ASSORTATIVE MATING PREFERENCES AMONG HYBRIDS OFFERS A ROUTE TO HYBRID SPECIATION

Maria C. Melo,[1,2] Camilo Salazar,[3,4] Chris D. Jiggins,[5,6] and Mauricio Linares[1,7]

[1]*Instituto de Genética, Universidad de los Andes, Carrera 1E No 18ª–10, P.O.Box 4976, Santafé de Bogotá D.C., Colombia.*

[2]*E-mail: mar-melo@uniandes.edu.co*

[3]*Smithsonian Tropical Research Institute. Apartado 0843-03092, Panamá, República dePanamá*

[4]*E-mail: salazarc@si.edu*

[5]*Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ. United Kingdom*

[6]*E-mail: c.jiggins@zoo.cam.ac.uk*

[7]*E-mail: mlinares@uniandes.edu.co*

Homoploid speciation generates species without a change in chromosome number via introgressive hybridization and has been considered rare in animals. *Heliconius* butterflies exhibit bright aposematic color patterns that also act as cues in assortative mating. *Heliconius heurippa* has a color pattern that can be recreated by introgression of the *H. melpomene* red band into an *H. cydno* genetic background. Wild *H. heurippa* males show assortative mating based on color pattern and we here investigate the origin of this preference by studying first-generation backcross hybrids between *H. melpomene* and *H. cydno* that resemble *H. heurippa*. These hybrids show assortative mating preferences, showing a strong preference for their own color pattern over that of either parental species. This is consistent with a genetic basis to wing pattern preference and implies, first, that assortative mating preferences would facilitate the initial establishment of a homozygous hybrid color pattern by increasing the likelihood that early generation hybrids mate among themselves. Second, once established such a lineage would inherit assortative mating preferences that would lead to partial reproductive isolation from parental lineages.

KEY WORDS: Homoploid speciation, introgression, mating preference, pleiotropy, transgressive segregation.

Homoploid hybrid speciation is the establishment of a reproductively isolated lineage through hybridization without a change in chromosome number (Coyne and Orr 2004). It is therefore distinct from the numerous examples of hybrid speciation through allopolyploidization in plants (Ramsey and Schemske 2002). Although skepticism about the importance of this process in animal speciation remains, several cases of homoploid hybrid speciation have recently been reported (Mallet 2007; Mavárez and Linares 2008).

The best-studied case of homoploid hybrid speciation is in *Helianthus* sunflowers. Large blocks of the genome of *Helianthus anomalus* are derived in similar proportions from each of its puta-tive parental species (Ungerer et al. 1998). In this case, genes involved in epistatic hybrid incompatibilities contribute to isolation of the hybrid lineage from its parental taxa (Buerkle et al. 2000). Moreover, the generation of novel ecological traits through hybridization has also facilitated establishment of the hybrid linage (Rieseberg et al. 2003). In contrast to the situation in *Helianthus*, hybrid species might also form through introgression of just a few loci from one species into another. If such introgression were to generate a novel adaptive trait(s) with pleiotropic effects on reproductive isolation it could lead to rapid speciation (Jiggins et al. 2008). We have recently termed this model "hybrid trait speciation" (Jiggins et al. 2008). This process might play an

important role in recent radiations in which genetic incompatibilities between hybridizing species are absent. In effect, "hybrid trait speciation" is an extension of the "magic trait" model of speciation, in which divergence in a single trait under ecological selection also causes reproductive isolation as a side effect, facilitating speciation (Gavrilets 2004). Examples include beak morphology in Darwin finches, which is correlated with vocalizations, shifts in color patterns and female preferences in sticklebacks, and disruptive selection in predatory mimicry associated with assortative mating in hamlet fish (Boughman 2001; Podos 2001; Puebla et al. 2007).

Heliconius butterflies are known for their aposematic color pattern diversity and usually form Müllerian mimicry rings both with other *Heliconius* species and more distantly related taxa (Jiggins et al. 2001). The genetic basis of these color patterns can be studied through artificial hybridization (Naisbit et al. 2003), and such experiments show that few loci of major effect and some modifiers that improve the mimetic resemblance are involved in controlling color pattern diversity (Naisbit et al. 2003). Natural hybridization and gene flow can occur at various levels of divergence, from races through species (Mallet et al. 2007), suggesting that hybridization could have a role in shaping diversity in this genus.

