

Review

Hybrid trait speciation and *Heliconius* butterflies

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Homoploid hybrid speciation (HHS) is the establishment of a novel species through introgressive hybridization without a change in chromosome number. We discuss different routes by which this might occur and propose a novel term, ‘hybrid trait speciation’, which combines the idea that hybridization can generate adaptive novelty with the ‘magic trait’ model of ecological speciation. *Heliconius* butterflies contain many putative examples of hybrid colour patterns, but only recently has the HHS hypothesis been tested explicitly in this group. Molecular data has shown evidence for gene flow between many distinct species. Furthermore, the colour pattern of *Heliconius heurippa* can be recreated in laboratory crosses between *Heliconius melpomene* and *Heliconius cydno* and, crucially, plays a role in assortative mating between the three species. Nonetheless, although the genome of *H. heurippa* shows evidence for hybridization, it is not a mosaic of the two parental species. Instead, ongoing hybridization has likely blurred any signal of the original speciation event. We argue that where hybridization leads to novel adaptive traits that also cause reproductive isolation, it is likely to trigger speciation.

Keywords: mimicry; homoploid hybrid speciation; biodiversity

1. INTRODUCTION

Homoploid hybrid speciation (HHS) is the formation of a novel, reproductively isolated population through hybridization, without a change in chromosome number (Coyne & Orr 2004). The process is distinct from hybrid speciation through polyploidization, which is a well established route to speciation, particularly in plants (Stebbins 1950; Hegarty *et al.* 2008). HHS has commonly been considered rare or absent in animals (but see Stebbins 1959; Templeton 1981), although there have been several putative examples published recently (Nolte *et al.* 2005; Schwarz *et al.* 2005; Gompert *et al.* 2006; Mavárez *et al.* 2006; Meyer *et al.* 2006). The reasons for its apparent rarity are probably partly real and partly historical. First, there are theoretical reasons why it is hard to imagine a hybrid lineage becoming established, primarily because such a lineage is likely to be eliminated by its parents either through backcrossing or ecological competition, which might suggest hybrid speciation is genuinely a rare phenomenon (Mallet 2007). Second, there is a logistical problem of detection inherent in the genetic and morphological methodologies available. Specifically, determining that novel adaptive traits (as opposed to neutral genetic variation) are a result of hybridization can be challenging. Finally, however, there has also been an inherent scepticism of the importance of hybrids in the animal

literature, perhaps because of the widespread adoption of the biological species concept, which specifically precludes hybridization between ‘good’ species (Mallet 2005, 2008). This scepticism is in marked contrast to the plant literature and is an attitude that is changing rapidly in the light of new evidence. In particular, hybridization has been resurrected as a major force in adaptive animal radiations (Rieseberg *et al.* 1999; Seehausen 2004).

(a) *Models of homoploid hybrid speciation*

Hybrid speciation has recently been defined as the ‘process in which natural hybridization results in the production of an evolutionary lineage that is at least partially reproductively isolated from both parental lineages, and which demonstrates a distinct evolutionary and ecological trajectory’ (Arnold 2006). Importantly, hybridization must lead to at least some reproductive isolation from both parental species, allowing the daughter population to diverge independently and this is the definition that we adopt here. HHS has been best studied in the sunflower genus *Helianthus*, where the stabilization of hybrid species has involved the establishment of a lineage with genomic blocks derived from one or other parent, distributed throughout the genome (Ungerer *et al.* 1998). The stabilization of such hybrid species takes a number of generations during which incompatible genomic regions are rapidly eliminated by selection. Incompatibility between parental and daughter species is thus rapidly established because the resultant hybrid genome possesses large numbers of genes that cause

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One contribution of 12 to a Theme Issue ‘Speciation in plants and animals: pattern and process’.

