. However our recent work has shown that even here there is strong evidence that host choice is acting as a *pleiotropic assortment trait*: host choice has a very strong influence on cross-mating between the host races, in spite of the predominant effect of pheromone composition on mate choice.

Zeiraphera diniana is distributed widely across the Palaearctic, where it feeds on a variety of conifers in the family Pinaceae, particularly pine (*Pinus*), larch (*Larix*) and spruce (*Picea*). We have studied this species in the Swiss and French Alps, where two forms are often found sympatrically, feeding on European larch (*Larix decidua*) and cembran pine (*Pinus cembra*) where they grow in mixed forest stands. The two forms of the moth are strongly differentiated in colour pattern of larvae, pheromone blend, and at a number of genetic marker loci (Baltensweiler *et al*., 1977; Emelianov *et al*., 1995). The larch form is well known for its 9–10-year outbreak cycles near the tree-line in the Alps, where populations can cycle through 10^5-fold changes in density, a phenomenon that has been studied for many years (Baltensweiler *et al*., 1977).

Although the two forms are strongly differentiated, none of the genetic differences are completely fixed, with colour polymorphisms, pheromone blends and allozyme loci all showing some evidence of transfer between the two forms via hybridization. For this reason, the two forms are considered to be ‘host races’ rather than separate species. When we studied the probability of hybridization in the field and laboratory, we found that assortative mating mediated by pheromones was not perfect. We baited traps with live females to attract wild males; using an allozyme-based identification procedure, and we found that the probability of cross-attraction between the two forms varied between 3% and 38% (Emelianov *et al*., 2001). In the laboratory at close range, cross-mating was readily achievable (20–30% of matings), occurring only slightly less often than at random (50%) (Drès, 2000). In fact, males will attempt to copulate with any moth-like object and homosexuality is quite common (Benz, 1973). Thus there is ample possibility for cross-mating at close range, and pheromone attraction is the most important selective agent in assortative mating. Overall, we expect the species to hybridize at the rate of a few percent per generation in sympatric populations (Emelianov *et al*., 2003), and we now have good genetic data to show the existence of hybrids in the wild, and for gene flow between the two host races in some regions of the genome (Emelianov *et al*., 2004). The implication is that selection must counterbalance the homogenizing effect of gene flow and maintaining between-race divergence in certain genomic regions (Emelianov *et al*., 2004).

We were interested in whether there could be an influence of the host plant on reproductive isolation between the two races. To test this, traps baited with

