Title

Time-dependent effects of species interactions at macroevolutionary scale shape the diversity of mimetic butterflies.

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Short running title

Macroevolutionary signature of mimicry

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Statement of authorship

NC, TGA, VL and ME conceptualized the study. KW collected data. NC and MPB performed the analyses. NC wrote the first draft of the manuscript, and all authors contributed substantially to revisions.

Data accessibility statement

Color pattern classification used for this study is available at 10.5281/zenodo.10073539.

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Abstract

Identifying how species interactions may translate into macroevolutionary dynamics of trait and species diversification is challenging. Here, we test how Müllerian mimicry shapes macroevolutionary patterns of diversification in the Ithomiini butterflies. We show that the age of color patterns is the primary determinant of species richness within mimicry rings. We find pervasive phylogenetic signal in mimicry rings and in color patterns associated within polymorphic species. Only a small set of mimicry rings show high phylogenetic diversity, rings that often involve distantly-related butterflies. We identify patterns of saturation in the accumulation of new mimicry rings and in the number of evolutionary convergences towards the most species-rich mimicry rings. Those saturation patterns are likely caused by niche filling along various ecological dimensions, within and among the mimetic communities living in sympatry. The time-dependent effects detected in our study illustrate how neutral processes and ecological interactions interact and shape species and phenotypic diversification.

Introduction

Identifying how microevolutionary processes and ecological interactions occurring at community level and can affect macroevolutionary dynamics has been a major focus of evolutionary biology for some time (Reznick & Ricklef, 2009, Rolland et al. 2023). Despite this long interest, the evidence of a link between ecological interactions and macroevolutionary patterns of species diversification is scarce. Limitations lie primarily in the difficulty of testing such effects, in particular because species interactions are usually difficult to characterize, and because the effects of microevolutionary processes on macroevolutionary dynamics are most of the time not straightforward (Rolland et al. 2023). Besides, different ecological interactions (e.g. competition, parasitism, mutualism) may involve one or several guilds of species and may therefore result in different outcomes at the macroevolutionary scale.

Here, we focus on Müllerian mimicry, a widespread and well-documented mutualistic interaction that offers a remarkable opportunity to study the link between micro- and macroevolutionary processes (Kunte et al. 2021). The evolutionary convergence of warning signals shared by co-occurring defended species that are Müllerian mimics is one of the most compelling examples of natural selection. Müllerian mimicry among co-occurring species arises through positive frequency-dependent selection exerted by predators that learn and avoid locally abundant warning signals (Müller 1879, Sherratt 2006). Because the cost of predator learning is diluted among individuals of all the species that share the same warning signal, Müllerian mimicry is described as a mutualistic interaction; individuals from a given species benefit from co-occurring with individuals belonging to co-mimetic species, thereby promoting wing color pattern convergences. The resulting groups of species that share a common warning color pattern are referred to as mimicry rings.

Müllerian mimicry is widespread across the animal kingdom, and has evolved independently in multiple lineages, including, among others, frogs (e. g., Symula et al. 2001), snakes (e. g., Sanders et al. 2006), catfish (Alexandrou et al. 2011), butterflies and moths (e. g., Beccaloni 1997), bees and wasps (e. g., Wilson et al. 2012, Ezray et al. 2019) and beetles (e. g., Muñoz-Ramírez et al. 2016). The phenomenon is particularly well-studied in butterflies, especially in the species-rich neotropical tribe Ithomiini (396 species).