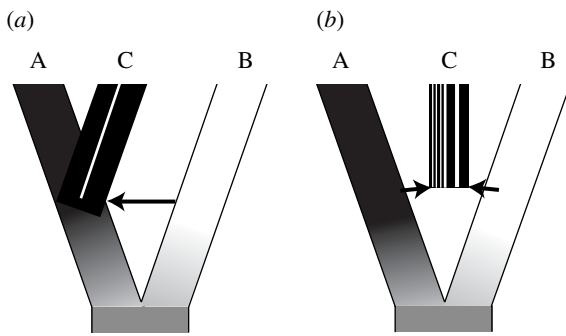


Figure 1. Two possible routes to hybrid speciation. (a) Hybrid trait speciation involves backcrossing of genes from lineage B into the genome of A. If those genes control a trait with adaptive potential that also causes reproductive isolation (similar to a magic trait, *sensu* Gavrillets 2004), then this might give rise to establishment of the novel species C. (b) In contrast, under mosaic genome hybrid speciation the novel species C is formed by the establishment and stabilization of a recombinant lineage combining compatible regions of the genome in similar proportions from A and B.

epistatic hybrid breakdown when crossed back to either parent (Ungerer *et al.* 1998).

This mode of hybrid speciation is unlikely to apply in more recent adaptive radiations, such as cichlid fish, where hybridizing species have not accumulated genetic incompatibility at multiple genetic loci. In such lineages speciation typically does not result from intrinsic hybrid incompatibility at large numbers of loci, but rather through sexual and ecological isolation at a few traits driven by strong selection. Recent species pairs can be strongly isolated in the wild but produce completely compatible hybrid offspring in laboratory crosses (Seehausen *et al.* 1997). Commonly, adaptive divergence at a single or a few traits causes reproductive isolation as a side effect of divergence (i.e. magic traits, Gavrillets 2004; Jiggins *et al.* 2004). Examples include beak size and vocal signature in Darwin's finches (Podos 2001), body size and colour in stickleback fish (Boughman 2001) and colour patterns in *Hypoplectrus* fish (Puebla *et al.* 2007). Pleiotropic effects of ecological traits on mating can be divided into three classes (Jiggins *et al.* 2004): *Pleiotropic assortment traits*, in which a single trait leads directly to assortative mating, the classic example being host choice in phytophagous insects; *Pleiotropic mating cues*, in which a cue used in mate choice diverges under ecological selection, such as butterfly colour patterns that are under selection for signalling to predators as well as to potential mates; and finally *pleiotropic mating preferences* in which adaptation of the sensory system leads to pleiotropic divergence in mate preference because of ecological selection (Jiggins *et al.* 2004).

Given the long-standing suggestion that hybridization might contribute to adaptively significant variation, either within species or in novel lineages (Stebbins 1959; Lewontin & Birch 1966; Templeton 1981; Arnold 1997, 2006), it seems probable that novel magic traits might also arise through hybridization. Thus, a hybrid lineage might colonize a new habitat by combining one or more ecological traits from two divergent species, and those novel traits would also confer reproductive isolation from the parents. The

hybrid characteristics must necessarily be controlled by two or more genes, such that both parents contribute adaptively significant variation to the novel lineage, but could involve just a handful of genetic loci. This process might also be facilitated by 'transgressive segregation' of an ecological trait. This is the phenomenon whereby hybrid trait values lie outside the range of the two parents, and is a surprisingly frequent occurrence (Rieseberg *et al.* 2003; Hegarty *et al.* 2008).

It might therefore be helpful to distinguish different modes of HHS. We propose the terms *hybrid trait speciation* and *mosaic genome hybrid speciation* to describe this distinction (figure 1). To summarize the main characteristics, hybrid trait speciation involves colonization of a novel adaptive peak through introgression of genes controlling adaptive traits (Mallet 2007). The novel adaptive trait must also confer a degree of reproductive isolation from the parental lineages to qualify as an example of HHS. In contrast, mosaic genome hybrid speciation involves stabilization of a hybrid genome, where the parental species differ in multiple loci conferring intrinsic hybrid incompatibility. Thus, the distinction is primarily based on the nature of selection acting during the establishment of the hybrid lineage (ecological versus intrinsic incompatibility), but the two models are also likely to differ in the proportion of the genome involved in hybridization. The former might involve just a handful of adaptively important genes introgressing into a related species, whereas the latter will commonly involve establishment of a hybrid lineage comprising similar proportions from two or more parental genomes (figure 1). These two modes of hybrid speciation are not so much mutually exclusive alternatives as opposite ends of a continuum and, indeed, in *Helianthus* hybrid lineages novel ecological characteristics are important as well as intrinsic incompatibility from their parents (Rieseberg *et al.* 2003).

