Correlations between adult mimicry and larval host plants in ithomiine butterflies

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The apparent paradox of multiple coexisting wing pattern mimicry 'rings' in tropical butterflies has been explained as a result of microhabitat partitioning in adults. However, very few studies have tested this hypothesis. In neotropical forests, ithomiine butterflies dominate and display the richest diversity of mimicry rings. We show that co-mimetic species occupy the same larval host-plant species significantly more often than expected in two out of five communities that we surveyed; in one of these, the effect remains significant after phylogenetic correction. This relationship is most probably a result of a third correlated variable, such as microhabitat. Host-plant microhabitat may constrain adult movement, or host-plant choice may depend on butterfly microhabitat preferences and mimicry associations. This link between mimicry and host plant could help explain some host-plant and mimicry shifts, which have been important in the radiation of this speciose tropical group.

1. INTRODUCTION

A logical extension of Müllerian mimicry theory (Müller 1879) is that all aposematic species in a community should converge on a single warning signal. The reality is quite different: in neotropical ithomiine butterflies (Lepidoptera: Nymphalidae: Ithomiinae), eight or more distinct mimetic patterns, or 'mimicry rings,' occur in the same community (Papageorgis 1975; Beccaloni 1997*a*; Joron & Mallet 1998). Plausible explanations for some of this mimetic diversity include developmental constraints on wing pattern, weak selective pressure for convergence of abundant rings, rapid evolution of novel patterns (Joron & Mallet 1998) and, perhaps, 'escape' by better-protected species from Batesian or more poorly protected Müllerian mimics (Pough *et al.* 1973; but see Turner 1987). However, mimetic polymorphisms—even within single species—suggest that additional ecological factors may be important, and another hypothesis, that mimicry rings occupy distinct microhabitats, has also been invoked (Papageorgis 1975; Mallet & Gilbert 1995; Beccaloni 1997*a,b*; DeVries *et al.* 1999).

At least some ithomiine mimicry rings are stratified by flight height (Papageorgis 1975; Medina *et al.* 1996; Beccaloni 1997*b*), which correlates with the height of host plants (Beccaloni 1997*b*). DeVries *et al.* (1999) found that co-mimics tended to occur in the same areas of forest, and anecdotal observations suggest that co-mimics and host plants may occur in similar microhabitats (Haber 1978; K. R. Willmott, personal observation). Given that predators such as birds also partition forest microhabitats (e.g. Walther 2002), this could contribute to the stable coexistence of multiple mimicry rings (Beccaloni 1997*b*).

So far, no study has investigated links between larval host plant microhabitat and adult mimicry, but, if the two are correlated, co-mimics might also occur on the same species of host plant. Although it is difficult to obtain quantitative data on butterfly and larval host plant microhabitat associations, it is easy to obtain data enumerating larval host plants. Here, we use new and previously published data to test for a correlation between mimicry and larval host-plant species.

2. METHODS

(a) *Host-plant records*

Host-plant data for ithomiines were compiled from two lowland and three lowland-montane neotropical communities that are reasonably well studied. Host-plant records from elsewhere were excluded because butterfly and plant communities vary between sites. The communities analysed are: Campinas, southeastern Brazil (Brown 1987); east Ecuadorian lowlands (Drummond & Brown 1987); northern and southern Ecuador montane (this study); and Monteverde, Costa Rica (Haber 1978, 2003). These five communities yield a total of 110 ithomiine species (128 taxa) from all tribes and *ca.* 120 host-plant species (table 1; electronic Appendix A). Host plants were identified by S. Knapp (Natural History Museum, London) and ithomiine taxa were identified by K.R.W. for the montane Ecuador datasets. Data for the remaining communities were taken unaltered from the cited publications.

