

Three-butterfly system provides a field test of müllerian mimicry

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In 1879, Müller proposed that two brightly coloured distasteful butterfly species (co-models) that share a single warning-colour pattern would benefit by spreading the selective burden of educating predators^{1–5}. The mutual benefit of sharing warning signals among distasteful species, so-called müllerian mimicry, is supported by comparative evidence^{2,3}, theoretical studies^{5,6} and laboratory simulations⁷; however, to date, this key exemplar of adaptive evolution has not been experimentally tested in the field. To measure natural selection generated by müllerian mimicry, I exploited the unusual polymorphism of *Heliconius cydno* (Lepidoptera: Nymphalidae)⁸. Here I show increased survival of *H. cydno* morphs that match locally abundant monomorphic co-model species. This study demonstrates müllerian mimicry in the field. It also shows that müllerian mimicry with several co-models generates geographically divergent selection, which explains the existence of polymorphism in distasteful species with warning coloration⁹.

The importance of warning coloration in *Heliconius* butterflies has been examined by marking out wing colour-pattern elements or by transferring colour-pattern races across an inter-racial hybrid zone^{10,11}. These studies showed that there is strong, positive frequency-dependent (aposematic) selection¹² acting on colour pattern in a species, but failed to measure directly the benefit of müllerian mimicry. This is because they measured purifying selection on colour pattern in a numerically dominant co-model species *Heliconius erato* (W. W. Benson, personal communication)^{10,11,13} rather than selection for colour-pattern convergence between species (Fig. 1). The results of both studies demonstrate how natural selection operates on intraspecific variation in warning coloration (uninitiated predators rapidly eliminate rare warning-

colour variants within a species⁹), and can explain the observation that distasteful *Heliconius*^{14,15} species are generally monomorphic within a given geographic area⁹.

This study tests the evolution of müllerian mimetic coloration (in *Heliconius*) by exploiting an unusual system of colour-pattern polymorphism in one species of *Heliconius* butterfly. In western Ecuador, two colour morphs of the unpalatable *H. cydno* butterfly (yellow and white) resemble two different unpalatable species, *Heliconius eleuchia* (yellow) and *Heliconius sapho* (white) (Fig. 1; the proposed co-models of *H. cydno*⁸). Where all three species occur together, the two monomorphic co-models vary in relative abundance (see Methods)⁸.

I used the distasteful polymorphic *H. cydno* to test two predictions of müllerian mimicry theory: first, that unfamiliar experimental *H. cydno* morphs whose wing colour does not match the locally dominant co-model suffer more attacks by predators compared with control morphs whose wing colour does match the co-model¹; second, that differences in survival between experimental and control butterflies decreases with increasing release density. Predators must attack a number of butterflies to learn to avoid an unfamiliar experimental colour pattern¹⁵. These attacked butterflies will represent a lower fraction of the experimental butterflies when more experimental butterflies are released^{3,10,11}. In fact, predator education may have thwarted previous warning-colour experiments that failed to detect differences between experimental and control treatments because, in an attempt to achieve significant samples sizes, they used high-density releases, long release times or re-released butterflies at the same study site^{10,11,16–18}.

To test the first prediction, I captured pairs of yellow and white *H. cydno* butterflies from two source sites and released them at four other sites that were dominated by yellow or white co-models (Table 1, *H. eleuchia* or *H. sapho*). In contrast to previous experiments, this reciprocal transplant design measures the benefit that a rare unpalatable morph of *H. cydno* derives from müllerian mimicry with either of its more common co-model species (Fig. 1). I tested simultaneously the second prediction by releasing these *H. cydno* pairs at different densities: low density (1 pair every 163–194 m of trail) in three sites (Table 1, Manta Real, Agua Caliente and Tinalandia) and high density (1 pair every 40 m of trail) at one site (Table 1, Maquipucuna; also see Methods)⁸.