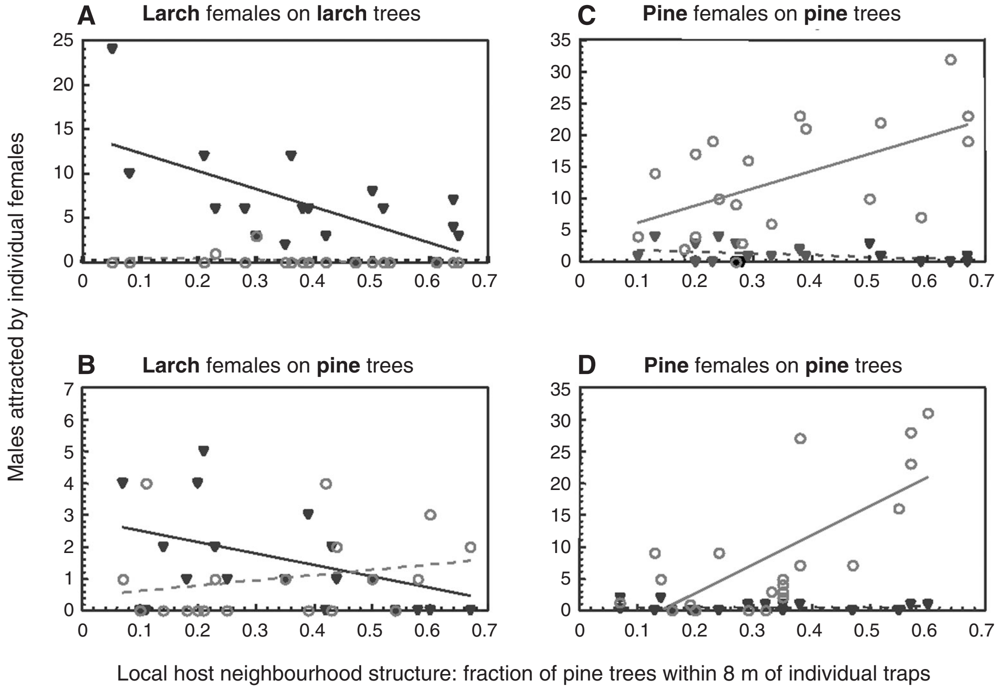

Fig. 16.1. Effect of host neighbourhood on assortative attraction. Panels **A**–**D** represent the four types of traps (larch females on larch trees, larch females on pine trees, pine females on pine trees and pine females on larch trees). In each panel, the numbers of males of each race attracted by individual females are plotted against the fraction of pine trees in local host neighbourhoods (i.e. 8 m radius circular patches of larch–pine forest surrounding individual traps). Larch male data are shown as *solid triangles*, and pine male data are shown as *hollow circles*. *Solid regression lines* represent males of the same race as the calling female (that is, larch males in panels **A** and **B**, and pine males in panels **C** and **D**. Dotted lines represent catches of alien males.

live females were put out on different hosts (Fig. 16.1). Larch-race females tended to attract significantly more pine-race males when the traps were placed on pine than when placed on their own host. However, the reciprocal effect was not evident; pine females attracted slightly fewer larch males when placed on larch than when placed on pine, although the effect was not significant.

A more striking result was the influence of the immediate surroundings of the trap. As is true for most plant species, the distributions of larch and pine trees in mixed forests are significantly clumped, with fractions of larch and pine trees varying between individual patches within the forest. Females cross-attracted many more males of the other host race when placed in an environment surrounded by the other host than if surrounded by their own host species (Emelianov *et al*., 2001). Thus, in spite of the very strong assortative attraction induced by the pheromone differences, assortativeness was strongly enhanced when females called from their own host tree, or from areas with a high proportion of their natural host; cross-attraction was much more likely if they called from the other host or in neighbourhoods dominated by the other host.

This effect of local host abundance on mate attraction might be caused solely by female host preferences, such that females will typically be calling from within clumps of their own host. Hence, each host clump would produce a clump of pheromone signals emitted by a 'chorus' of simultaneously calling same-race females. Such a clumped distribution of pheromone signals could amplify the power of the race-specific pheromone signal by combining 'voices' from individual members of the 'chorus'. This clumping might reduce the level of noise relative to signal, thus limiting the probability of male cross-attraction even in the absence of male host preferences. This would imply a role for pleiotropy in enhancing and maintaining *existing* racial differences, but it seems unlikely to have had a role before pheromone divergence.

Alternatively, specific host preference of calling females might be complemented by host-specific cues used by males searching for females. Host choice in insect parasites is usually thought of as a female trait, and there are no previously published data on host preference in male Lepidoptera. However, it would not be surprising if host choice is found in males as well as females: although males do not lay eggs, they do need to find females and a good way to do this would be to use the inherited ability that females use to choose hosts for oviposition. To test for divergence of male and female host preference in *Zeiraphera*, we studied the distribution of adults in mixed forests. Emelianov *et al*. (2003) collected live adult *Zeiraphera* from branches in mixed forests early in the morning, when moths do not fly. Both sexes had a strongly biased tendency to rest on their own hosts in the field (86% and 82%, respectively, versus random settling of 50%). The distribution of males in the wild will then be affected both by their host and by locally calling females; for this reason, we also tested male and female host preferences in the laboratory and found a significant and heritable tendency for each sex and host race to alight on their own host (Emelianov *et al*., 2003).