(b) *Mimetic classification*

Taxa were assigned to mimicry complex based on several criteria, including: parallel minor geographical pattern variation between taxa (e.g. the white forewing band of certain 'clearwing' species shows parallel variation in width and opacity); close geographical and elevational range congruence; and possession of distinctive (unique or differentiating) pattern characters for that mimicry complex (Beccaloni 1997*a*; K. R. Willmott, unpublished data; electronic Appendix B). These criteria often resulted in a finer-grained classification than that found by previous authors (see electronic Appendix B). It should be noted that some patterns with different names in different communities represent geographical variants, and that the sexes within species may differ in mimicry ring.

(c) *Phylogenetic correction*

Both wing pattern and larval host plant may be influenced by phylogeny and cannot necessarily be regarded as independent data for each species. Phylogenetic tests of pattern and host-plant correlation are currently not possible, however, because of the lack of knowledge of species-level phylogeny in ithomiines. Nevertheless, both mimicry pattern and larval host plant evolve rapidly, usually varying even within species, so the assumption that both traits are independent of phylogeny for pairs of species in different species groups within genera, as well as between genera, is reasonable. For pairs of species within species groups, where traits might be shared through ancestry and cause incorrect rejection of the null hypothesis (type I error), the raw data were reanalysed, with records of shared host plants of co-mimetic ithomiines excluded. By 'within-species goups' we mean species where morphological (particularly genitalic) traits have not yet ruled out the possibility that they might be sister taxa or members of monophyletic clades in which mimicry and host-plant traits are invariant (K. R. Willmott, unpublished data). This definition is conservative because it also excludes congeneric species that are almost certainly distantly related but which lack structural differences, like some *Ithomia* (C. Jiggins, personal communication, unpublished molecular phylogenetic analysis). In five out of the seven excluded pairs, geographical variation within at least one of the species shows that the local similarity in pattern is almost certainly a result of mimicry rather than common ancestry.

Table 1. Results from randomization analyses. Significant *p*-values are in bold, indicating communities where matches between host plant and mimicry occurred more often than expected by chance alone.
(Abbreviations: ith., ithomiine; no. ith. spp., number of ithomiine species with host plant records; est. no. ith. spp., estimated total number of ithomiine species in community; no. M, F mim., number of mimicry rings for males, females; no. hosts, number of host plants used by all species; empir. M, F m, empirical value of m, the number of matches of mimicry pattern and host plant, for males and females; no. recs, number of recorded interactions of mimicry and host plant; p male, female, proportion of the 100 000 simulated datasets in which m equalled or exceeded the empirical value; min., max., mean sim. M, F m, minimum, maximum and mean values for m in the 100 000 simulations, for males and females.)

analysis	no. ith. spp.	est. no. ith. spp.	no. M mim.	no. F mim.	no. hosts	empir. M m	empir. F m	no. recs	p male	p female	min. sim. M m	min. sim. F m	max. sim. M m	max. sim. F m	mean sim. M m	mean sim. F m
Ecuador north montane																
raw data	24	90	10	8	20	8	10	37	**0.006**	**0.003**	0	0	12	13	2.50	3.25
corrected	24	90	10	8	20	8	9	36	**0.005**	**0.006**	0	0	13	15	2.39	3.11
Ecuador south montane	24	90	14	12	18	1	1	29	0.751	0.839	0	0	9	11	1.24	1.59
Ecuador lowland																
raw data	25	65	8	9	33	2	2	40	0.311	0.257	0	0	7	7	1.15	1.00
corrected	25	90	8	9	33	1	1	39	0.593	0.532	0	0	6	6	0.98	0.84
southeast Brazil																
raw data	16	25	7	7	41	7	7	73	0.367	0.368	0	0	33	37	5.71	5.72
corrected	16	25	7	7	41	6	6	72	0.452	0.453	0	0	35	33	5.48	5.48
Costa Rica																
raw data	46	55	9	9	55	22	21	102	**0.008**	**0.042**	0	1	33	42	10.65	12.57
corrected	46	55	9	9	55	11	10	93	0.131	0.326	0	0	24	29	7.09	8.38