Helianthus cydno and *H. melpomene* are good examples of naturally hybridizing species (Mallet et al. 2007). Although sympatric across much of their range, they show distinct ecology and color pattern phenotype (Estrada and Jiggins 2002). *Heliconius melpomene* usually has a red and yellow color pattern and mimics *H. erato*, whereas *H. cydno* has white and yellow patterns and mimics species in the sara-sapho clade. This shift in color pattern due to mimicry also acts as a cue in mating preference between these species (Jiggins et al. 2001). Interestingly, three species belonging to the *H. cydno* species complex, *H. timareta, H. pachinus*, and *H. heurippa* have all been proposed as hybrid species (Linares 1989; Mallet 1999; Gilbert 2003). In particular, the color pattern of *H. heurippa* was reconstructed by laboratory crosses between the adjacent geographical races of *H. cydno* and *H. melpomene* (Mavárez et al. 2006). *Heliconius cydno cordula* and *H. melpomene melpomene* have yellow and red bands, respectively, and are found in sympatry near to the current range of *H. heurippa* (Mavárez et al. 2006). Previous work has shown that *H. heurippa* uses its color pattern as a cue in assortative mating leading to reproductive isolation (Mavárez et al. 2006). However, nothing is known of the origin of this mate preference. It has been speculated that the distinctive mate preferences of *H. heurippa* might have arisen subsequent to the establishment of the novel color pattern, in populations already fixed for the *H. heurippa* pattern (Mavárez et al. 2006). Alternatively, combining mate preference alleles inherited from the two parental species might have led to the establishment of the novel pattern of assor-

tative mating more directly through the process of hybridization. Here we investigate the latter possibility by testing the mating preference of first-generation backcross hybrids with a color pattern similar to that of *H. heurippa*.

Methods

EXPERIMENTAL POPULATIONS AND RECONSTRUCTION OF *HELICONIUS HEURIPPA*

Heliconius melpomene melpomene, *H. heurippa*, and *H. c. cordula* were collected in Virgen de Chirajara (Colombia, 4°12′48′′N, 73°47′70′′W), Buenavista (Colombia, 4°10′30′′N, 73°40′41′′W) and San Cristobal (Venezuela, 7°47′56′′N, 72°11′56′′W), respectively. These sites are located in the eastern Colombian Andes. Experimental populations for each species were established from field mated females in $2 \times 3 \times 2$ m insectaries in La Vega, Colombia following methods described previously (Jiggins et al. 2001). Here we study first-generation backcross hybrids that phenotypically resemble *H. heurippa*. First, a female *H. c. cordula* was mated with an *H. m. melpomene* male. F_1 males were then backcrossed to *H. c. cordula* females and the phenotypic class of backcross offspring most resembling *H. heurippa* was used for mate choice experiments. The selected hybrids had $Bb\ N^N N^N\ Brbr$ color pattern genotypes (following the Mavárez et al. 2006 loci nomenclature), being heterozygous for the red forewing band (B), homozygous for the yellow forewing band (N), and heterozygous for the brown pincer-like mark (Br, see Mavárez et al. 2006). Note that these are not the "reconstructed" *H. heurippa* phenotypes reported previously that are genetically homozygous for all major color pattern alleles, and they differ somewhat from *H. heurippa* in possessing the heterozygous brown pincer mark on the underside of the hindwing (see fig. 2 in Mavárez et al. 2006). Nonetheless, we are specifically interested in these individuals for two reasons. First, the most direct route to generation of a homozygous *H. heurippa* pattern is through intercrossing these phenotypes, so we are interested in the probability that these phenotypes would mate in the wild (Mavárez et al. 2006). Although the relative probability of establishment of this particular pattern would require knowledge of mating preferences among all hybrid phenotypes, nonetheless the establishment of any hybrid pattern would be facilitated if hybrids mate assortatively. Most importantly however, these are the first generation of hybrids to show the combined red and yellow forewing band characteristic of *H. heurippa*, so we are interested in the establishment of assortative mating preferences among individuals that possess this phenotype.

