

Disruptive sexual selection against hybrids contributes to speciation between *Heliconius cydno* and *Heliconius melpomene*

Russell E. Naisbit¹*, Chris D. Jiggins^{1,2} and James Mallet^{1,2}

¹The Galton Laboratory, Department of Biology, University College London, 4 Stephenson Way, London NW1 2HE, UK

²Smithsonian Tropical Research Institute, Apartado 2072, Balboa, Panama

Understanding the fate of hybrids in wild populations is fundamental to understanding speciation. Here we provide evidence for disruptive sexual selection against hybrids between *Heliconius cydno* and *Heliconius melpomene*. The two species are sympatric across most of Central and Andean South America, and coexist despite a low level of hybridization. No-choice mating experiments show strong assortative mating between the species. Hybrids mate readily with one another, but both sexes show a reduction in mating success of over 50% with the parental species. Mating preference is associated with a shift in the adult colour pattern, which is involved in predator defence through Müllerian mimicry, but also strongly affects male courtship probability. The hybrids, which lie outside the curve of protection afforded by mimetic resemblance to the parental species, are also largely outside the curves of parental mating preference. Disruptive sexual selection against F_1 hybrids therefore forms an additional post-mating barrier to gene flow, blurring the distinction between pre-mating and post-mating isolation, and helping to maintain the distinctness of these hybridizing species.

Keywords: Lepidoptera; Nymphalidae; hybridization; mate choice; post-mating isolation; pre-mating isolation

1. INTRODUCTION

Studies of recently diverged species are increasingly producing examples of sympatric species that hybridize in the wild yet remain distinct (Grant & Grant 1992; Mallet *et al.* 1998). Understanding the fate of hybrids is therefore critical to our understanding of the nature of the species boundary and of the forces that drive speciation.

Given incomplete assortative mating, the fitness of hybrids will determine the extent to which gene flow occurs between species. Hybrid fitness can be reduced by (i) sterility or inviability ('genomic' incompatibility), (ii) disruptive ecological selection, or (iii) disruptive sexual selection. Most attention has been focused on the first effect, hybrid sterility and inviability, with the majority of studies using *Drosophila*. That work has yielded a number of consistent generalizations about speciation, for instance Haldane's rule, which states that where one sex of F_1 hybrid is absent, rare or sterile, it is the heterogametic sex (Haldane 1922; Coyne 1992; Sperling 1993). However, these studies of genomic incompatibility arguably tell us little about the early stages of speciation: a growing body of work is demonstrating that speciation can proceed in the absence of this form of post-mating isolation (Feder *et al.* 1994; Bradshaw *et al.* 1995; Grant & Grant 1997; McMillan *et al.* 1997; Seehausen *et al.* 1997; Hatfield & Schlüter 1999). More recently, there has been an upsurge in theoretical and empirical interest in the second source of selection against hybrids: ecological forms of disruptive selection (McMillan *et al.* 1997; Schlüter 1998; Dieckmann & Doebeli 1999; Kondrashov & Kondrashov 1999).

Rather less experimental work has investigated mate choice during speciation and the possibility of the third type of selection against hybrids: disruptive sexual selection. Recent theory suggests that disruptive sexual selection could be extremely important in speciation (Payne & Krakauer 1997; Higashi *et al.* 1999). Several recent examples have shown a reduced mating probability of F_1 hybrids between a pair of butterfly species (Davies *et al.* 1997), preference for conspecific over F_1 hybrid males in sticklebacks (Vamosi & Schlüter 1999), strong mating discrimination against hybrids in lacewings (Wells & Henry 1998) and almost complete behavioural sterility of both sexes of F_1 hybrid in wolf spiders (Stratton & Uetz 1986). This form of selection against hybrids provides an additional element of speciation for which Haldane's rule might hold, as in the effects on vigour or choosiness of female hybrids seen in *Anartia* butterflies (Davies *et al.* 1997). Sexual selection will limit the extent to which introgression is possible following hybridization and, if mating asymmetries exist, they will influence the direction in which gene flow might proceed.

