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Maintaining mimicry diversity: optimal warning colour patterns differ among microhabitats in Amazonian clearwing butterflies

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Mimicry is one of the best studied examples of adaptation, and recent studies have provided new insights into the role of mimicry in speciation and diversification. Classical Müllerian mimicry theory predicts convergence in warning signal among protected species, yet tropical butterflies are exuberantly diverse in warning colour patterns, even within communities. We tested the hypothesis that microhabitat partitioning in aposematic butterflies and insectivorous birds can lead to selection for different colour patterns in different microhabitats and thus help maintain mimicry diversity. We measured distribution across flight height and topography for 64 species of clearwing butterflies (Ithomiini) and their co-mimics, and 127 species of insectivorous birds, in an Amazon rainforest community. For the majority of bird species, estimated encounter rates were non-random for the two most abundant mimicry rings. Furthermore, most butterfly species in these two mimicry rings displayed the warning colour pattern predicted to be optimal for anti-predator defence in their preferred microhabitats. These conclusions were supported by a field trial using butterfly specimens, which showed significantly different predation rates on colour patterns in two microhabitats. We therefore provide the first direct evidence to support the hypothesis that different mimicry patterns can represent stable, community-level adaptations to differing biotic environments.

Introduction

One of the most intensively studied examples of adaptation is Müllerian mimicry, where groups of unpalatable species display a common warning colour pattern and thereby share the cost incurred through predator learning [1]. Butterflies provide many examples of mimicry "rings" comprising multiple species with extremely similar patterns (e.g., [2]) that have evolved through convergence [3]. Furthermore, numerous field experiments have demonstrated very strong stabilising selection, which explains this convergence as predicted by classical Müllerian mimicry theory [1, 4]. However, surprisingly, mimicry patterns are also highly diverse [5], both across space and within communities, with more than ten butterfly mimicry rings occurring at a single Amazonian locality [2]. Shifts between mimicry patterns have long been considered a likely cause of ecological speciation [6], since sexual selection on colour pattern and natural selection against hybrid individuals can rapidly result in reproductive isolation, even in the presence of gene flow [7-15].

Spatial variation in predator communities over distances of a few kilometres to hundreds of kilometres is a likely factor in maintaining intraspecific variation in warning colour patterns [16-21], with strong natural selection driving narrow geographic colour pattern clines [17, 22, 23]. Seasonal variation in predators also facilitates the maintenance of alternative defensive strategies [24]. However, the processes responsible for maintaining mimicry diversity within communities are less well understood. Two classes of hypotheses have been proposed. In the first, colour pattern diversity is viewed as an unstable phenomenon, resulting either from geographic overlap between largely allopatric mimicry patterns or from rapid evolution of new patterns that once abundant experience only weak selection for convergence [5]. Such communities represent a dynamic equilibrium, either because mimicry rings offering less protection are continuously "rescued" by immigration [25, 26], or because selection constantly drives convergence but is counter-balanced by rapid diversification.

By contrast, the second class of hypotheses views distinct mimicry rings as adaptations to varying abiotic or biotic environments, and thus as representing stable niches related to predator defence. Several studies have confirmed height stratification of mimicry rings in ithomiine [3, 27, 28] and nocturnally roosting *Heliconius* butterflies [29]. Mimicry rings may also be segregated with respect to forest disturbance [3, 29-32] and topography [3]. Species that share hostplants often mimic each other, most likely because adults are constrained to fly in similar microhabitats by the distribution of their hostplants [27, 33].

There are several possible explanations for such microhabitat segregation in mimicry rings. Papageorgis [34] proposed that diverse warning colour patterns in Amazonian butterflies might be maintained by different patterns having a "dual-signal" function of both camouflage and warning against different vegetation backgrounds related to flight height and ambient light. An alternative hypothesis is that different patterns represent adaptations not to physical variation among microhabitats, but to ecological variation in the predator community. If predators show microhabitat segregation similar to mimicry rings, then different predator species may be most familiar with different mimicry patterns and selection for convergence across microhabitats will be low [5, 27, 29, 30].

Gompert *et al.* [35] provided theoretical support for the hypothesis that microhabitat preferences in predators and prey can promote mimicry diversity. In that study, strong microhabitat segregation in predators drove microhabitat segregation among mimicry rings, and thereby fostered coexistence of several mimicry rings [35]. Furthermore, on a broader scale it has been shown that habitat patches several kilometres apart can represent alternative mimicry optima, supporting polymorphisms within species [16, 26, 36]. However, the optimality of mimicry patterns in finer scale microhabitats has never been tested. Here, we test this hypothesis for the first time by studying ithomiine butterflies (Nymphalidae: Ithomiini) and their avian predators in a diverse Amazonian rainforest community in eastern Ecuador. Ithomiines, known as "clearwing butterflies" after the transparent wings of many species, inhabit Neotropical forests below 3000 m, with some 60 species in the most diverse communities of the western Amazon. All species are believed to be unpalatable [37] and participate in mimicry "rings" with other ithomiines or putatively unpalatable butterflies, especially the Heliconiinae [38], in addition to presumed palatable Batesian mimics. Ithomiine butterflies dominate these mimicry rings in both species diversity and abundance [2]. Although there are almost no published observations of predation on ithomiines, the primary predators driving the evolution of mimicry are believed to be insectivorous birds. These are the only abundant predators with sufficiently developed colour vision to explain precise mimicry [13, 39-42].

We adopted two approaches to test our hypothesis. Firstly, in our study community we measured the distribution of butterflies and birds with respect to two principal microhabitat axes, flight height and topography, which are known to influence ithomiine mimicry pattern abundance at the study site [3]. We then used these data to estimate the relative encounter rates between mimicry patterns and individual bird species and therefore test whether butterfly species were, on average, most likely to encounter birds that were most familiar with their colour pattern. Secondly, we conducted an experimental field trial with dead butterfly specimens to directly measure predation rates on colour patterns in different microhabitats. We use the resulting data to address the question of whether microhabitat segregation in birds and butterflies can lead to the stable coexistence of multiple mimicry patterns.