In *Zeiraphera*, therefore, a pleiotropic effect of host adaptation even today adds to mate choice chiefly regulated via pheromonal divergence, and there is no evidence that pheromone production or preferences are in any way dependent directly on host-plant use (Emelianov *et al*., 2003). As host preference is expressed in both sexes, it is easy to imagine that host race formation began with a host choice shift, which led to some initial reproductive isolation via host choice/mate choice pleiotropy, enhanced by the clumped host distribution. Then, if even slightly divergent pheromonal signals and preferences become associated with the two host races, the degree of assortative mating, and thus host adaptation, would be enhanced in areas of sympatry, allowing further improvements in association of mate choice with host choice (see Johnson *et al*., 1996, for a model of a similar scenario). This example therefore demonstrates how, in nature, assortative mating may often result from a complicated series of factors involving both pleiotropic effects of ecological divergence and non-pleiotropic mate choice.

2.2 Speciation in *Heliconius*

Brightly coloured and slow-flying heliconiine butterflies are among the most striking insects in neotropical rainforests. Similar colour patterns shared by heliconiine, ithomiine and pierid butterflies were initially explained by Bates (1862) as a deceptive strategy whereby rare palatable or unpalatable species gain from looking similar to common unpalatable 'model' species. Later, Müller (1879) showed how both members of a pair of unpalatable species might benefit from mimicry, because the cost of teaching predators to associate a particular pattern with unpalatability is thereby shared. *Heliconius* feed on *Passiflora* and related host plants from which they sequester cyanogenic glycosides (Brown *et al*., 1991). In addition, the adults can synthesize the same chemicals *de novo* (Nahrstedt and Davis, 1983; Engler *et al*., 2000). All species in the genus *Heliconius* that have been tested are distasteful and are avoided by bird predators such as jacamars (Brower *et al*., 1963; Chai, 1986, 1988). Hence, pattern convergence between *Heliconius* species is most likely to be mutualistic Müllerian mimicry (Mallet, 1999).

The wing patterns of *Heliconius* are under strong mimetic selection (Benson, 1972; Mallet and Barton, 1989; Kapan, 2001) that leads to local convergence of pattern between 'mimicry rings' consisting of two or more species. At first sight, mimicry, a theory of convergence, is an unlikely candidate for a causative agent in speciation. However, in nature there is a great diversity of patterns at both a local and a regional scale. There are sympatric mimicry rings with distinct patterns in most areas of the neotropics, whose distinctness is maintained by segregation in both vertical and horizontal dimensions (Mallet and Gilbert, 1995; Beccaloni, 1997; Joron *et al*., 1999). On a broader spatial scale, there are striking switches in the dominant mimetic patterns between regional faunas, both within and between species (Brown, 1979).

The phylogenetic distribution of mimicry shifts among species seems to follow the classic pattern of 'adaptive radiation', as though closely related species can avoid competition by switching to a new mimicry ring (Turner, 1976). This suggests that mimetic adaptation and diversification might play a role in speciation. Here we review our work on diversification in *Heliconius*, from the formation of colour pattern races through to good species.

2.2.1 How do novel patterns arise?

The diversity of mimicry patterns both within and between regions gives rise to ample opportunity for populations to interact with novel potential model species. Wherever a population finds itself sympatric with a mimicry ring that is numerically either more abundant or more toxic than its own, there will be selection favouring a switch to a new pattern, albeit across an adaptive valley. There are many examples of this process having occurred. For example, *Heliconius hecale* mimics the ithomiine species *Melinaea idae idae* in Panama, but switches to a different ithomiine, *Tithorea tarricina*, in Costa Rica, where *M. idae* does not occur (DeVries, 1986).

A second possibility that must have occurred frequently in *Heliconius* is that switches occur to entirely novel patterns. The frequency-dependent nature of mimetic selection means that any pattern, provided it is suitably effective in being seen and remembered by predators, could potentially go to fixation once it has become sufficiently common in a population. Presumably, the initial rise of a novel pattern has to occur through drift and/or temporary local relaxation of selection, but once established will be maintained by frequency-dependent selection. For example, the Ecuadorean Pacific coast races, *Heliconius erato cyrbia* and *H. melpomene cythera* have a highly idiosyncratic pattern not seen in any other species (Brown, 1979). This most probably arose completely *de novo* in one of the species which was then mimicked by the other (Mallet, 1999). Newly evolved races of *Heliconius* have been considered as some of the best examples of Wright's shifting balance (Mallet, 1993). Distinct patterns clearly lie on alternative adaptive peaks separated by a fitness valley, caused by selection against hybrid patterns. Furthermore, recent evidence suggests that hybrid zones between races are mobile, providing evidence for the key and contested Phase III of the shifting balance (Barton, 1992; Blum, 2002).