Three processes are likely to contribute to divergence in mating preference: (i) pleiotropic or otherwise genetically correlated effects of ecological selection, (ii) disruptive sexual selection, and (iii) reinforcement. First, assortative mating can arise as a by-product of disruptive natural selection (Schlüter 1998). Assortment will result if a trait under ecological selection also forms the basis of mate choice or through the recruitment of other traits involved in mate choice by the build-up of linkage disequilibrium with the ecological character (Dieckmann & Doebeli 1999; Kondrashov & Kondrashov 1999). This will generate populations with divergent ecology that are also reproductively isolated and, therefore, that are able to coexist. Second, recent theory suggests that the genetic

*Author for correspondence (r.naisbit@ucl.ac.uk).

correlations between preferences and traits generated purely by disruptive sexual selection can also drive sympatric speciation (Turner & Burrows 1995; Payne & Krakauer 1997, 2000; Higashi *et al.* 1999). However, the daughter populations from such a process may be able to coexist genetically, but without adaptive differentiation they will not be able to coexist ecologically (but see Lande & Kirkpatrick 1988; Van Doorn *et al.* 1998). Reinforcement provides a third process that can strengthen pre-mating isolation, however produced, between differentiated populations through selection for assortative mating if hybridization produces offspring with low fitness (Liou & Price 1994). Interestingly, once mating divergence occurs, hybrids are potentially left in an adaptive valley between the ranges of mating preference of the two parental species, which itself can promote additional divergence by further reinforcement.

Heliconius cydno and *Heliconius melpomene* (Lepidoptera: Nymphalidae) are sister species that are sympatric across much of Central and Andean South America below 1500 m (Brown 1979; Brower 1996). Both are unpalatable and warningly coloured, and speciation has accompanied a shift in their Müllerian mimicry. *Heliconius melpomene* is black with red and yellow markings and mimics *Heliconius erato*, while *H. cydno* is black with yellow or white markings and usually mimics members of the *Heliconius sapho/eleuchia* clade (Linares 1997; Jiggins *et al.* 2001). The two species have diverged in habitat use, with *H. melpomene* in second growth and *H. cydno* in forest understorey, which matches the distribution of their co-mimetic species (Smiley 1978a; Waage *et al.* 1981; Mallet & Gilbert 1995). However, there is considerable overlap and the two species can be found flying together. They also differ in their degree of host plant specialization within the *Passiflora* (Smiley 1978a,b) and female F₁ hybrids are sterile (Linares 1989). Despite these differences the two species do hybridize in the wild and F₁ and backcross hybrids are known from across their range, although they probably form less than 0.1% of natural populations (Mallet *et al.* 1998).

In this paper, we investigate mating interactions between these two species and estimate the extent of sexual selection against hybrids.

2. METHODS

The work was carried out with *Heliconius melpomene rosina* and *Heliconius cydno chioneus* and their F₁ hybrids. Crosses and mating trials were performed in outdoor insectaries in Gamboa, Republic of Panama, between October 1999 and March 2000. This area lies close to Pipeline Road in Soberanía National Park where stocks of both species were collected. The two species will cross in the insectary although rarely, and one direction of cross, *H. melpomene* female \times *H. cydno* male, could not be obtained to supply hybrids for these experiments: males of *H. cydno* are consistently more reluctant to mate with heterospecifics than are *H. melpomene* males. Although the *H. melpomene* female \times *H. cydno* male cross has been produced on two earlier occasions, in both cases the female died before laying eggs.

Mating behaviour was analysed using no-choice trials. Each female was left to eclose in a 1 m \times 1 m \times 2 m insectary in the presence of a single male and the pair observed at 30 min intervals during the daylight hours for a minimum of 2 days. Males

were allowed to mature for at least 5 days beforehand with access to pollen sources (*Psiguria* and *Lantana*) and artificial nectar (10% sugar solution) (McMillan *et al.* 1997). All individuals were reared without access to the opposite sex and were used once only. Pairs stay coupled for at least 1 h and so all matings were observed. In addition, females were dissected in order to check for the presence of a spermatophore. The pair were disturbed at each observation period if perching and, once they had come into contact, male behaviour such as chasing the female, fluttering courtship or attempted mating was recorded. This no-choice experimental design was adopted in order to mimic the natural situation, where males patrol larval host plants and mate teneral females soon after eclosion (Mallet 1986). Male choice is almost certainly the primary determinant of mating probability at this stage. Females use their wings for fending off unwanted males, but the wings of teneral females are too soft to be used in this way. A female can use the sperm from a single spermatophore to fertilize several eggs each day over her 6 month lifespan, although a fraction of females do remate in the wild (Boggs 1979).

