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SHORT COMMUNICATION

Host plant adaptation has not played a role in the recent speciation of *Heliconius himera* and *Heliconius erato*

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Introduction

Herbivorous insects are often highly specialized in their utilization of host plants. This strong host specificity has been implicated as a driving force in speciation (Tauber & Tauber, 1989; Bush, 1994) and may be responsible for the huge species diversity present among herbivorous taxa (Bush, 1993). The association between *Heliconius* and their Passifloraceae host plants is one of the most widely cited examples of host–herbivore coevolution, in which there is clear evidence for the evolution of host plant defences on the one hand, and their subsequent defeat by herbivores on the other (Gilbert, 1971; Smiley, 1978; Brown, 1981). Previous authors have used phylogenetic associations between *Heliconius* and *Passiflora* to support the hypothesis that adaptive radiation has occurred as a result of host–herbivore coevolution (Ehrlich & Raven, 1965; Benson *et al*., 1975; but see Mitter & Brooks, 1983). Furthermore, detailed studies of heliconiine communities demonstrate that coexisting species of *Heliconius* partition host plant resources (Gilbert, 1991), as expected if speciation is driven by host plant divergence. However, despite the important evolutionary interactions that undoubtedly take place between *Heliconius* and *Passiflora*, there is little direct evidence for host-plant-mediated speciation.

There are essentially two ways in which host plant adaptation can precipitate speciation directly. Firstly co-speciation, whereby a speciation event in the host results directly in speciation of an obligate herbivore. Secondly, and perhaps more common, are host plant shifts whereby new herbivore races arise that are adapted to alternative host species. In the extreme case this can lead to divergent evolutionary pathways between sympatric forms, as has occurred in *Rhagoletis pomonella* (Feder *et al*., 1988, 1994; McPheron *et al*., 1988). The observed result of both processes will be strong differences in host plant niche between closely related sympatric species, as is the case in *Heliconius*. However, such a pattern might also be observed if speciation were unrelated to host plant ecology, but necessary to allow sympatry of daughter species. To test these possibilities, an experiment was carried out to investigate the causes of speciation in a pair of parapatric sister species of *Heliconius*.

Heliconius himera and *H. erato* are closely related species that hybridize occasionally in narrow zones of parapatric contact (Mallet, 1993; Brower, 1994; Jiggins *et al*., 1996). *Heliconius himera* occurs in the dry thorn scrub woodlands of south-western Ecuador, north-western Peru and the Marañon valley, whilst the various colour pattern races of *H. erato* are widespread in wet forest across South and Central America. Although they have previously been considered as races (Eltringham, 1916; Lamas, 1976; Brown, 1979; Sheppard *et al*., 1985; but see Kaye, 1916 and Emsley, 1965), they are considered here as separate species because their genetic identity is maintained in the contact zones. In the centre of the contact zone intermediate forms occur at a frequency of 5–10% in the population, a pattern that is confirmed by both colour pattern and presumed neutral genetic markers (Jiggins *et al*., 1997). The reduced frequency of hybrids is primarily a result of strong assortative mating behaviour, with no evidence of reduced fertility or viability in hybrids (McMillan *et al*., 1996). These species therefore provide an ideal opportunity to investigate the importance of host plant adaptation in the early stages of speciation.

Here the results of an experiment to investigate whether *Heliconius erato* and *H. himera* differ in their host plant ecology are presented. Differences in host plant use between these sister species could suggest speciation by host plant shifts. On the other hand, if the species do not differ then the possibility that speciation was driven by host plant adaptation can confidently be excluded.

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Materials and methods

Heliconius larvae feed exclusively on the family Passifloraceae (Brown, 1981). Field investigations (Jiggins *et al.*, 1996) have shown that both *H. himera* and *H. erato cyrbia* utilize two species, *Passiflora rubra* and *P. punctata,* in the contact zone region. *Passiflora rubra* and *punctata* were similarly abundant in Balsas and Piñas where *erato* females were collected, whilst *rubra* was the only species found in Vilcabamba where most of the *himera* females were collected. Previous studies have shown that *H. erato* also feeds on other species, especially *P. auriculata* (Brown, 1981), which is abundant in wet forest throughout western Ecuador but does not occur in the dry forest habitat of *H. himera.* Although *P. auriculata* has not been recorded as a host plant in this area (Jiggins *et al.*, 1996), it is potentially interesting as it occurs only on the *erato* side of the contact zone, so is a possible host of *H. erato* but not *H. himera. Passiflora rubra, P. punctata* and *P. auriculata* are in the subgenus *Plectostemma* (Killip, 1938; Holm-Nielsen *et al.*, 1988).