(**d**) *Statistical analysis*

We used as a test statistic the number of matches between pairs of co-mimics and host plants (m). One ithomiine species feeding on one host-plant species constitutes a single record, regardless of the number of feeding observations. Under the null hypothesis, mimicry and host-plant traits are randomly distributed among species. Therefore, to obtain an appropriate null frequency distribution for m, both mimicry complex and host plant were randomized 100 000 times with respect to ithomiine species, keeping the numbers of records for each plant and ithomiine species constant (we used a specially written program RANDMIM, available at http://abacus.gene.ucl.ac.uk/jim/bin/software.html). The frequency with which the empirical value of m was equalled or exceeded in the simulated (random) datasets was interpreted as a measure of significance (*p*-values in table 1).

3. RESULTS AND DISCUSSION

Among 281 host-plant–mimicry interactions, there were 190 possible pairwise combinations of ithomiine species feeding on the same host plant, of which 40 male and 41 female pairs matched in mimicry pattern (see electronic Appendix C). The proportion of such possible pairwise combinations with mimicry matches in the five communities varied from 37% in montane northern Ecuador to 8% in montane southern Ecuador. Three out of the five communities studied had more empirical matches than the mean of the 100 000 random datasets (table 1). Out of the observed matching pairs of species (30; electronic Appendix D), almost half were in different genera (20% within the same tribe and 27% from different tribes), with 13% of the remainder being unrelated species within a genus (figure 1; electronic Appendix D). Thus, shared descent alone could account for at most 40% of matches, and many of these may prove to be independent of phylogeny when species relationships are resolved. In the randomization analysis with the raw data, two out of the five communities—montane northern Ecuador and Costa Rica—showed a significant association between mimicry ring and host plant, rejecting the null hypothesis that these traits are randomly distributed among species (table 1: $p < 0.01$ for males, $p < 0.05$ for females). The null hypothesis was not rejected in the remaining communities. With phylogenetic correction (deletion of matches within species groups), the null hypothesis was still rejected for montane northern Ecuador (table 1: $p < 0.01$), but not for other communities.

A link between host-plant species and mimicry complex is most plausibly caused by a correlated third variable, microhabitat. In Ecuador, for example, *Solanum* species eaten by co-mimics *Dircenna adina* and *Hyalyris ocna* occur in secondary growth at forest edges, while *Cestrum* 'sp1' favours the shady open understorey of undisturbed forest, where its herbivore co-mimics, *Godyris panthyale* and *Greta enigma*, usually fly. Searching in particular microhabitats may help female ithomiines to locate host plants and male ithomiines to find mates (Courtney 1984; Beccaloni 1997*b*). The slow flight of ovipositing females and the stationary display behaviour of courting males (Haber 1978) may further increase the chances of predation in these microhabitats. Alternatively, existing microhabitat preferences in adult butterflies, perhaps constrained by mimicry, may result in oviposition on particular host-plant species.