Mating probabilities were estimated using likelihood in order to test between hypotheses differing in complexity, as well as obtaining measures of reliability (McMillan *et al.* 1997). A binomial mating probability $P_{i \times j}$ was obtained for each combination of *i*-type female and *j*-type male, thereby maximizing the expression for log_e-likelihood given by

$$m \log_e P_{i \times j} + n \log_e (1 - P_{i \times j}), \quad (1)$$

where *m* and *n* are the numbers of trials in which the pair mated or remained unmated, respectively. The log_e-likelihoods for the $P_{i \times j}$ values were maximized using the SOLVER algorithm supplied with Microsoft EXCEL. Support limits for $P_{i \times j}$, which are asymptotically equivalent to 95% confidence intervals, were obtained at the parameter values that led to a decrease in the log_e-likelihood of two units (Edwards 1972). In the case of the parameters *a*, *b*, *c* and *e*, which are multiplicatively combined in the final model (see below), support limits were obtained while maximizing the likelihoods for the other parameters. Fitting models with different numbers of parameters allowed a test for differences in the mating probability across trials using a likelihood ratio test with $G = 2\Delta \log_e L$, which asymptotically follows a χ^2 -distribution (Edwards 1972).

The likelihood model was fitted in a stepwise manner by adding the parameters to an initial null model with a single mating probability (*a* = *b* = *c* = *d* in table 2) across all trials. Estimating the mating probability separately for interspecific trials (parameter *c* versus *a* = *b* = *d* for the rest) gave a significant improvement ($G = 30.12$, d.f. = 1 and $p < 0.01$). Further improvement was achieved in a three-parameter model estimating separate probabilities for trials among individuals of like genotype (parameter *a* in table 2), hybrid \times parental (*b* = *d*) and interspecific mating (parameter *c*) ($G = 10.14$, d.f. = 1 and $p < 0.01$). Males of *H. cydno* displayed a consistently poor performance throughout the period of the experiment, with reduced courtship effort and mating success in all trials including those with a conspecific female. This was not the case in a different set of experiments performed the year before (Jiggins *et al.* 2001). It was apparent in the offspring of several wild-caught females and seemed to reflect poor adaptation of *H. cydno* to the cage environment. Here we account for the poor performance of *H. cydno* males by estimating an extra parameter (parameter *e*) ($G = 14.25$, d.f. = 1 and $p < 0.01$). Separate mating probabilities

Table 1. Results from the no-choice mating trials.

(The expected number of matings were estimated from the five-parameter model using the parameters in the final column.)

Female	Male	number of trials with mating occurring	total number of trials	expected number of matings	parameters
<i>H. melpomene</i> × <i>H. melpomene</i>		15	17	13.4	<i>a</i>
<i>H. melpomene</i> × <i>F</i> ₁		5	14	4.2	<i>d</i>
<i>H. melpomene</i> × <i>H. cydno</i>		0	10	0.0	<i>c</i> × <i>e</i>
<i>F</i> ₁ × <i>H. melpomene</i>		8	18	8.2	<i>b</i>
<i>F</i> ₁ × <i>F</i> ₁		11	16	12.6	<i>a</i>
<i>F</i> ₁ × <i>H. cydno</i>		3	16	2.7	<i>b</i> × <i>e</i>
<i>H. cydno</i> × <i>H. melpomene</i>		0	20	0.0	<i>c</i>
<i>H. cydno</i> × <i>F</i> ₁		4	16	4.8	<i>d</i>
<i>H. cydno</i> × <i>H. cydno</i>		5	18	5.3	<i>a</i> × <i>e</i>

(parameters *b* and *d*) were estimated for the reciprocal trials of hybrid with parental individual because, although this did not significantly improve the model ($G = 1.29$, d.f. = 1 and $p > 0.05$), it allowed the mating success of the three genotypes to be compared for each sex. The adoption of a full nine-parameter model with separate probabilities estimated for each genotype × genotype combination gave no significant improvement over this five-parameter model ($G = 2.39$, d.f. = 4 and $p > 0.05$).

3. RESULTS

There was very strong assortative mating between the species, with no interspecific mating occurring in 30 trials (table 1). Hybrid males had a high mating success with hybrid females, but both sexes of hybrids had considerably reduced mating probabilities with the parental species (table 1). Male courtship in the first 2 h after female eclosion was a good predictor of male mating success in the trials (figure 1): male mating probability is highly correlated with male courtship probability ($r^2 = 0.65$ and $p < 0.01$).

