

# Mutualistic Interactions Drive Ecological Niche Convergence in a Diverse Butterfly Community

Marianne Elias<sup>1,2\*</sup>, Zachariah Gompert<sup>3</sup>, Chris Jiggins<sup>2</sup>, Keith Willmott<sup>4</sup>

**1** Institute of Evolutionary Biology, University of Edinburgh, Edinburgh, United Kingdom, **2** Department of Zoology, University of Cambridge, Cambridge, United Kingdom, **3** Department of Botany, University of Wyoming, Laramie, Wyoming, United States of America, **4** McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, University of Florida, Gainesville, Florida, United States of America

**Ecological communities are structured in part by evolutionary interactions among their members. A number of recent studies incorporating phylogenetics into community ecology have upheld the paradigm that competition drives ecological divergence among species of the same guild. However, the role of other interspecific interactions, in particular positive interactions such as mutualism, remains poorly explored. We characterized the ecological niche and inferred phylogenetic relationships among members of a diverse community of neotropical Müllerian mimetic butterflies. Müllerian mimicry is one of the best studied examples of mutualism, in which unpalatable species converge in wing pattern locally to advertize their toxicity to predators. We provide evidence that mutualistic interactions can drive convergence along multiple ecological axes, outweighing both phylogeny and competition in shaping community structure. Our findings imply that ecological communities are adaptively assembled to a much greater degree than commonly suspected. In addition, our results show that phenotype and ecology are strongly linked and support the idea that mimicry can cause ecological speciation through multiple cascading effects on species' biology.**

Citation: Elias M, Gompert Z, Jiggins C, Willmott K (2008) Mutualistic interactions drive ecological niche convergence in a diverse butterfly community. *PLoS Biol* 6(12): e300. doi:10.1371/journal.pbio.0060300

## Introduction

A recent review of community ecology literature has suggested that “there is a dynamic interplay between ecology and evolution within communities” [1], such that understanding community structure requires study of both the evolutionary history and recent interactions between constituent species [2–4]. In this context, competition is usually seen as the major force shaping community ecology [5,6], causing ecological displacement among interacting species in communities or biasing community assembly towards more ecologically divergent species [7–10]. However, recent work has uncovered the importance of positive interspecific interactions in influencing the composition and ecological structure of communities [11–15], suggesting that such interactions could outweigh competition [16], for example by facilitating colonization by invasive species [17]. Here we explore the hypothesis that mutualistic interactions among species that are ecologically similar in a broad sense (i.e., members of the same guild) can result in convergence along multiple fine-scale ecological variables.

Müllerian mimicry is a spectacular example of ecological adaptation that is mutually beneficial to multiple species [18,19]. Butterflies are perhaps the best studied Müllerian mimetic organisms [20], where unpalatable species have evolved brightly colored wing patterns that advertize their toxicity to predators. Multiple co-occurring species converge in wing pattern, forming mimicry complexes (i.e., sets of species sharing the same wing pattern), and thus share the cost of educating predators [21]. However, although Müllerian mimicry favors convergence in warning signal locally, ten or more distinct mimicry complexes may coexist in rainforest butterfly communities [22,23]. There is evidence that mimicry complexes are partially segregated by microhabitat such that co-mimics (species that belong to the same mimicry complex)

tend to occur in similar microhabitats [24–27]. Key predators including insectivorous birds are likely segregated in a similar way [28], which in turn should reduce selection for convergence in wing pattern across microhabitats, facilitating the stable coexistence of several mimicry complexes in communities [18,29]. Mimicry should therefore promote adaptive convergence in microhabitat niche among co-mimic species to maximize warning signal overlap, thereby counteracting competition within mimicry complexes. To date, however, the microhabitat niche of mimetic butterflies has never been studied in a phylogenetic context, and ecological similarity among co-mimics could be largely due to common ancestry.

Here we investigate the evolution of microhabitat use among a diverse community of Müllerian mimetic butterflies and conduct the first test of adaptive ecological convergence at the community level. By examining multiple niche axes simultaneously for butterfly species belonging to the same and different mimicry complexes we are able to disentangle the interplay between mimicry, common ancestry and competition in the evolution of microhabitat niche. Our study group is the ithomiines (Nymphalidae: Ithomiinae), the most diverse (~350 species [30]) and abundant Müllerian mimetic butterflies in the neotropics, which act as mimicry models for many other Lepidoptera species [24,31]. Ithomiine larvae feed almost exclusively on Solanaceae plants [32], and adult males of nearly all species actively seek sources of

**Academic Editor:** Anurag Agrawal, Cornell University, United States of America

**Received:** July 24, 2008; **Accepted:** October 22, 2008; **Published:** December 2, 2008

**Copyright:** © 2008 Elias et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

\* To whom correspondence should be addressed. E-mail: melias2008@gmail.com

## Author Summary

What governs the composition of communities of species? Competition promotes divergence in behavior and habitat, allowing species to co-exist. But the effects of other interactions, such as mutualism, are less well understood. We examined the interplay between mutualistic interactions, common ancestry and competition in mimetic butterflies, one of the best studied examples of mutualism, in which species converge in wing pattern to advertize their toxicity to predators. We showed that mutualism drives convergence in flight height and forest habitat, and that these effects outweigh common ancestry (which should lead related species to be more similar) and competition (which promotes ecological divergence). Our findings imply that species that benefit from one another might evolve to form more tightly knit local communities, suggesting that adaptation is a more important process affecting community composition than is commonly suspected. Our results also support the idea that mimicry can cause speciation, through its multiple cascading effects on species' biology.

pyrrolizidine alkaloids to provide chemical protection from predators [33] as well as sex pheromone precursors [34]. Exploitation of such a relatively narrow range of resources among diverse taxa makes ithomiines an ideal system in which to investigate the relative roles of competition and mutualistic interactions in shaping community structure.

## Results/Discussion

The study community in the upper Amazon contained 58 ithomiine species [35] distributed among eight mimicry complexes (Figure 1). A complete species phylogeny for the community was generated from 3,511 bp of mitochondrial and nuclear DNA sequence (Figure 1, Table S1). Microhabitat variables representing forest structure, topography (ridge versus valley) and flight height were recorded during a 3-month field study of the ithomiine community. Forest structure was assessed using 16 variables designed to capture variation in vegetation density, height and light levels, which were subsequently summarized by three principal components, FS1, FS2 and FS3 (Table S2).