By contrast, the lack of correlation between mimicry and host-plant species in other communities does not prove that mimicry and microhabitat are uncorrelated there. In other words, there is no *a priori* reason to suppose that
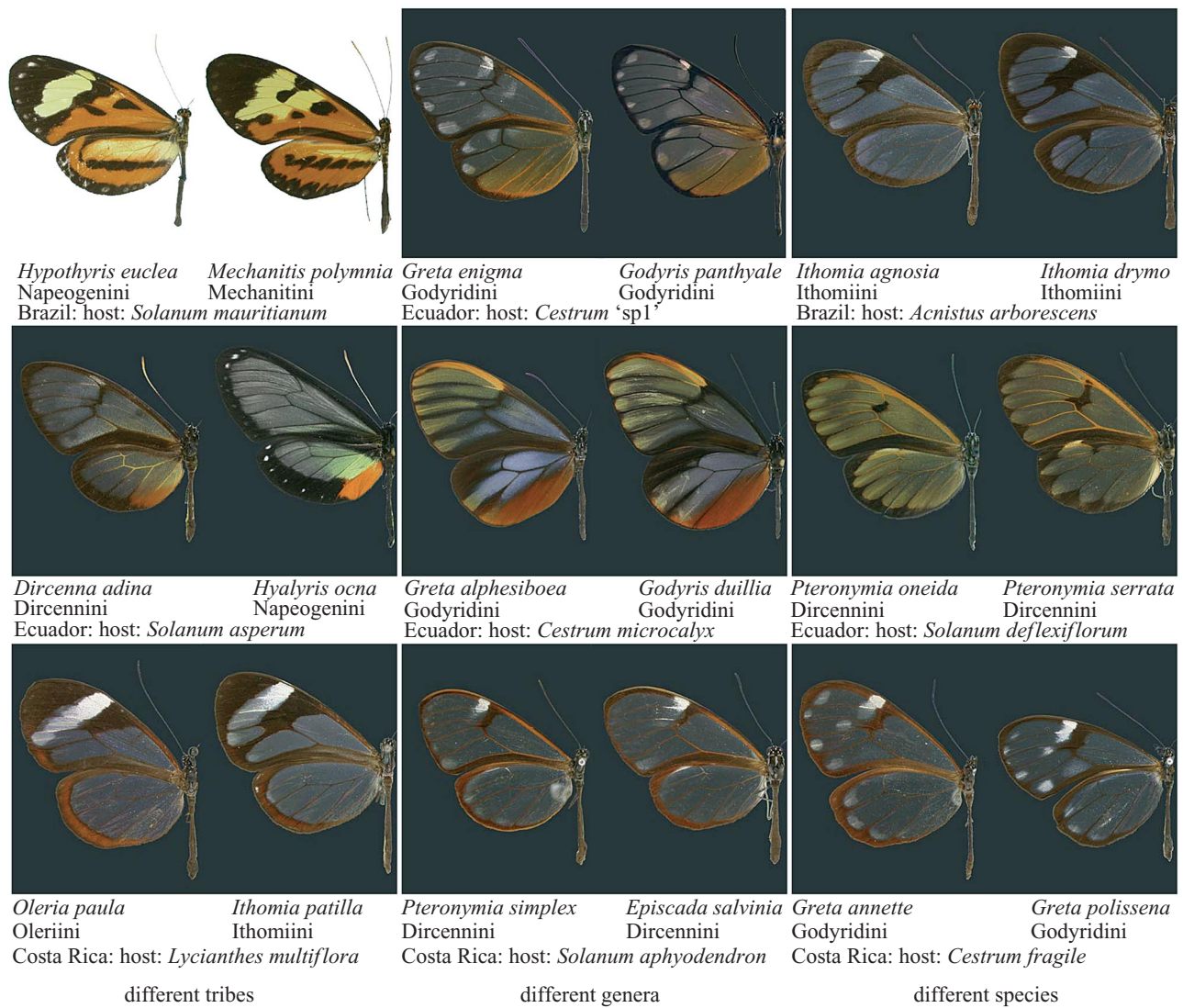
Hypothyris euclea	*Mechanitis polymnia*	*Greta enigma*	*Godyris panthyale*	*Ithomia agnosia*	*Ithomia drymo*
Napeogenini	Mechanitini	Godyridini	Godyridini	Ithomiini	Ithomiini
Brazil: host: *Solanum mauritianum*		Ecuador: host: *Cestrum* 'sp1'		Brazil: host: *Acnistus arborescens*	

Dircenna adina	*Hyalyris ocna*	*Greta alphesiboea*	*Godyris duillia*	*Pteronymia oneida*	*Pteronymia serrata*
Dircennini	Napeogenini	Godyridini	Godyridini	Dircennini	Dircennini
Ecuador: host: *Solanum asperum*		Ecuador: host: *Cestrum microcalyx*		Ecuador: host: *Solanum deflexiflorum*	

Oleria paula	*Ithomia patilla*	*Pteronymia simplex*	*Episcada salvinia*	*Greta annette*	*Greta polissena*
Oleriini	Ithomiini	Dircennini	Dircennini	Godyridini	Godyridini
Costa Rica: host: *Lycianthes multiflora*		Costa Rica: host: *Solanum aphyodendron*		Costa Rica: host: *Cestrum fragile*	

different tribes   different genera   different species

Figure 1. Examples of ithomiine co-mimics, from different tribes, genera and species, with the same species of larval host plant.

that species that occur in the same microhabitat must feed on the same host plant: empirically, many co-mimics do feed on different host plants and, in other butterfly groups, like *Heliconius* (Nymphalidae), overlap in host plants among micro-sympatric species is probably actively reduced by competition (Benson, 1978; Gilbert 1991; see below).