Interpretation of the likelihood model is complicated by the poor performance of the *H. cydno* males in both courtship effort and mating success (figure 1 and parameter *e* in table 2). Since courtship appears to determine male mating performance, it is reasonable to treat the performance parameter (parameter *e*) as an adjustment for the effect of the poor performance by male *H. cydno*. We believe this to be an artefact of this experiment, since a different study with the same pair of species showed similar conspecific mating probabilities within *H. cydno* and within *H. melpomene* (Jiggins *et al.* 2001). This variation between experiments places doubt on the precision of some of the parameter estimates, but the relationships between parameters hold up for the males of each pure species (for example *a* > *b* > *c*), regardless of whether the performance correction for *H. cydno* is used. We can then compare the four mating parameters (parameters *a*–*d* in table 2). There is very strong assortative mating of the parental species (test of *a* = *c*, $G = 44.33$, d.f. = 1 and $p < 0.01$) and there is good evidence of discrimination against female hybrids (test of *a* = *b*, $G = 6.41$, d.f. = 1 and $p < 0.05$). Males of the parental species are therefore most likely to mate with conspecific females, less so with

hybrids, and are extremely unlikely to pair with heterospecifics. For hybrid males, pairing with hybrid females is more likely than with either parental species (test of *a* = *d*, $G = 15.79$, d.f. = 1 and $p < 0.01$): the probability of mating between hybrid males and parental females is estimated to be less than half that of parental × parental or hybrid × hybrid matings (table 2). As noted previously, the mating success of male and female hybrids with parental partners does not differ significantly (test of *b* = *d*, $G = 1.29$, d.f. = 1 and $p > 0.05$).

4. DISCUSSION

There is extremely strong assortative mating between *H. cydno* and *H. melpomene* and reduced mating success of *F*₁ hybrids of both sexes with the parental species. The probability of mating is strongly correlated with the probability of courtship, suggesting that, while female receptivity may play a role, male choice is the primary determinant of mating probability. Although hybrids were not tested in earlier work, a similar correlation was obtained in a study of male courtship and mate choice in *H. cydno* from Panama and *H. melpomene* from Panama and French Guiana: in both cases, mating success seems mainly a result of male courtship interest due largely to the males' colour pattern preference (Jiggins *et al.* 2001). Male choosiness is not unexpected since the spermatophore of *Heliconius* represents a considerable nutrient investment, providing the female with amino acids used in egg production (Boggs 1979). In the experiments described here, the mating probabilities for male and female parents are intermediate when tested with hybrid individuals (conspecific > hybrid > heterospecific) and hybrid success is greatest with hybrids (hybrid > parental). Although the precise nature of mating cues is uncertain, the hybrid male preferences and hybrid female signals from our data seem to be intermediate between those of the two parental species, suggesting an approximately additive genetic basis of both mating cue and response. Similar cases of intermediate signals and preferences of hybrids exist in other species, for example in tree frogs (Doherty & Gerhardt 1983) and lacewings (Wells & Henry 1998), but there are also instances where the mating success of hybrids is similar to that of the

