BIOTROPICA 31(4): 661–668 1999

Behavioral and Physiological Differences between Two Parapatric *Heliconius* Species[1]

Angus Davison[2], **W. Owen McMillan**, **Ashleigh S. Griffin**[3], **Chris D. Jiggins**, and **James L. B. Mallet**

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

ABSTRACT

The behavior and physiology of two parapatric sibling species, *Heliconius erato cyrbia* Godt. and *H. himera* Hew., were investigated to assess if environmental adaptation enabled stable morphological, genetic, and ecological differences to exist in the face of hybridization. Morning and evening activity, egg production, and larval development time of *H. himera* and *H. erato* in insectaries were recorded; individuals were collected in allopatry and in sympatry from a hybrid zone in which the species overlapped. Studies were performed at ambient conditions within the natural range of *H. himera*. *H. himera* was considerably more active than *H. erato,* flying earlier in the morning and later in the evening, even when both species were collected in sympatry. Similarly, *H. himera* laid more eggs, and the hatched larvae developed more rapidly. The results suggest that physiological constraints are an important selective force that may have been important in speciation and counteracts hybridization in the maintenance of the *H. himeralH. erato* contact zone. Ecological selection, arising from adaptation to low temperatures, may help explain the competitive exclusion of *H. erato* by *H. himera* in the drier, cooler montane habitat favored by the latter species.

RESUMEN

Se investiga la conducta y fisiología de dos especies gemelas parapátricas, *Heliconius erato cyrbia* Godt. y *H. himera* Hew., a fin de dilucidar como la adaptación al ambiente puede contribuir a diferencias morfológicas, genéticas y ecológicas a pesar de la hibridación de las mismas. Se registró la actividad matinal y vespertina, la producción de huevos y tiempo de desarrollo larvario de *H. himera* y *H. erato* en insectarios utilizando individuos recolectados en alopatría y en zonas de hibradación donde ambas especies se solapan. Los estudios se realizaron en condiciones ambientales dentro del rango natural de *H. himera*. *H. himera* es considerablemente más activa que *H. erato* volando desde mas temprano por la mañana y hasta más tarde al atardecer, incluso cuando ambas especies se recolectan en zonas de simpatría. Igualmente *H. himera* pone mayor número de huevos, y el desarrollo larvario es mas rápido. Los resultados sugieren que restricciones fisiológicas pueden actuar a modo de fuerza selectiva importante que contrarreste la hibridación en el mantenimiento de *H. himera* y *H. erato* en zonas de solapamiento. Una selección ecológica en relación a una adaptación a bajas temperaturas puede ayudar a explicar la exclusión competitiva de *H. erato* por *H. himera* en habitats montanos más frios y secos que favorecen a esta última especie.

Key words: competitive exclusion; Heliconius; *hybridization; hybrid zone; Lepidoptera; parapatry; speciation.*

SPECIATION IS STUDIED MOST USEFULLY BY INVESTIGATING INTERMEDIATE CASES, races or species that maintain morphological, genetic, and ecological differences despite hybridization (Endler 1977; Szymura & Barton 1986, 1991; Barton & Hewitt 1989; Hewitt 1989; Harrison 1993). Reproductive differences between sibling species have been studied intensively (Coyne & Orr 1989), but much less is known about the role of ecological adaptation in speciation. Parapatric contact zones between geographic races and species are very common, especially in the tropics (MacArthur 1972, Brown 1979, Barton & Hewitt 1989, Harrison 1993). Bull (1991) reported that many coincide with ecotones, which suggests that ecological factors are important in maintaining species differences. Species differences can be maintained in the face of liberal gene flow, implying that ecological differences can stabilize adaptive peaks (Grant & Grant 1992). In this study, we assessed the extent of ecological differentiation between two butterfly sibling species, *Heliconius erato cyrbia* Godt. and *Heliconius himera* Hew., and their hybrids (Descimon & Mast de Maeght 1984).

Heliconius erato is common in light gaps and second growth forest from sea level to 1500 m

---

[1] Received 18 November 1997; revison accepted 12 June 1998.

[2] Corresponding author: Department of Genetics, University of Nottingham, Nottingham NG7 2UH, England; Fax: 0115 970 9906; a.davison@hgmp.mrc.ac.uk.