Nonetheless, the motivation for highlighting this distinction is twofold. First, it is done to emphasize that hybridization can generate novel adaptive variation that might facilitate both colonization of novel habitats and speciation at a faster rate than through mutation alone, potentially accelerating the rate of adaptive radiation (Seehausen 2004; Mallet 2007). One possible example of this is the establishment of the apple race of *Rhagoletis pomonella* through introgression of genetic variation from more southerly populations (Feder *et al.* 2003). The colonization of this novel niche was facilitated by combining existing genetic variation rather than waiting for novel mutation. Second, it is also done to highlight the fact that detection of putative hybrid species will be challenging following hybrid trait speciation, as there will not necessarily be a strong signal of overall hybridity in the genome of the novel species. The difficulty of both defining and identifying HHS when only a small proportion of the genome is involved was also highlighted in a recent review (Mallet 2007). Thus, the study of a handful of neutral molecular markers will not necessarily give a strong signal of hybridization in the putative hybrid species. Instead it will require the study of the genetic basis of traits with a putative hybrid origin and, ideally, genealogical study of the genes controlling such

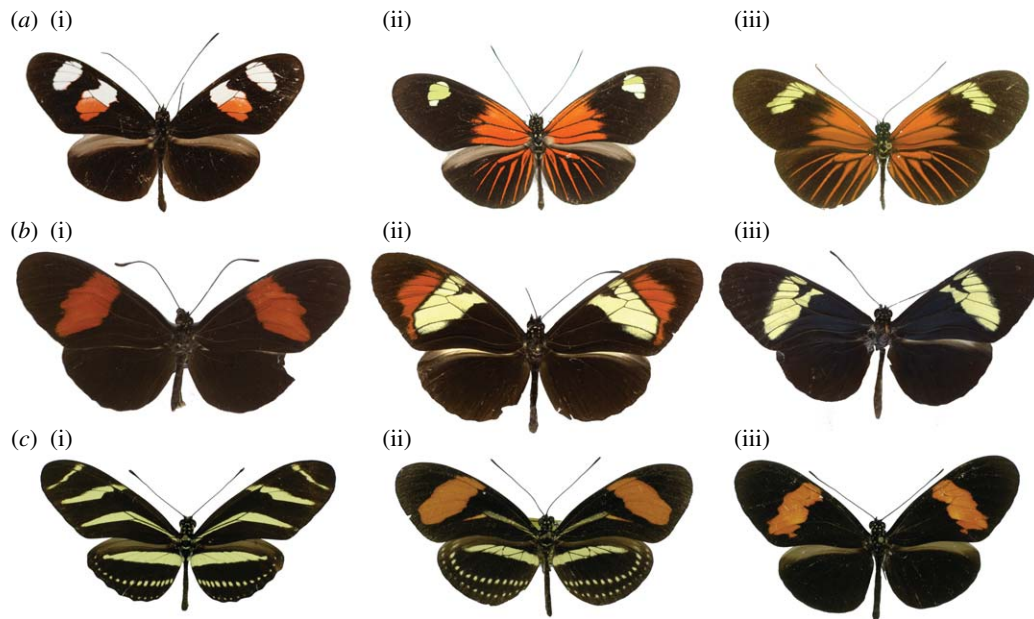


Figure 2. Some putative examples of hybrid patterns in *Heliconius* and their progenitors. (a) (i) *Heliconius erato notabilis*, (ii) *Heliconius erato etylus* and (iii) *Heliconius erato emma*. The position of the forewing spot in *H. erato etylus* is apparently homologous to that of the most distal spot of *H. erato notabilis*. (b) (i) *Heliconius melpomene melpomene*, (ii) *Heliconius heurippa* and (iii) *Heliconius cydno cordula*. Crosses have shown that the *H. heurippa* pattern can be recreated by crossing the other two forms. (c) (i) *Heliconius charithonia*, (ii) *Heliconius hermathena renatae* and (iii) *Heliconius erato hydara*. *Heliconius hermathena* has yellow bands and spots similar to that of *H. charithonia* and a red band similar to that of *H. erato hydara*.

traits—a difficult task but one that is becoming increasingly feasible (Colosimo *et al.* 2005). Furthermore, the key distinction between hybrid trait speciation and merely adaptive introgression is the role of the novel trait in reproductive isolation, which needs to be demonstrated experimentally. Nonetheless, we strongly feel that there is a need to move away from studying putative hybrid species at neutral markers, and for a stronger emphasis on documenting the hybrid origin of the adaptive traits that allow those lineages to persist.

(b) Hybrid speciation in *Heliconius* butterflies