We first investigated whether mimicry complexes were segregated by microhabitat. The abundance of individuals in distinct mimicry complexes was significantly segregated along each variable (ANOVAs; FS1:  $F_{7,947} = 72.277$ , FS2:  $F_{7,947} = 7.791$ , FS3:  $F_{7,947} = 5.226$ , Topography:  $F_{7,597} = 28.318$ , Flight height:  $F_{7,1215} = 24.891$ ;  $p \leq 0.0001$  for all variables) and in the multidimensional microhabitat space constituted by the five variables measured (hereafter, global microhabitat; MANOVA: Pillai–Bartlett statistic<sub>35,4735</sub> = 1.594,  $p < 0.0001$ ). Thus, individuals are more likely to be found alongside others that share the same color pattern than expected by chance. Furthermore, the same is true at a species level, such that segregation is not solely driven by the most abundant species. Indeed, regardless of relative abundance, species were segregated according to mimicry complex along FS1 (ANOVA:  $F_{7,46} = 5.519$ ,  $p = 0.0013$ ) and flight height ( $F_{7,46} = 14.648$ ,  $p < 0.0001$ ), and for the global microhabitat (MANOVA: Pillai–Bartlett statistic<sub>35,230</sub> = 1.594,  $p < 0.0001$ ), meaning that co-mimic species were ecologically more similar than expected at random. Thus, mimicry complexes were segre-

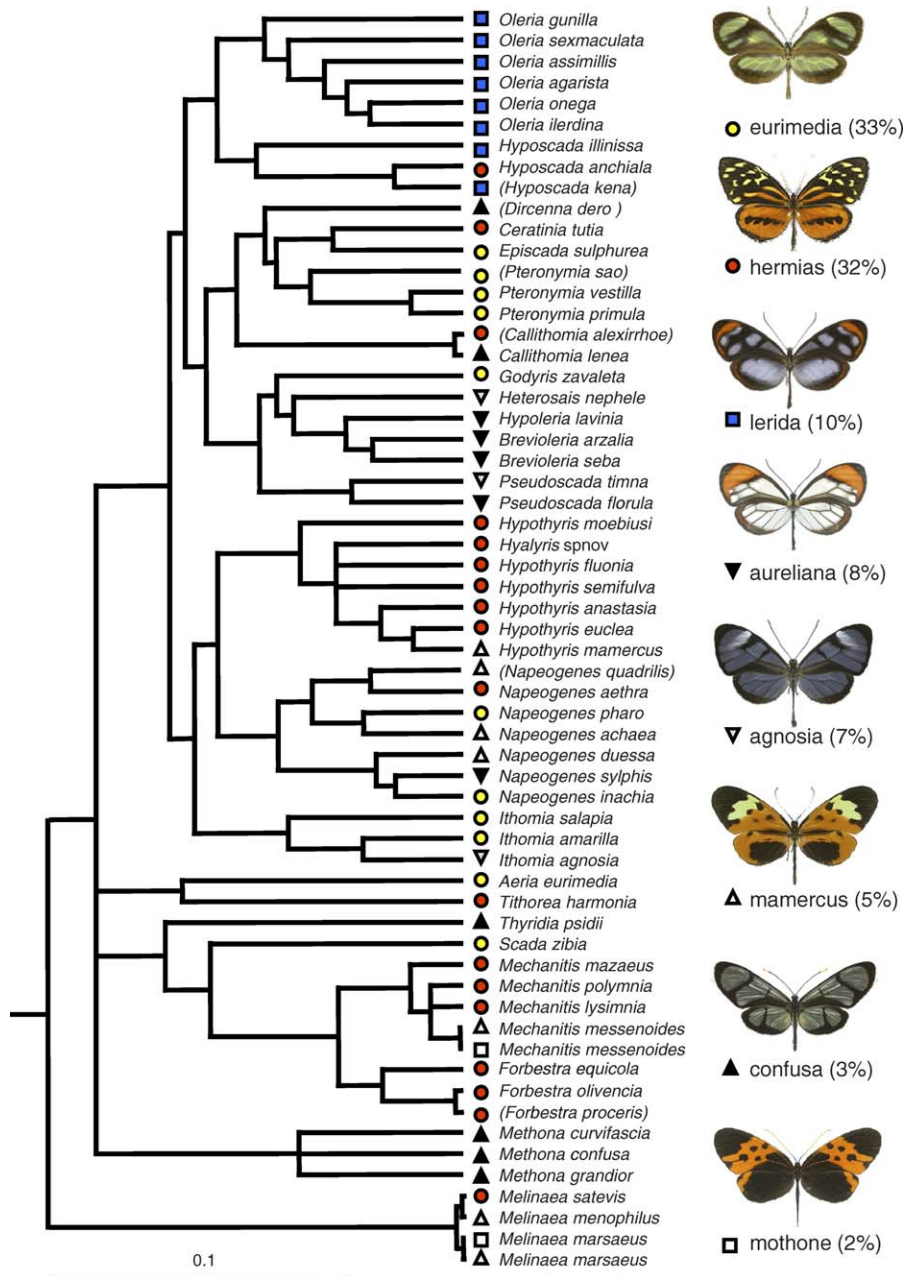
gated in multiple microhabitat dimensions in the study community, in line with previous observations in other communities [24–27].

We then investigated the influence of phylogeny on microhabitat use. Both global microhabitat and distances along each individual microhabitat variable correlated positively with phylogenetic distances (Figure 2), but correlations were significantly supported only for flight height ( $r = 0.168$ ,  $n = 1,431$ ,  $p = 0.0032$ ) and global microhabitat ( $r = 0.137$ ,  $n = 1,431$ ,  $p = 0.0262$ , Figure 2). Thus, as species diverge genetically they also diverge ecologically, as might be expected, but the phylogenetic signal for our measures of microhabitat use is rather weak.