As predicted, experimental individuals had a lower frequency of being refound on subsequent visits (resighted) compared with controls for each study site (Fig. 2). From resight data, I used

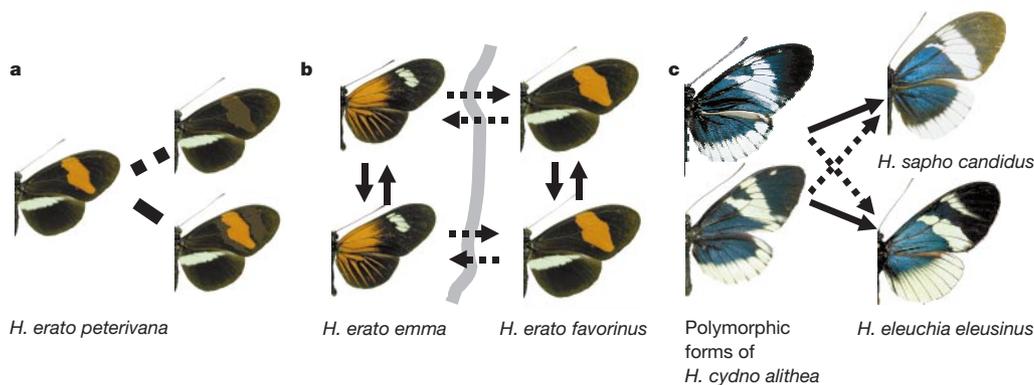


Figure 1 Experimental designs of aposematic selection and müllerian mimicry in *Heliconius* butterflies. **a**, Experimental (dashed line) and control butterflies (solid lines) of a single Costa Rican population of *H. erato peterivana*. The red forewing patch is darkened for experimentals and an equivalent amount of ink is applied to dark parts of the forewing for controls¹⁰. **b**, Experimental (dashed arrows) and control (solid arrows) butterflies of *H. erato emma* and *H. erato favorinus*. Experimental butterflies were transferred across their common hybrid zone (grey line) and control butterflies were transferred parallel to the

hybrid zone¹¹. In both studies abundant *H. erato* were manipulated at sites where they occurred with the rare co-model *H. melpomene* (W. W. Benson, personal communication)^{10,11,30}. **c**, Reciprocal transfer of polymorphic *H. cydno*. White *H. cydno* are control butterflies (solid arrow) when transferred to sites dominated by the white co-model (*H. sapho*) and experimental butterflies (dashed arrow) when transferred to sites dominated by the yellow co-model (*H. eleuchia*). The opposite is true for the yellow *H. cydno* butterfly.

maximum likelihood methods^{19,20} to estimate survival differences between experimental and control butterflies²¹. This involved partitioning butterfly disappearance into two phases: disappearance during the first 24 h (to estimate the probability of establishment, P_E) and subsequent disappearance (to estimate the exponential mortality rate, λ) (see Methods)¹¹. Disappearance rates of released butterflies were in the predicted direction: experimental butterflies had higher initial disappearance rates (lower P_E) and higher subsequent disappearance rates (Fig. 3, λ).

The greater disappearance rate of experimental butterflies is not due to emigration from release sites, as the dispersal distances of experimental and control butterflies were very similar. The mean maximum distance moved by each resighted butterfly did not differ between experimental (105 ± 35.9 m; mean \pm s.e.m.) and control (118 ± 37.4 m) butterflies ($t_{41} = 0.41$, $P = 0.68$; on ln-transformed distance data). Very few released butterflies flew long distances. Only visually oriented predators are expected to affect experimental butterflies while leaving the controls alone. It is therefore reasonable to attribute differences in disappearance rate to differences in survival between morphs.

Morphs differed in survival (life expectancy, P_E/λ): the maximum likelihood estimate of life expectancy across all sites was 5 days for experimental butterflies and 14 days for control butterflies (Fig. 3b, $G_4 = 10.72$, $P = 0.03$). These differences in life expectancy for *H. cydno* butterflies, which have a fairly constant reproductive

output²², are equivalent to a selective coefficient (s) of 0.64 against experimentals (see Methods)¹¹.

Release density affects survival differences. At high release density, selection was not detected: life expectancies for experimental and control butterflies were similar (16 and 17 days respectively, $G_4 = 1.72$, $P = 0.79$). In contrast, life expectancy was 2 days for experimental and 12 days for control butterflies at the three sites of low-density release combined (Fig. 3a, $G_2 = 8.92$, $P = 0.012$).