Materials and Methods

Study groups: Our study group included all ithomiines and co-mimetic butterflies (co-mimics). In the absence of data on butterfly palatability to a range of insectivorous birds, we assumed that all non-ithomiine co-mimics might potentially be Müllerian mimics. Eight mimicry rings involving ithomiines were recognised based on similarity in wing pattern characters and parallel geographic variation in wing pattern [2, 3, 33, 43]. Although human and bird vision differ, we assume that shared wing pattern characters visible to us and used to classify mimicry must also be important cues for predators since these characters show convergent evolution. Moreover, experiments show that birds learn, after attacking unpalatable butterflies, to avoid palatable butterflies that humans classify as co-mimics [44, 45], and models of animal vision suggest that birds are unlikely to be able to discriminate between butterflies that are regarded as mimetic by humans [46].

Several bird species present are known to be predators of butterflies, such as jacamars (Galbulidae: [47, 48], pers. obs.) and some flycatchers ([42, 49, 50], but these are species characteristic of forest edges, large light gaps or forest canopy. We know of no published evidence of insectivorous birds that are regular predators of butterflies in the understory, yet some must be to drive the evolution of understory mimicry rings. We therefore assumed that all potentially insectivorous birds could be important selective agents, and used Ridgely and

Greenfield [51] to determine such insectivorous birds (see S3). Manakins (Pipridae) are predominantly frugivorous but also eat insects and were thus included in our analyses.

Study location: The study was conducted at the Napo Wildlife Center, Orellana, Ecuador, a topographically variable area with relatively undisturbed forest (see S1). Data on the topographic distribution of butterflies were obtained by KW, ME and CJ sampling eight 30 m-diameter plots, located in pairs with one on a ridge and one in the adjacent valley, along the "Parrot Trail" (240-300m, 0˚31'S, 76˚23'W). Birds were sampled by JRW in the same eight plots as butterflies and during additional timed transect walks along ridge and valley trails between and near the plots.

Species abundance and distribution: Fieldwork was conducted from 16 October to 15 December 2005. We recorded butterfly distribution and abundance during 30 min sampling and observation periods in each plot, from 0800-1700 hrs. Ridge plots were surveyed for a total of 23 hr among all plots, and the same for valley plots. Plots were patrolled continuously during the 30 min sampling period and attempts made to capture all mimetic butterflies using hand-nets, up to 9 m above the ground. Specimens were either killed and retained or marked and released, and we recorded the time of day, species or mimicry pattern (if unidentified), sex, and initial flight height. Observers carried entomological nets with metal handles composed of up to 12 sections each 0.6 m in length, with these gradated handles facilitating flight height estimation.

Birds were recorded in the same plots from 0420 hrs to 1800 hrs, concentrating in particular between 0630-1100 hrs and 1500-1700 hrs when bird activity was greatest. A total of 44 hr observation time was spent in ridge plots and the same time in valley plots. Birds were also recorded during timed walks along ridge and stream trails between Parrot trail plots (15 hr spent in ridge walks and the same in valley walks). Species were identified by sight and call using prior experience and Ridgely and Greenfield [51]. For all individuals we recorded time of day, species, and initial flight or perch height.

Mimicry pattern encounter rates for bird species: To characterise butterfly and bird distribution we assigned each butterfly and bird individual to one of eight microhabitats, representing combinations of two topography categories (ridge and valley) with four flight height categories (0-1 m, 1-2 m, 2-3 m, above 3 m). Flight height intervals were based on our observations of significant differences in mimicry rings between the ground and 3 m, and a sharp decline in observed numbers of butterfly individuals above 3 m. These data were used to estimate the relative frequency of encounters between each bird species and each of the eight mimicry patterns. For a given bird species (k) and mimicry pattern (i), we summed the product of bird abundance (I_{kj}) and butterfly abundance (B_{ij}) in each of the eight microhabitats (j) and divided by the sum of these products across all mimicry patterns, as an estimate of the relative encounter rate (M_{ik}) of that mimicry pattern in comparison with others.

Therefore, the relative mimicry pattern encounter rate for bird species k and mimicry pattern i is:

$$M_{ik} = \frac{\sum_{j=1}^{8} I_{kj} * B_{ij}}{\sum_{i=1}^{8} \sum_{j=1}^{8} I_{kj} * B_{ij}}$$

We then used a permutation approach to test for non-random encounter rates between different mimicry patterns and individual bird species, which might occur due to microhabitat segregation. We permuted mimicry pattern among butterfly individuals to generate 500 "null" communities, maintaining the same numbers of individuals in each mimicry ring and the same butterfly abundance distribution among microhabitats. For each null community, we calculated relative mimicry encounter rates (M_{ik}) as above, for the 25 most abundant bird species (> 10 individuals recorded) in the community. We then compared our empirical values of M_{ik} to those in the 500 null communities to address two questions:

H1. Within individual bird species, do the encounter rates of different mimicry patterns differ significantly from those expected if there were no microhabitat segregation of mimicry patterns? We focused on the mimicry pattern encountered most frequently by each bird species since that is the pattern that species is most likely to avoid, and therefore the pattern that should be optimal for co-existing butterflies to display. For each bird species k and mimicry pattern i, the frequency of null communities with the highest M_{ik} equal or greater than the highest empirical M_{ik} represents the probability of such a high encounter rate being the result of chance (a one-tailed test).