Weak overall phylogenetic signal appears to result from heterogeneity between co-mimics and non-co-mimics. When considering only pairs of non-co-mimic species, distances along four of the five measured variables (FS2, FS3, topography, and flight height) were significantly positively correlated with phylogenetic distances (Figure 2), resulting in a significant phylogenetic signal in global microhabitat ( $r = 0.176$ ,  $n = 1,206$ ,  $p < 0.0001$ ). By contrast, among co-mimics we failed to detect a significant positive correlation for any of the microhabitat variables (Figure 2). Mimicry thus obscures patterns of phylogenetic signal in microhabitat niche.

We carried out a series of tests to specifically investigate the hypothesis that this pattern was due to greater ecological similarity among co-mimics than expected given phylogenetic relationships. Testing this hypothesis requires controlling for phylogeny. Phylogenetic signal can either be removed from the ecological data (regression-based methods) or incorporated into the expected distribution of type I error (simulation-based methods). The first category of methods showed a significant positive correlation between mimicry distances and ecological distances for FS1, flight height, and global microhabitat when phylogeny was controlled for in partial Mantel tests (Table 1), indicating that species in the same mimicry groups tend to be ecologically more similar than species in different mimicry groups. A follow-up analysis, in which ecological distances were regressed onto phylogenetic distances and the resulting residuals tested for convergence (negative values) and divergence (positive values) among co-mimics and non-co-mimics, respectively, confirmed that this pattern of ecological similarity among co-mimics and ecological dissimilarity among non-co-mimics was stronger than expected given the phylogeny (Table 1). Finally, simulations of evolution of the microhabitat variables on the phylogeny under both gradual and speciation models of character evolution also showed that co-mimics were more similar and non-co-mimics more dissimilar than expected assuming no selection and given the phylogeny along FS1, flight height, and global microhabitat (Table 1). Thus, the observation of convergent microhabitat use among co-mimics is robust to both simulation and regression analyses, each based on a different set of assumptions.

Increased ecological similarity among co-mimics is likely to be due initially to mimicry evolving among spatially co-occurring species (i.e., species with at least partial microhabitat niche overlap), followed by further ecological convergence to maximize the efficiency of the warning signal. Divergence among non-co-mimics could be a byproduct of convergence among co-mimics, but could also be enhanced by competitive interactions (niche partitioning). Although



**Figure 1.** Phylogeny of the Ithomiine Species of the Community

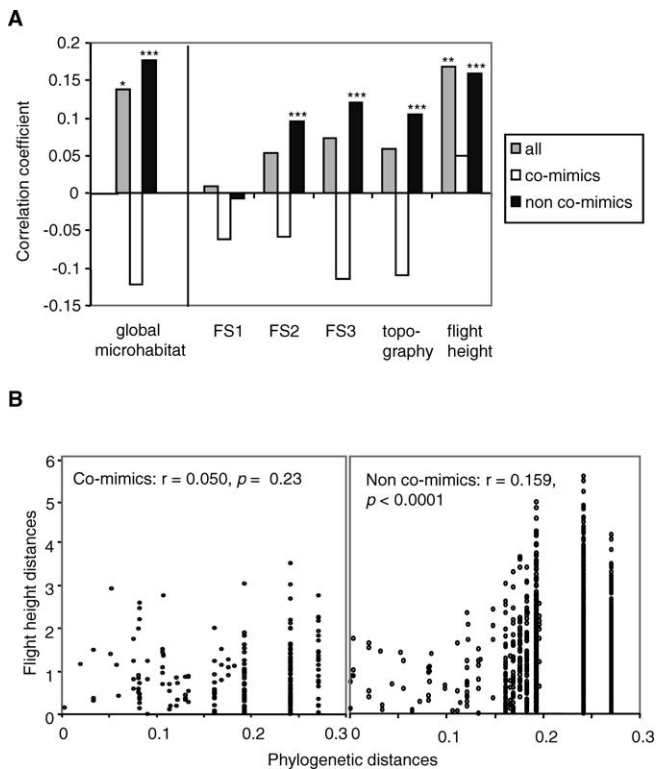
The relaxed-clock tree (maximum clade credibility tree resulting from a Bayesian phylogenetic analysis using a mitochondrial region and a nuclear gene) shows the 60 ithomiine taxa (58 species) of the community, after pruning 20 additional taxa not present in the community. All nodes have a posterior probability above 0.90. Brackets indicate rare species that were excluded from the analyses because not all microhabitat variables could be measured. The eight mimetic patterns are shown on the right with their names and relative abundance in the community, and indicated by colored symbols at the tips of the tree (see Table S1 for the list of taxa and corresponding mimetic patterns). There was a significant phylogenetic signal in the mimicry structure of the community ( $r = 0.162$ ,  $n = 1,431$ ,  $p < 0.0001$ ), confirming that closely related species share color patterns more often than expected at random.

doi:10.1371/journal.pbio.0060300.g001

the microhabitat variables measured here do not represent resources per se, they are likely correlated with key limiting resources, including adult pyrrolizidine alkaloids and food sources, lek sites or larval host-plants [24,27].

To investigate whether interspecific competition occurred along the variables measured and to determine the interplay between competition and mimicry we used niche complementarity, whereby species that are similar along one niche axis diverge along another [36], as an indicator of competi-

tion. As only co-mimics benefit from occurring together, niche complementarity is expected to be greater among non-co-mimics. In agreement with these predictions, significant negative correlations between two microhabitat variables were detected only among non-co-mimics, for FS1–FS3, FS2–FS3 and FS1–flight-height (Table 2). Corresponding correlations for co-mimics were not significantly different from zero and correlation coefficients were positive (Table 2). This provides evidence for competitive interactions between non-



**Figure 2.** Correlation Between Ecological and Phylogenetic Distances (A) Correlation coefficients among all species (gray,  $n = 1,431$ ), among co-mimics (white,  $n = 225$ ) and among non-co-mimics (black,  $n = 1,206$ ) for the global microhabitat and for each ecological variable. One-tailed  $p$ -values for positive correlation are shown for all comparisons with a significant correlation: \*,  $p < 0.05$ , \*\*,  $p < 0.01$ , \*\*\*,  $p < 0.001$  (tests performed using 10,000 permutations, all significant after correction for multiple tests). (B) Scatterplots showing the relationship between flight-height distances and phylogenetic distances for co-mimics and for non-co-mimics, as an example illustrating the results above. The correlation is significant for non-co-mimics only. doi:10.1371/journal.pbio.0060300.g002

co-mimic species only, showing that mimicry counteracts the effects of competition along the variables measured by promoting co-occurrence. Nonetheless, although all tests performed produced similar correlation coefficients, the significance of niche complementarity among non-co-mimics was not robust to the different tests. This was most probably due to reduced power to detect effects in the simulation analysis caused by the inherent increase in complexity and stochasticity associated with this method (unlike the previous tests, here two variables were simulated simultaneously). This also suggests that niche complementarity along microhabitat variables is a weak phenomenon relative to the convergence observed between co-mimics.