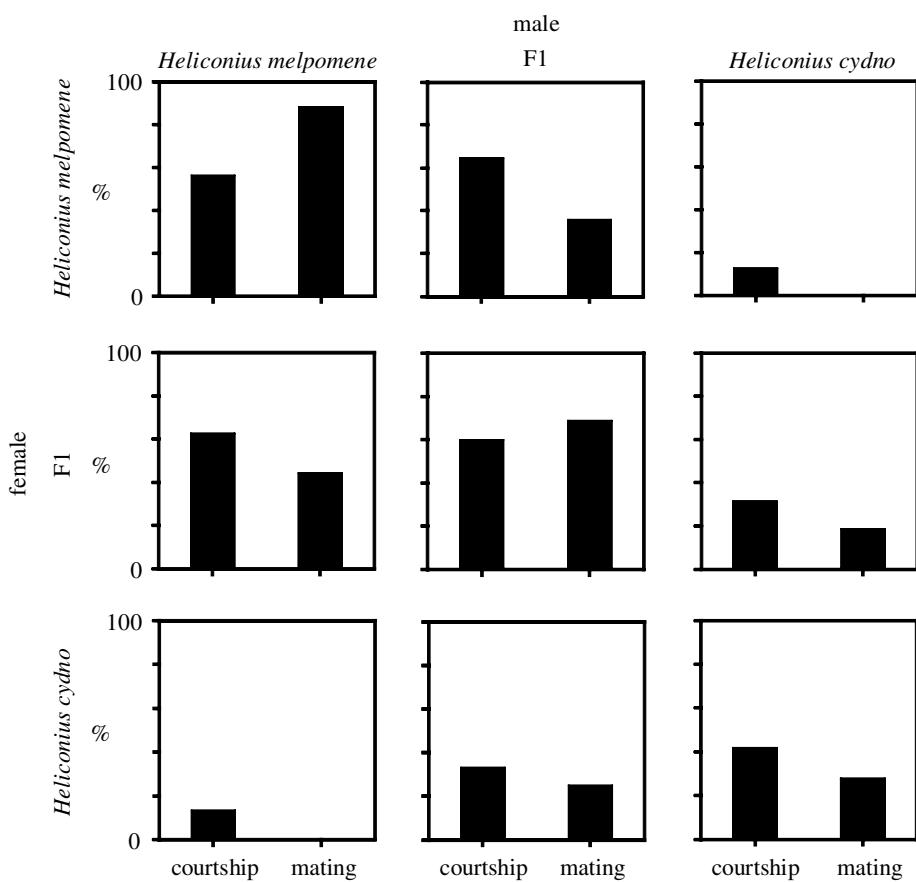


Figure 1. Courtship and mating in the no-choice trials. Each cell shows the percentage of males displaying courtship interest towards the female during four observations in the first 2 h after eclosion and the percentage mating over the whole trial period. Courtship was taken to include chasing the female, fluttering courtship above her and attempted or actual mating.

Table 2. Multiplicative mating probability parameters from the five-parameter model with support limits.

type of parameter	parameter	maximum likelihood value	support limits
pure \times pure and hybrid \times hybrid mating probability	<i>a</i>	0.786	0.625–0.903
pure male \times hybrid female mating probability	<i>b</i>	0.455	0.257–0.672
interspecific mating probability	<i>c</i>	0.000	0.000–0.081
pure female \times hybrid male mating probability	<i>d</i>	0.300	0.155–0.479
<i>H. cydno</i> male mating performance parameter	<i>e</i>	0.371	0.176–0.648

parentals (Davies *et al.* 1997; McMillan *et al.* 1997). In only one published case that we could find, namely that of a spider, was hybrid mating success non-additive, being very low with all genotypes (Stratton & Uetz 1986).

The concept of the 'shape' of mating preference provides a unifying theme in the study of sexual selection and species recognition (Ryan & Rand 1993; Ritchie 1996). The shape of mating preference is the relationship between the values of a trait and their probability of acceptance by a mating partner (Ritchie 1996). It determines how sexual selection acts within a population, as well as the probability of hybridization between populations. The distribution of trait values within a population and the shape of preference are expected to coevolve closely, although recognition may be elicited by trait values beyond the normal range of the population, as found for syllable number in the song of the cricket *Euphippiger euphippiger* (Ritchie 1996). In the initial stages of

divergence the preference distributions of two incipient species will overlap. The trait values found in hybrids will depend on the genetic architecture of those traits, but if a number of cues combine additively to form the basis for recognition, hybrids will lie at some intermediate point. Hybrids may therefore be recognized as potential mates by both species and could even have superior mating success to parents in mixed populations (dashed line in figure 2a). Another pair of sister species, *Heliconius himera* and *H. erato*, illustrate a possible intermediate stage of divergence as in figure 2a, with strong assortative mating but little discrimination against hybrids (McMillan *et al.* 1997). At this point, mate choice on its own merely provides stabilizing selection, thereby opposing further divergence. However, if hybrids are selected against, reinforcement can lead to a narrowing of the preference function of the parental species, which might reduce the extent to which hybrids are accepted as mates (figure 2b).