H2. Within the entire bird community, do a significant number of bird species most frequently encounter a mimicry pattern other than the most abundant pattern? The two most abundant mimicry patterns recorded were ‘eurimedia’ (37%) and ‘hermias’ (31%) (see Results, S4), so to simplify analyses we focused on encounter rates of these two patterns. Empirically, 19 out of 25 bird species had the highest empirical encounter rate for the less abundant pattern 'hermias' (see Results, S5). The frequency of null communities with 19 or more bird species having $M_{hermias\ k}$ as the highest pattern encounter rate represents the probability of such a biased community being the result of chance (a one-tailed test).

Birds encountered by butterfly species: We then examined whether butterfly species tend to most often encounter birds that are most familiar with their colour pattern, and hence that are most likely to avoid them. First, we calculated the weighted average rate of encounters ($\overline{M}_{ij}$) of each of the eight mimicry rings (i) within each of the eight microhabitats (j) across all bird individuals (all bird species, k=1-129) observed in a given microhabitat (I_j).

The average relative encounter rate for mimicry pattern i of birds occurring in microhabitat j is:

$$\overline{M}_{ij} = \frac{\sum_{k=1}^{129} M_{ik} * I_{kj}}{\sum_{k=1}^{129} I_{kj}}$$

Finally, for each butterfly species (h), we calculated weighted average mimicry encounter rates ($\overline{M}_{hi}$) of birds occurring in the microhabitats where that butterfly species was recorded, by weighting bird-mimicry encounter frequencies ($\overline{M}_{ij}$) with observed butterfly abundance (B_{hj}) in each microhabitat and summing across microhabitat.

The average relative encounter rate for mimicry pattern i, of birds encountering butterfly species h, is:

$$\overline{M}_{hi} = \frac{\sum_{j=1}^{8} \overline{M}_{ij} * B_{hj}}{\sum_{j=1}^{8} B_{hj}}$$

These final data indicate the mimicry pattern that will be most familiar to birds encountering a given butterfly species, and the pattern with the highest ($\overline{M}_{hi}$) should be the optimal pattern for that butterfly species. Since we found that the second most abundant pattern 'hermias' was predicted to be optimal for 15 of the 21 species with that colour pattern (see Results, S6), we tested whether the association between predicted and actual optimal patterns was greater than expected by chance, by permuting predicted optimal patterns among all butterfly species 500 times.

Differential predation: We tested whether predation rates differed between mimicry patterns and microhabitats using dead butterfly specimens. Because we found differences in the two predominant mimicry patterns ('eurimedia' and 'hermias') across topography (between ridges and valleys), we designed the experiment to test whether topography had an effect on predation rate of these two patterns. Ithomiines belonging to 'eurimedia' and 'hermias' mimicry rings were collected outside of predation study plots and at random with respect to species, killed, and attached with cyanoacrylate glue to the tips of sticks that were driven into the ground (S7A,B). The bottom of each stick was sprayed with locally obtained insect repellent up to 10 cm above the ground to deter terrestrial scavengers. Butterflies were placed alternately with wings open or closed (both natural postures for resting ithomiines), from 1-1.5 m above the ground. At each of ten sites (five on a ridge and five in the adjacent valley, each separated by 100-200 m) we placed two pairs of 'eurimedia' and 'hermias' butterflies *ca*. 5 m apart, with each pair containing one 'eurimedia' and one 'hermias' individual *ca*. 1 m apart (S7D), with individuals randomised with respect to species. Predation study sites were located outside of study plots but in similar microhabitats, where ithomiines were observed flying. A total of ten butterfly individuals of each mimicry pattern were thus distributed across five ridge sites, and a further ten individuals of each mimicry pattern were distributed across five adjacent stream sites. The study was conducted during the latter part of the same fieldwork period in which butterfly and bird surveys were conducted and sites were checked twice daily, once at dawn and once at dusk. At each check, the number and pattern of predated individuals were recorded. Bird predation was inferred where wings were observed to be torn (e.g., S7C) or entirely missing, with the body intact, or when the body was observed to be torn consistent with a bird (rather than arthropod) attack. No other scavengers were observed attacking the specimens. Damaged or missing specimens were replaced, and sites were moved *ca.* 20 m each day to reduce predator habituation. Specimens were checked 34 times in total.

We used a maximum likelihood approach to test for differences in predation with respect to the two mimicry patterns and the two microhabitats, pooling data for all specimens within these four categories. Given the low numbers of predation events, we felt that it would be unreasonable to attempt to include additional parameters (such as study site and wing position) into modelling variation in predation rate. We calculated the values of the predation probabilities of the two patterns within one microhabitat, or of one pattern across two microhabitats, that maximised the log-likelihood functions for the observed predation results, and we computed the corresponding maximum log-likelihood value. Next, we calculated the maximum log-likelihood under the assumption that these predation probabilities were the same (the null hypothesis) and compared that to the maximum log-likelihood where predation probabilities were allowed to differ. A likelihood ratio test was used to test for the significance of the difference between the two log-likelihood scores, with df=1. See S8 for further details.

Results

In our eight study plots (S1) we recorded 656 individuals of 64 species of butterflies, distributed across eight mimicry rings (Fig. 1, S2). Very similar relative abundances were recorded for the most common mimicry rings by different observers. Dominant groups were Ithomiini (49 species) and Heliconiinae (5 species). A total of 127 species and 893 individuals of birds were recorded and identified as potential predators of Lepidoptera (see S3). Dominant families included Tyrannidae, Thamnophilidae, Furnariidae, Thraupidae, Bucconidae and Picidae, representing 63% of all species.