[3] Present address: ICAPB, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT, Scotland.

throughout much of the Neotropics. Wing pattern variation within the unpalatable *H. erato* is extensive and there are > 30 different racial types, each a Müllerian mimic of a similar race of *H. melpomene* (Brown 1981, Brower 1994). Parapatric races often differ strikingly in wing patterns, but mate randomly in contact zones composed largely of hybrids (Mallet 1993). Müllerian mimicry between species and narrow boundaries between color pattern races of *H. erato* are maintained by frequency dependent predation against rare warning color variants (Turner 1971; Benson 1982; Mallet 1986, 1993; Mallet *et al.* 1990).

Heliconius himera replaces its sibling species *H. erato* in dry forest and thorn scrub of southern Ecuador and northeastern Peru (Brown 1979, Sheppard *et al.* 1985). Contact zones between *H. himera* and three distinct color pattern races of *H. erato* have been located (Jiggins *et al.* 1996). In the Río Puyango drainage (Loja and El Oro provinces of Ecuador), a relatively abrupt transition occurs over a *ca* 5-km distance from *H. erato cyrbia* to *H. himera.* Unlike the contact zones between races of *H. erato,* hybrids between *H. himera* and *H. erato* are much rarer than the pure species, making up only 8 to 10 percent of the population (Jiggins *et al.* 1996). These hybrids survive well and mate freely with either parental type (McMillan *et al.* 1997); however, the transition between *H. erato* and *H. himera* is considerably narrower than in previously studied contact zones between color pattern races of *H. erato,* which implies stronger selection (Jiggins *et al.* 1996, Mallet *et al.* 1998). Frequency dependent selection by predators against rare hybrid phenotypes, similar to the mimetic selection known from interracial hybrid zones of *H. erato* (Turner 1971, Benson 1982, Mallet 1986, 1993, Mallet *et al.* 1990), is probably important in the *erato/himera* contact zone. The larvae of the two species, however, use identical larval host plants in the genus *Passiflora,* and adults do not differ in oviposition preference (Jiggins, McMillan, & Mallet 1997). Genetic differences between *H. himera* and *H. erato* are stable and there is little evidence of introgression at color pattern loci, allozyme markers, or mtDNA (Jiggins, McMillan, King, & Mallet 1997). Extensive crosses have failed to show any hybrid inviability or sterility, and the scarcity of hybrids relative to pure forms is caused mainly by strong assortative mating, balanced by some unidentified form of ecological selection (McMillan *et al.* 1997, Mallet *et al.* 1998). The transition between *H. erato* and *H. himera* coincides very closely with a steep ecotone between wet forest and dry thorn scrub (Jiggins *et al.* 1996). It therefore seems likely that adaptive environmental differences between the species (other than frequency dependent predation or host-plant adaptation) play a role in maintaining species differences and the position of the hybrid zone.

This paper describes experiments investigating potential behavioral and ecological differences between *H. erato, H. himera,* and their hybrids to explain the narrow contact zones with little introgression, their position on an ecotone, and also the divergence that led to speciation. Specifically, we examined differences in morning and evening activity, egg production, and larval developmental time.

METHODS

Sources of butterflies.—Butterflies were collected in Loja and El Oro provinces, southwest Ecuador (Jiggins *et al.* 1996). *Heliconius erato* were captured in two wet forest sites near Balsas and Piñas (*ca* 800–1000 m elev.), while *H. himera* were collected in dry forest near Vilcabamba (*ca* 1500–1600 m elev.). These sites were far from the hybrid zone, *i.e.*, species were allopatric. In addition, individuals of both species were collected in sympatry within the narrow contact zone. This region, near Chaguarpamba (*ca* 1000 m elev.), marks a rapid transition between wet and dry forest habitat types (Jiggins *et al.* 1996).