Although previous studies have shown that competitive interactions among species can alter community-level patterns of phylogenetic niche conservatism and cause overdispersion [7–9,37], we provide evidence for the opposite pattern, namely adaptive, increased ecological similarity locally between species that benefit from co-occurrence. Mutualistic and commensal interactions that could lead to similar ecological convergence are widespread among plants [38], animals [39–41], and microorganisms [11]. For example, vertebrates commonly form mixed-species groups or flocks, thereby enhancing protection against predators [40,42–44] and foraging efficiency [40]. Species involved in mixed-species groups should have similar habitat requirements [40] and are therefore a nonrandom subset of species of the community. Furthermore, such associations should prevent habitat divergence and could also promote increased habitat similarity, provided that the costs of competition are outweighed by the benefits of coexistence. Facilitative interactions among plants via shared pollinators, shared seed dispersers, or shared seed predators might also promote adaptive convergence in several ways. Plant species that are pollinated by the same pollinator guild often show dramatic convergence in flower morphology [45,46], in a manner analogous to Müllerian mimicry, because coexisting species

**Table 1.** Tests of Adaptive Association Between Mimicry and Ecological Variables

Type of Test	Test	Statistic	Global Microhabitat	FS1	FS2	FS3	Topography	Flight Height
<b>Correlation between mimicry and ecological distances controlling for phylogeny<sup>a</sup></b>		$r$	0.137	0.129	-0.025	0.016	0.020	0.204
		$p$	<b>0.0012</b>	<b>0.0013</b>	0.7357	0.3713	0.2777	< <b>0.0001</b>
<b>Tests of convergence among co-mimics</b>		Ecological distance	0.873	0.681	1.073	0.997	1.006	0.644
		Simulations <sup>b</sup>						
		$p$ (gradual evolution)	<b>0.0300</b>	<b>0.0023</b>	0.9023	0.6398	0.6351	<b>0.0005</b>
		$p$ (speciational evolution)	0.1236	<b>0.0096</b>	0.9049	0.6973	0.6837	<b>0.0025</b>
		Sign of residuals <sup>c</sup>	-0.468	-0.476	0.099	0.008	0.000	-0.454
		$p$	< <b>0.0001</b>	< <b>0.0001</b>	0.9315	0.5443	0.5104	< <b>0.0001</b>
<b>Tests of divergence among non-co-mimics</b>		Ecological distance	1.037	1.062	1.010	1.012	1.022	1.076
		Simulations <sup>b</sup>						
		$p$ (gradual evolution)	<b>0.0013</b>	0.0555	0.9229	0.889	0.6613	0.0142
		$p$ (speciational evolution)	<b>0.0002</b>	0.0322	0.9196	0.8930	0.7042	<b>0.0042</b>
		Sign of residuals <sup>c</sup>	0.0487	0.061	-0.017	-0.018	0.004	0.064
		$p$	<b>0.0012</b>	< <b>0.0001</b>	0.9546	0.9230	0.4014	< <b>0.0001</b>

Bold  $p$ -values indicate tests that remain significant after correction for multiple independent tests.

<sup>a</sup>Partial Mantel test of correlation between ecological and mimicry distances, controlling for phylogenetic distances ( $r$ , correlation coefficient; test performed using 10,000 permutations).

<sup>b</sup>Tests of ecological convergence among co-mimics and divergence among non-co-mimics based on simulations (ecological distance: average standardized ecological distance among co-mimics and non-co-mimics, respectively;  $p$  (gradual evolution) and  $p$  (speciational evolution): one-tailed  $p$ -values with simulations based on gradual and speciational model, respectively; 10,000 simulations).

<sup>c</sup>One-tailed tests on sign of residuals of regression of ecological distances on phylogenetic distances for co-mimics (test for negative residuals) and non-co-mimics (test for positive residuals); tests performed using 10,000 permutations. Sample sizes are  $n = 1,431$  for all pairs of species,  $n = 225$  for pairs of co-mimic species and  $n = 1,206$  for pairs of non-co-mimic species. doi:10.1371/journal.pbio.0060300.t001