COLOR PATTERN MODELS

In butterflies males generally search for females using visual cues (Rutowski 1991; Vane-Wright and Boppré 1993). In particular,

Heliconius males are attracted by bright colors at distances of 20 m or more. Thus, males appear adapted to use bright color patterns as a long-range attraction signal to detect mates (Jiggins et al. 2001, 2004). At close quarters, males also show preferences based in part on color pattern, in general preferring their own color pattern. In addition, pheromonal and/or tactile signals are also important at a close range (Jiggins et al. 2001; Jiggins et al. 2004).

The preference of Heliconius heurippa *for the hybrid color pattern*

Five males of *H. c. cordula* were exposed to female wing pattern models in 2 × 2 × 2 m insectaries. In each trial, one model was a control, having the same pattern as the males being tested. The other corresponded to the experimental color pattern, in this case the hybrid wings described above. Models were made from dissected natural wings and hung by transparent nylon lines in the center of spherical area of 60 cm (Jiggins et al. 2001). Entering to the sphere was considered an approach, whereas a sustained fluttering directed toward the model was a courtship (Jiggins et al. 2001). Approaches and courtships were recorded in 25 replicates of 5 min each. Males were replaced in each replica. This experiment was repeated for *H. heurippa* and *H. m. melpomene* males.

Reconstructed Heliconius heurippa *mating preference*

The backcross hybrid males obtained here were then tested for their preference using the same experimental design except that individuals were tested individually (i.e., each reconstructed male was tested with its own pattern and with a different pattern at the same time). Hybrid males were separately tested for their preference for *H. heurippa*, *H. c. cordula*, and *H. m. melpomene* patterns, relative to a control (see results for details).

Statistical analysis

For both experiments the corresponding probabilities of approach and courtship were calculated using a likelihood. The model used here was developed and maximized following Jiggins et al. (2001) and Mavárez et al. (2006). For all experiments, a probability value of one was assigned to the control color pattern model with the same pattern as the males being tested, with the objective being to estimate relative probabilities of experimental approach and courtship. A likelihood-ratio test (LRT) with $G = 2\Delta \log_e L$, which asymptotically follows a χ^2 distribution (Edwards 1972), was used to determine whether probabilities of approach/courtship directed toward the experimental model differed from probabilities of approach/courtship directed toward the control pattern model.

Results

Heliconius heurippa males did not distinguish between their own color pattern and that of the backcross hybrid model, that is the

laboratory created $BbN^N N^N Brbr$ hybrid with the pattern similar to *H. heurippa* (hereafter the backcross model, Fig. 1). Neither approach nor courtship probabilities differed between control and experimental patterns ($G_1 = 1.363, P > 0.05$ and $G_1 = 0.981, P > 0.05$, respectively). In contrast both *H. melpomene* and *H. cydno* males were significantly less likely to approach the backcross model as compared to their own pattern ($G_1 = 67.736, P < 0.05$ and $G_1 = 32.825, P < 0.05$, respectively). This reflects the pattern seen when these two species were evaluated for their courtship behavior toward the wild *H. heurippa* color pattern (Mavárez et al. 2006). Assortative mate preferences for courtship behavior were even stronger than that for approach (Fig 1; $G_1 = 60.64, P < 0.05$ and $G_1 = 51.741, P < 0.05$ respectively).