Perhaps because of the abundance of natural hybrids, there has long been an emphasis on the potential importance of hybridization in the evolution of these brightly coloured butterflies (Linares 1989; Gilbert 2003; Mallet 2005, 2007). *Heliconius* are known for their bright warning colour patterns and mimicry between divergent species, and the genetic basis of these diverse patterns has been widely studied through laboratory hybridization over many years. Furthermore, natural hybrid zones are common and provide a huge natural diversity of phenotypes, which have been avidly sought after by collectors. One of the observations made as a result of field and laboratory studies has been that the phenotypes of extant taxa often show marked similarity to hybrids between related species (figure 2). This has led to the suggestion by several *Heliconius* biologists that hybridization may play a significant constructive role in the generation of novel pattern diversity.

Natural hybridization is known to occur between races and species and shows an exponential decline with genetic distance (Mallet 2007). This demonstrates that speciation is a gradual process of decreasing compatibility and that introgression can potentially occur long

after speciation is considered complete (Mallet 2008). Putative hybrid entities occur at all levels of this continuum. At a racial level, the forewing yellow spot of *Heliconius erato etylus* appears homologous in location to the white spot found slightly further north in the race *Heliconius erato notabilis* (J. Mallet 1994, personal communication, figure 2). Thus *H. erato etylus* is putatively a hybrid between *H. erato notabilis* and the rayed Amazonian form *Heliconius erato lativitta* (see figure 2). This is supported by recent crossing experiments showing that in both *Heliconius erato* races this element is controlled by the same genetic locus, known as *Sd* (Papa *et al.* submitted). At the other end of the scale, the species *Heliconius hermathena* has a pattern that shows marked similarity on the hindwing to *Heliconius charithonia*, but on the forewing it has a red band similar to sympatric *H. erato* (figure 2). *Heliconius charithonia* and *H. erato* are rather distantly related, but a single hybrid specimen is known from Mexico (Mallet 2007); so, it is not implausible that *H. hermathena* might have arisen through hybridization between these two species or their ancestors. Furthermore, *H. hermathena* has mixed patterns of relationships in phylogenetic studies, and an unusual pupal morphology for the *H. erato* group (Brown & Benson 1977). This mixed phylogenetic signal might be further indicative of a hybrid origin (Beltrán *et al.* 2007), although an alternative hypothesis that has been proposed is that *H. hermathena* is ancestral to the *H. erato* and *H. charithonia* lineages (Brown & Benson 1977).