Our results also suggest a novel ecological mechanism that might further contribute to the evolution of mimicry. If host plants are patchily distributed within forest then the abundance of adults will also vary in space, with local aggregations where host-plant density is high. If predator home ranges are also restricted, such that the principal predators in areas of high host-plant density are quickly educated to avoid a particular pattern, all species feeding on the same host plant will benefit through mimicry, even in the absence of strong host-plant or butterfly microhabitat associations.

Mimicry diversity is no doubt also maintained by mechanisms unrelated to host-plant use, and this factor, in combination with random sampling effects, must explain at least some of the variation between communities studied here. Northern and southern montane Ecuador faunas

are similar but differ markedly in their overlap of co-mimics and host plants; only few pairs of co-mimics have been recorded in the latter, so that overlap is unlikely for the data so far gathered. By contrast, many ithomiines in the Brazilian community have multiple host-plant records. Some of these are probably rare hosts where links to adult mimicry may be slight, and, in addition, higher numbers of hosts per species increase the chance of matches in the randomized datasets, making rejection of the null hypothesis less likely.

Direct or apparent competition between ithomiines might also cause host-plant partitioning and thus reduce the overlap of mimicry and host-plant use in some ithomiine communities, as in *Heliconius* (Benson 1978; Gilbert 1991). In *Heliconius*, mimicry is instead correlated with microhabitat and height of adult roosts (Gilbert 1991; Mallet & Gilbert 1995), although examples of larval mimicry are known between species that do share host plants (Mallet & Longino 1982). Larvae of co-mimics in the Ithomiinae are often found feeding in numbers on the same plant individual, which suggests that competition may be less important in this group than in *Heliconius* (see also Haber 1978).

Even in the absence of direct host-plant-related competition, host-plant shifts may occur for a variety of reasons, including adaptation to features of the host plant, such as secondary defences or associated predators or parasitoids. If a host-plant shift places adult butterflies in a new mimetic environment, strong predator selection may drive wing pattern change. Alternatively, mimicry evolution may be driven by local variation in abundance of other co-mimics (Bates 1862), favouring subsequent shifts to new larval host plants. Correlated shifts in mimicry and flight microhabitat are likely to cause pre-mating isolation, because wing patterns function as courtship cues (Naisbit *et al.* 2001; Jiggins *et al.* 2001) and because males often locate females by waiting in particular microhabitats (e.g. Shields 1967; K. R. Willmott, personal observation). Mimicry shifts also cause disruptive selection because the unfamiliar wing patterns of hybrids are not recognized and therefore not avoided by predators (Mallet & Barton 1989). Marked geographical changes in ithomiine mimetic wing patterns occurring across the Amazon basin as if at 'an enchanter's wand' inspired Bates (1862) to formulate his theory of mimicry. Bates also saw changes in mimetic wing patterns as a prime example of how natural selection could drive speciation. Our study shows how a better knowledge of the ecology of mimicry and host-plant choice can provide insights into how adaptation may influence the diversification of these butterflies.

Bates, H. W. 1862 Contributions to an insect fauna of the Amazon Valley. Lepidoptera: Heliconidae. *Trans. Linn. Soc. Lond.* **23**, 495–566.

Beccaloni, G. W. 1997*a* Ecology, natural history and behaviour of ithomiine butterflies and their mimics in Ecuador (Lepidoptera: Nymphalidae: Ithomiinae). *Trop. Lepid.* **8**, 103–124.