Aposematic butterfly density declined sharply with height above 3 m, whereas bird density showed a peak at 2-3 m and a more gradual decline with height (Fig. 1). Overall, 'eurimedia' was the dominant pattern, comprising 241 (37%) of all individuals, followed by 'hermias' (203, 31%) and 'lerida' (91, 14%), but the fraction of the community occupied by these mimicry patterns varied across height. 'Lerida' and 'eurimedia' patterns were dominant from 0-1 m, 'eurimedia' and 'hermias' equally dominant from 1-2 m, and 'hermias' dominant in height categories above 2 m, reaching more than 60% of the community from 3-5 m (Fig. 1). With respect to topography, more than twice as many butterflies were recorded in valley sites compared to ridge sites, whereas bird abundance was similar across these two categories (Fig. 2). 'Eurimedia' was the dominant pattern (43% of individuals) in valley sites, followed by 'hermias' (23%), whereas 'hermias' was dominant (49%) in ridge sites, followed by 'eurimedia' (23%) (Fig. 2).

Based on the distribution of birds and butterflies (S4) among the eight topography-flight height microhabitats, 19 of the 25 most abundant bird species were estimated to encounter the second-most abundant mimicry pattern ('hermias') most frequently, and 16 of these species encountered 'hermias' significantly more often than expected by chance (H1, $p < 0.05$) (S5, e.g., Fig. 3). Out of the 6 bird species encountering 'eurimedia' most frequently, 2 encountered 'eurimedia' significantly more often than expected by chance (H1, $p < 0.05$) (S5, e.g., Fig. 3). As a community, the number of bird species encountering 'hermias' most frequently was significantly higher than in null communities without microhabitat segregation of mimicry patterns (H2, $p = 0.04$ of finding this number [19] or more in 500 permuted communities).

Estimates of the average mimicry encounter rates of predators co-occurring with each butterfly species resulted in 10 of 12 species in the most abundant mimicry ring ('eurimedia') being predicted to have the optimal colour pattern for predator avoidance (S6). In other words, the average bird predator encountering these 'eurimedia' species is more likely to have previously encountered that colour pattern than any other. Of the 21 species within the second-most abundant mimicry ring ('hermias'), 15 were predicted to have the optimal colour pattern for predator defence ($p<0.01$ of finding 15 or more 'hermias' with an optimal pattern when expected optimal patterns were permuted among all butterfly species 500 times). Within 'hermias', there was no correspondence between rarity and species identified as having sub-optimal patterns; most notably, the optimal pattern for the most abundant 'hermias' species, *Hypothyris semifulva*, was predicted to be 'eurimedia', as this was a relatively low-flying valley species that overlapped most with other non-mimic 'eurimedia' species. Species in all other mimicry rings were predicted to have sub-optimal patterns, since their optimal patterns were predicted to be either 'eurimedia' or 'hermias' (S6).

In the predation study, a total of 340 trials (checks for predation of specimens) were conducted for each combination of mimicry pattern and microhabitat. At ridge sites, 34 'eurimedia' and 19 'hermias' were predated, while at valley sites 24 'hermias' and 13 'eurimedia' were predated (Fig. 4). In terms of predation on different mimicry patterns, 'eurimedia' was significantly more predated on ridges than 'hermias' ($P_H = 0.056$ and $P_E = 0.100$; $p=0.03$), while 'hermias' was more predated at streams than 'eurimedia' ($P_H = 0.071$ and $P_E = 0.038$; p=0.06). Across microhabitats, 'eurimedia' was significantly more predated on ridges than in valleys (p=0.001), while predation rates on 'hermias', although higher at streams, did not differ significantly across microhabitats (p=0.4). Because the morning and evening checks of specimens were conducted in the same site, prior to moving specimens to another site, some independence of data is potentially lost because a single bird is more likely to be responsible for predation events recorded at those two times. We therefore analyzed the morning and evening data independently, as above. Most predation events were recorded at the morning check of sites (61 out of 90 predation events); 'eurimedia' was significantly more predated on ridges than 'hermias' ($P_H = 0.029$ and $P_E = 0.067$; p=0.020), while 'hermias' was significantly more predated at streams than 'eurimedia' ($P_H = 0.058$ and $P_E = 0.023$; p=0.020). Across microhabitats, 'eurimedia' was significantly more predated on ridges than in valleys (p=0.004), while predation rates on 'hermias', although higher at streams, did not differ significantly across microhabitats (p=0.06). All comparisons were non-significant for the smaller evening dataset.

Discussion

Our study provides the first empirical support for the hypothesis that microhabitat segregation in warningly coloured butterflies and avian insectivores can maintain a diversity of Müllerian mimetic warning colour patterns within communities [27, 29, 32, 35]. This is the first time that both mimetic butterflies and their predators have been studied together at the microhabitat scale, and both our analytical approach and experimental results support the idea that different warning colour patterns can be optimal for anti-predator defence in different microhabitats. Our study thus extends research on how variation in predator communities helps maintain warning colour pattern polymorphisms within prey species at larger spatial scales [16-22, 26], to show that predator community structure can also promote warning colour pattern diversity across species within a single prey community.

It is likely that height and topography, through their effects on microclimate variables such as temperature and humidity [52], affect two important aspects of ithomiine ecology: choice of hostplant and male mate-locating sites. Ithomiine caterpillars feed almost exclusively on Solanaceae plants, and different clades of butterflies have specialised on particular plant clades [53]. Hostplants are regarded as significant in determining ithomiine flight height [27] and spatial distribution [33], and we also documented marked preferences for ridge or valley sites among ithomiine hostplants (unpub. data) that may help explain specific preferences for topographic microhabitats. Furthermore, we also noted that males tended to maintain territories where they awaited females (termed "perching", Scott [54]) at similar heights and in similar topographic microhabitats to those where their mimicry rings typically fly.