The best-studied examples, however, are in the *Heliconius cydno* species complex. *Heliconius melpomene* and *H. cydno* are closely related species that hybridize both in the wild and the laboratory (Mallet 2007). They occur sympatrically in Central America and in the Andes, although they are ecologically and altitudinally

segregated to some degree (Smiley 1978; Estrada & Jiggins 2002). While female F_1 hybrids are sterile, males are fertile and can be used to introgress genes between the two species in the laboratory. Colour pattern genes, such as the yellow hindwing bar controlled by the *Yb* gene, appear homologous between the two species on the basis of identical gene action and linkage (Linares 1996; Naisbit *et al.* 2003; Joron *et al.* 2006). For example, when *Heliconius melpomene vulcanus* and *H. cydno* from west Colombia are crossed, the *H. melpomene* yellow bar behaves as another allelic variant at the same locus in the *H. cydno* genome (Linares 1989). The *H. cydno* complex actually consists of several species that are parapatric replacements of one another. All three geographical isolates of *H. cydno*, namely *Heliconius pachinus* found on the Pacific slopes of Costa Rica and western Panama, *Heliconius heurippa*, found on the eastern slopes of the Andes near Villavicencio in Colombia (figure 2), and *Heliconius timareta*, which occurs in the eastern Andes of Ecuador, have been suggested at some time as hybrid entities (Linares 1989; Mallet 1999; Gilbert 2003; Salazar *et al.* 2005). All three species are ecologically and genetically similar to *H. cydno* but share pattern elements with nearby races of *H. melpomene*, putatively acquired by introgression.

(c) Neutral marker surveys of introgression

The first major line of evidence demonstrating the evolutionary importance of hybridization are surveys of neutral genetic variation, which suggest widespread introgression in the wild even between fairly distantly related species (Bull *et al.* 2006; Kronforst *et al.* 2006b; Kronforst 2008). For example, strong positive evidence for introgression was found at one of the four loci sequenced in sympatric *H. melpomene* and *H. cydno* populations from Panama (Bull *et al.* 2006). An allopatric population of *H. melpomene* from French Guiana provided a natural control, showing that genetic similarity in sympatry was not simply a result of a slow evolutionary rate for a particular locus. A complementary genome-wide approach using large numbers of amplified fragment length polymorphism markers, in addition to sequence data for 16 genes, showed similar evidence for recent and persistent introgression between the same two species in Costa Rica (Kronforst *et al.* 2006b). These studies provide confirmation that hybrids are not an evolutionary dead end—hybridization is a genuine source of novel genetic variability.

However, such studies of putatively neutral genetic variation do not directly address the hypothesis proposed originally, which is that novel adaptive colour patterns have arisen through hybridization. The basic problem is that genetic markers used are not themselves informative regarding the origin of the colour pattern phenotype.

(d) Laboratory reconstruction of extant patterns

A better approach is to directly study the phenotypes. The easiest way to do this is through laboratory crosses. For example, the broad black band that divides the forewing yellow pattern into two thin bands in *H. pachinus* can be recreated by introgression of a forewing ‘shutter’ gene from *Heliconius melpomene*

rosina into *Heliconius cydno galanthus* (Gilbert 2003). Similarly, the unusual all-black forewing phenotype of *Heliconius cydno weymeri* can be created by introgression of genetic variation from *Heliconius melpomene vulcanus* into *H. cydno* races from the northern Cauca valley (Colombia; Linares 1989; Gilbert 2003). In Ecuador the variation observed in the polymorphic *Heliconius cydno alithea*, which mimics *Heliconius sapho* and *Heliconius eleuchia*, has also been recreated in the crosses between *H. melpomene* and *H. cydno* from Costa Rica (Gilbert 2003).