In some cases particular colours or patterns may be favoured in particular environments. For example the iridescent blue colour of *H. cydno chioneus* in Panama produces a polarized signal that may be more conspicuous in dark understorey habitats (Sweeney *et al*., 2003). Similarly, Amazonian forest habitats have a large mimicry ring of yellow and orange rayed patterns, while surrounding savanna regions to the north and south, in Venezuela, French Guiana and Brazil, more commonly have bold red and yellow patterns (Benson, 1982). Nonetheless, there are many exceptions to such generalizations and hybrid zones are rarely associated with obvious habitat features (Mallet, 1993), suggesting that the different patterns are acting as arbitrary alternative signals, each with similar overall memorability for predators.

Some of the genetic variability for this diversification might derive from hybridization (Gilbert, 2003). As expected under Müllerian mimicry, patterns are generally monomorphic within *Heliconius* populations. However, where divergent races meet in hybrid zones, recombination produces novel gene combinations giving rise to a high diversity of phenotypic patterns. Much rarer hybrids also occur between species and might similarly contribute variation on an evolutionary time-scale (Mallet *et al*., 1998; Gilbert, 2003). Furthermore, there are some obvious candidates for 'hybrid species', where naturally occurring taxa appear to share pattern elements from other species. For example *H. hermathena* has a red forewing band similar to that of *H. erato* and narrow yellow fore- and hindwing bands very similar to *H. charithonia* (Brown and Benson, 1977). Similarly, the red and yellow forewing band of *H. heurippa* can be recreated in crosses between *H. melpomene* and *H. cydno* (Linares, 1989; Naisbit *et al*., 2003). Hence it seems likely that hybridization plays a role in generating pattern diversity. Obviously, novel mutations must also be important, and separating the relative contributions of mutation and hybridization will only become possible with molecular characterization of switch gene loci.

2.2.2 Coevolution of pattern with mate preferences

Mimetic switches therefore usually generate parapatric populations with divergent colour patterns, maintained distinct by frequency-dependent mimetic selection (Turner, 1971). Most of these populations interbreed freely and so speciation is not considered to have occurred. Since colour pattern genes are generally in Hardy–Weinberg equilibrium where races of *Heliconius* meet, it had been assumed that there is no reproductive isolation between such races, aside from that generated by selection against colour pattern hybrids (Mallet, 1993; Mallet *et al*., 1998).

Nonetheless, it has long been known that colour pattern is an important cue in courtship behaviour in *Heliconius* (Crane, 1955), so mimetic patterns seem likely to be used as cues in mate detection, and as such are candidates as ecologically selected traits with pleiotropic effects on mate choice. To test this, we collected several races of *H. melpomene* with very distinct patterns and investigated their colour pattern preferences in the laboratory (Jiggins *et al*., 2004). Butterfly courtship generally involves an initial phase in which males detect females using visual cues, followed by close-range interactions between the sexes that involve visual, pheromonal and tactile communication (Vane-Wright and Boppré, 1993). In *Heliconius*, females mate when teneral and have little ability to reject males. As a result, mate choice is mainly exerted by males. We were primarily interested in the role of colour patterns in courtship and so concentrated on the use of visual cues by males. In order to test the preferences of males for different colour patterns, moving colour pattern models were presented to population cages of males in paired trials, consisting of a control model of the same pattern as the males being tested, and an experimental model with the pattern of a different population. Models were presented sequentially, with the order of presentation randomized. In total, five distinct races were tested in 20 pairwise comparisons. In virtually all the trials, males preferred to court their own patterns, with only a few comparisons showing a significant preference for another race (Fig. 16.2).