Sixteen reconstructed *H. heurippa* individuals were obtained from seven F_1 males backcrossed to *H. cydno* females. A total of 881 approaches and 132 courtships were recorded when these individuals were tested. Unsurprisingly, these individuals do not distinguish *H. heurippa* and backcross models ($G_1 = 0.397, P > 0.05$ and $G_1 = 0.581, P > 0.05$, respectively). Subsequently, *H. heurippa* models were used as controls in experiments with these hybrids that involve parental species models (approach and courtship data are shown in Table 1). In both experiments, the backcross hybrid males were significantly less likely to approach either parental pattern as compared to that of *H. heurippa* ($G_1 = 90.302, P < 0.05$ and $G_1 = 7.586, P < 0.05$ for approach and courtship of *H. cydno*, respectively and $G_1 = 65.267, P < 0.05$ and $G_1 = 14.699, P < 0.05$ for approach and courtship of *H. melpomene*, respectively). In addition, there was significant heterogeneity between individuals in their response to the parental patterns, ($G_{15} = 139.6, P < 0.05$ and $G_{15} = 95.0, P < 0.05$ for experiments with *H. cydno* and *H. melpomene*, respectively). Nonetheless, all individuals showed more approaches to the *H. heurippa* pattern so the direction of preference was consistent between individuals. In contrast, there was no heterogeneity among this same set of individuals when tested against *H. heurippa* vs. backcross hybrid models ($G_{15} = 5.4$, NS).

Discussion

We have shown that the first-generation backcross hybrids between *H. cydno* and *H. melpomene* that phenotypically resemble *H. heurippa* (*Bb $N^N N^N$ Brbr* color pattern genotypes) show similar mate preferences to wild *H. heurippa*. In addition, the parental species, *H. cydno* and *H. melpomene*, discriminate against the color pattern of these first-generation backcross hybrids in a manner similar to their response to wild *H. heurippa*. This suggests that hybrid offspring of interspecific crosses could become established as populations partially reproductively isolated from their parental species very rapidly, and therefore offers insights into the
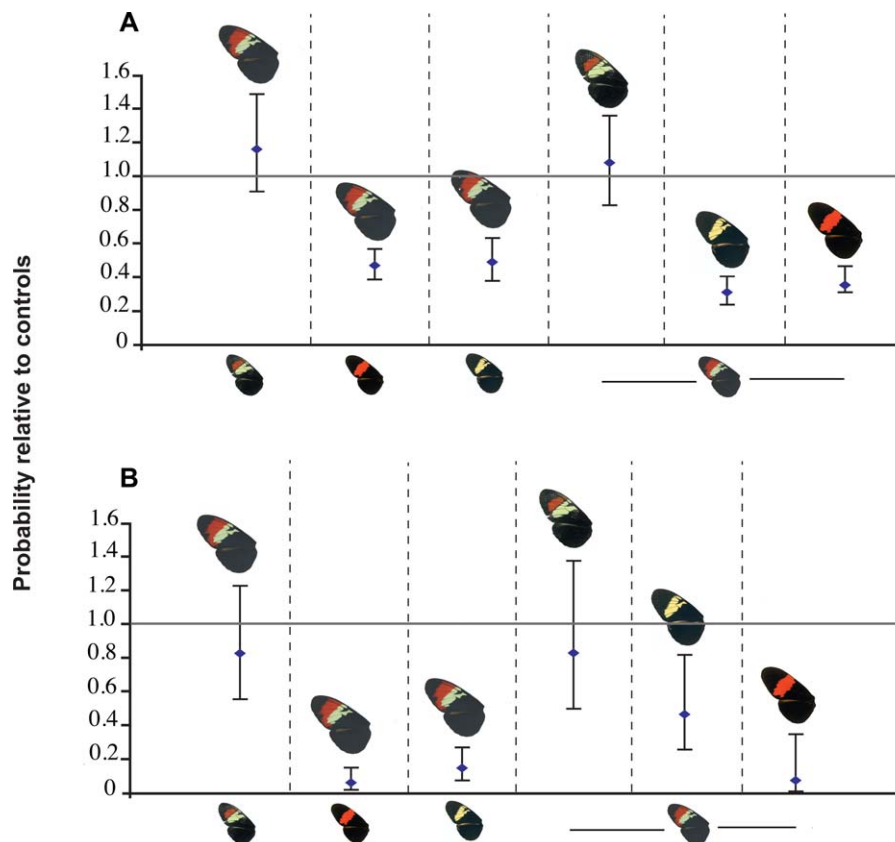
Figure 1. Results of mate preference experiments. The tested males are shown along the x-axis and represent *H. heurippa*, *H. melpomene melpomene*, *H. cydno cordula*, and the backcross hybrids, respectively. The y-axis shows the relative probabilities of (A) approach and (B) courtship toward the experimental model relative to that to a control with the same color pattern as the males being tested, which is set to 1 (horizontal line). Probabilities were calculated using likelihood and bars show support limits, equivalent to 95% confidence intervals. Both charts are divided into columns by dashed lines. The three first columns show the approach/courtship probabilities to the reconstructed *H. heurippa* model of *H. heurippa*, *H. melpomene*, and *H. cydno* males, respectively. The three last columns show the approach/courtship probabilities of the backcross hybrid males to *H. heurippa*, *H. cydno* and *H. melpomene*, models, respectively.