**Table 2.** Tests for Niche Complementarity (One-Tailed Tests for Negative Correlations)

Correlations Among	Pairs of Variables	Simulations			Residuals	
		<i>r</i> (raw)	<i>p</i> (grad.)	<i>p</i> (spec.)	<i>r</i> (residual)	<i>p</i>
<b>All species</b>	FS1–FS2	0.141	0.873	0.837	0.140	0.933
	FS1–FS3	–0.093	0.054	0.049	–0.094	0.172
	FS2–FS3	–0.040	0.228	0.203	–0.044	0.327
	FS1–topography	0.151	0.878	0.854	0.152	0.972
	FS2–topography	0.010	0.447	0.430	0.010	0.624
	FS3–topography	0.390	0.994	0.992	0.390	1.000
	FS1–height	–0.034	0.255	0.239	–0.035	0.378
	FS2–height	–0.014	0.343	0.323	–0.023	0.403
	FS3–height	0.181	0.926	0.896	0.172	0.967
	Topography–height	0.098	0.769	0.740	0.103	0.954
<b>Co-mimics</b>	FS1–FS2	–0.096	0.080	0.066	–0.090	0.086
	FS1–FS3	0.059	0.538	0.499	0.068	0.844
	FS2–FS3	0.015	0.387	0.355	0.034	0.692
	FS1–topography	0.150	0.774	0.736	0.159	0.985
	FS2–topography	0.110	0.690	0.645	0.110	0.939
	FS3–topography	0.293	0.945	0.923	0.290	1.000
	FS1–height	0.028	0.434	0.395	0.052	0.779
	FS2–height	0.022	0.415	0.378	0.059	0.811
	FS3–height	0.221	0.884	0.858	0.275	1.000
	Topography–height	0.080	0.601	0.558	0.102	0.925
<b>Non-co-mimics</b>	FS1–FS2	0.180	0.922	0.890	0.180	1.000
	FS1–FS3	–0.107	0.050	0.049	–0.108	<b>0.0001</b>
	FS2–FS3	–0.053	0.204	0.194	–0.063	<b>0.012</b>
	FS1–topography	0.153	0.879	0.859	0.153	1.000
	FS2–topography	–0.010	0.394	0.381	–0.009	0.389
	FS3–topography	0.406	0.995	0.992	0.407	1.000
	FS1–height	–0.061	0.178	0.170	–0.061	<b>0.013</b>
	FS2–height	–0.015	0.379	0.356	–0.030	0.140
	FS3–height	0.182	0.929	0.908	0.168	1.000
	Topography–height	0.103	0.789	0.761	0.105	1.000

Correlation between distances along one microhabitat variable and distances along another variable, tested by two different methods, simulation of microhabitat characters on the phylogeny (*r* (raw): correlation coefficient performed on raw variables; *p* (grad.) and *p* (spec.): one-tailed *p*-values with simulations based on gradual and speciation model, respectively; 10,000 simulations), and partial Mantel test controlling for phylogeny (*r* (residuals): correlation coefficient performed on the residuals of the regression of ecological distances against phylogenetic distances; *p*-value obtained from 10,000 permutations). Sample sizes are  $n = 1,431$  for all pairs of species,  $n = 225$  for pairs of co-mimic species and  $n = 1,206$  for pairs of non-co-mimic species. Bold *p*-values indicate tests that remain significant after correction for multiple tests. Note that distances along some variables are positively correlated, but these correlations are found among both co-mimics and non-co-mimics, and therefore represent genuine correlations that apply to the entire community. Niche complementarity occurs as long as distances along at least one pair of variables are negatively correlated (regardless of other correlations), which is the case for non-co-mimics only. doi:10.1371/journal.pbio.0060300.t002

benefit from the increased abundance of pollinators. Such species are also likely to show habitat convergence similar to that observed here, as well as increased synchrony of flowering time [47,48]. This would act to maximize overlap with pollinators, provided that the benefits outweigh the costs of increased competition and cross-pollination. In addition, synchrony in flowering time and seed set could also be promoted by limitation in seed dispersers [49] or to ensure satiation of generalist seed predators [47,49,50]. Our study demonstrates a tight link between the evolution of a mutualism and the evolution of microhabitat niche, which implies a long history of ecological interactions between members of local communities. If pervasive, such effects would imply that local ecological communities are adaptively assembled to a greater and more complex degree than is commonly considered.

Determining the factors that shape local species assemblages is a hot topic in modern community ecology [51,52]. In addition to interactions among species, local assemblages may also be determined by neutral processes, such as dispersal limitation and drift, or by habitat filtering, whereby only species adapted to a certain habitat can colonize, and

each of these processes can have different relative importance. This field has recently benefited from the development of neutral theories of diversity [53–56] and the incorporation of phylogenetic information into community ecology [4]. Both approaches facilitate the detection of non-neutral processes [57]. However, despite positive interactions among species being widespread [12,14,15,40,41], the majority of theoretical and empirical studies that incorporate phylogenetic information have considered only habitat filtering and competitive interactions [4,8,10,57]. One notable exception is a recent study of Mexican woody plant communities, which demonstrated that facilitation turns to competition with increased phylogenetic relatedness [58]. This involved a study of the relationship between phylogenetic relatedness and temporal patterns of co-occurrence among species from the same community. The approach was very different to that taken here, in which species of similar levels of relatedness are either mutualistic (co-mimics) or not (non-co-mimics). More generally, community ecology theory would greatly benefit from expanding current phylogenetic models [57] to incorporate positive interactions alongside neutral processes,



habitat filtering, and competition in order to derive testable predictions and disentangle these processes.

Finally, our findings further support the idea of mimicry as a driver of speciation through ecological adaptation [59]. The implications of mimicry evolution extend far beyond the evolution of warning signals, with links to microhabitat use, including flight height and forest structure [24–26], but also to mate choice [60], flight physiology [61], and larval host-plant use [24,27]. Here we show that the links between mimicry and microhabitat use are a clear result of adaptation. Our results therefore support the hypothesis that shifts in mimicry trigger shifts in microhabitat and vice versa. Divergence in both factors contributes to prezygotic reproductive isolation and ultimately speciation through assortative mating [60,62], while shifts in mimicry additionally cause postzygotic isolation through selection against hybrids with intermediate, non-mimetic wing patterns [63,64]. Hence multiple coevolved factors may act together to generate reproductive isolation.

## Materials and Methods

**Ecological data.** The field site for this study covered an area of ~15 km<sup>2</sup> on the south bank of the Napo River, Ecuador (~0°32'S, 76°24'W) [35]. Field work was conducted between October and December 2005. Sixteen variables representing forest structure (Table S2) were measured in 18 circular plots (diameter: 30 m) established in different areas of the study site. A principal component analysis was then performed on these variables to extract meaningful information on forest structure (Table S2). The first three principal components (FS1: dense understorey, abundance of vines, 25.1%; FS2: abundance of palms, absence of trees, 18.1%; and FS3: open canopy, palms variable, 14.5%) were retained. Eight of these plots comprised paired adjacent ridge and valley plots to measure topographic influence. Ithomiines were surveyed in each study plot, captured with a hand net, identified, and either marked and released or kept for genetic analyses. Height at initial observation was recorded [65], and the individual was attributed measures of forest structure and topography (0: valley, 1: ridge) for the plot of capture. A total of 1,231 individual butterflies were surveyed and included in the analyses. Averages were calculated for each variable for each species, and distance matrices for each variable computed. Global microhabitat distances were calculated as the Euclidean distances between species in the 5-dimensional ecological space defined by the five centered and scaled microhabitat variables measured. Each species was classified among eight mimicry complexes [27,59] to generate a mimicry distance matrix (0: co-mimics, 1: non-co-mimics).