Moreover, a recent study has demonstrated a route for the formation of *H. heurippa*, by reconstructing its pattern through laboratory crosses of *H. melpomene* and *H. cydno* (Mavárez *et al.* 2006). Earlier crossing experiments had suggested that the *H. heurippa* pattern could arise through hybridization of these species, which led to experiments specifically with the races geographically adjacent to the current range of *H. heurippa* (Linares 1989). The colour pattern of *H. heurippa* essentially consists of the N^N allele from *H. cydno* controlling the yellow forewing band, combined with the *B* and *br* alleles from *H. melpomene* controlling the red forewing band (*B*), hindwing red spots and the absence of forceps-shaped brown marking on the hindwing ventral surface (*br*). These loci have to be combined on an *H. cydno* genetic background in order for the full expression of the yellow band; hence, the reconstruction involved backcrossing with *H. cydno*. The *B* and *br* loci are tightly linked, which facilitates their introgression into *H. cydno*, and led to the surprising result that this reconstruction only required three generations of crosses. *Heliconius heurippa* shares one other pattern trait with *H. melpomene*, whose genetics is not well understood, namely the lack of structural blue iridescent colour. The reconstructed pattern is remarkably similar, but not an exact match to wild *H. heurippa*—the only difference being a thin black line between the red and yellow bands, which is only occasionally faintly seen in laboratory hybrids, perhaps implying additional pattern evolution subsequent to the speciation event.

Although such a reconstruction experiment in itself cannot prove a hybrid origin for *H. heurippa*, this hypothesis is strongly supported by the geographical distribution of the species involved. Of all the genetic variation present in *H. melpomene* and *H. cydno*, natural overlap between the red forewing band of *H. melpomene* and the yellow forewing of *H. cydno* only occurs geographically adjacent to the current range of *H. heurippa*. Nonetheless, there are two alternative hypotheses to hybrid origin that should be considered. First, the *H. heurippa* pattern might be ancestral and have given rise to *H. melpomene* and *H. cydno* lineages that inherited different aspects of the ancestral pattern. This seems unlikely as the *H. heurippa* pattern is not seen in any other species in the genus, and is not a plausible ancestor in any parsimony or likelihood ancestral state reconstructions based on a recent phylogeny of the genus (Beltrán *et al.* 2007; C. D. Jiggins 2006, personal observation). Second, the pattern alleles might have arisen through mutation rather than hybridization. In principle it might be possible to estimate the

relative probability of a particular pattern arising through mutation versus hybridization. We have never observed novel pattern mutants in many thousands of lab-reared butterflies and the probability of them arising and being convergent to patterns found locally in related species is low. Of course *Heliconius* do frequently evolve convergent patterns, but this is driven by mimicry selection, which does not apply to the non-mimetic *H. heurippa*. This contrasts with the ease of generating novel hybrid patterns through laboratory crosses (see above). In the wild the relative probability of hybrids versus novel pattern mutants is harder to estimate, but the great majority of 'aberrant' wild phenotypes are most readily explained as hybrids (Mallet *et al.* 2007). Furthermore, a large proportion of known wild hybrids are between *H. melpomene* and *H. cydno*. It seems probable that novel patterns generated through hybridization are an order of magnitude more common than those generated through mutation. On balance, the relative frequency of occurrence of hybrids versus novel mutants in both laboratory and wild populations provides further evidence to support the hybrid origin hypothesis for the formation of *H. heurippa*.

In summary, therefore, the genetic data show that hybrid introgression is rife between *Heliconius* species, and provide the most probable scenario for the origin of the *H. heurippa* pattern. Ultimately, however, the positional cloning of loci controlling pattern diversity offers the potential to reconstruct the genealogical history of colour pattern alleles and flanking markers and, hence, estimate directly the importance of hybridization in pattern evolution.

(e) *The origin of reproductive isolation*

Importantly, what makes *H. heurippa* a possible case of hybrid speciation, rather than merely adaptive introgression, is that the hybrid colour pattern itself has a direct influence on reproductive isolation. Previous work has shown that species in this group use colour in mate recognition (Jiggins *et al.* 2001; Kronforst *et al.* 2006a). This was confirmed with mate choice experiments on *H. heurippa* and adjacent races of *H. melpomene* and *H. cydno*. Males of all three species are about half as likely to approach the colour pattern of another species. Furthermore, when the *H. heurippa* pattern was manipulated, both red and yellow elements were necessary to stimulate approach and courtship by *H. heurippa* males (Mavárez *et al.* 2006). Thus, the novel colour pattern contributes to partial reproductive isolation from both the parental lineages, as required by our definition of hybrid speciation.