route by which *H. heurippa* could have become established as a hybrid species.

Although we show strong mating preferences among hybrid individuals, our experiments do not resolve exactly how these came about. It seems most likely that preferences have a genetic basis, but we cannot rule out a learned response given our current data. Nonetheless, previous experiments have shown no evidence that *H. melpomene* males learn their color pattern preferences from contact with other individuals (Jiggins et al. 2004). It remains possible that individuals might be able to somehow determine their own color pattern and thereby learn an assortative mating preference, although this is perhaps unlikely. Previous experiments in which males were allowed to emerge in the dark and prevented from visual inspection of their own patterns had no effect on mating behavior (Crane 1955). In addition, *H. cydno* and *H. pachinus* are predominantly white and yellow, respectively. Their F_1 hybrids are phenotypically similar to the *H. cydno* parents, but show intermediate mate preferences as expected for a geneti-

cally determined, rather than a learned response (Kronforst et al. 2006). The genetic mapping of preference would otherwise be consistent with individuals learning to prefer their own patterns, which would make "preference" a direct pleiotropic effect of color pattern phenotype (Kronforst et al. 2006). Similarly, F_1 hybrids between *H. melpomene rosina* (red forewing band) and *H. cydno chioneus* (white forewing band) from Panama prefer mating with themselves more frequently than with either species, despite being phenotypically most similar to *H. melpomene* (Naisbit et al. 2001). Hence, the most likely explanation for the hybrid mate preferences observed here is that they have a genetic basis, such that genes for preferring yellow from *H. cydno*, and those for preferring red from *H. melpomene* combine to produce the hybrid mate preference for a yellow and red pattern. This could be considered a form of transgressive segregation, whereby hybrid trait values lie outside the range of the two parents (Mallet 2007). Hence, a new aposematic signal coupled with a novel mating preference could have been rapidly generated by hybridization.

Table 1. Results of experiments on individual backcross males involving pure species models. The number of approach (App.) and courtship (Co.) events to control and experimental males are shown.

Male ID	*H. cydno* model				*H. melpomene* model			
	cydno		control		melpomene		control	
	App.	Co.	App.	Co.	App.	Co.	App.	Co.
1	5	0	14	3	4	0	10	1
9	3	0	3	0	2	1	6	1
8	2	1	9	2	6	0	9	0
10	8	0	13	1	1	0	7	0
21	2	0	12	1	2	0	14	0
3	2	0	17	0	6	0	11	1
4	2	0	12	0	3	0	11	1
5	4	1	8	7	4	0	11	0
16	3	2	5	3	2	0	5	5
104	5	0	28	2	6	0	20	1
103	2	0	15	0	2	0	14	0
105	4	10	0	10	0	0	12	0
10–1	2	0	23	0	2	0	10	0
10–2	16	2	27	6	13	0	27	4
10–3	5	1	36	1	12	0	15	0
10–4	7	0	12	1	5	0	18	1
Total	72	17	234	37	70	1	200	15
Mean	4.50	1.06	14.6	2.31	4.38	0.063	12.50	0.938
SD	3.60	2.49	9.68	2.94	3.67	0.250	5.59	1.48

Further crosses will be required to determine the genetic basis of preference that leads to this pattern.