Beccaloni, G. W. 1997*b* Vertical stratification of ithomiine butterfly (Nymphalidae: Ithomiinae) mimicry complexes: the relationship between adult flight height and larval host plant height. *Biol. J. Linn. Soc.* **62**, 313–341.

Benson, W. W. 1978 Resource partitioning in passion vine butterflies. *Evolution* **32**, 493–518.

Brown Jr, K. S. 1987 Chemistry at the Solanaceae/Ithomiinae interface. *Ann. Missouri Bot. Gdn* **74**, 359–397.

Courtney, S. P. 1984 Habitat versus food plant selection. In *Biology of butterflies* (ed. R. I. Vane-Wright & P. R. Ackery), pp. 89–90. London: Academic.

DeVries, P. J., Lande, R. & Murray, D. 1999 Associations of co-mimetic ithomiine butterflies on small spatial and temporal scales in a neotropical rainforest. *Biol. J. Linn. Soc.* **67**, 73–85.

Drummond, B. A. & Brown, K. S. 1987 Ithomiinae (Lepidoptera: Nymphalidae): summary of known larval food plants. *Ann. Missouri Bot. Gdn* **74**, 341–358.

Gilbert, L. E. 1991 Biodiversity of a Central American *Heliconius* community: pattern, process, and problems. In *Plant–animal interactions: evolutionary ecology in tropical and temperate regions* (ed. P. W. Price, T. M. Lewinsohn, G. W. Fernandes & W. W. Benson), pp. 403–427. New York: Wiley.

Haber, W. A. 1978 Evolutionary ecology of tropical mimetic butterflies (Lepidoptera: Ithomiinae). PhD thesis, University of Minnesota, USA.

Haber, W. A. 2003 *Clearwing butterflies of Costa Rica (Ithomiinae)*. See http://www.cs.umb.edu/~whaber/Monte/Ithomid/Ithomid-fram.html.

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

Joron, M. & Mallet, J. L. B. 1998 Diversity in mimicry: paradox or paradigm? *Trends Ecol. Evol.* **13**, 461–466.

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. & Longino, J. T. 1982 Host plant records and descriptions of juvenile stages for two rare species of *Eueides* (Nymphalidae). *J. Lepid. Soc.* **36**, 118–126.

Medina, M. C., Robbins, R. K. & Lamas, G. 1996 Vertical stratification of flight by ithomiine butterflies (Lepidoptera: Nymphalidae) at Pakitza, Manu National Park, Perú. In *Manu. The biodiversity of southeastern Peru* (ed. D. E. Wilson & A. Sandoval), pp. 211–216. Washington, DC: Smithsonian Institution.

Müller, F. 1879 *Ituna* and *Thyridia*: a remarkable case of mimicry in butterflies. *Proc. Entomol. Soc. Lond.* **1879**, xx–xxix.

Naisbit, R. E., Jiggins, C. D. & Mallet, J. 2001 Disruptive sexual selection against hybrids contributes to speciation between *Heliconius cydno* and *Heliconius melpomene*. *Proc. R. Soc. Lond.* B **268**, 1849–1854. (DOI 10.1098/rspb.2001.1753.)

Papageorgis, C. 1975 Mimicry in neotropical butterflies. *Am. Scient.* **63**, 522–532.

Pough, H. F., Brower, L. P., Meck, H. R. & Kessell, S. R. 1973 Theoretical investigations of automimicry: multiple trial learning and the palatability spectrum. *Proc. Natl Acad. Sci. USA* **70**, 2261–2265.

Shields, O. 1967 Hilltopping. *J. Res. Lepid.* **6**, 69–178.

Turner, J. R. G. 1987 The evolutionary dynamics of Batesian and Muellerian mimicry: similarities and differences. *Ecol. Entomol.* **12**, 81–95.

Walther, B. A. 2002 Vertical stratification and use of vegetation and light habitats by neotropical forest birds. *J. Ornithol.* **143**, 64–81.

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