Birds also showed distinct preferences for vertical foraging stratum and topography, consistent with previous studies (e.g., [55]). The similar height and topographic distributions of birds and mimicry patterns resulted in the encounter rates of different mimicry patterns being significantly different for individual bird species. The great majority of the most abundant insectivorous birds occurred in the midstorey and canopy, and thus were estimated to preferentially encounter the second-most abundant but highest flying mimicry pattern ('hermias'). A smaller number of understory birds were estimated to most frequently encounter the most common understory mimicry ring ('eurimedia'). Topographic preferences among predators and prey strengthened these patterns, since high-flying species of both birds and butterflies also tended to occur more commonly on ridge-tops. As a consequence, both the most abundant ('eurimedia') and the second-most abundant pattern ('hermias') were predicted to be optimal in different microhabitats for the majority of species in each mimicry ring.

If colour patterns serve as microhabitat-specific anti-predator defences, then the differing abundance in ridge and valley sites of the two most common patterns, 'hermias' and 'eurimedia', leads to clear predictions of relative predation rates in the field trials. In all comparisons (between mimicry patterns within a single microhabitat and between microhabitats within a single mimicry pattern) empirical predation rates were, as expected, inversely related to observed abundances of mimicry patterns. Most notably, at ridge sites, 'hermias' was approximately twice as abundant as 'eurimedia', and predation rates on 'eurimedia' were overall 1.8 times as high as for 'hermias'. The opposite was observed in valleys, where 'eurimedia' was approximately twice as abundant as 'hermias', and predation rates on 'hermias' were 1.9 times as high as for 'eurimedia'. These data thus support the conclusion that the 'eurimedia' pattern was optimal in valley sites and the 'hermias' pattern optimal on ridges, despite these microhabitats being only 100-300 m apart (see S1).

The overall dominance of the two most abundant mimicry patterns and segregation by flight height that we observed were consistent with three other studies at Ecuadorian Amazonian sites spaced across a 3 decade period [27, 32, 56]. These broad patterns of abundance and flight height partitioning thus seem to be a general feature of ithomiine communities in this region. However, even though microhabitat segregation can help maintain the coexistence of these two most common mimicry patterns, the remaining six patterns were never predicted to be optimal in our analysis of butterfly and bird microhabitat distributions. It is likely that at least some patterns, such as 'agnosia', might prove to be optimal in marginal microhabitats, such as forest edges and secondary growth, that were not well represented in our study. In addition, we did not consider temporal changes in bird and butterfly distribution. For example, the lowest-flying mimicry ring, 'lerida', was most active early in the morning, perhaps exposing it to a distinct suite of predators in comparison with later-flying mimicry rings. Furthermore, temporal partitioning of mimicry rings also occurs throughout the year [30], which may also be coincident with seasonal changes in the predatory bird fauna, perhaps selecting for different optimal defences at different times of year (e.g., [24]). Our first analysis also assumes equivalence among insectivorous birds and among mimicry patterns. Neither of these are likely to be true, and some bird species are likely to account for a disproportionate number of attacks. Nevertheless, our data suggest that the majority of bird species, regardless of abundance, are sufficiently restricted in microhabitat as to encounter particular mimicry patterns at non-random rates. Furthermore, we made the simplifying assumption that the most abundant patterns are the best protected, but differences in unpalatability, detectability and escaping ability are also likely to be significant [57]. Finally, because of predator generalization, some rare patterns (e.g., 'mamercus') may be avoided by predators that have been educated by encounters with similar but more abundant (e.g., 'hermias) mimicry rings.

Alternatively, sub-optimal patterns may be maintained because there is little selection for a mimicry switch [5, 32]. If community composition shifts over time, weak selective pressures for convergence may not persist long enough to effect change and similarly protected patterns may coexist indefinitely [58]. Similar shifts in community composition may occur over space; some of the rarer mimicry rings in our study community may be maintained by continuous migration from source regions where they are more abundant, such that patterns that are apparently sub-optimal in one area are optimal in another [16, 17, 19, 20, 26, 36, 59].

Our study supports the idea that any ecological shift that results in a mimetic butterfly species being exposed to new suites of predators, such as a change in hostplant or mate-locating strategy linked to different microhabitats, likely initiates selection for phenotypic change that could ultimately lead to speciation. Previous studies of Neotropical butterflies have shown that predation has morphological, physiological and behavioural consequences [38, 60]. Here, we confirm that shifts in microhabitat are likely to also result in strong selection on warning colour patterns. Concerted changes in wing pattern and microhabitat have indeed apparently occurred multiple times in the ithomiine community that we studied [3], driving ecological convergence. Furthermore, shifts in mimetic wing pattern are associated with both pre-zygotic and postzygotic reproductive isolation in mimetic butterflies [10, 12, 13, 15]. Our results suggest that at least some coexisting mimetic patterns can also be considered ecological niches in their own right [61], rather than by-products of processes operating at larger spatial and temporal scales. Partitioning of species among these niches should help to maintain community species richness in some of the most biologically diverse ecosystems in the world.

Finally, our research contributes to knowledge of the complexity of ecological interactions linking plants, herbivores and predators, and adds to a growing body of literature showing that diversity in anti-predator defence can be maintained by differing abiotic and biotic microenvironments [62-69]. Although competition has usually been seen as the principal driving force for ecological divergence and adaptive radiation [70], predation is likely to be just as important, if not more so in communities where competition is minimal [71-75].

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Data accessibility

Distribution databases for butterflies and birds recorded in this study are included as supplementary material.

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Figure captions

Fig. 1. Relative butterfly mimicry pattern abundance (bars) and butterfly and bird density (numbers of individuals per 1 m height band) (lines) recorded at different heights above the ground.

Fig. 2. Relative butterfly mimicry pattern abundance (bars) and numbers of butterfly and bird individuals recorded (lines) in valley and ridge sites. Black and white dots represent relative abundances of mimicry patterns that would be expected if butterflies were distributed randomly with respect to topography.