**Phylogenetic analyses.** We generated a phylogeny of the 58 ithomiine species (24 genera) of the community using Bayesian Inference under the uncorrelated lognormal relaxed clock model implemented in BEAST [66], using a 2,296 bp mitochondrial region spanning the *Col*, *tRNA leu* and *CoII* genes, as well as a 1,215 bp fragment of *Elongation Factor 1a*, a nuclear gene (Table S1) [35]. Each region followed a GTR+ $\Gamma$  model of substitution, and two MCMC chains were run for 200 million generations (sampling every 1,000 generation, 10% burn-in). Two species had two subspecies each with different color patterns in the community, which were considered as separate taxa in both cases (Figure 1). To increase the accuracy of the tree topology we added published sequences of genera that were not represented in the community [67,68], but these additional taxa were pruned from the tree prior to the analyses presented below. Six rare ithomiine species were also excluded from the analyses because of missing ecological data (Figure 1).

**Statistical analyses.** All analyses on the phylogeny were performed on the maximum clade credibility tree with average branch lengths, computed by TreeAnnotator [66] from the 360,000 trees retained. To account for phylogenetic uncertainty we collapsed all nodes that had a posterior probability less than 0.90. Analyses were conducted on all 54 taxa for which sufficient ecological data was recorded. All statistical analyses were performed with R [69], using packages APE 2.1–3 [70], Geiger 1.2–06 [71], Vegan 1.11–0 [72], and Cluster 1.11.10 [73].

To investigate ecological segregation of mimicry complexes we performed a MANOVA on all variables and an ANOVA for each

individual variable. These analyses were performed on the entire data set to test whether mimicry complexes are ecologically segregated (regardless of species identity), and on species' averages to test whether co-mimetic species are more similar than species picked at random (regardless of species abundance). The significance of ANOVA/MANOVA test statistics was assessed by permuting the observed ecological data (10,000 permutations).

Correlations between phylogenetic and ecological distances among all species, among co-mimics and among non-co-mimics were computed and their significance was tested by permuting the observed ecological data among species (10,000 permutations, one-tailed test for positive correlation). We performed a partial Mantel test for the correlation between ecological and mimicry distances while controlling for phylogeny. We did this by first regressing ecological and mimicry distances against phylogenetic distances and then assessing the significance of correlations between residual ecological and mimicry distances by permutation of the residual distances among species pairs (logistic regression for mimicry distances; 10,000 permutations, one-tailed test).

Tests for ecological convergence and divergence for each microhabitat variable and for the global microhabitat were performed in two additional ways: (1) simulated character evolution, which incorporates phylogeny into the expected distribution of type I error, and (2) regression-based methods, which remove the phylogenetic component of variation in ecological characters. In the first way, we calculated the average ecological distance among co-mimics standardized by the total tree ecological distance (tests for ecological convergence among co-mimics), or the average distance among all non-co-mimics standardized by the total tree ecological distance (tests for ecological divergence between mimicry complexes). Character evolution was then simulated on the phylogeny assuming gradual and speciation character evolution (10,000 simulations), and the actual value of the parameters of interest compared with the distribution of the same parameter generated by the simulations to obtain *p*-values. As we wanted to test whether scaled ecological distances were smaller than expected among co-mimics and greater than expected among non-co-mimics, one-tailed tests were used in each case. In the second way, ecological distances were regressed against phylogenetic distances using all species. We then permuted these residual ecological distances among species pairs to determine whether the observed residuals were more negative or positive than expected by chance for co-mimics and non-co-mimics, respectively (10,000 permutations).

Niche complementarity was investigated by calculating the correlation coefficient of distances along one ecological variable with distances along another variable, for all pairs of variables. The significance of correlation coefficients was tested in two ways. First, as above, the actual value was compared with the distribution of the same parameter generated from 10,000 replicate simulations of character evolution under both gradual and speciation models. Second, a partial Mantel test was carried out for each pair of ecological variables, in which distances along each of the two variables were regressed against phylogenetic distances. We assessed the significance of correlations between residuals for each pair of ecological variables by permutation of the residual distances among species pairs. The tests were one-tailed (negative correlations) and the significance of each test was based on 10,000 permutations.

We controlled for multiple tests using the false discovery rate procedure [74], a powerful alternative to the Bonferroni correction that seeks to minimize both type I and type II errors, with the allowed proportion of false positives set at 0.05. Unless stated otherwise, all tests presented in the text, tables and figures are significant after this correction.

## Supporting Information

**Table S1.** List of Specimens Used, with GenBank Accession Numbers Found at doi:10.1371/journal.pbio.0060300.st001 (36 KB PDF).

**Table S2.** Principal Component Analysis of Forest Structure Variables Found at doi:10.1371/journal.pbio.0060300.st002 (28 KB PDF).

## Acknowledgments

We thank Raúl Aldaz, Alexandre Toporov, Julia Robinson-Willmott, Aniko Zölei, Gabor Papp, and Carlos Giraldo Sanchez for help with collecting data in the field. Andrew Rambaut provided support with

BEAST. We thank the Ecuadorian Ministerio del Ambiente and Museo Ecuatoriano de Ciencias Naturales for providing research permits and the Napo Wildlife Center for logistic support in the field. We thank George Beccaloni and Phil DeVries for inspiration, Jim Mallet and Jérôme Chave for stimulating discussions, and Nick Davies, James Fordyce, Mathieu Joron, Graham Stone, Bill Sutherland, and Joe Tobias for comments on this paper.

**Author contributions.** ME, CJ, and KW conceived and designed the

experiments. ME, CJ, and KW performed the experiments. ME and ZG analyzed the data. ME, ZG, CJ, and KW wrote the paper.

**Funding.** This work was funded by the Royal Society (UK), the Leverhulme Trust F/00158/AK, F/09 364F (UK), and the National Science Foundation DEB 0103746 (USA).