Müllerian mimicry is an ideal ecological process to study at a macroevolutionary level, because mutualistic interactions between species can easily be inferred from the color patterns displayed within species and from their overlapping geographic ranges: when sympatric species share a similar color pattern, the individual mortality risk decreases (Kunte

et al. 2021) and species are thereby engaged in mutualistic interactions. Numerous theoretical, experimental, genomic and ecological studies have already shown that predation is a strong selective force driving the convergent evolution of warning signals such as color patterns, as well as shaping the structure of local communities of defended species (e. g., Sherratt 2006, Elias et al. 2008, Gompert et al. 2011, Willmott et al. 2017, Birskis-Barros et al. 2021). The strength of frequency-dependent selection resulting from predation has been convincingly shown empirically in *Heliconius* (e. g., Mallet and Barton 1989, Kapan 2001, Chouteau et al. 2016) and ithomiine (Willmott et al. 2017) butterflies, and has led to striking convergence among species sometimes belonging to distantly related families, such as that observed between erebid moths and nymphalid butterflies (Beccaloni 1997).

Finally, several lines of evidence show that mimicry drives the evolution of multidimensional ecological niches, with convergence in multiple traits (e.g. host-plant preference, flight height, wing morphology, altitudinal and climatic niche) among co-mimetic species (Willmott and Mallet 2003, Elias et al. 2008, Gompert et al. 2011, Hill 2010, Hill 2021, Chazot et al. 2014, Aubier and Elias 2020, Doré et al. 2023). Furthermore, assortative mating based on similarity in wing color pattern observed in mimetic species (Jiggins et al. 2001, Chamberlain et al. 2009, Merrill et al. 2011), as well as the increased predation targeting non-mimetic hybrids between taxa belonging to distinct mimicry rings (Merrill et al, 2012), suggest that switches in mimetic patterns may act as a driver of speciation in mimetic lineages. Mimetic color patterns are thus often considered so-called magic traits (*i. e.*, a trait used as the basis of mate choice that is also under ecologically divergent selection, Gavrilets 2004, Servedio et al. 2011), and one of the factors put forward to explain the high diversification rates measured in some clades of mimetic butterflies (Jiggins et al. 2006, Kozak et al. 2015). Another way mimicry can fuel diversification is through the formation of a spatial mosaic of mimicry rings (Aubier et al. 2017), because such mosaic allows the maintenance of different species occupying similar ecological niches in different patches of the mosaic.

Given the multiple effects in structuring local communities, selecting for particular color patterns, driving ecological niche evolution and even fostering diversification, we suggest that mimetic interactions could affect macroevolutionary dynamics of colour pattern evolution and species diversification. In order to test this hypothesis, we define a framework of eight predictions describing how mimetic interactions should (1) affect the dynamics of color pattern diversification through time, (2) structure polymorphism within species, and (3) explain the variation in species richness among mimicry rings (Figure 1). We tested these

predictions for the largest radiation of mimetic butterflies, namely the tribe Ithominii, by combining phylogenetic information with a dataset of color patterns for 339 Ithomiini butterfly species (Chazot et al. 2019, Doré et al. 2021, 2022).

MATERIAL AND METHODS

Data

We used the time-calibrated phylogeny of Ithomiini from Chazot et al. (2019), which contains 339 species of Ithomiini (85% of species). Species classification into color patterns and mimicry rings followed Doré et al (2021) definitions, resulting in 44 mimicry rings (Figure 2, Table S1). Mimicry ring species richness in Ithomiini ranges from a single to over 70 species, and at least half of Ithomiini species belong to two or more mimicry rings.

Ancestral state estimations

In our dataset, 200 out of the 339 species studied show color pattern polymorphism. We therefore estimated ancestral color patterns across the Ithomiini tree using the method described in Braga et al. (2020). This method, initially designed for estimating ancestral host-parasite associations on a phylogenetic tree, allows allowing current and ancestral species to belong to multiple mimicry rings, due to wing color pattern polymorphism. We used the 2-state model and the inference strategy of Braga et al. (2020) as implemented in RevBayes (Höhna et al., 2016) to infer the history of color pattern evolution, based on the color patterns known for each extant species. Model parameters and ancestral states were inferred using Markov Chain Monte Carlo (MCMC). We ran two chains for 8 x 10^4 iterations, discarding the first 10% as burn-in and sampling every 50 iterations, then confirmed that the chains converged to the same posterior distribution by calculating the Gelman diagnostic (Gelman and Rubin, 1992) in the R package *coda* (Plummer et al., 2006). Results from the MCMC chain with highest effective sample size are presented (Supplementary Figure S1). All RevBayes and R scripts are available at github.com/maribraga/Ithomiini_color.
Because polymorphic states can be inferred as ancestral states, the complexity of the evolutionary history estimated greatly increased. We thus took advantage of the Bayesian framework, by performing all follow-up analyses on a posterior distribution of evolutionary histories.