This strong preference for the hybrid pattern in the *H. heurippa* lineage raises the question of how such a preference would have arisen. There are essentially two scenarios—either the mating preference came about through evolution of mate-finding subsequent to the establishment of the novel colour pattern as a 'race' of *H. cydno*, or it arose as a direct result of the hybridization event. The former is fairly trivial to envisage, but the latter would imply a greater and more direct role for hybridization in speciation and seems less likely *a priori*. Under the first hypothesis, hybridization plays a role basically equivalent to mutation at multiple loci in generating a novel colour pattern phenotype. Under the

second hypothesis there is a more central role for hybridization in producing a genetic association between the novel colour pattern and mate preferences in the novel lineage. In support of the latter hypothesis, earlier work indeed suggests that F₁ hybrids between *H. melpomene* and *H. cydno* actually prefer to mate with each other rather than their parents (Naisbit *et al.* 2001). Furthermore, a recent quantitative trait locus analysis of the genetics of mate preference in *H. cydno* and *H. pachinus* showed a genetic association between a colour pattern shift (from yellow to white) and the preference for that pattern (Kronforst *et al.* 2006a). This might imply that preference genes could be introgressed between species alongside patterning genes. Between them, these studies imply that it is plausible to imagine that hybrids in which a novel red pattern element was introgressed into an *H. cydno* genome might actually also show a mate preference for their novel pattern. This remains to be demonstrated experimentally.

(f) *Patterns of post-mating isolation*

One of the major barriers to HHS is hybrid breakdown. In the case of *H. melpomene* and *H. cydno*, F₁ female hybrids are sterile, while males are fully fertile (Naisbit *et al.* 2002). Sterility segregates in backcross broods and provides a partial barrier to introgression. Nonetheless, as described previously it is possible to introgress colour pattern genes from one species into another and therefore overcome the sterility barrier. In addition, numerous backcross hybrids have been collected in San Cristobal, Venezuela, and shown to be genetically indistinguishable from *H. cydno* at microsatellite markers. This clearly demonstrates multiple generations of natural backcrossing and shows that this hybrid sterility can be overcome in the wild as well. *Heliconius heurippa* itself is completely compatible with *H. cydno*, but shows unidirectional F₁ female hybrid sterility with *H. melpomene*—in other words female offspring of a male *H. melpomene* crossed with a female *H. heurippa* are sterile, while offspring of the reciprocal cross are fully fertile (Salazar *et al.* 2005). This is in contrast to the bi-directional hybrid sterility between *H. melpomene* and *H. cydno* (although male *H. cydno* rarely mate with female *H. melpomene*, so crosses in this direction are very poorly documented). The pattern is therefore compatible with a hybrid origin, as *H. heurippa* is somewhat intermediate between the other two species in the expression of hybrid sterility. Nonetheless, the same pattern could equally be generated by an entirely bifurcating speciation scenario, in which additional incompatibility alleles were acquired along the *H. cydno* lineage subsequent to divergence of *H. heurippa*. These data do not therefore provide positive support for hybrid speciation, but are consistent with the hypothesis.

(g) *Evidence for hybrid genomes?*

Unfortunately, the large amount of shared neutral genetic variation between related species actually makes it difficult to identify hybrid taxa. A study of *H. pachinus* failed to show any positive evidence for a hybrid origin at neutral loci, despite identifying a substantial number of markers diagnostic for *H. melpomene* and *H. cydno*. Instead, *H. pachinus* appears as a divergent form of

H. cydno, consistent with a bifurcating origin for this species (Kronforst *et al.* 2007). The power of such a study is weakened however when the putative parents themselves share such a large amount of genetic variation. The *H. melpomene* and *H. cydno* genomes are clearly far less differentiated than, for example, those of *Helianthus annuus* and *Helianthus petiolaris* (Ungerer *et al.* 1998).