**Competing interests.** The authors have declared that no competing interests exist.

## References

- Johnson MTJ, Stinchcombe JR (2007) An emerging synthesis between community ecology and evolutionary biology. *Trends Ecol Evol* 22: 250–257.
- Cavender-Bares J, Wilczek A (2003) Integrating micro- and macroevolutionary processes in community ecology. *Ecology* 84: 592–597.
- Schluter D (2000) *The Ecology of Adaptive Radiation*. New York: Oxford University Press.
- Webb CO, Ackerly DD, McPeck MA, Donoghue MJ (2002) Phylogenies and community ecology. *Annu Rev Ecol Syst* 33: 475–505.
- Begon M, Townsend CA, Harper JL (2000) *Ecology: from individuals to ecosystems*. Oxford: Blackwell Publishing.
- Chesson P (2000) Mechanisms of maintenance of species diversity. *Annu Rev Ecol Syst* 31: 343–366.
- Cavender-Bares J, Ackerly DD, Baum DA, Bazzaz FA (2004) Phylogenetic overdispersion in Floridian oak communities. *Am Nat* 163: 823–843.
- Cavender-Bares J, Keen A, Miles B (2006) Phylogenetic structure of floridian plant communities depends on taxonomic and spatial scale. *Ecology* 87: S109–S122.
- Losos JB, Leal M, Glor RE, de Queiroz K, Hertz PE, et al. (2003) Niche lability in the evolution of a Caribbean lizard community. *Nature* 424: 542–545.
- Lovette IJ, Hochachka WM (2006) Simultaneous effects of phylogenetic niche conservatism and competition on avian community structure. *Ecology* 87: S14–S28.
- Anderson TM, Lachance MA, Starmer WT (2004) The relationship of phylogeny to community structure: the cactus yeast community. *Am Nat* 164: 709–721.
- Brooker RW, Maestre FT, Callaway RM, Lortie CL, Caviries LA, et al. (2008) Facilitation in plant communities: the past, the present, and the future. *J Ecol* 96: 18–34.
- Bruno JF, Stachowicz JJ, Bertness MD (2003) Inclusion of facilitation into ecological theory. *Trends Ecol Evol* 18: 119–125.
- Valiente-Banuet A, Rumebe AV, Verdu M, Callaway RM (2006) Modern quaternary plant lineages promote diversity through facilitation of ancient tertiary lineages. *Proc Natl Acad Sci U S A* 103: 16812–16817.
- Valiente-Banuet A, Verdu M (2007) Facilitation can increase the phylogenetic diversity of plant communities. *Ecol Lett* 10: 1029–1036.
- Gross K (2008) Positive interactions among competitors can produce species-rich communities. *Ecol Lett* 11: 929–936.
- Bulleri F, Bruno JF, Benedetti-Cecchi L (2008) Beyond competition: incorporating positive interactions between species to predict ecosystem invasibility. *PLoS Biol* 6: e162. doi:10.1371/journal.pbio.0060162
- Mallet J, Joron M (1999) Evolution of diversity in warning colour and mimicry: Polymorphisms, shifting balance and speciation. *Annu Rev Ecol Syst* 30: 201–233.
- Rowland HM, Ihalainen E, Lindstrom L, Mappes J, Speed M (2007) Co-mimics have a mutualistic relationship despite unequal defences. *Nature* 448: 64–67.
- Sherratt TN (2008) The evolution of Mullerian mimicry. *Naturwissenschaften* 95: 681–695.
- Müller F (1879) *Ituna* and *Thyridia*: a remarkable case of mimicry in butterflies. *Trans Entomol Soc Lond* 1879: xx–xxix.
- Beccaloni GW (1997) Ecology, natural history and behaviour of ithomiine butterflies and their mimics in Ecuador (Lepidoptera: Nymphalidae: Ithomiinae). *Trop Lepid* 8: 103–124.
- Joron M, Mallet JLB (1998) Diversity in mimicry: paradox or paradigm? *Trends Ecol Evol* 13: 461–466.
- Beccaloni G (1997) 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.
- DeVries PJ, 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.
- Papageorgis C (1975) Mimicry in neotropical butterflies. *Am Sci* 63: 522–532.
- Willmott KR, Mallet J (2004) Correlations between larval hostplants and adult mimetic wing pattern in neotropical ithomiine butterflies. *Proc Biol Sci* 271: S266–S269.
- Walther BA (2002) Vertical stratification and use of vegetation and light habitats by Neotropical forest birds. *J Ornithol* 143: 64–81.
- Mallet J, Gilbert LE (1995) Why are there so many mimicry rings - correlations between habitat, behavior and mimicry in *Heliconius* butterflies. *Biol J Linn Soc* 55: 159–180.
- Lamas G (2004) Ithomiinae. In: Heppner JB, editor. *Atlas of Neotropical Lepidoptera Checklist: Part 4A Hesperioidea - Papilionoidea*. Gainesville: Association for Tropical Lepidoptera/Scientific Publishers.
- Joron M, Wynne IR, Lamas G, Mallet J (1999) Variable selection and the coexistence of multiple mimetic forms of the butterfly *Heliconius numata*. *Evol Ecol* 13: 721–754.
- Beccaloni GW, Vilorio AL, Hall SK, Robinson GS (2008) *Catalogue of the hostplants of the Neotropical butterflies. Catálogo de las plantas huésped de las mariposas neotropicales*. London: Natural History Museum.
- Brown KS (1984) Adult-obtained pyrrolizidine alkaloids defend ithomiine butterflies against a spider predator. *Nature* 309: 707–709.
- Schulz S, Beccaloni G, Brown KS, Boppre M, Freitas AVL, et al. (2004) Semicheicals derived from pyrrolizidine alkaloids in male ithomiine butterflies (Lepidoptera: Nymphalidae: Ithomiinae). *Biochem Syst Ecol* 32: 699–713.
- Elias M, Hill RI, Willmott KR, Dasmahapatra KK, Brower AVZ, et al. (2007) Limited performance of DNA barcoding in a diverse community of tropical butterflies. *Proc Biol Sci* 274: 2881–2889.
- Hector A, Schmid B, Beierkuhnlein C, Caldeira MC, Diemer M, et al. (1999) Plant diversity and productivity experiments in European grasslands. *Science* 286: 1123–1127.
- Silvertown J, McConway K, Gowing D, Dodd M, Fay MF, et al. (2006) Absence of phylogenetic signal in the niche structure of meadow plant communities. *Proc Biol Sci* 273: 39–44.
- Moeller DA (2004) Facilitative interactions among plants via shared pollinators. *Ecology* 85: 3289–3301.
- Mann CA (1985) Permanent canopy and understory flocks in Amazonia: Species composition and population density. *Ornithological Monographs* 36: 683–711.
- Stensland E, Angerbjorn A, Berggren P (2003) Mixed species groups in mammals. *Mamm Rev* 33: 205–223.
- Wiley RH (1971) Cooperative roles in mixed flocks of antwrens (Formicariidae). *Auk* 88: 881–892.
- Beauchamp G (2004) Reduced flocking by birds on islands with relaxed predation. *Proc Biol Sci* 271: 1039–1042.
- Bshary R, Noe R (1997) Red colobus and Diana monkeys provide mutual protection against predators. *Anim Behav* 54: 1461–1474.
- Thiollay JM, Jullien M (1998) Flocking behaviour of foraging birds in a neotropical rain forest and the antipredator defence hypothesis. *Ibis* 140: 382–394.
- Grant V (1994) Historical development of ornithophily in the Western North-American flora. *Proc Natl Acad Sci U S A* 91: 10407–10411.
- Thomson JD, Wilson P (2008) Explaining evolutionary shifts between bee and hummingbird pollination: Convergence, divergence, and directionality. *Intl J Plant Sci* 169: 23–38.
- Boulter SL, Kitching RL, Howlett BG (2006) Family, visitors and the weather: patterns of flowering in tropical rain forests of northern Australia. *J Ecol* 94: 369–382.
- Sakai S (2002) General flowering in lowland mixed dipterocarp forests of South-east Asia. *Biol J Linn Soc* 75: 233–247.
- Mduma SAR, Sinclair ARE, Turkington R (2007) The role of rainfall and predators in determining synchrony in reproduction of savanna trees in Serengeti National Park, Tanzania. *J Ecol* 95: 184–196.
- Kelly D (1994) The evolutionary ecology of mast seeding. *Trends Ecol Evol* 9: 465–470.
- Gewin V (2006) Beyond neutrality - Ecology finds its niche. *PLoS Biol* 4: 1306–1310. doi:10.1371/journal.pbio.0040278
- Leibold MA (2008) Ecology - Return of the niche. *Nature* 454: 39–41.
- Bell G (2001) Ecology - Neutral macroecology. *Science* 293: 2413–2418.
- Chave J (2004) Neutral theory and community ecology. *Ecol Lett* 7: 241–253.
- Hubbell SP (2001) *The Unified Neutral Theory of Biodiversity and Biogeography*. Monographs in Population Biology. Princeton (New Jersey): Princeton University Press. 375 p.
- Leibold MA, McPeck MA (2006) Coexistence of the niche and neutral perspectives in community ecology. *Ecology* 87: 1399–1410.
- Kraft NJB, Cornwell WK, Webb CO, Ackerly DD (2007) Trait evolution, community assembly, and the phylogenetic structure of ecological communities. *Am Nat* 170: 271–283.
- Valiente-Banuet A, Verdu M (2008) Temporal shifts from facilitation to competition occur between closely related taxa. *J Ecol* 96: 489–494.
- Jiggins CD, Mallarino R, Willmott KW, Bermingham E (2006) The phylogenetic pattern of speciation and wing pattern change in neotropical Ithomia butterflies (Lepidoptera; Nymphalidae). *Evolution* 60: 1454–1466.