Dynamics of color pattern evolution through time (Predictions 1-2)

We investigated the dynamics of color pattern evolution, and specifically tested two main predictions.

Prediction 1: The rate of emergence of new patterns decreases through time, because the advantage provided to species sharing the same color pattern should favor convergence rather than innovation.

Prediction 2: The rate of color pattern shifts increases through time as the overall number of color patterns rises, because an increasing number of color patterns implies new opportunities for convergence.

To test these predictions, we extracted the timing of color pattern evolutionary events (*i.e.*, gains and loss) for each independent history sampled from the posterior distribution of the ancestral state estimations. We extracted the earliest timing of emergence of each color pattern to calculate the cumulative sum of new color patterns through time within 1 My time bins. Using a sliding-window analysis, we also counted the total number of color pattern shifts (irrespective of color pattern identity) occurring within 1 My time bins to estimate the number of shifts through time. Since both statistics are expected to increase proportionally with branch length, we obtained the rate of new pattern emergence (Prediction 1) and the rate of color pattern shift (Prediction 2) for each 1 My time-bin by dividing the number of new patterns and the number of shifts, respectively, by the sum of branch length in that bin.

Color pattern combinations within polymorphic species (Prediction 3)

We then tested a prediction regarding the non-random combinations of color patterns within polymorphic species.

Prediction 3: There is a significant phylogenetic signal in color pattern associations, caused by phylogenetically conserved developmental pathways of color patterns and/or parallel co-evolution of color patterns across species, since polymorphic species can simultaneously shift to different mimicry rings.

We evaluated whether polymorphic species exhibit random color pattern associations or instead, whether they represent repeated association of the same color patterns. We used statistical tools designed for ecological network analyses to test for modularity in the network of color pattern associations; modules representing clusters of color patterns frequently associated in polymorphic species. We used the Beckett (2016) algorithm to identify the network configuration maximizing the proportion of interactions within modules. We repeated the module identification 100 times and calculated the frequency of pairwise

associations of color patterns within modules. We used the package *qgraph* to build the network of pairwise associations.

We then tested whether species composition of each module is phylogenetically random or instead phylogenetically structured. We measured phylogenetic diversity in the modules by comparing the mean phylogenetic distance (MPD) between species belonging to the same module to the distribution of mean phylogenetic distances after permutations of color patterns between modules. High MPD score indicates a module comprising more distantly related species than expected by random community assemblage, *i.e.* resulting from color pattern convergence between distantly related lineages. Conversely, a low MPD indicates a module shared by species more closely related than a random combination of species. MPD values falling into the 95% interval of the distribution obtained from the permutations indicate no departure from random evolution of color pattern, *i.e.* driven neither by convergence events nor by phylogenetic proximity. We performed the MPD test one iteration of the module search algorithm.

Phylogenetic diversity within mimicry rings (Prediction 4)

First, we explored the phylogenetic diversity found in mimicry rings. We used an MPD test similar to the tests performed within modules, by comparing the mean phylogenetic distance between species belonging to the same mimicry ring to the distribution of mean phylogenetic distances after permutations. Five color patterns were unique to a single species and therefore we did not perform the MPD test for these color patterns.

Second, we tested for phylogenetic signal of color patterns in relation with their age. We predicted that *the phylogenetic signal within a mimicry ring decreases with the age of the mimicry ring (Prediction 4)*. Greater time since the emergence of a color pattern allows for color pattern convergence involving more distantly related species (Kunte et al. 2021).