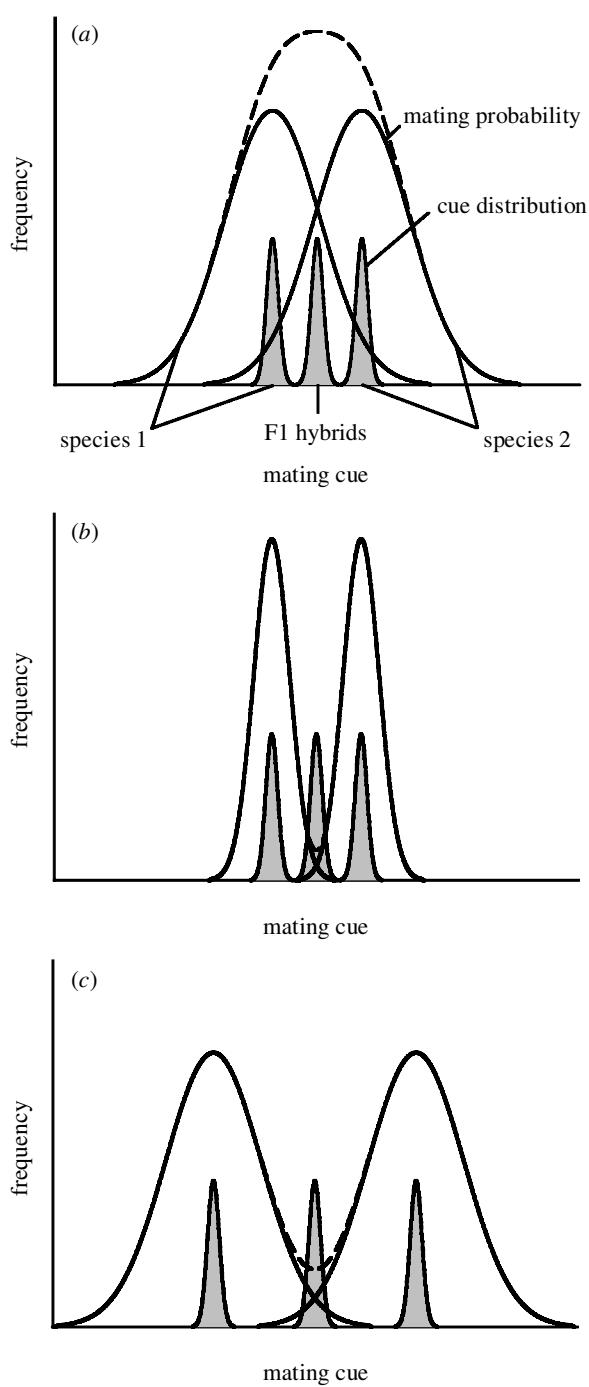


Figure 2. Hypothetical example of variation in the mating cues of two species and their F_1 hybrids (shaded) and the shape of mating preference of each species (solid lines). (a) after initial divergence with strong assortative mating, but little discrimination against hybrids, (b) following reinforcement of assortative mating and (c) an alternative source of discrimination, after further divergence but without reinforcement. The dashed line in each figure shows the mating probability for both parental species combined, assuming equal population sizes.

Alternatively, discrimination may result from further trait divergence of the parental species and coevolution of their mating preference (figure 2c). In either case, disruptive sexual selection against hybrids is generated. This is exactly what we see in *H. melpomene* and *H. cydno*, with an additive pattern of hybrid mating, very strong assortative

mating and a reduction in the mating success of hybrids with the parental species of over 50%. This sexual selection may only delay mating for hybrid females, but should result in a strong reduction in lifetime reproductive success for male hybrids. The strength of this sexual selection depends on an Allee effect (Allee *et al.* 1949) for hybrids: male mating opportunities are only scarce because female hybrids are rare. Hybrids between *H. melpomene* and *H. cydno* are always at extremely low density (less than one in 1000 individuals) (Mallet *et al.* 1998), so they are unlikely to encounter other hybrids as compatible mates. This creates what is in effect a novel form of post-mating isolation due to poor hybrid mating success.

Ecological adaptation in this pair of species initiated a chain of divergence that led to speciation. Changes in habitat use exposed populations to different suites of potential Müllerian co-mimics (Waage *et al.* 1981; Mallet & Gilbert 1995), thereby selecting for a shift in mimetic allegiance. This switch in mimicry then led to pleiotropic changes in mate choice, as assortative mating coevolved with colour pattern (Jiggins *et al.* 2001). Reduced production of hybrids created further disruptive predator selection, leading to low fitness of non-mimetic hybrids, probably in the order of 50% as seen in *H. erato* hybrid zones (Mallet & Barton 1989). Selection within each nascent species after separation almost certainly led to the acquisition of female hybrid sterility (Linares 1989), presumably via Muller's (1940) classical pleiotropic route for the evolution of genomic incompatibility between populations. These ecological and genomic post-mating barriers created the conditions necessary for reinforcement, which appears to have strengthened the assortative mating in sympatric populations: allopatric *H. melpomene* court and mate with *H. cydno* more readily than the same species pair in sympatry (Jiggins *et al.* 2001). This greater choosiness in sympatry very probably resulted in still stronger disruptive sexual selection against hybrids. Thus, it is possible to see how simple ecological divergence can trigger a cascade of further changes that lead to full speciation. Each step in divergence we have documented leads to conditions that promote further divergence by reducing gene flow and creating additional disruptive selection pressures in a series of examples of positive feedback in the speciation process. Of course not every species is mimetic or has such clear selective forces at work, but mimicry is a particularly good example where an ecological change that has pleiotropic effects on both pre-mating isolation and post-mating isolation may ultimately cause speciation. Many other examples of similar pleiotropy probably exist.