The insectaries.—After capture, butterflies were placed in envelopes and transported to Vilcabamba (30 km south of Loja). During transit, butterflies were fed a 10 percent sugar solution every two to three hours. Survival using this strategy was high. Butterflies were allowed to acclimatize in outdoor insectaries for 2–3 days prior to data collection. Each insectary was partitioned into six 1 × 1 × 2 m^3 cages that housed one or two butterflies (Jiggins, McMillan, & Mallet 1997, McMillan *et al.* 1997). Individual cages were provided with ample artificial nectar, pollen resources, and larval food plants. Artificial nectar consisted of a sugar solution displayed in small red and yellow plastic cups. Sugar solution was checked daily and replenished as necessary. In addition, fresh nectar and pollen resources (critical for egg production) were provided by cut *Lantana* sp. flowers. *Passiflora rubra* and *P. punctata,* the larval hosts of both *erato* and *himera* (Jiggins *et al.* 1996; Jiggins, McMillan, & Mallet 1997) were provided for oviposition. Eggs were collected daily and larvae were raised individually in small plastic pots to prevent cannibalism. Developing larvae were fed ample new growth of *P. rubra* and *P. punctata* (Jiggins, McMillan, & Mallet 1997). Larval containers were kept at ambient shade temperature, cleaned daily, and new food was added as needed. A small twig taped to the top of each pot provided a suitable pupation site. Pupae were then housed together by brood in larger plastic cages until emergence. Sex and date of egg collection, pupation, and eclosion were recorded for each individual. We avoided inbreeding by crossing only unrelated individuals (McMillan *et al.* 1997).

Behavioral observations.—Within the outdoor insectaries, we monitored butterflies for five behavioral criteria: (1) roosting—butterfly hanging in inverted position, wings together; (2) perching—butterfly at rest, upright, wings together; (3) sunning—butterfly at rest, upright, wings open at *ca* 180°, facing sun; (4) flying; and (5) feeding. Clustered cages allowed the activity of up to 18 butterflies to be recorded simultaneously. Observations were carried out every 10 min from 0710 to 1100 h, and from 1600 to 1830 h between October and December 1994. Except under unusual circumstances, butterflies were still roosting at 0710 h (sunrise: *ca* 0600–0620 h), and by 1830 h (sunset: *ca* 1800 h) all had roosted again for the night.

The times of four behavioral events were recorded: (1) time of departure from the roost in the morning; (2) morning activity—time of second observation of flying or feeding. The second time of flying, rather than the time the butterfly actually left the roost, was used to indicate the earliest point in the day when adults consistently were active. Adults frequently left the roost, but then perched on the ground or sides of the cage for some time before flying again. In contrast, the "second flying time" adults remained more or less continuously active; (3) evening activity—latest time that the butterflies actively were flying and feeding. Again, we used the time of the second to last observation period when adults were flying or feeding, because *Heliconius* frequently remained perched for some time in the late afternoon before slowly fluttering about and flying into the roost; (4) the time when adults roosted.

Most observations were carried out on females of the two species collected from allopatry in either wet forest (*H. erato*) or dry forest (*H. himera*) biotopes, but a smaller group of males collected from the same locations and a group of males of both species collected in sympatry from the Chaguarpamba contact zone were also included. Unfortunately, females were rare in the hybrid zone; thus studies with sympatric individuals could be done only with males.

Paired design.—Adult behavior and larval developmental time were clearly sensitive to temporal fluctuations in temperature, solar intensity, and rainfall. We therefore compared only individuals kept together and experiencing the same ambient conditions. For the behavioral observations, five types of paired comparison were made: (1) female *himera* against female *erato* (allopatric); (2) female allopatric *himera* against female F1; (3) female allopatric *erato* against female F1 (In both 2 and 3, the F1 was the offspring of a mating between *himera* and *erato* individuals collected from allopatric populations.); (4) male *himera* against male *erato* (allopatric); and (5) male *himera* against male *erato* (sympatric). When possible, individuals were used only in a single paired comparison; however, because of a lack of material, some F1 butterflies were compared to *himera* and *erato* adults previously used in interspecific comparisons.

Analyses.—The difference in time between the first and second individual of a pair (*e.g.*, *himera*/*erato*, *himera*/F1) completing one of the four behavioral events was measured for each day. An arbitrary ranking was used—*H. himera* > hybrid F1 > *H. erato*—so a positive difference in time indicated that highest ranking individual carried out that activity first. In most paired trials, activities were recorded over more than one day. When this was the case, the mean of the difference in paired timed observations between any two individuals over a number of days was used as the paired time difference. The mean paired time difference and 95 percent confidence limits (Student's *t*-distribution) of the sample mean were calculated for each set of comparisons to test for a significant deviation from zero.