We have previously proposed a scenario for the origin of *H. heurippa* whereby *H. cydno* and *H. melpomene* initially overlapped and hybridized near to the current range of *H. heurippa*. A period of reduced intensity of stabilizing selection on color pattern could have led to an increase in the number of hybrids, as observed currently in a natural population in San Cristobal, Venezuela (Mavárez et al. 2006). As selection was established again, whichever novel hybrid form was most abundant and had a successful aposematic signal would have become favored by positive frequency-dependent selection and been driven to fixation. We previously speculated that the proto-*H. heurippa* would have gained assortative mating preferences, and hence reproductive isolation from parental species, subsequent to its establishment as a geographic population with a distinct color pattern phenotype. However, the data presented here suggest a much more direct route whereby assortative mating would arise as an instantaneous result of the hybrid origin of the new color pattern. Furthermore, the data also imply that in the early stages of hybridization, sexual selection would have favored the establishment of proto-*H. heurippa* hybrids as they would have mated most readily with other such hybrids. Nonetheless, determining the relative prob-

ability of establishment of the *H. heurippa* pattern, as opposed to alternative hybrid phenotypes, would require knowledge of the mating preferences for all hybrid genotypes, combined with an estimation of the frequency with which they are generated in natural populations. Similar modeling of other hybrid speciation scenarios suggests that this is likely to be a fruitful approach if further mate preference data can be obtained (Buerkle et al. 2000; Gavrilets et al. 2007).

Several examples of animal homoploid hybrid species have been proposed but in most cases little is known about how they attained reproductive isolation from their parental species (Mallet 2007; Mavárez and Linares 2008). In *Rhagoletis* sp., *Lycaeides* sp., and *Cottus gobio* group, novel ecological adaptations have been identified that might have contributed to reproductive isolation in sympatry/ parapatry (Mallet 2007; Mavárez and Linares 2008). However, not all homoploid hybrid species have shown ecological differentiation from parents, suggesting other mechanisms such as sexual selection could be involved in reproductive isolation. Studies on *Lycaeides* sp., *Rhagoletis* sp., and cichlid fish have shown that hybrid species exhibit assortative mating with their parents (Mavárez and Linares 2008). In the case of *Xiphophorus clemensiae*, a parental female mating preference for long-tailed males enhances the opportunity for hybridization, but it is not clear how the intermediate tail length hybrid would be reproductively isolated from long- and short-tailed parents. Assortative mating was also recently observed in a homoploid hybrid *Pogonomyrmex* ant species and one of its parental species, but nothing is known of how these mate preferences arose (Schwander et al. 2008). Our experiments therefore extend previous results by showing how hybridization could have had a direct role in generating assortative mating in an incipient hybrid species.

LITERATURE CITED

Boughman, J. W. 2001. Divergent sexual selection enhances reproductive isolation in sticklebacks. Nature 411:944–948.

Buerkle, C. A., R. J. Morris, M. A. Asmussen, and L. H. Rieseberg. 2000. The likelihood of homoploid hybrid speciation. Heredity 84:441–451.

Coyne, J. A., and H. A. Orr. 2004. Speciation. Sinauer Associates, Sunderland, MA.

Crane, J. 1955. Imaginal behaviour of a Trinidad butterfly, *Heliconius erato hydara* Hewitson, with special reference to the social use of color. Zool., N. Y. 40:167–196.

Edwards, A. W. F. 1972. Likelihood. Cambridge Univ. Press, Cambridge.

Estrada, C., and C. D. Jiggins. 2002. Patterns of pollen feeding and habitat preference among *Heliconius* species. Ecol. Entomol. 27:448–456.

Gavrilets, S. 2004. Fitness landscapes and the origin of species: monographs in population biology vol. 41. Princeton Univ. Press, Princeton, NJ.

Gavrilets, S., A. Vose, M. Barluenga, W. Salzburger, and A. Meyer. 2007. Case studies and mathematical models of ecological speciation. 1. Cichlids in a crater lake. Mol. Ecol. 16:2893–2909.