To test Prediction 4, we computed the D statistics (Fritz & Purvis 2010) for each mimicry ring (and merging all other mimicry rings) with more than 3 species, with permutations to identify significantly conserved and overdispersed mimicry rings. A D value of 0 indicates Brownian motion evolution, and a value of 1 random evolution. D<0 indicates phylogenetic clustering while D>1 indicates phylogenetic overdispersion. We tested for a relationship between color pattern age and D statistics by fitting a linear model.

To obtain the age of color patterns, we extracted the timing of color pattern gain and loss events for each independent history sampled from the posterior distribution of the

ancestral state estimation analysis. The oldest timing of emergence of a color pattern, in absolute value, was considered as the age of the color pattern.

Species richness of mimicry rings (Predictions 5-8)

Mimicry ring species richness (i. e., the number of species in a mimicry ring) in Ithomiini ranges from a single species to over 70 species (Figure 2). To explain such disparity, we tested four processes by which variation in species richness may arise and we tested how much each variable contributed to the species richness of mimicry rings (Figure 1).

Prediction 5: Species richness of a mimicry ring increases with the age of the color pattern associated (Figure 1). As a null hypothesis, species richness should increase with time through the accumulation of either speciation without color pattern change or speciation driven by convergence of color pattern to occur. We tested for a relationship between the oldest timing of emergence of color patterns and species richness. In contrast, *Predictions 6-8* imply that differences in species richness stem from differences in the properties of color patterns or associated features, such as habitats, leading to different rates of color pattern shifts or diversification.

Prediction 6: *Species richness increases with the number of evolutionary gains of color patterns, but decreases with the number of evolutionary losses (Figure 1)*. Differences in evolutionary gains among mimetic color patterns may be observed, either (1) because they confer different advantages such as a higher protection against predation (e. g., because they are locally abundant, or more memorable) or benefits unrelated to aposematism (e.g., thermoregulation, crypsis), or (2) because fewer mutations are required for this pattern to emerge from an ancestral one, for example when the capacity to produce certain pigments and pattern elements is already present as an ancestral state.

We summed the total number of species gain and loss events for each mimicry ring across the posterior distribution of ancestral state scenarios, similar to the calculation of the rate of shifts through time. However, even under stochastic evolutionary processes, the number of gains and losses increase with time. Thus, we divided the gains and losses by the age of the color pattern to obtain a rate of gains and a rate of losses.

Prediction 7: *Species richness of a mimicry ring increases if lineages belonging to that mimicry ring exhibit higher rate of color pattern evolution (Figure 1).* Such lineages may

lead to a high number of convergence events towards the same color pattern, thereby increasing species richness via convergence and subsequent diversification. While Prediction 6 focuses on tree-wide association between rates of gains and losses, Prediction 7 tests for clade-specific rates of evolution that may lead to higher species richness.

We estimated a tip rate of color pattern evolution – species-specific rate of evolution – by first calculating the number of shifts occurring along each branch in the tree across the posterior distribution of ancestral state estimations. The length of each branch of the phylogenetic tree was then rescaled to this number of events. We calculated for every tip in the tree the total rescaled branch length connecting the root to the tips. Because the number of possible evolutionary changes is expected to increase with the number of nodes connecting the root and the tips, we divided the sum of branch length of each tip by the number of connecting nodes to obtain the tip rate of color pattern evolution. Finally, we calculated the median tip rate of evolution for each mimicry ring.

Prediction 8: Species richness of mimicry rings increases with diversification rate of their members (Figure 1). Although the reasons why a given color pattern could directly affect the probability of a speciation event (without color pattern shift) are unclear, it is indirectly associated with higher diversification through correlation with other phenotypical, ecological or geographical traits e.g., habitats that favor speciation or limit extinction, such as high-altitude Andean forests (Chazot et al. 2016, 2018, 2019).