Fig. 3. Estimated relative encounter rates of eight ithomiine butterfly mimicry rings by two potential bird predators during the study. Black squares show observed values and standard box-plots represent a distribution of values generated under a null model of no microhabitat segregation of mimicry rings (500 simulations). The low-flying, Spot-backed Antbird was estimated to encounter more 'eurimedia' and fewer 'hermias' in the field than expected under the null model, while the opposite was true for Wedge-billed Woodcreeper, which showed a preference for midstorey and ridge-tops where the 'hermias' mimicry ring tended to fly.

Fig. 4. Results of experimental field predation study. Relative butterfly mimicry pattern abundance recorded in surveys (solid bars), fraction of butterflies attacked in field trials (hatched bars), and numbers of butterfly and bird individuals recorded in surveys (lines) in valley and ridge sites.

Supplementary material

S1. Map showing the location of the study region within South America, and the location of sampling plots at the study site.

S2. Microhabitat distribution data for butterflies at Río Añangu study site, eastern Ecuador.

S3. Microhabitat distribution data for birds at Río Añangu study site, eastern Ecuador. The classification follows Remsen *et al.* [76].

S4. Butterfly abundance in eight mimicry patterns across eight microhabitats.

S5. Results of the permutation test comparing empirical estimated relative encounter rates of eight mimicry patterns for most abundant 25 bird species with those in 500 permuted datasets (H1).

S6. Weighted average mimicry pattern encounter rates ($\overline{M}_{hi}$) for birds co-occurring with butterfly species. The predicted optimal pattern is that with the highest ($\overline{M}_{hi}$), representing the pattern with which birds encountering a given butterfly species are most familiar and therefore most likely to avoid.

S7. Field predation study. **A.** Example of a 'eurimedia' pattern butterfly (*Napeogenes inachia*). **B**. Example of a 'hermias' pattern butterfly (*Mechanitis mazaeus*). **C**. Predated 'eurimedia' pattern butterfly (*Pteronymia primula*). **D**. Valley study site, with four butterflies, two 'eurimedia' and two 'hermias'.

S8. Maximum likelihood approach for comparing predation probabilities between wing patterns and microhabitats.

Competing Interests

We have no competing interests.

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Authors' contributions

CJ, KW and JRW conceived the study; all authors contributed to the design of the field experiments; KW, ME and CJ collected field data on butterflies and microhabitats, JRW collected field data on birds; KW and ME carried out statistical analyses; KW wrote the first draft of the manuscript and all authors contributed to significantly revising and improving the draft. All authors gave final approval for publication.

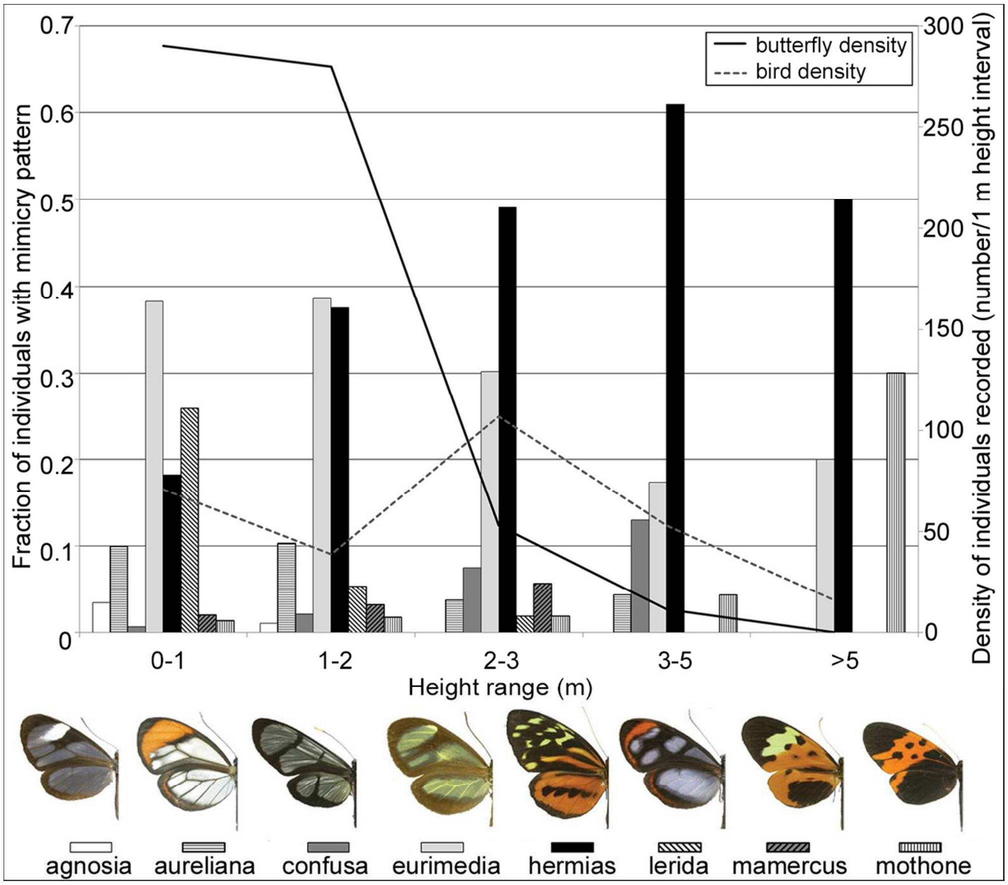

Fig. 1. Relative butterfly mimicry pattern abundance (bars) and butterfly and bird density (numbers of individuals per 1 m height band) (lines) recorded at different heights above the ground.