The experiments were initially carried out with wings dissected from female butterflies. To confirm that preferences were based on colour pattern and not some other aspect of the wing such as cuticular pheromones, we repeated comparisons showing significant preferences with paper models printed using a standard inkjet printer. Paper models produced results that were in general strikingly concordant with those from the real wings (Fig. 16.2; Jiggins *et al*., 2004). The level of responsiveness to models was generally reduced compared with that observed with dissected wings, and we rarely observed any courtship. Nonetheless, the fact that attraction to a model can be replicated using crude paper models provides strong support for the idea that initial preference is indeed based largely on visual cues.

In *H. melpomene* there was an exception to the general rule that populations prefer their own patterns: the broad red forewing band of the ‘postman’ pattern in *H. m. melpomene* and *H. m. rosina* was attractive to males of all races, leading, in a few cases, to significant preference for a pattern other than their own (Fig. 16.2). This might represent a constraint on the evolution of

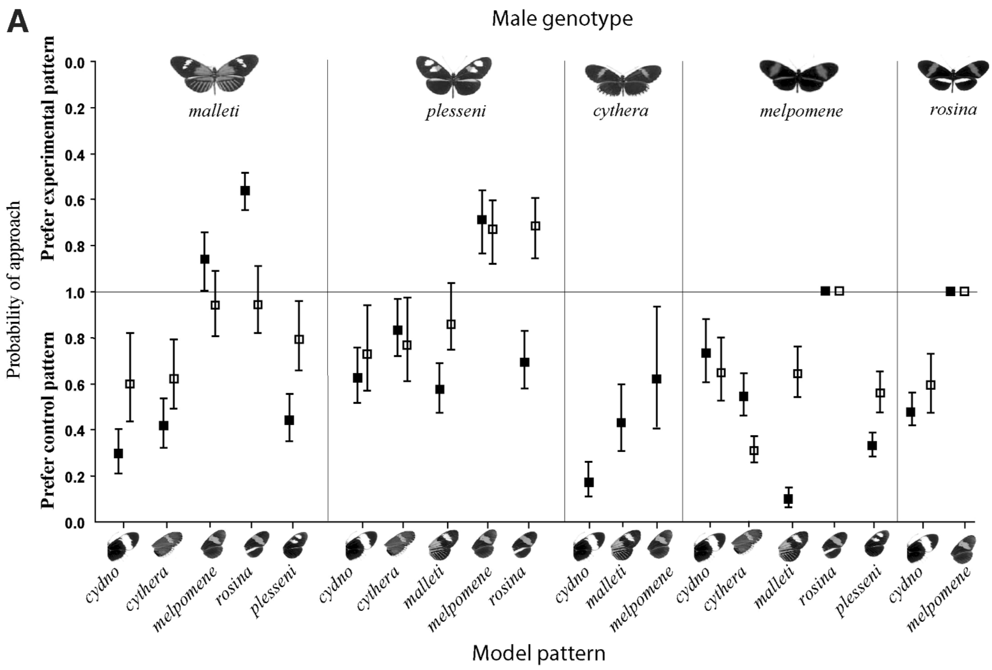

Fig. 16.2. Mate preferences among races of *H. melpomene*. The data show the probability that males **A** are attracted to and **B** attempt to court an experimental wing pattern model (model pattern), relative to a similar model with the same pattern as the males themselves. *Filled points* show experiments using real dissected butterfly wings, *hollow points* show experiments using paper models. In all comparisons except those involving the red forewing band 'postman' patterns, males show strong and significant preferences for their own pattern over that of other races. Probabilities were calculated using likelihood and bars show support limits, equivalent to 95% confidence intervals. Non-significant values from Jiggins *et al.* (2001a,b) are shown as values of 1 without support limits.

preferences, perhaps due to phylogenetically conserved neural physiology (Autumn *et al.*, 2002), or perhaps due to an ecological requirement for attraction to red and orange flowers, such as *Cephaelis tomentosa* and *Psiguria* spp., that provide pollen to adult *Heliconius melpomene*. Such constraints might therefore restrict or bias the direction of the evolution of mimicry.