Host choice experiments were carried out as part of a series of breeding experiments to investigate hybridization between *himera* and *erato.* All experiments were carried out in Vilcabamba, Loja province, Ecuador, which lies at 1600 m a.s.l. in a central valley of the Andes, in a dry forest region. Data from ninety-three females are presented, representing a total of 3948 eggs laid. Females used were either captured in the wild already mated, or were reared in laboratory conditions and mated with males in cages. Laboratory-reared butterflies were fed a mixture of *P. rubra* and *P. punctata* as larvae. All the females tested in this paper are 'pure types', i.e. non-hybrids, although in some instances they may have been mated with a male hybrid as part of the breeding experiments. Experimental females were kept individually in outdoor, wire-mesh cages with dimensions 2 × 1 × 1 m. Each cage was kept supplied with artificial nectar (∼10% sugar solution) in plastic feeders and fresh cut *Lantana* flowers. These provide a source of pollen that is essential for egg production in *Heliconius* (Boggs, 1981). *Passiflora* were grown in pots in each insectary cage. All cages contained both *Passiflora rubra* and *P. punctata* and a few also contained *P. auriculata.*

Every day the location of each egg was recorded and it was then collected by removing a small portion of the leaf or shoot on which it was laid. Data for all females that laid ten or more eggs are included in the analysis. The data are divided into two separate experiments, involving a choice of either two or three host plant species, and each is analysed separately. The number of eggs laid by each female on each species was recorded. A nested *G*-test was then used to test for heterogeneity between individuals and species. Comparisons were also made between wild-caught and laboratory-reared females. Unfortunately the experimental design did not allow the effects of individual cages or host plants to be tested.

Results and Discussion

Heliconius himera and *H. erato* showed virtually identical patterns of oviposition behaviour. Both species laid almost equal numbers of eggs on *P. rubra* and *P. punctata* (Fig. 1a). However, both *H. himera* and *H. erato* laid fewer eggs on *P. auriculata* than on the other two species (Fig. 1b). This result suggests that the lack of field observations of either butterfly species utilizing *P. auriculata* in the contact zone area (Jiggins *et al.*, 1996) was at least partially due to preferences for *P. rubra* and *P. punctata* over *P. auriculata.* However, the fact that both species laid on *P. auriculata* confirms field records from other areas that have shown that this species is a host of *H. erato* (Benson *et al.*, 1975; Brown, 1981). It seems probable that *P. auriculata* is an occasional host in the field, and the lack of records is simply due to a paucity of data. It is interesting that *H. himera* laid on *P. auriculata* as frequently as *erato,* even though this plant is not known to occur in the dry forest habitat of *himera.*