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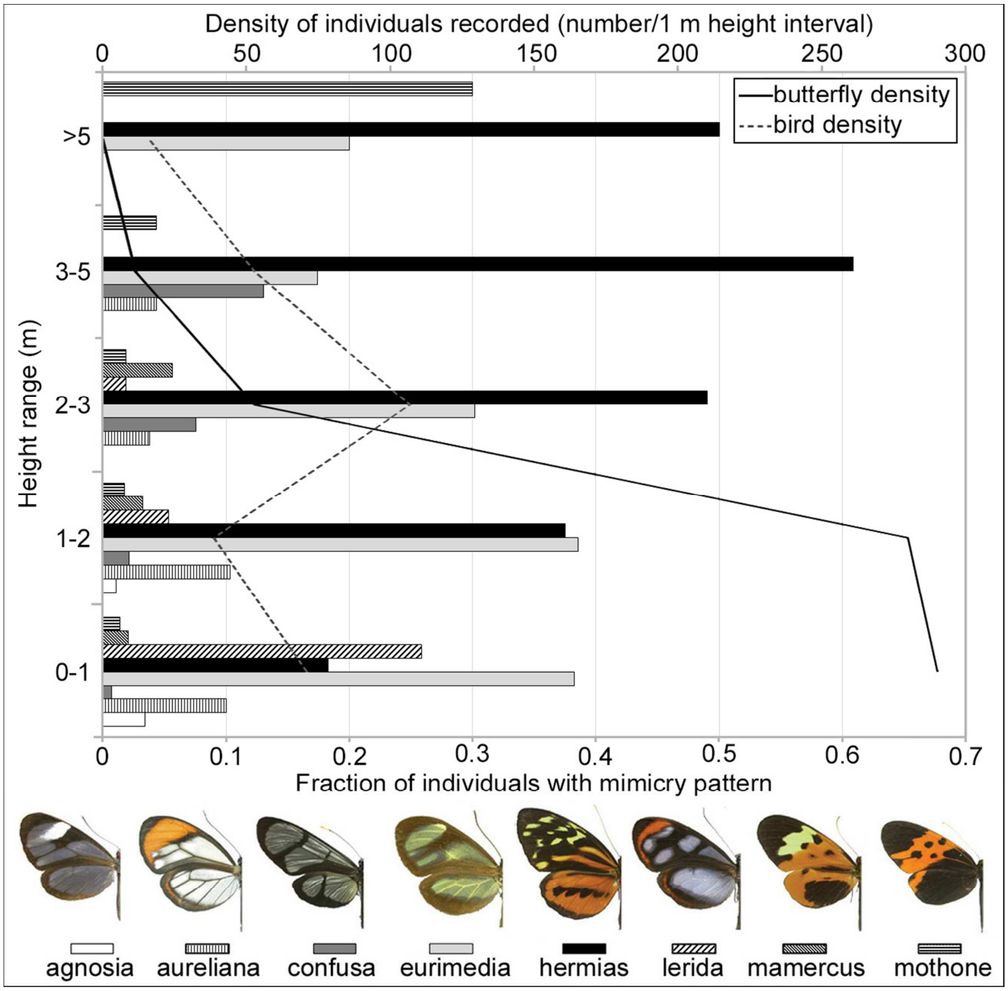

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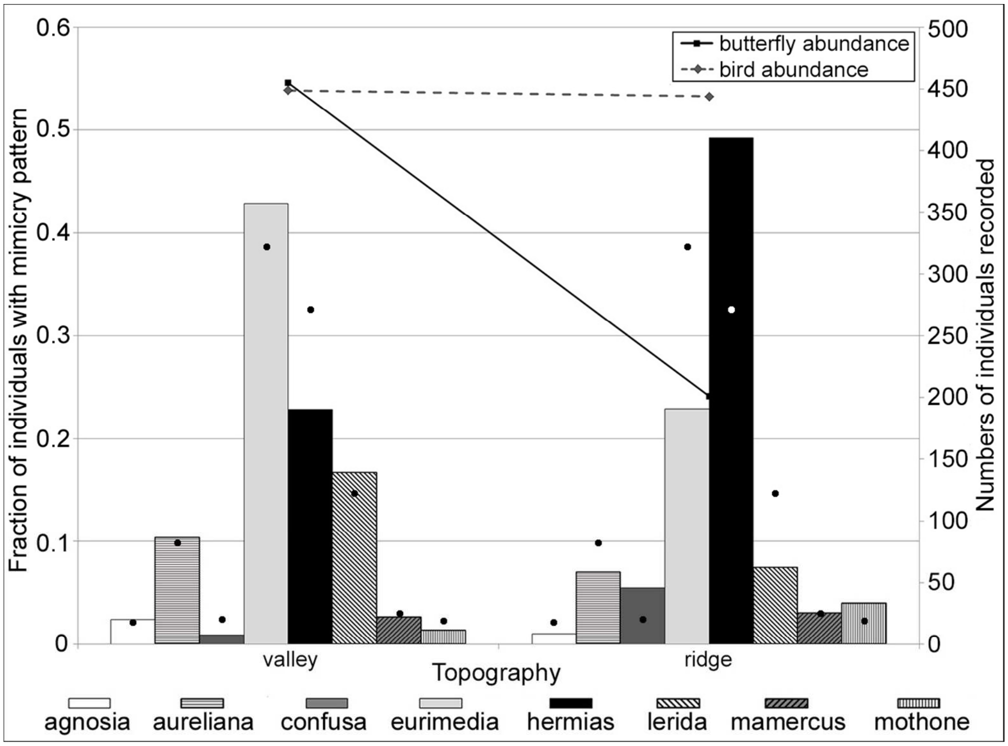

Fig. 2. Relative butterfly mimicry pattern abundance (bars) and numbers of butterfly and bird individuals recorded (lines) in valley and ridge sites. Black and white dots represent relative abundances of mimicry patterns that would be expected if butterflies were distributed randomly with respect to topography.