To test whether species-rich mimicry rings are those distributed among rapidly diversifying lineages, we calculated the Diversification Rate (DR) statistics (Jetz et al., 2012) for each tip in the tree. DR is a tip rate of diversification calculated as the inverse of the equal-split measure (Redding & Mooers 2006). We calculated the median DR for each mimicry ring from the all species belonging the same mimicry ring.

Statistical analyses – First, we tested for relationships between pairs of explanatory variables using linear regressions. Second, we identified the significant combinations of predictors of species richness within mimicry rings by fitting linear models with all possible sets of variables included and using hierarchical partitioning to estimate the proportion of variance explained by each variable. However, the age of color patterns correlated with many variables. Both species richness and the rate of shifts increased with age. Therefore, we calculated the residuals of the linear model between species richness and color pattern age, then repeated the statistical analyses using those residuals as the dependent variable. All

statistical tests were performed using the median predictor scores calculated over 100 posterior evolutionary histories.

RESULTS

Color pattern evolution through time

The number of color pattern shifts increased over time, but the rate of shifts remained nearly stable (Figure 3A and 3B), providing no support for Prediction 2. The rate of new pattern emergence decreases through time, moderately until about 15 My ago, and more abruptly after that, with hardly any new pattern appearing during the last 5 My (Figure 3C and 3D). These results confirm Prediction 1.

Patterns of species richness and modularity

There is high heterogeneity in extant species richness among mimicry rings, with a mean of 16.3 species but ranging from 74 species sharing the same warning signal (color pattern AGNOSIA*)*, to a single species (ACRISIONE, DERCYLLIDAS, HUMBOLDT, PRAESTANS, VESTILLA) (Figure 2). We found modularity in the network of interactions, indicating that certain combinations of color patterns within polymorphic species occur more often than expected at random. Based on a single iteration of the Beckett (2016) algorithm, we identified nine modules (Figure 4A). We found significantly low MPD scores for four of these modules (module 2, 3, 4, 9, Figure 4A). A single module had significantly high MPD (module 6). This module contained most of the patterns with black, orange and yellow opaque colors (often referred to as 'tiger' color patterns). These results provide partial support for Prediction 3. The most species-rich mimicry rings were not concentrated in a single module. For example, the six most species-rich mimicry rings were distributed across four different modules. These modules were not all supported 100 iterations of the Beckett algorithm. Module 2, 4 and 6 were well supported and module 3 partly supported (Figure 5).

Phylogenetic structure of mimicry rings

Using the MPD test, we found significantly low phylogenetic diversity within 20 mimicry rings (Figure 4B). By contrast, only four mimicry rings showed significantly high phylogenetic diversity (EXCELSA, HERMIAS, MAELUS, MAMERCUS, 'tiger' color patterns with black, orange and yellow opaque colors).

Using the D statistics, we found significant phylogenetic signal for 14 color patterns and overdispersion for 12 mimicry rings (Figure 4C). The D statistics increased as color patterns emerged more recently, indicating higher phylogenetic signal in older mimicry rings, i.e. opposite to Prediction 4 (Figure 4C).

Predictors of mimicry ring species richness

When tested independently, we found that the age of color patterns and the rate of color pattern losses predicted mimicry ring species richness (Figure 6A and 6B). The relationship between the rate of gains and species richness was not significant (p-value = 0.052, Figure 6C). Diversification rate alone did not predict species richness within mimicry ring (Figure 6C and D). Overall, species-rich mimicry rings were older and experienced higher rate of color pattern loss.