The pattern in *H. heurippa* is more unusual, with sequence data for two loci, fragments of the genes *Invected* and *Distalless*, showing a striking pattern in which *H. melpomene* and *H. cydno* are differentiated, but *H. heurippa* shares allelic variation with both the species. Of the three further loci sampled since, one showed a similar pattern, the other showed the three species intermixed and the third showed the three species mutually differentiated (Salazar *et al.* 2008). This data initially led us to state that *H. heurippa* has a hybrid genome (Mavárez *et al.* 2006, in which this result was erroneously attributed to Salazar *et al.* 2005). However it actually seems probable that this allele sharing can also be explained by ongoing introgression between the three species. Thus, *H. melpomene* and *H. cydno* sampled from Costa Rica also show evidence for introgression at the *Distalless* locus, with a strong frequency difference between the species but a few very similar shared alleles (Kronforst *et al.* 2006b). *Heliconius heurippa* is likely to show higher levels of introgression with *H. melpomene* and *H. cydno* than occurs between those two species, given the intermediate colour pattern and, hence, mating signal.

Distinguishing the alternate hypotheses of a branching speciation event from that of hybrid founding, with the likelihood of ongoing gene flow under either scenario, is a challenge. Previous models of divergence population genetics were designed for the study of two species rather than three and primarily developed to distinguish shared ancestral polymorphism from introgression (Wakeley 2000). Our own attempts to develop a coalescent model incorporating three species and the possibility of either branching or hybrid founding speciation have failed to provide a good estimate of parameter values for *H. heurippa* based on our sequence data (J. Taylor 2007, personal communication). The only finding was that parameter estimates for introgression between the species were consistently non-zero. In the future we aim to focus our sequencing efforts on regions surrounding colour pattern loci in order to directly test the hypothesis that the *B* locus has been introgressed from *H. melpomene* into the *H. cydno* genome. In summary, the data are probably consistent with either a hybrid founding or a bifurcating origin for *H. heurippa*, and more than anything provide evidence for ongoing introgression.

The evidence for hybrid origin is based largely on the three Mendelian loci that control colour pattern and their direct role in reproductive isolation through mate choice. Thus, our basic hypothesis for the origin of *H. heurippa* argues for the role of a hybrid trait in the speciation event, but makes no specific prediction regarding the rest of the genome. In the recently discovered Venezuelan hybrid zone between *H. melpomene* and *H. cydno*, the hybrids are all indistinguishable from pure *H. cydno* at 12 microsatellite loci (Mavárez *et al.* 2006) and must therefore represent greater than fifth

generation backcrosses to this species. If a similar scenario were involved in the origin of *H. heurippa*, then it would be very difficult to detect a significant signal of the hybrid founding event—the genome would primarily be derived from *H. cydno* despite the crucial role of introgression of the patterning genes in the formation of the novel lineage. We therefore propose *H. heurippa* to be an example of hybrid trait speciation.

2. CONCLUSIONS

We primarily envisage the role of hybridization as a means of instantaneously generating novel variants at multiple genetic loci, providing the raw material for the establishment of lineages with adaptive potential (Seehausen 2004). While the adaptive traits in question also contribute to reproductive isolation with parental lineages, this can lead to hybrid trait speciation. In the case of *H. heurippa*, there is evidence from laboratory crosses and the biogeographic distribution of colour patterns that its pattern has arisen through hybridization. Crucially, the colour pattern is also involved in mate choice and leads to reproductive isolation between *H. heurippa* and its close relatives. We predict that the new era in which the genetic basis of adaptive traits can be understood at the DNA sequence level will increasingly uncover examples of the role of hybridization in the generation of novel adaptive diversity and species.

We would like to thank the Royal Society, BBSRC and Leverhulme Trust for funding to C.D.J.; M.L. and C.S. were funded by the Fondo Colombiano de Investigaciones Científicas y Proyectos Especiales Francisco Jose de Caldas COLCIENCIAS grants 1204-05-11414 and 7155-CO, Banco de la Republica and private donations from Continuos, S. A., Proficol El Carmen, S. A., Didacol, S. A. and Arango, F. Colombia. We thank O. Delgado, Miza-UCV, for the photographs of Venezuelan specimens.

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