60. Jiggins CD, Naisbit RE, Coe RL, Mallet J (2001) Reproductive isolation caused by colour pattern mimicry. *Nature* 411: 302–305.
61. Chai P, Srygley RB (1990) Predation and the flight, morphology, and temperature of neotropical rain-forest butterflies. *Am Nat* 135: 748–765.
62. Via S (2001) Sympatric speciation in animals: the ugly duckling grows up. *Trends Ecol Evol* 16: 381–390.
63. Kapan DD (2001) Three-butterfly system provides a field test of Müllerian mimicry. *Nature* 409: 338–340.
64. Mallet J, Barton NH (1989) Strong natural-selection in a warning-color hybrid zone. *Evolution* 43: 421–431.
65. Joron M (2005) Polymorphic mimicry, microhabitat use, and sex-specific behaviour. *J Evol Biol* 18: 547–556.
66. Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol Biol* 7: 214.
67. Brower AVZ, Freitas AVL, Lee MM, Silva-Brandao KL, Whinnett A, et al. (2006) Phylogenetic relationships among the Ithomiini (Lepidoptera: Nymphalidae) inferred from one mitochondrial and two nuclear gene regions. *Syst Entom* 31: 288–301.
68. Whinnett A, Brower AVZ, Lee MM, Willmott KR, Mallet J (2005) Phylogenetic utility of Tektin, a novel region for inferring systematic relationships among Lepidoptera. *Ann Entom Soc Am* 98: 873–886.
69. Development Core Team (2008) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available: <http://www.R-project.org> and <http://cran.r-project.org/>. Accessed 31 October 2008.
70. Paradis E, Claude J, Strimmer K (2004) APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics* 20: 289–290.
71. Harmon LJ, Weir JT, Brock CD, Glor RE, Challenger W (2008) GEIGER: investigating evolutionary radiations. *Bioinformatics* 24: 129–131.
72. Dixon P (2003) VEGAN, a package of R functions for community ecology. *J Veg Sci* 14: 927–930.
73. Maechler M, Rousseeuw P, Struyf A, Hubert M (2008) Cluster analysis basics and extensions, version 1.11.11. Available: <http://cran.r-project.org/>. Accessed 31 October 2008.
74. Verhoeven KJF, Simonsen KL, McIntyre LM (2005) Implementing false discovery rate control: increasing your power. *Oikos* 108: 643–647.