2.2.3 Learning and speciation

In birds it has been suggested that imprinting might play an important role in speciation, by allowing species-specific recognition cues to be learnt through contact with parents or other conspecifics (Irwin and Price, 1999). *Heliconius* are known to have well-developed learning abilities, and for example rapidly learn the position of floral and host-plant resources within their home ranges

(Gilbert, 1975; Mallet, 1986). If colour-pattern-based mate preferences were similarly learnt, this would profoundly change the dynamics of preference evolution, perhaps making speciation more rapid. Experiments were therefore carried out to test for learnt pattern preferences. Males of *H. m. malleti* were raised in the laboratory and separated into two groups after eclosion. One was exposed to females of its own race and the other only to females and males of *H. m. plesseni* for a week before experiments were carried out. If males learnt the patterns of the females with whom they had contact, then a difference between the two groups in pattern preference was predicted. In fact both groups retained a strong preference for the *H. m. malleti* pattern, suggesting that preferences were genetic. The one possible caveat is that if males could somehow determine their own colour pattern, they may be able to learn and prefer it, irrespective of experience of females. A small number of *H. erato* individuals were tested by Crane (1955) to investigate this possibility, and provided no support for the idea; but clearly more experiments are warranted. Such self-learning seems unlikely, but if it were important it would make speciation rather easy, as novel colour patterns would automatically be preferred in mating; a similar possibility has already been suggested for Darwin's finches mentioned above.

Heliconius adults are highly social insects that interact strongly at times other than during courtship. Individuals of many species roost gregariously, and spend long periods of time hovering near one another prior to roosting (Mallet, 1986). Gregarious roosting is probably a means of clustering to avoid predators, and it seems likely that preferences measured in our experiments result from selection for social as well as sexual interactions. In fact, there is a significant tendency for individuals to roost at similar heights, in similar habitats, and even within the same roosts as their co-mimics, as well as of their own species (Mallet and Gilbert, 1995). Hence, colour preferences might in part be selected to increase gregariousness between mimetic species. What is important from the point of view of speciation, however, is that such preferences do lead straightforwardly to assortative mating (Jiggins *et al.*, 2001b, 2003).

2.2.4 From races to species

In summary, colour pattern races of *H. melpomene* differ in mimetic colour patterns, and the same patterns are also used as cues in mate finding. Mate choice by males coevolves strongly with these colour patterns. Hence wing pattern differences generate a degree of both pre- as well as post-mating isolation (because of selection against non-mimetic hybrids). However these parapatric and conspecific populations hybridize freely, and therefore have not yet speciated. The pre-mating isolation measured in our experiments is clearly not sufficient to maintain stable sympatric coexistence. In order to understand how coexisting 'good' species arise, we have also studied two species pairs that still hybridize occasionally in nature, one in each of the two major clades within the genus *Heliconius* (Jiggins *et al.*, 1996, 2001b; Mallet *et al.*, 1998). In both cases species differ in colour pattern, such that rare intermediate hybrid colour patterns are selected against due to mimetic selection mediated by predators. Furthermore, both species pairs also show strong assortative mating that is responsible for the rarity of hybrids. In the case of *H. melpomene* and *H. cydno*, mimetic patterns play a similar role in mate recognition as between races (Jiggins *et al*., 2001, 2004). Hence the pleiotropic effects of mimetic pattern on mate choice are similar between species and between races, and contribute to reproductive isolation between these species in the wild.