Differences in life expectancy between experimental and control butterflies vary predictably with the density of release, suggesting that avian predators such as Jacamars (Galbulidae), motmots (Motmotidae) and tyrant flycatchers (Tyrannidae) are capable of learning and can select against unfamiliar coloration of experimental butterflies in the field¹⁵. The lower life expectancy of experimental relative to control butterflies at sites of low-density release indicates that looking like an abundant co-model increases the probability of survival. In contrast, at high release density, life-expectancy differences between treatments are negligible. Predators attack a number of experimental colour morphs to learn avoidance of an unfamiliar colour pattern, but at high release density this number will be a lower fraction of the total experimentals released. Even if predators are responsible for the high initial loss of experimental butterflies (low P_E) across all sites (Fig. 3), they would be expected to learn to avoid experimental morphs when frequent, accounting for the decreased difference between experimental and control λ observed at the high-density release site, Maquipucuna.

There are several implications of this study. The benefit of müllerian mimicry is very high. Predators quickly eliminate rare phenotypes that deviate from the local fitness peak provided by abundant co-models. Given this, polymorphism in distasteful

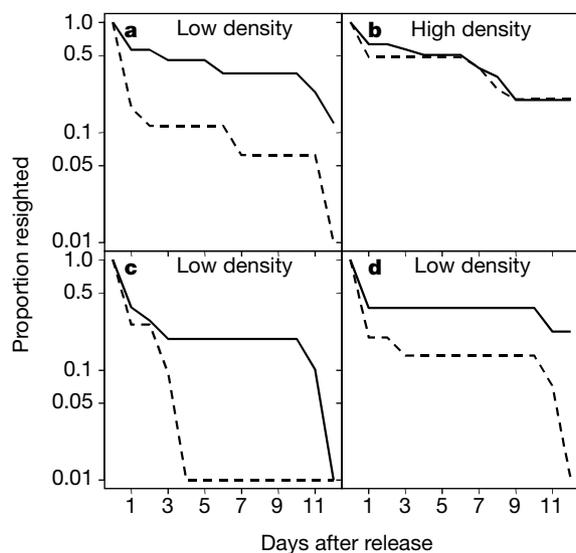


Figure 2 Observed proportion (log₁₀ scale) of control and experimental *H. cydno* butterflies resighted after initial release. Control (solid line) and experimental butterflies (dashed line) resighted at Manta Real (a), Maquipucuna (b), Agua Caliente (c) and Tinalandia (d). To ensure even coverage, all possible locations to resight butterflies were visited daily on a rotating basis (except for Tinalandia where these sites were visited every 1.5 days).

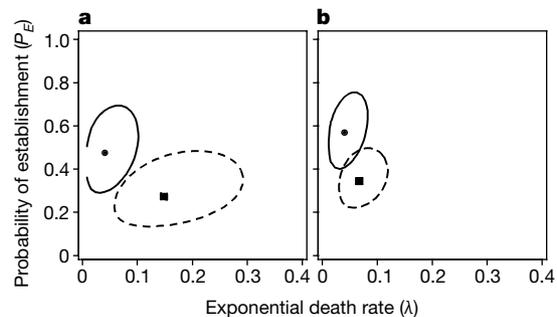


Figure 3 Probability of establishment (P_E) versus exponential death rates (λ) estimated for released butterflies at low-density sites Manta Real, Agua Caliente and Tinalandia summed (a), and at all sites combined (b). Butterflies with lower life expectancies have lower estimates of P_E and higher estimates of λ . The maximum likelihood estimates for P_E and λ for control (circles) and experimental (squares) butterflies are shown. In-likelihood profiles for these two parameters are shown by the two-unit support ellipses (control (solid line) and experimental (dotted line) butterflies) that are roughly equal to the 95% confidence limits for any one parameter¹⁹.

Table 1 Details of release experiments

Release site*	Source site	Dates	Treatments†	Release density	Total released	Total resighted
Manta Real	El Copal	15/16–28 July, 1993	C (yellow)	Low (2 per 163 m)	9	5
Manta Real	El Copal	15/16–28 July, 1993	E (white)	Low (2 per 163 m)	18	3
Maquipucuna	El Padrino	3–23 Aug, 1994	C (yellow)	High (2 per 40 m)	16	10
Maquipucuna	El Padrino	3–23 Aug, 1994	E (white)	High (2 per 40 m)	21	10
Agua Caliente	El Copal	23 Nov–5 Dec, 1994	C (yellow)	Low (2 per 164 m)	11	4
Agua Caliente	El Copal	23 Nov–5 Dec, 1994	E (white)	Low (2 per 164 m)	12	3
Tinalandia	El Copal	24 June–7 July, 1995	C (white)	Low (2 per 193 m)	14	5
Tinalandia	El Copal	24 June–7 July, 1995	E (yellow)	Low (2 per 193 m)	16	3
		Overall	Control		50	24
			Experimental		67	19
			Total		117	43