RESULTS

Behavioral analyses.—Butterflies appeared to acclimatize rapidly to our insectaries; they soon were flying and feeding during the day and roosting from small twigs during the night. *Heliconius himera, H. erato,* and F1s left the roost at similar times during the morning (Fig. 1a; Table 1). In paired comparisons, however, both male and female *H. erato* were active significantly later in the morning than *H. himera* (Fig. 1b). Female *H. himera* actively flew and fed, on average, 40 min ear-

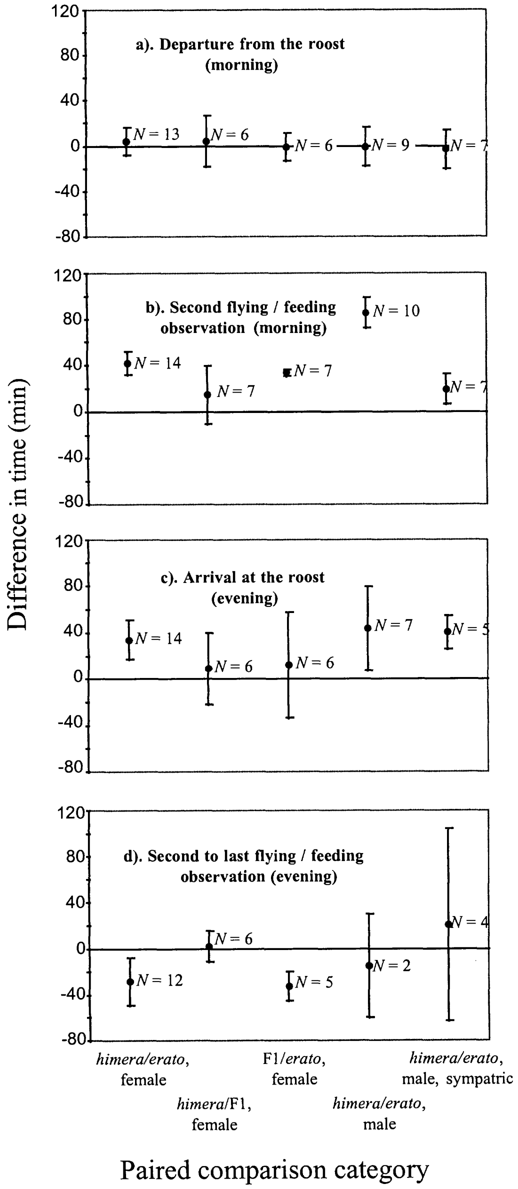

FIGURE 1. A–D Paired comparisons among *Heliconius himera, H. erato cyrbia,* and hybrid F1s for the four behavioral categories. All butterflies were of allopatric origin except where stated. A positive (t value shows that the highest ranking species carried out that activity first (ranking *H. himera* > hybrid F1 > *H. erato*). The bars indicate ± 95 percent confidence limits.

lier than *H. erato* (Fig. 1b). Differences between males were even more pronounced, with allopatric male *H. himera* flying 90 min earlier that male *H. erato* (Fig. 1b). Female F1s were similar to *H. himera* in morning activity. A difference in morning activity between *H. himera* and *H. erato* was also evident (although less pronounced) among males collected in sympatry in the Chaguarpamba contact zone (Fig. 1b).

In the evening, allopatric female *H. himera* roosted 30–40 min earlier than *H. erato,* but ceased flying and feeding 25 min later than *H. erato* females (Fig. 1c–d; Table 1). For males, no significant differences were observed in the evening flying and feeding times, but both allopatric and sympatric *H. himera* males again tended to roost, on average, 40 min earlier. Again, F1 females were active in the evening like *H. himera,* but they did not differ significantly in roosting time. The direction of the cross (*i.e.*, male *himera* × female *erato* or vice versa) did not affect morning and evening activity of hybrids (data not shown).