Apart from Haldane's-Rule sterility in male hybrids between *H. melpomene* and *H. cydno* (Jiggins *et al*., 2001a), and likely close-range pheromone differences in both pairs, a key factor that makes them different from races seems to be habitat and host-plant use. For example, *H. himera* and *H. erato* are parapatric species which hybridize relatively infrequently in narrow hybrid zones where they meet (Jiggins *et al*., 1996; Jiggins and Mallet, 2000). Hybrid zones are associated with a transition from the wet forest habitat of *H. erato* to the much dryer regions where *H. himera* is found. This ecological shift appears to be the main difference between *H. himera* and the other races of *H. erato*, suggesting that ecological selection may play a major role in maintaining the species differences in the hybrid zone. However, these species have almost identical host-plant use, which probably explains their inability to coexist in broad sympatry (Jiggins *et al*., 1997). In the *H. melpomene* group, the closest sister taxon to *H. melpomene* is the *H. cydno* species complex, with mtDNA suggesting a divergence time of 1.5 million years, similar to that between *H. himera* and *H. erato* (Beltrán *et al*., 2002). However unlike the latter pair, *H. melpomene* and *H. cydno* are broadly sympatric. *Heliconius melpomene* and *H. cydno* differ chiefly in microhabitat preference and host-plant use, rather than in adaptations to non-overlapping habitats, such as wet and dry forest as in *H. erato* and *H. himera*. Hence, their ecologies allow sympatric ecological segregation, and broad overlap. This ecological segregation also maximizes the advantages of mimicry: *H. melpomene* occurs with its co-mimic, *H. erato*, in open areas, while *H. cydno* and co-mimic *H. sapho* are both found in closed canopy forest (Gilbert, 1991; Estrada and Jiggins, 2002). Thus, genes for habitat preference are co-adapted with those for colour pattern.

If mimetic patterns are also used as mating cues, this raises the question of how co-mimics with identical patterns recognize their own species (Brower *et al*., 1963; Vane-Wright and Boppré, 1993). When male *H. melpomene* were presented with their own patterns and those of their sympatric co-mimic, *H. erato*, they could not distinguish between them (C. Estrada and C.D. Jiggins, unpublished). Similarly, in the wild it is common to see mimetic butterflies of different species, such as *H. cydno* and *H. sapho*, approach one another and interact briefly before flying on. It seems likely, therefore, that confusion does occur between co-mimics in nature, leading to time wasted in pursuit of unsuitable partners. Time-wasting may be minimized because mimicry is most common between rather distantly related species in the genus *Heliconius* (Turner, 1976). Distantly related species may have diverged sufficiently in close-range pheromonal or other mating signals so that conspecifics can be readily distinguished from co-mimics.

2.2.5 What drives divergence in mate preference?

Having reviewed the empirical evidence, we now attempt to place the *Heliconius* data in the context of the speciation theory described at the beginning of the chapter. This requires a certain amount of speculation regarding the genetic basis of mate preference, but the assumptions are testable. Assuming that preference is genetic and due to alleles for a behavioural rule such as ‘prefer individuals with orange rays’, they will follow a ‘two-allele’ model whereby alleles evolve to different frequencies in each population. So how do preference genes become associated with their respective colour pattern? Given the strong selection on pattern, and monomorphism of patterns in populations outside narrow hybrid zones, it seems most likely that when a novel pattern arises it goes to fixation very rapidly in the local population.

Thus, a novel pattern phenotype, *A*, goes to fixation locally and is followed by an increase in frequency of *B*, a choice phenotype causing individuals to prefer pattern *A* over the ancestral pattern, *a*. Is *B* favoured by direct or indirect selection? Since hybrid zones are very narrow relative to the range of each race, and there is no evidence for assortative mating in hybrid zone populations, reinforcement seems unlikely, at least initially. This implies that preferences are favoured not by selection to reduce the production of unfit hybrids, but rather by direct selection on preference alleles. This most probably occurs through improved mate-finding efficiency (Dawkins and Guilford, 1996). A population that has recently acquired a novel pattern *A* still has the ancestral choice phenotype *b* that prefers the *a* pattern as a mating cue. Individuals therefore waste time courting the wrong species, or waiting for individuals with *a* patterns. Thus, *B* is favoured, since less time is wasted finding potential mates. This process will be driven by sexual selection, since *B* individuals are likely to achieve more matings than *b*. It could also be driven purely by natural selection if mating success is similar for *B* and *b*, but the former has more time to feed or avoid predators. Some combination of the two is likely. When considered in this way, the fixation of alternative preference alleles between populations with distinct patterns seems almost inevitable.