Gilbert, L. E. 2003. Adaptive novelty through introgression in *Heliconius* wing patterns: evidence for shared genetic "tool box" from synthetic hybrid zones and a theory of diversification. Pp. 281–318 *in* C. L. Boggs, W. B. Watt and P. R. Ehrlich, eds. Ecology and evolution taking flight: butterflies as model systems. Univ. of Chicago Press, Chicago.

Jiggins, C. D., R. E. Naisbit, R. L. Coe, and J. Mallet. 2001. Reproductive isolation caused by colour pattern mimicry. Nature 411:302–305.

Jiggins, C. D., C. Estrada, and A. Rodrigues. 2004. Mimicry and the evolution of pre-mating isolation in *Heliconius melpomene*. J. Evol. Biol. 17:680–691.

Jiggins, C. D., C. Salazar, M. Linares, and J. Mavárez. 2008. Hybrid speciation in *Heliconius* butterflies. Philos. Trans. R. Soc. Lond. B 363:3047–3054.

Kronforst, M. R., L. G. Young, D. D. Kapan, C. McNeely, J. R. O'Neill, and L. E. Gilbert. 2006. Linkage of butterfly mate preference and wing color preference cue at the genomic location of *wingless*. Proc. Natl. Acad. Sci. USA 103:6575–6580.

Linares, M. 1989. Adaptive microevolution through hybridization and biotic destruction in the neotropics. Univ. of Texas, Austin.

Mallet, J. 1999. Causes and consequences of a lack of coevolution in Müllerian mimicry. Evol. Ecol. 13:777–806.

———.2007. Hybrid speciation. Nature 446:279–283.

Mallet, J., M. Beltrán, W. Neukirchen, and M. Linares. 2007. Natural hybridization in Heliconiine butterflies: the species boundary is a continuum. BMC Evol. Biol. 7:28.

Mavárez, J., and M. Linares. 2008. Homoploid hybrid speciation in animals. Mol. Ecol. 17:4181–4185.

Mavárez, J., C. Salazar, E. Bermingham, C. Salcedo, C. D. Jiggins, and M. Linares. 2006. Speciation by hybridization in *Heliconius* butterflies. Nature 441:868–871.

Naisbit, R. E., C. D. Jiggins, and J. Mallet. 2001. Disruptive sexual selection against hybrids contributes to speciation between *Heliconius cydno* and *Heliconius melpomene*. Proc. R. Soc. Lond. B 268:1849–1854.

———. 2003. Mimicry: developmental genes that contribute to speciation. Evol. Dev. 5:269–280.

Podos, J. 2001. Correlated evolution of morphology and vocal signal structure in Darwin's finches. Nature 409:185–188.

Puebla, O., E. Bermingham, F. Guichard, and E. Whiteman. 2007. Colour pattern as a single trait driving speciation in *Hypoplectrus* coral reef fishes. Proc. R. Soc. Lond. B 274:1265–1271.

Ramsey, J., and D. W. Schemske. 2002. Neopolyploidy in flowering plants. Annu. Rev. Ecol. Syst. 33:589–639.

Rieseberg, L. H., O. Raymond, D. M. Rosenthal, Z. Lai, K. Living-stone, T. Nakazato, J. L. Durphy, A. E. Schwatzbach, L. A. Donovan, and C. Lexer. 2003. Major ecological transitions in wild sunflowers facilitated by hybridization. Science 301:1211–1216.

Rutowski, R. L. 1991. The evolution of mate-locating behavior in butterflies. Am. Nat. 138:1121–1139.

Schwander, T., S. S. Suni, S. Helms Cahan, and L. Keller. 2008. Mechanisms of reproductive isolation between an ant species of hybrid origin and one of its parents. Evolution 62:1635–1643.

Ungerer, M. C., S. J. E. Baird, J. Pan, and L. H. Rieseberg. 1998. Rapid hybrid speciation in wild sunflowers. Proc. Natl. Acad. Sci. USA 95:11757–11762.

Vane-Wright, R. I., and M. Boppré. 1993. Visual and chemical signalling in butterflies: functional and phylogenetic perspectives. Philos. Trans. R. Soc. Lond. B 340:197–205.

Associate Editor: W. O. McMillan