* Elevations and locations of release sites: Manta Real, (400–500 m) 2° 34.73' S, 79° 20.92' W; Maquipucuna, (1,150–1,300 m) 0° 7.42' N, 78° 37.77' W; Agua Caliente, (200–350 m) 2° 37.07' S, 79° 28.99' W; Tinalandia, (600–800 m), 0° 17.88' S, 79° 3.26' W. Site elevations and locations for source sites: El Copal, (885 m) 0° 52.81' S, 79° 0.4.96' W and El Padrino, (775 m) 0° 0.73' S, 78° 59.21' W. † Treatments are determined by the colour of the dominant co-model species: C, controls; E, experimentals (see Methods).

species with warning coloration is a challenge to müllerian mimicry theory: distasteful butterflies should converge on a single, shared warning coloration with their müllerian co-model³. However, this experiment shows that polymorphic *H. cydno* from western Ecuador seem to be under strong geographically variable selection alternately favouring mimicry with two different co-model species. It also seems that selection against unfamiliar phenotypes may be relaxed at higher density. Thus, geographical variation in selection for mimicry coupled with weak selection against morphs when they are common⁸ explains the puzzling existence of this and other polymorphic müllerian mimics²³. Furthermore, multiple co-models coupled with relaxed selection at higher density may allow a shifting balance favouring new warning-colour patterns^{8,24–26}. This phenomenon may be particularly important not only in the origin and maintenance of new warning colours but also in the adaptive radiation of new mimetic species^{24,25,27}. □

Methods

Butterfly capture and release

I captured *H. cydno* at polymorphic source sites and transported them to release sites in glassine envelopes in a moist cooler (Table 1). I numbered, sterilized⁸ and fed the butterflies a sucrose solution at the release sites, and held them overnight in large cages. I released healthy butterflies the next day in pairs (one experimental and one control) or in triplets (two experimentals and one control, to increase the likelihood of resighting experimentals) at even intervals along release trails (Table 1). The geometry of release trails is similar at all sites (trails parallel a creek or river). I calculated the release density from the number of butterflies divided by the trail distance (m). I reduced known handling effects on recaptured butterflies²⁸ by resighting individual butterflies with 10 × 42 binoculars or a 20 × 60 spotting scope. I began resighting 12 h after release and continued until nearly all of the butterflies had disappeared from the release sites.

Treatments

At all release sites, co-models outnumber *H. cydno* (typically in a 4:1 ratio, range 2:1–36:1)⁸. At three release sites (Manta Real, Maquipucuna and Agua Caliente), abundant yellow co-models *H. eleuchia* co-occur with exclusively yellow *H. cydno*. At these sites, transferred yellow *H. cydno* butterflies are controls and white butterflies are experimentals⁸. At the fourth release site, Tinalandia, both co-model species co-occur with white and yellow morphs of *H. cydno*⁸. However, before and during the release at Tinalandia white *H. sapho* outnumbered yellow *H. eleuchia* by 5:1, thus providing the reciprocal treatment of a ‘white’ dominated release site⁸.

Data analysis

The daily probability of resighting a butterfly (θ_t) is estimated as a function of daily resighting effort E_t (total native butterflies of the three species encountered in a given day i , $[\theta_t = \alpha E_t]$)¹¹. I measured survival differences²¹ using maximum likelihood to estimate parameters of a simple survival model, $P_E e^{-\lambda t}$. P_E indicates the day 0 to day 1 probability of establishment or survival, and $e^{-\lambda t}$ indicates the proportion of these butterflies still alive (or present) at day t with death rate λ . Statistical analysis of survival data also takes into account θ_t (equations not shown). The complete model equations used are found in the appendix of ref. 11. Life expectancy (LE) is estimated as P_E/λ (ref. 11). Selection against the unfamiliar colour pattern ($s = 1 - LE_{\text{exp}}/LE_{\text{con}}$) where exp is experimental and con is control is directly proportional to the reduction in experimental life expectancy relative to controls¹¹.