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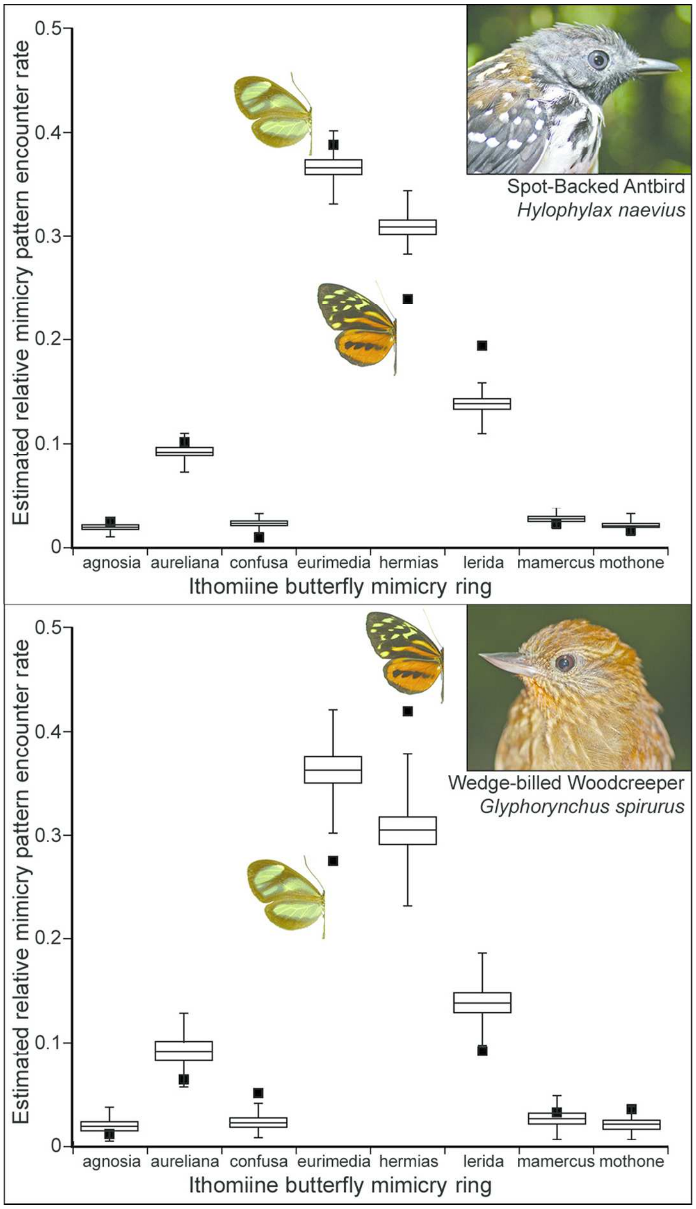

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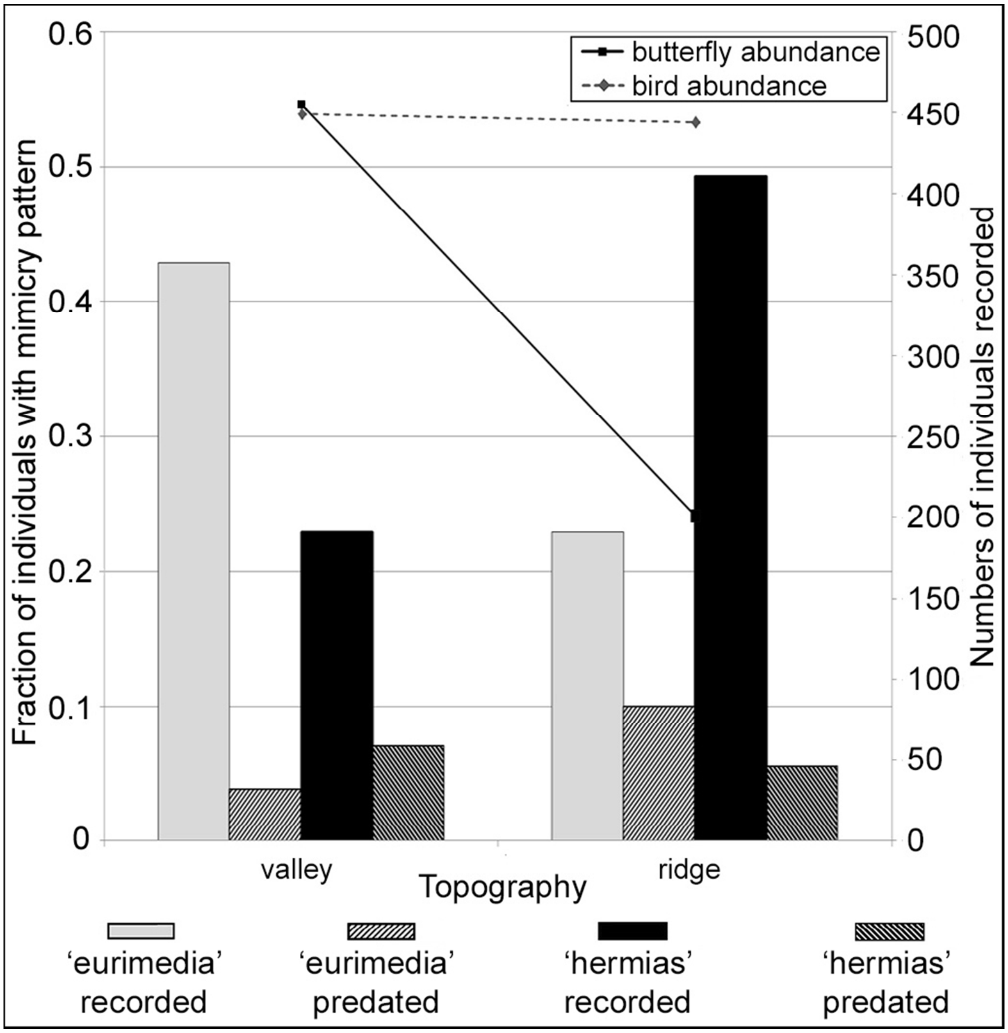

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