EGG LAYING AND DEVELOPMENTAL RATE.—Differences in daytime activity between *H. himera* and *H. erato* also were reflected in rates of egg production. In these paired experiments, females were allowed to lay eggs for two weeks to one month, and laid, on average, 50 eggs over the observation period (McMillan *et al.* 1997). In 19 paired comparisons (12 reared and 7 wild-caught pairs), *H. himera* laid significantly more eggs per day than *H. erato* (mean difference = 0.74 eggs/day; $t = -2.66$; $P = 0.016$; df = 18). As expected, the number of eggs laid per day in hybrid crosses depended largely on the genotype of the mother. *Heliconius himera* females mated by *H. erato* males laid fewer (not significantly) eggs per day than conspecific mated *H. himera* (mean difference = 0.1 eggs/day; $t = -0.33$; $P = 0.753$; df = 7). *H. erato* females mated by *H. himera* males showed significantly reduced egg production relative to *H. himera* females mated by conspecifics (mean difference = 0.5 eggs/day; $t = -2.47$; $P = 0.031$; df = 11).

There were also differences in egg to adult development time between *H. himera* and *H. erato.* Using a paired comparison between broods raised together, *H. erato* took, on average, 1.3 d longer to develop into an adult than *H. himera* ($t = 12.9$; $P = 0.0001$; df = 11). Significant differences, however, were not detected without this paired comparison, probably because of changing weather conditions throughout the study (mean egg to adult developmental time 29.1 ± 0.7 d for *H. himera* [$N = 26$], and 29.9 ± 1.0 d for *H. erato* [$N = 12$]). Development time for larvae from both directions of hybrid crosses were similar and took significantly longer to develop than *H. himera* × *H. himera* crosses (1.2 d longer for offspring of *H.*

TABLE 1. *Estimated mean times of departure from the roost, morning/evening activity, and arrival at the roost for allopatric* H. himera *and* H. erato *(including data from males and females). These behavioral events are defined in the methods. Values given are the mean time ± SE in minutes (* N *in parentheses).*

	Departure from roost	Active in morning	Active in evening	Arrival at roost
H. himera	0753 h ± 0:04 (22)	0841 h ± 0:04 (24)	1712 h ± 0:06 (14)	1718 h ± 0:06 (21)
H. erato	0755 h ± 0:04 (22)	0937 h ± 0:04 (24)	1645 h ± 0:05 (14)	1749 h ± 0:08 (21)

himera females mated by *H. erato* males [$t = 4.32$; $P = 0.005$; df = 6] and 0.8 d longer for the offspring of matings between *H. erato* females and *H. himera* males [$t = 3.08$; $P = 0.011$; df = 11]).

DISCUSSION

In *Heliconius,* closely related pairs of species usually differ strongly in their ecology. For example, the species most closely related to *H. erato,* such as *H. himera, H. clysonymus, H. charithonia, H. hermathena, H. hortense, H. telesiphe,* and *H. hecalesia,* are all found in more arid or montane environments than *H. erato.* In contrast, races within *H. erato* differ far less from each other ecologically (Benson 1982, Mallet 1993). This contrasting pattern in the habitat associations of races and species suggests that adaptation to different biotopes could play a crucial role in the transition from color pattern races to species. Subsequently, parapatric distributions may be maintained along ecotones because habitat adaptation geographically restricts distinct species by competitive exclusion, until eventually, ecological divergence (particularly the evolution of host plant ecology) permits coexistence. Few attempts, however, have been made to investigate how speciation might result from adaptive differences in ecology, physiology, or behavior. We performed experiments to investigate the ecological parameters by which *H. himera* and *H. erato* must have diverged; we examined how the species acquired enough differences to form sharp parapatric contact zones with little introgression, but not enough to invade the others range. By excluding classical intrinsic hybrid inviability and sterility as causes of the lack of introgression, we were aware that this selection must be largely ecological; however, the precise mode of selection was previously unclear (Jiggins *et al.* 1996; Jiggins, McMillan, King, & Mallet 1997; McMillan *et al.* 1997; Mallet *et al.* 1998).

In Vilcabamba, *H. himera* was considerably more active than *H. erato.* It flew both earlier in the morning and later in the evening (except the males in the evening for which there was a lack of data), despite the facts that adults left the roost at similar times in the morning and *H. himera* roosted earlier in the evening. Physiological differences perhaps are expected between individuals collected from different localities and altitudes, simply due to local adaptation; however, this cannot be the sole explanation because both morning behavior and evening roosting differences were maintained between *H. himera* and *H. erato* collected in sympatry. F1 hybrid behavior was either intermediate or like that of *H. himera.*

Egg laying and developmental rates of *H. himera* and *H. erato* closely corresponded to differences in flying, feeding, and roosting. The more active *H. himera* laid more eggs than *H. erato,* and egg–adult development time was one to two days less in *H. himera* than in *H. erato.* Hybrids were again intermediate.