When testing all variables together, we found the combination of color pattern age, gain rate and loss rate to be the best model predicting species richness within a ring. Loss rate explained 49.3% of species richness variance, while color pattern age and gain rate explained 42.6% and 5.6%, respectively (Figure 6, Table S2). Based on the AIC score, two other models adding either DR or the tip rate of evolution to the previous variables were not significantly different. When repeating the analyses using the residuals of the regression between species richness and color pattern ages, the same combinations of predictors (age excluded) were best fitting models (Figure 6, Table S3). Loss rate predicted 52.5% of the residual species richness and gain rate 27.8%. However, the rate of color pattern shifts and DR only explained 13.7% and 5.8% of the variance respectively. The rate of losses and gains, as well as DR increased with residual species richness (Figure 6E, 6F and 6G), while the rate of color pattern shift decreased with species richness residuals.

Overall, our results provide strong support for prediction 5, partial support for prediction 6 when considering the rate of gains but opposite to prediction 6 for losses, results opposite to predictions 7 and only poorly supported results for prediction 8.

DISCUSSION

Strong phylogenetic signal and striking convergences in the formation of mimicry rings

Despite selection by predation favoring convergence among distantly-related species, we found significant phylogenetic signal in 14 mimicry rings. Modularity in the network of mimetic interactions also indicates that there is a non-random co-occurrence of color patterns

in polymorphic species. Out of the nine detected modules, half were phylogenetically clustered. Furthermore, polymorphic states at nodes were generally estimated from our analyses. This suggests that phylogenetic inheritance is not limited to single color patterns but also frequently affects the ability to produce sets of different patterns, maybe generated by similar developmental pathways. We predicted that phylogenetic diversity should increase with color pattern age (Prediction 4, Kunte et al. 2021) but we found the opposite result. Even though the number of convergence events also increased with time (in line with Prediction 4), it is probably outweighed by speciation without color pattern changes.

Phylogenetic diversity was significantly higher than a random distribution of the color patterns in four mimicry rings (EXCELSA, HERMIAS, MAELUS, MAMERCUS). Besides, all of them but EXCELSA are part of the same module, the only module also phylogenetically overdispersed. While wing transparency is very common in Ithomiini, with most of the mimicry rings harboring wings with transparent patches, the four highly convergent color patterns mentioned above are all non-transparent and happen to be frequently found outside of Ithomiini, such as in heliconiine butterflies (Nymphalidae: Heliconiini), danaines (Nymphalidae: Danainae; Danaini) or in pericopine tiger-moths (Erebidae: Pericopina). The elevated number of distantly-related species displaying these color patterns, both within and outside Ithomiini butterflies, may stem from several developmental or selective factors: 1- the retention of genetic abilities that could be ancestral to all Lepidoptera (phylogenetic signal hypothesis), 2- these patterns can be produced by a variety of genetic toolkits, thus making them more likely to appear independently (evolutionary convergence hypothesis), 3 – opaque patterns are more conspicuous and therefore more easily learnt by predators (natural selection hypothesis).

The age of mimicry rings predicts species richness

In our results, time appears as the main driver underlying mimicry ring composition. Time also predicted the number and rate of evolutionary events (both gains and losses of color patterns), as expected under stochastic evolution of color patterns. However, we found strong time-dependency effects in the other evolutionary processes tested here and we highlight below their implications for the dynamics of diversification.

Color pattern evolution through time: evidence for ecological saturation?

We initially predicted that the frequency of color pattern shifts would increase through time as a result of the increasing number of possibilities for convergence provided by the increasing

number of color patterns with time (Prediction 2). Yet, across all color patterns, we found hardly any variation in the dynamics of shifts through time (when accounting for the increasing number of lineages through time). Therefore, the increasing complexity of the network of interactions over time did not result in an increase in the overall rate of evolutionary shifts. If the overall rate of shifts remained stable while the number of patterns increased, this implies that the rate of gains and losses per pattern decreased through time.