The analysis proceeded in two steps: model parameter estimation for all combinations of treatments, release sites and different sexes (the ‘full’ model), followed by reduction to include only statistically significant effects^{19–21,29}. The full model fitted the observed number of butterflies resighted on a given day (goodness-of-fit test, $\chi^2_{66} = 59.13$, $P = 0.71$) and thus represents a reasonable starting point for the analysis²¹. Because the full model fit the data well I used likelihood ratio tests (LRT)^{19,21,29} and Akaike information criteria (AIC)²⁰ to reduce it to include only statistically significant main effects (and interactions), such as sex or site differences in α , P_E or λ , before testing for differences in these parameters between experimental and control butterflies²¹.

I considered first statistically significant variation in the resighting coefficient α (as suggested by ref. 21). Maximum likelihood estimates of α were higher for experimentals ($\alpha = 0.0103$) than for controls ($\alpha = 0.00798$; LRT, $G_1 = 4.59$, $P = 0.032$; AIC = 309.16, 2 parameters)^{19,20}. This indicates that experimental butterflies may stand out to human observers because they differ from butterflies with local warning colour patterns (natives and controls). Experimentals were easier to resight than controls at Maquipucuna, Agua Caliente and Tinalandia but not at Manta Real. At Manta Real, females were more likely to be resighted than males ($G_1 = 4.08$, $P = 0.0435$). To avoid potential resighting biases²¹, I estimated an intermediate resighting model that included treatment-based differences in α (control $\alpha = 0.00743$, experimental $\alpha = 0.0106$) from the three sites combined (Maquipucuna, Agua Caliente and Tinalandia, $G_1 = 5.63$, $P = 0.018$) and sex-based differences in α at Manta Real (female $\alpha = 0.013$, male $\alpha = 0.0069$). Other than effects on resighting probability at Manta Real, sex did not affect parameter estimates. I used this resighting model with the lowest AIC (306.0, four parameters) to calculate the daily probability of capture (θ_t) for the analysis of survival parameters²¹. For survival the

combined LRT and AIC analysis indicated that the best combination of P_E and λ include additive effects of site and treatment with 12 parameters (site + treatment model). Sex had no main effect on survival parameters ($G_4 = 6.60$, $P = 0.16$), and none of the interactions was statistically significant^{21,29}. I used this final model to assess the experimental effects of treatments on survival.

Programming

I programmed likelihoods using Visual Basic (Microsoft, Redmond, Washington, 1994). I located global maxima by passing ln likelihoods to the SOLVER function in Microsoft Excel (Microsoft, Redmond, Washington, 1994). All runs using different initial parameter values converged on the same maximum likelihood estimate. Visual inspections showed that all likelihood profiles were smooth and continuous with a single global maximum.

Received 14 June; accepted 10 October 2000.