We are very grateful to the Smithsonian Tropical Research Institute where this work was carried out and to the Autoridad Nacional del Ambiente for permission to work in Panama. Thanks are also due to Vanessa Bull and Cris Thompson for company and help with the butterflies. The project was funded by a Natural Environment Research Council grant and a studentship from the Biotechnology and Biological Sciences Research Council.

REFERENCES

- Allee, W. C., Emerson, A. E., Park, O., Park, T. & Schmidt, K. P. 1949 *Principles of animal ecology*. Philadelphia, PA: W. B. Saunders.

- Boggs, C. L. 1979 Resource allocation and reproductive strategies in several Heliconiine butterfly species. Doctoral dissertation, University of Texas at Austin, Austin, TX, USA.
- Bradshaw, H. D., Wilbert, S. M., Otto, K. G. & Schemske, D. W. 1995 Genetic mapping of floral traits associated with reproductive isolation in monkeyflowers (*Mimulus*). *Nature* **376**, 762–765.
- Brower, A. V. Z. 1996 Parallel race formation and the evolution of mimicry in *Heliconius* butterflies: a phylogenetic hypothesis from mitochondrial DNA sequences. *Evolution* **50**, 195–221.
- Brown, K. S. 1979 *Ecologia geográfica e evolução nas florestas neotropicais*. São Paulo, Brasil: Universidade Estadual de Campinas.
- Coyne, J. A. 1992 Genetics and speciation. *Nature* **355**, 511–515.
- Davies, N., Aiello, A., Mallet, J., Pomiankowski, A. & Silberglied, R. E. 1997 Speciation in two Neotropical butterflies: extending Haldane's rule. *Proc. R. Soc. Lond. B* **264**, 845–851.
- Dieckmann, U. & Doebeli, M. 1999 On the origin of species by sympatric speciation. *Nature* **400**, 354–357.
- Doherty, J. A. & Gerhardt, H. C. 1983 Hybrid tree frogs: vocalizations of males and selective phonotaxis of females. *Science* **220**, 1078–1080.
- Edwards, A. W. F. 1972 *Likelihood*. Cambridge University Press.
- Feder, J. L., Opp, S. B., Wazlo, B., Reynolds, K., Go, W. & Spisak, S. 1994 Host fidelity is an effective pre-mating barrier between sympatric races of the apple maggot fly. *Proc. Natl Acad. Sci. USA* **91**, 7990–7994.
- Grant, P. R. & Grant, B. R. 1992 Hybridization of bird species. *Science* **256**, 193–197.
- Grant, P. R. & Grant, B. R. 1997 Genetics and the origin of bird species. *Proc. Natl Acad. Sci. USA* **94**, 7768–7775.
- Haldane, J. B. S. 1922 Sex ratio and unisexual sterility in hybrid animals. *J. Genet.* **12**, 101–109.
- Hatfield, T. & Schlüter, D. 1999 Ecological speciation in sticklebacks: environment-dependent hybrid fitness. *Evolution* **53**, 866–873.
- Higashi, M., Takimoto, G. & Yamamura, N. 1999 Sympatric speciation by sexual selection. *Nature* **402**, 523–526.
- Jiggins, C. D., Naisbit, R. E., Coe, R. L. & Mallet, J. 2001 Reproductive isolation caused by colour pattern mimicry. *Nature* **411**, 302–305.
- Kondrashov, A. S. & Kondrashov, F. A. 1999 Interactions among quantitative traits in the course of sympatric speciation. *Nature* **400**, 351–354.
- Lande, R. & Kirkpatrick, M. 1988 Ecological speciation by sexual selection. *J. Theor. Biol.* **133**, 85–98.
- Linares, M. 1989 Adaptive microevolution through hybridization and biotic destruction in the Neotropics. Doctoral dissertation, University of Texas at Austin, Austin, TX, USA.
- Linares, M. 1997 Origin of Neotropical mimetic biodiversity from a three-way hybrid zone of *Heliconius cydno* butterflies. In *Tropical biodiversity and systematics. Proceedings of the International Symposium on Biodiversity and Systematics in Tropical Ecosystems* (ed. H. Ulrich), pp. 93–108. Bonn, Germany: Zoologisches Forschungsinstitut und Museum Alexander Koenig.