While the selection against hybrids and the narrowness of the hybrid zone can be explained in part by predator selection against hybrid color patterns, the strong correlation between wet/dry ecotone and the contact zone in three separate contacts between *H. erato* and *H. himera* in Ecuador and Peru suggest that the ecological differences we found are adaptive (Descimon & Mast de Maeght 1984, König 1986, Mallet 1993, Jiggins *et al.* 1996). Our results show that the species differ in the amount of time spent foraging during the morning and afternoon, the number of eggs laid, and their consequent developmental rate. These traits must impact directly the relative fitness of individuals. Butterflies that forage more will be able to collect more pollen, and thus produce more eggs (Dunlap-Pianka *et al.* 1977, Boggs 1981). In addition, more active females will be able to search for scarce, cryptic (even mimetic) new shoots of their host plants and lay more eggs on them (Gilbert 1975). Males will be able to forage more effectively for newly eclosing females (Deinert *et al.* 1994). Finally, rapidly developing larvae will outcompete slower ones, especially since new growth of the host *Passiflora* may be limiting and larvae are cannibalistic (Gilbert 1975).

Just what are the differences between habitats that might cause the observed differences in the butterflies? The experiments were carried out in the dry habitat of *H. himera* near the upper limit of the altitudinal range for *H. erato.* The higher altitude and dryer climate in the range of *H. himera* resulted in cooler average temperatures and greater diurnal temperature fluctuation (Jiggins *et al.* 1996). Every increase in altitude of 100 m coincides with a temperature decrease of 0.6 to 1.0°C in the Neotropics (MacArthur 1972). In our study, between October and November 1994, temperatures at 1500 m in Vilcabamba varied considerably between a daytime maximum of > 30°C and a nighttime minimum of 11°C. In contrast, lowland wet forest rarely falls below 20°C at night and average daily temperature fluctuation may be as low as 4°C (Wallace 1853, Mabberley 1992). Therefore, the most probable explanation is that *H. himera* is physiologically adapted to lower temperatures early and late in the day. This is entirely concordant with the faster development time of *H. himera* larvae in Vilcabamba, particularly since it is difficult to imagine other factors that could have such an adverse effect in the relatively controlled, humid environment of our larval pots. The existence of performance differences in larvae protected from direct solar radiation implies that the effect is not a result of differences in the ability to absorb heat. Other species of butterflies are able to use dark areas of their wings and bodies to heat up more rapidly during basking, and this has a direct effect on the flight capacity (Watt 1968, Douglas & Grula 1978). As the temperature rose in the early morning, *H. himera* became active faster than *H. erato,* and remained active later during cooling in the evening. Increased foraging resulted in more eggs laid and shorter development times of the larvae in the cold-resistant species.

Because larval host plant adaptation plays no role in *H. himera/H. erato* speciation, and because overlapping species of *Heliconius* almost always differ in their food-plant choice, competition for hosts probably prevents the coexistence of the two species (Jiggins, McMillan, & Mallet 1997). *Heliconius himera* may forage more sustainedly for pollen-donor plants, larval host plants, and mates in dry areas, which could result in the competitive exclusion of *H. erato* from the cooler, drier upland forests. The converse experiment to test if *H. erato* has the competitive advantage in warm, wet habitats was not logistically possible. We predict, however, that *H. erato* can forage better in the less sunny but more equable, humid conditions with lower diurnal temperature fluctuations, of the rain forests and cloud forests in which it occurs. The competitive switch between the species across the transition from wet to dry forest could lead to a very sudden changeover between the species even if the ecotone were fairly broad (Bull 1991). The hybrid zone between the two species, however, may not necessarily be a result of mutual competitive exclusion. Neither may be able to sucessfully reproduce in the range of the other, or else a single species may exclude the other.