- Müller, F. *Ituna* and *Thyridia*; a remarkable case of mimicry in butterflies. *Trans. Ent. Soc. Lond.* **1879**, xx–xxix (1879).
- Wickler, W. *Mimicry in Plants and Animals* (Wiedenfeld and Nicolson, London, 1968).
- Turner, J. R. G. Butterfly mimicry—the genetical evolution of an adaptation. *Evol. Biol.* **10**, 163–206 (1977).
- Fisher, R. A. *The Genetical Theory of Natural Selection* (Dover Publications, New York, 1958).
- Holmgren, N. M. & Enquist, M. Dynamics of mimicry evolution. *Biol. J. Linn. Soc.* **66**, 145–158 (1999).
- Gavrilets, S. & Hastings, A. Coevolutionary chase in two-species systems with applications to mimicry. *J. Theor. Biol.* **191**, 415–427 (1998).
- Alatalo, R. & Mappes, J. Tracking the evolution of warning signals. *Nature* **382**, 708–710 (1996).
- Kapan, D. D. *Divergent Natural Selection and Müllerian Mimicry in Polymorphic Heliconius cydno (Lepidoptera: Nymphalidae)*. Thesis, Univ. of British Columbia (1998).
- Joron, M. & Mallet, J. L. Diversity in mimicry: paradox or paradigm. *Trends Ecol. Evol.* **13**, 461–466 (1998).
- Benson, W. W. Natural selection for Müllerian mimicry in *Heliconius erato* in Costa Rica. *Science* **176**, 936–939 (1972).
- Mallet, J. L. & Barton, N. H. Strong natural selection in a warning-colour hybrid zone. *Evolution* **43**, 421–431 (1989).
- Endler, J. A. Frequency-dependent predation, crypsis and aposematic coloration. *Phil. Trans. R. Soc. Lond. B* **319**, 459–472 (1988).
- Gilbert, L. E. in *Plant-Animal Interactions: Evolutionary Ecology in Tropical and Temperate Region* (eds Lewinsohn, P. W., Wilson, T. M., Fernandes, G. & Benson, W. W.) 403–427 (John Wiley and Sons, New York, 1991).
- Brower, L. P., Brower, J. V. Z. & Collins, C. T. Experimental studies of mimicry. 7. Relative palatability and Müllerian mimicry among Neotropical butterflies of the subfamily Heliconiinae. *Zoologica* **48**, 65–83 (1963).
- Chai, P. Field observations and feeding experiments on the responses of rufous-tailed jacamars (*Galbula ruficauda*) to free-flying butterflies in a tropical rainforest. *Biol. J. Linn. Soc.* **29**, 161–189 (1986).
- Brower, L. P., Cook, L. M. & Croze, H. J. Predator responses to artificial Batesian mimics released in a neotropical environment. *Evolution* **21**, 11–23 (1967).
- Cook, L. M., Brower, L. & Alcock, P. J. An attempt to verify mimetic advantage in a neotropical environment. *Evolution* **23**, 339–345 (1969).
- Waldbauer, G. P. & Sternburg, J. G. Saturniid moths as mimics: an alternative interpretation of attempts to demonstrate mimetic advantage in nature. *Evolution* **29**, 650–658 (1975).
- Edwards, A. W. F. *Likelihood* 2nd edn (Johns Hopkins Univ. Press, Baltimore, 1992).
- Akaike, H. in *International Symposium on Information Theory* 2nd edn (eds Petran, B. N. & Csáki, F.) 267–281 (Akadémiai Kiadó, Budapest, 1973).
- Lebreton, J. D., Burnham, K. P., Clobert, J. & Anderson, D. R. Modeling survival and testing biological hypotheses using marked animals: a unified approach with case studies. *Ecol. Monogr.* **62**, 67–118 (1992).
- Dunlap-Pianka, H., Boggs, C. L. & Gilbert, L. E. Ovarian dynamics in heliconiine butterflies: programmed senescence versus eternal youth. *Science* **197**, 487–490 (1977).
- Brown, K. & Benson, W. W. Adaptive polymorphism associated with multiple Müllerian mimicry in *Heliconius numata* (Lepid. Nymph.). *Biotropica* **6**, 205–228 (1974).
- Mallet, J. L. & Joron, M. Evolution of diversity in warning colour and mimicry: Polymorphisms, shifting balance, and speciation. *Annu. Rev. Ecol. Syst.* **30**, 201–233 (2000).
- Mallet, J. L., Mallet & Singer, M. C. Individual selection, kin selection, and the shifting balance in the evolution of warning colours: the evidence from butterflies. *Biol. J. Linn. Soc.* **32**, 337–350 (1987).
- Turner, J. R. & Mallet, J. L. Did forest islands drive the diversity of warningly coloured butterflies? Biotic drift and the shifting balance. *Phil. Trans. R. Soc. Lond. B* **351**, 835–845 (1996).
- Mallet, J. L., McMillan, W. & Jiggins, C. in *Endless Forms: Species and Speciation* (eds Berlocher, S. & Howard, D.) 390–403 (Oxford Univ. Press, New York, 1998).
- Mallet, J. L., Longino, J. T., Murawski, D., Murawski, A. & Gamboa, A. S. Handling effects in *Heliconius*: Where do all the butterflies go? *J. Anim. Ecol.* **56**, 377–386 (1987).
- Skalski, J. R., Hoffman, A. & Smith, S. G. in *Marked Individuals in the Study of Bird Populations* (eds Lebreton, J. D. & North, P. M.) 9–28 (Birkhäuser, Basel, 1993).
- Mallet, J. L. *et al.* Estimates of selection and gene flow from measures of cline width and linkage disequilibrium in *Heliconius* hybrid zones. *Genetics* **124**, 921–936 (1990).

Acknowledgements

I thank D. Schluter, L. Gilbert, M. Kirkpatrick, M. Singer, R. Dudley, U. Mueller, P. Schappert, W. O. McMillan and S. Bennett for valuable discussion and critical review of this manuscript, and H. Knechtel, C. Chapman, J. Page, S. Zaklan and K. Holston for field assistance. This research is supported by Earthwatch and its volunteer corps, by L. Gilbert and, in part, by a National Science and Engineering Research Council grant to D. Schluter.

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