We have some data about the form of selection on mate preference from experiments on hybrid matings (Naisbit *et al*., 2001). Hybrids between *H. melpomene* and *H. cydno* suffer a mild mating disadvantage; matings are more probable within parental types than between hybrids and either parental. In addition, F1 hybrids prefer other F1 hybrids, suggesting that the genetic basis of mate preference is largely additive. These experiments also demonstrate that, once strong mating preferences have evolved, disruptive sexual selection against rare hybrids acts as an additional pleiotropic effect that further contributes to reproductive isolation (Naisbit *et al*., 2001). In contrast, between *H. erato cyrbia* and *H. himera*, we could not detect disruptive sexual selection against hybrids (McMillan *et al*., 1997). The differences between these two systems may be due to reinforcement, which appears to have narrowed the mating preferences of *H. melpomene* where it overlaps with *H. cydno* (Jiggins *et al*., 2001a,b).

Fixation of alternative habitat preference alleles should similarly be favoured between parapatric populations in order to maximize overlap with co-mimics. Speciation of *H. melpomene* and *H. cydno* is therefore likely to have been parapatric, with abutting populations diverging in mimicry, colour-based mate preference, and habitat preference. Genes affecting all of these traits would be strongly epistatic. Hence, mate preference alleles for white and iridescent blue colour patterns and habitat-preference alleles for living in forest understorey would have been favoured in the incipient *H. cydno* population that was mimetic with forest-dwelling *H. sapho*. Meanwhile, in the adjacent incipient *H. melpomene* population the red-band mate preference alleles and open habitat ecological preference alleles would have been favoured to maximize overlap with its co-mimic, *H. erato*.

The order in which these changes occurred is perhaps impossible to determine, but the study of *H. melpomene* races described above suggests that perhaps the first step in divergence is a change in colour pattern and associated mate preferences. The races of *H. melpomene* have not yet speciated, perhaps because they all mimic *H. erato*, with correspondingly similar preferences for open habitat.

In summary, habitat preference, mate preference and mimetic pattern form co-adapted gene complexes in *Heliconius* populations, in a manner similar to the co-adaptation of communication systems to the environment described in the 'sensory drive' hypothesis (Endler, 1992; Endler and Basolo, 1998). Our proposed model for speciation in *Heliconius* therefore requires an initial leap across an adaptive valley, represented by the evolution of a novel colour pattern form. Subsequent adaptation of habitat preference and mate preference alleles can occur through the gradual accumulation of additive genetic changes, in a manner similar to that proposed in the Dobzhansky–Müller model for the evolution of post-mating incompatibilities.

3. Conclusions

Our results from these two lepidopteran systems suggest that genes selected by particular ecological conditions may commonly have some pleiotropic effect on mating. We have highlighted the distinction between *pleiotropic assortment traits*, where a single ecologically selected trait leads automatically to assortative mating between divergent populations; *pleiotropic mating cues*, where the ecologically selected loci also control a trait chosen during mating; and *pleiotropic mating preferences*, where mate preferences are influenced by ecological selection. In the latter two, the ecologically selected traits influence the mating system but do not lead directly to assortative mating; subsequent coevolution of mate choice and mating cues performs this task. There are many examples of *pleiotropic assortment traits*, as any form of habitat choice where mating occurs in the habitat chosen (including temporal habitat, or phenology) will lead to a degree of assortative mating. In the pheromone-signalling host races of *Zeiraphera* a *pleiotropic assortment trait* is combined with habitat-independent mating cues. In contrast, good examples of *pleiotropic mating cues* and *pleiotropic mate preferences* are fewer, presumably because they require a more detailed understanding of the genetic basis of ecological traits, mating signals and mating preferences. *Heliconius* butterflies provide a good example of *pleiotropic mating cues*, where mimetic patterns are used as cues in mate-finding, leading to coevolution of mate choice and assortative mating. However, divergence in *Heliconius* mimicry does not automatically lead to completion of speciation; for that, further ecological divergence seems necessary. In both cases, more information is needed on the genetics of mate preferences to fully understand their origin. Nonetheless, we hope that our review and the current focus on the importance of ecology in speciation will stimulate further studies to illuminate the role of pleiotropic effects in species formation.

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