- Liou, L. W. & Price, T. D. 1994 Speciation by reinforcement of pre-mating isolation. *Evolution* **48**, 1451–1459.
- McMillan, W. O., Jiggins, C. D. & Mallet, J. 1997 What initiates speciation in passion-vine butterflies? *Proc. Natl Acad. Sci. USA* **94**, 8628–8633.
- Mallet, J. 1986 Dispersal and gene flow in a butterfly with home range behavior: *Heliconius erato* (Lepidoptera: Nymphalidae). *Oecologia* **68**, 210–217.
- Mallet, J. & Barton, N. H. 1989 Strong natural selection in a warning-color hybrid zone. *Evolution* **43**, 421–431.
- Mallet, J. & Gilbert, L. E. 1995 Why are there so many mimicry rings? Correlations between habitat, behaviour and mimicry in *Heliconius* butterflies. *Biol. J. Linn. Soc.* **55**, 159–180.
- Mallet, J., McMillan, W. O. & Jiggins, C. D. 1998 Mimicry and warning color at the boundary between races and species. In *Endless forms: species and speciation* (ed. D. J. Howard & S. H. Berlocher), pp. 390–403. New York: Oxford University Press.
- Muller, H. J. 1940 Bearings of the 'Drosophila' work on systematics. In *The new systematics* (ed. J. S. Huxley), pp. 185–268. Oxford University Press.
- Payne, R. J. H. & Krakauer, D. C. 1997 Sexual selection, space, and speciation. *Evolution* **51**, 1–9.
- Payne, R. J. H. & Krakauer, D. C. 2000 Disruptive sexual selection. *Trends Ecol. Evol.* **15**, 419–420.
- Ritchie, M. G. 1996 The shape of female mating preferences. *Proc. Natl Acad. Sci. USA* **93**, 14 628–14 631.
- Ryan, M. J. & Rand, A. S. 1993 Species recognition and sexual selection as a unitary problem in animal communication. *Evolution* **47**, 647–657.
- Schlüter, D. 1998 Ecological causes of speciation. In *Endless forms: species and speciation* (ed. D. J. Howard & S. H. Berlocher), pp. 114–129. New York: Oxford University Press.
- Seehausen, O., Van Alphen, J. J. M. & Witte, F. 1997 Cichlid fish diversity threatened by eutrophication that curbs sexual selection. *Science* **277**, 1808–1811.
- Smiley, J. T. 1978a The host plant ecology of *Heliconius* butterflies in Northeastern Costa Rica. Doctoral dissertation, University of Texas at Austin, Austin, TX, USA.
- Smiley, J. T. 1978b Plant chemistry and the evolution of host specificity: new evidence from *Heliconius* and *Passiflora*. *Science* **201**, 745–747.
- Sperling, F. A. H. 1993 Mitochondrial DNA variation and Haldane's rule in the *Papilio glaucus* and *P. troilus* species groups. *Heredity* **71**, 227–233.
- Stratton, G. E. & Uetz, G. W. 1986 The inheritance of courtship behavior and its role as a reproductive isolating mechanism in two species of *Schizocosoma* wolf spiders (Araneae; Lycosidae). *Evolution* **40**, 129–141.
- Turner, G. F. & Burrows, M. T. 1995 A model of sympatric speciation by sexual selection. *Proc. R. Soc. Lond. B* **260**, 287–292.
- Vamosi, S. M. & Schlüter, D. 1999 Sexual selection against hybrids between sympatric stickleback species: evidence from a field experiment. *Evolution* **53**, 874–879.
- Van Doorn, G. S., Noest, A. J. & Hogeweg, P. 1998 Sympatric speciation and extinction driven by environment dependent sexual selection. *Proc. R. Soc. Lond. B* **265**, 1915–1919.
- Waage, J. K., Smiley, J. T. & Gilbert, L. E. 1981 The *Passiflora* problem in Hawaii: prospects and problems of controlling the forest weed *P. mollissima* (Passifloraceae) with Heliconiine butterflies. *Entomophaga* **26**, 275–284.
- Wells, M. M. & Henry, C. S. 1998 Songs, reproductive isolation, and speciation in cryptic species of insects: a case study using green lacewings. In *Endless forms: species and speciation* (ed. D. J. Howard & S. H. Berlocher), pp. 217–233. New York: Oxford University Press.