One of the most interesting aspects of this system for understanding speciation was that these two species could maintain their genetic integrity in the face of hybridization. A number of previous studies have shown that even in cases in which there was no evidence for intrinsic genetic incompatibilities between taxa, disruptive environmental selection could eliminate intermediate phenotypes (Grant & Grant 1989, 1992; Schluter 1993). In association with partial assortative mating, this disruptive ecological selection can maintain two distinct genotypic clusters in sympatry. This represents an important intermediate stage in the speciation process. Our example was slightly different than those studied by Schluter (1993) and Grant and Grant (1989, 1992) as the two species and the two niches were parapatric, rather than broadly sympatric. The ecotone itself, in which intermediate phenotypes perhaps might be ecologically favored, may be too narrow relative to dispersal, and the rare intermediate phenotypes too strongly selected against via Müllerian mimicry, to cause a spreading of the contact zone.

This study contributes evidence to the hypothesis that speciation and parapatry in *Heliconius* is strongly associated with divergence in habitat adaptation (Mallet 1993). It provides evidence for behavioral differences between *H. himera* and *H. erato* that must translate into large fitness differences in the field. The results provide a partial explanation for a switch in competitive advantage between the species in the center of the wet/dry ecotone, and also may provide a key to how the gene pools of these two species remain distinct through selection against hybrids.

ACKNOWLEDGMENTS

We would like to thank all those in Ecuador who helped the project, in particular INEFAN for granting permission to carry out research in the country, the Museo de Ciencias Naturales in Quito, and Fundación Arcoiris in

Loja for their support. Help in raising butterflies was provided by Jose Carpio Ayala, Carlos Roberto Carpio Ayala, María del Carmen Cabrera, and Maria Soledad Arias Diaz. This project was funded by a research grant and a studentship from the BBSRC. Thanks also to two reviewers for their helpful comments.

LITERATURE CITED

Barton, N. H., and G. M. Hewitt. 1989. Adaptation, speciation, and hybrid zones. Nature 341: 497–503.

Benson, W. W. 1982. Alternative models for infrageneric diversification in the humid tropics: tests with passion vine butterflies. *In* G. T. Prance (Ed.). Biological diversification in the tropics, pp. 608–640. Columbia University Press, New York, New York.

Boggs, C. L. 1981. Nutritional and life-history determinants of resource allocation in holometabolous insects. Am. Nat. 117: 692–709.

Brower, A. V. Z. 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. Proc. Natl. Acad. Sci. U.S.A. 91: 6491–6495.

Brown, K. S. 1979. Ecologia geografica e evolucáo nas florestas neotropicais, 2 vols. (Livre de Docencia) Universidade Estadual de Campinas, Campinas, Brazil.

———. 1981. The biology of *Heliconius* and related genera. Annu. Rev. Entomol. 26: 427–456.

Bull, C. M. 1991. Ecology of parapatric distributions. Annu. Rev. Ecol. Syst. 22: 19–36.

Coyne, J. A., and H. A. Orr. 1989. Two rules of speciation. *In* D. Otte and J. A. Endler (Eds.). Speciation and its consequences, pp. 180–207. Sinauer Associates, Sunderland, Massachusetts.

Deinert, E. I., J. T. Longino, and L. E. Gilbert. 1994. Mate competition in butterflies. Nature 370: 23–24.

Descimon, H., and J. Mast de Maeght. 1984. Semispecies relationships between *Heliconius erato cyrbia* Godt. and *H. himera* Hew. in southwestern Ecuador. J. Res. Lepid. 22: 229–239.

Douglas, M. M., and J. W. Grula. 1978. Thermoregulatory adaptations allowing ecological range expansion by the pierid butterfly *Nathalis iole* Boisduval. Evolution 32: 776–783.

Dunlap-Pianka, H. L., C. L. Boggs, and L. E. Gilbert. 1977. Ovarian dynamics in heliconiine butterflies: programmed senescence versus eternal youth. Science (Wash.DC) 197: 487–490.

Endler, J. A. 1977. Geographic variation, speciation, and clines. Princeton University Press, Princeton, New Jersey.

Gilbert, L. E. 1975. Ecological consequences of a coevolved mutualism between butterflies and plants. *In* L. E. Gilbert and P. R. Raven (Eds.). Coevolution of animals and plants, pp. 210–240. University of Texas Press, Austin, Texas.