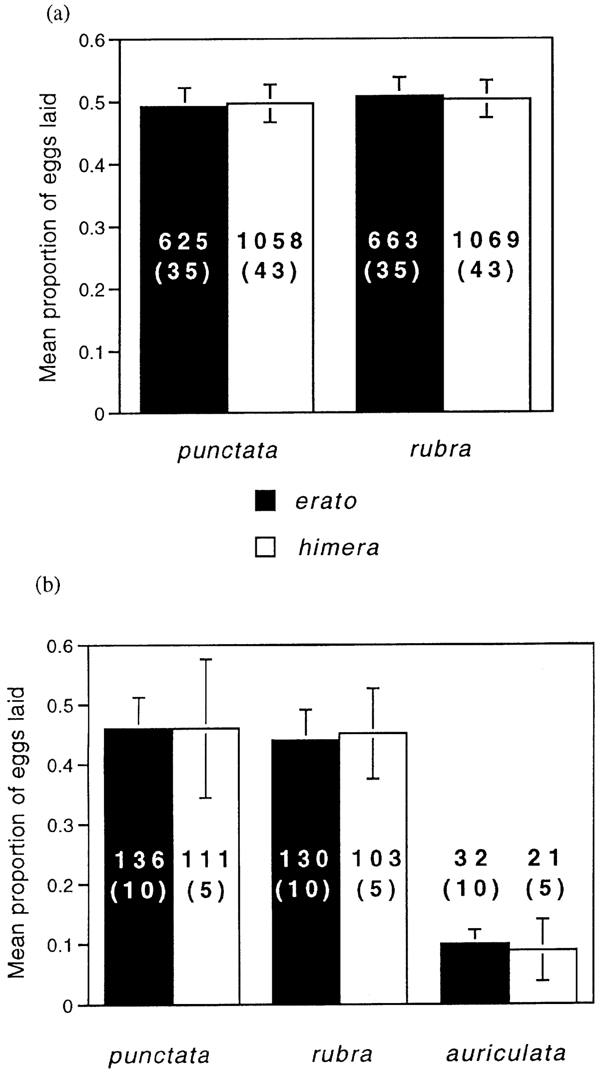

Fig. 1. Proportion of eggs laid on each host plant species averaged over all females. Data from (a) *rubra* vs. *punctata* and (b) *rubra* vs. *punctata* vs. *auriculata* experiments. Standard error bars are shown. Number of eggs laid and, in parentheses, number of females are also shown for each column.

Table 1. Nested *G*-test to investigate heterogeneity of host plant use between individuals, female type and species in the experiment comparing (a) *rubra* and *punctata* and (b) *rubra*, *punctata* and *auriculata*. The test indicates that there are highly significant differences between individual females within all classes, but no significant differences between species or female type. The wild vs. laboratory comparison was not carried out for the second experiment due to the small sample sizes involved (only two *erato* females and one *himera* were raised in the laboratory, the remainder all being wild caught).

	G	d.f.	*P*
(a) *rubra* and *punctata*			
Comparison among *erato* females			
Within laboratory reared	288.71	24	< 0.001
Within wild caught	45.84	9	< 0.001
Wild vs. laboratory	0.57	1	NS
Within *erato*	335.12	34	< 0.001
Comparison among *himera* females			
Within laboratory reared	294.25	33	< 0.001
Within wild caught	22.99	8	< 0.01
Wild vs. laboratory	0.03	1	NS
Within *himera*	317.27	42	< 0.001
erato vs. *himera*	0.48	1	NS
Total heterogeneity	652.87	77	< 0.001
(b) *rubra*, *punctata* and *auriculata*			
Comparison among all females			
Within *erato*	57.04	18	< 0.001
Within *himera*	55.72	8	< 0.001
erato vs. *himera*	0.51	1	NS
Total heterogeneity	113.27	27	< 0.001

There was strong heterogeneity between individual females within both butterfly species (Table 1), with some females showing strong preferences for one or other host plant. To some extent this could have been the result of differences in shoot availability between individual plants. Eggs were almost invariably laid singly on the newest shoots, so differences in the growth form of plants would affect the choices made by particular females. Whilst not affecting the overall comparisons between species, this could have been partly responsible for the large heterogeneity between individuals. However, in general, females did have access to healthy plants of both *P. punctata* and *P. rubra,* and it seems likely therefore that there was actual variation in individual preferences. Further experiments would be needed to test this hypothesis explicitly. If they exist, individual preferences are almost never absolute, as only two individual females showed complete rejection of *P. rubra* and two of *P. auriculata* (data not shown). In a natural situation host plant use of the two primary *Passiflora* species may be controlled more by the availability of new shoots than by slight individual preferences.

There were no significant differences between wild-caught and laboratory-reared females in either species (Table 1a), indicating that choice was not affected by the plant on which any individual was reared. Laboratory individuals were raised on a mixture of *P. punctata* and *P. rubra,* whilst any individual caught in the field could have fed only on a single host plant species. *Passiflora* are generally very widely spaced in the habitat and it is therefore extremely unlikely that larvae migrate from one plant to another. In particular, wild *H. himera* females were all, with one exception, caught in the Vilcabamba area where the only host plant available is *P. rubra.* Therefore all of these wild females almost certainly fed on *P. rubra* as larvae, but the adults showed no significant preferences in any direction. In the experiments there was therefore no evidence for any effects of larval conditioning on the host plant preferences of adults, as has been postulated for other taxa (Corbet, 1985).