Grant, P.R., and B. R. Grant. 1989. Sympatric speciation and Darwin's finches. *In* D. Otte and J. A. Endler (Eds.). Speciation and its consequences, pp. 433–457. Sinauer Associates, Sunderland, Massachusetts.

———.1992. Hybridization of bird species. Science 256: 193–197.

Harrison, R. G. 1993. Hybrids and hybrid zones: a historical perspective. *In* R. G. Harrison (Ed.). Hybrid zones and the evolutionary process, pp. 3–12. Oxford University Press, New York, New York.

Hewitt, G. M. 1989. The subdivision of species by hybrid zones. *In* D. Otte and J. A. Endler (Eds.) Speciation and its consequences, pp. 85–110. Sinauer Associates, Sunderland, Massachusetts.

Jiggins, C. D., W. O. McMillan, and J. Mallet. 1997. Host plant adaptation has not played a role in the recent speciation of *Heliconius himera* and *H. erato* (Lepidoptera: Nymphalidae). Ecol. Entomol. 22(3): 361–365.

———, ———, P. King, and J. L. B. Mallet. 1997. The maintenance of species differences across a *Heliconius* hybrid zone. Heredity 79(5): 495–505.

———, ———, W. Neukirchen, and J. Mallet. 1996. What can hybrid zones tell us about speciation? The case of *Heliconius erato* and *H. himera* (Lepidoptera: Nymphalidae). Biol. J. Linn. Soc. 59: 221–242.

König, F. 1986. Ein *Heliconius erato himera*—hybrid aus Nord-Peru (Lepidoptera, Heliconiinae). Zeitschrift Arbeitsgemeinschaft der Österreichischen Entomologen 38: 49–50.

Mabberley, D. J. 1992. Tropical rainforest ecology, 2nd Edition. Chapman and Hall, New York, New York.

MacArthur, R. H. 1972. Geographical ecology. Harper and Row, New York, New York.

Mallet, J. 1986. Hybrid zones in *Heliconius* butterflies in Panama, and the stability and movement of warning colour clines. Heredity 56: 191–202.

———. 1993. Speciation, raciation and color pattern evolution in *Heliconius* butterflies: evidence from hybrid zones. *In* R. G. Harrison (Ed.). Hybrid zones and the evolutionary process, pp. 226–260. Oxford University Press, New York, New York.

———, C. D. Jiggins, and W. O. McMillan. 1998. Mimicry and warning colour at the boundary between microevolution and macroevolution. *In* S. Berlocher and D. Howard (Eds.). Endless forms: species and speciation, pp. 390–403. Oxford University Press, New York, New York.

———, N. Barton, G. Lamas, J. Santisteban, M. Muedas, and H. Eeley. 1990. Estimates of selection and gene flow from measures of cline width and linkage disequilibrium in *Heliconius* hybrid zones. Genetics 124: 921–936.

McMillan, W. O., C. D. Jiggins, and J. Mallet. 1997. What initiates speciation in passion-vine butterflies? Proc. Natl. Acad. Sci. U.S.A. 94(16): 8628–8633.

Schluter, D. 1993. Adaptive radiation in sticklebacks: size, shape, and habitat use efficiency. Ecology 74: 699–709.

Sheppard, P. M., J. R. G. Turner, K. S. Brown, W. W. Benson, and M. C. Singer. 1985. Genetics and the evolution of Müllerian mimicry in *Heliconius* butterflies. Phil. Trans. Roy. Soc. Lond. (B) 308: 433–613.

Szymura, J. M., and N. H. Barton. 1986. Genetic analysis of the hybrid zone between the fire-bellied toads *Bombina bombina* and *B. variegata* near Cracow in southern Poland. Evolution 40: 1141–1159.

———, and ———. 1991. The genetic structure of the hybrid zone between the fire-bellied toads *Bombina bombina* and *B. variegata*: comparisons between transects and between loci. Evolution 45: 237–261.

Turner, J. R. G. 1971. Two thousand generations of hybridisation in a *Heliconius* butterfly. Evolution 25: 471–482.

Wallace, A.R. 1853. A narrative of travel on the Amazon and Rio Negro. Reeve & Co., London, England.

Watt, W. B. 1968. Adaptive significance of pigment polymorphisms in *Colias* butterflies. I. Variation of melanin pigment in relation to thermoregulation. Evolution 22: 437–458.