The analysis of heterogeneity within and between species shows that there were no significant differences in host plant choice between the two butterfly species, in any of the experiments (Table 1). In laboratory conditions larvae of *H. himera* and *H. erato* were reared successfully to adulthood on each of the three *Passiflora* species, although relative success rates on different host species were not examined explicitly. Therefore it seems reasonable to conclude that there are no strong biochemical or behavioural differences between *H. himera* and *H. erato,* with respect to these three *Passiflora* species. This conclusion is supported by field data (Jiggins *et al*., 1996) showing that both species use *P. rubra* and *P. punctata* in the contact zone. These experiments provide no evidence that host plant adaptation has played a role in the speciation of *himera* and *erato*. The strong pattern of assortative mating between *himera* and *erato* is clearly not related to host plant use (McMillan *et al*., 1997). In this case the strong association of all three known *himera*/*erato* hybrid zones with transitions between dry and wet forest (Mallet, 1993; Jiggins *et al*., 1996) suggests that ecological divergence has played a role in speciation, but that a broadly different biotope, rather than different host plants, has played the major part.

This is not to say that there is no evidence for host-plant-induced speciation in *Heliconius*. There are many examples, particularly within the *H. melpomene* group, of closely related species with divergent patterns of host plant use (Smiley, 1978; Gilbert, 1991), which would seem to support a model of host-shift-mediated diversification. For example *H. cydno* is, by *Heliconius* standards, a generalist that feeds on five *Passiflora* species at La Selva, Costa Rica. Its sympatric sister species, *H. melpomene* (Brown, 1981; Gilbert, 1991; Brower, 1994), is extremely specialized and feeds only on *Passiflora oerstedii*. Other species in the *melpomene* group include *H. hecale*, which feeds on *P. vitifolia*, and *H. ismenius* on *P. alata* and *P. ambigua*. In the case of *H. melpomene* this host plant specialization must be due to female oviposition behaviour and not biochemical adaptation, as the larvae survive equally well on all the hosts of *H. cydno* (Smiley, 1978). Similarly *Heliconius erato* and *charitonia* are closely related species that live sympatrically in secondary forest. In this case, *H. charitonia* has overcome the defences of *Passiflora adenopoda*, a species with hooked trichomes that kill other *Heliconius* larvae (Gilbert, 1971, 1991). It is therefore protected from competition with other *Heliconius* by the defences of its host. In any community there is not a one-on-one relationship between *Heliconius* and *Passiflora* species, but rather a diversity of different strategies – a few *Heliconius* are generalists (within the family Passifloraceae), some are microhabitat specialists and use the species found in that habitat, whilst most others are highly specialized on one or a few hosts (Benson, 1978; Thomas, 1990b; Gilbert, 1991).

However, these interspecific comparisons do not provide convincing evidence that host plant shifts lead to speciation in *Heliconius*. The results of this study show that speciation can occur without host shifts. In addition evidence from other butterflies, in particular *Euphydryas* spp., shows that host plant shifts may occur frequently without leading to speciation (Singer *et al*., 1993). The partitioning of host plant use between *Heliconius* species may instead result from competitive interactions. The overall diversity of *Heliconius* species in any area is highly correlated with the number of host plant species (Gilbert & Smiley, 1978; Thomas, 1990a), and this implies that the coexistence of species is dependent on divergent patterns of host plant use. *Heliconius himera* and *H. erato* provide a complementary example to this, where an inability to coexist is associated with shared host plants. What is most striking about these species is the extremely abrupt transition between the two. The contact zone between *H. himera* and *H. e. cyrbia* in southern Ecuador is very narrow (⊤ 5 km) and cannot be explained solely in terms of hybrid inviability or disruptive selection on colour pattern (Jiggins *et al*., 1996). It seems likely that competitive exclusion on larval hosts is in part preventing these species from becoming sympatric. Competitive interactions almost certainly explain patterns of divergent host plant use between sympatric *Heliconius* species, and parapatric contact between other closely related species that share host plants (Benson, 1978; Gilbert, 1991).

There exists a perennial problem in the study of speciation; adaptations acquired subsequent to the speciation event will become confounded with those that actually led to the initial divergence. In spite of the strong division of the host plant niche among *Heliconius* species, the evidence presented here shows that speciation is not necessarily driven by host plant adaptation. Instead, divergence in host plant use after speciation may be necessary to permit coexistence and persistence of the daughter species. Whilst the results presented here certainly do not preclude host shift speciation in other *Heliconius* species, they do suggest an alternative explanation for the observed patterns of host plant use.

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