In parallel, we found a strong dampening in the rate of appearance of new color patterns, with very few new patterns appearing over the last 10 My, suggesting the existence of a large-scale upper limit to the number of color patterns. Such dampening could stem from a mimicry saturation effect. While novel color patterns tend to be eliminated by positive frequency-dependent selection, they may still pass through the sieve of selection and become established due to drift followed by selection (shifting balance, Mallet and Joron 1999). Evolution of such novel color patterns is especially favored when selection from predation is relaxed (Chouteau and Angers 2012), as may be the case in habitats devoid of an established mimetic community (Sherratt 2006). Therefore, mimicry diversity can increase as long as there are empty ecological niches (e. g., new geographical regions, new elevations, new microhabitats), with communities of naive predators, to colonize (Aubier et al. 2017). When all niches have been colonized by mimetic butterflies, even if there is still room to accommodate more species because resources are not limiting yet, we can expect a strong filtering on color patterns, which are under selection to match the local mimetic community (Kunte et al. 2021, Aubier et al. 2017, Sherratt 2006, Mallet & Barton 1989). In Ithomiini, all geographical regions and likely most altitudinal niches were already colonized five million years ago, but the number of ithomiine species at that time was only about 25% of the number of extant species (Chazot et al 2019). Such conditions likely provided opportunities for further species accumulation in those different regions and habitats, either through speciation or colonization (Chazot et al. 2016, 2018), but mimetic communities may have been well established already, thereby favoring the establishment of species with an already existing color pattern locally. Therefore, as species continued to be formed, novel color patterns were likely selected against.

We also found evidence of saturation in the balance between the rates of color pattern gains and losses (Supplementary Figure S2). The rate of losses increased with the age of mimicry rings and in species-rich mimicry rings (opposite to our Prediction 6). However, while the rate of losses increased linearly with species richness and equaled the rate of gains

for recent/species-poor mimicry rings, the rate of gains in the oldest/species-rich mimicry rings were disproportionally low (Supplementary Figure S2). Species richness of mimicry rings may increase despite a high rate of evolutionary losses when the rate of speciation without color pattern changes (or without pattern losses) exceeds the rate of color pattern shift and loss. This result further supports the retention of color patterns over evolutionary time. It is also consistent with the significant decrease of tip rate of color pattern evolution in species-rich mimicry rings, a significant predictor of residual species richness, but in a direction opposite to Prediction 7. The rate of color pattern evolution declined in lineages belonging to species-rich mimicry rings, and the decline in the rate of gains highlighted above is probably responsible for that. Altogether, our results point at a saturation effect within mimicry rings, which could be due to resource limitation; co-mimetic species tend to share habitats (Chazot et al. 2014, Doré et al. 2023), microhabitats (Elias et al. 2008, Willmott et al. 2017) and even larval hostplants (Wilmott and Mallet 2003), and after a mimicry ring becomes too species-rich the cost of competition among co-mimics may outweigh the benefits of joining a very large mimicry ring, in terms of protection from predation.

Rate of species diversification also contributes to species richness

When we repeated the analyses on the residual species-richness within rings after removing the effect of time, species diversification rate emerged as a secondary predictor of residual species richness (alongside the tip rate of color pattern evolution discussed previously). This result indicates that diversification rate heterogeneity across the tree influences mimicry ring species richness (Prediction 8), maybe both directly and indirectly. Species-rich mimicry rings contain lineages diversifying faster, suggesting that certain color patterns might promote higher diversification rates within lineages, although the underlying mechanisms remain unknown. But this trend may also stem from correlated factors: for instance, geographic distribution of lineages may affect diversification rate and in turn increase or decrease the rate of accumulation of species within a mimicry ring.

Conclusion

Mimicry is undoubtedly a remarkable example of an interaction evolving through natural selection. How such selection translates into the dynamics of phenotypic evolution and species accumulation at macroevolutionary scale has previously been poorly studied (but see Aubier et al. 2017). Our results on Ithomiini support mechanisms of diversification that are independent from mimicry. Time alone is a strong predictor of the number of evolutionary

events and species richness in mimicry rings. We found phylogenetic signal in twelve mimicry rings and in color pattern associations in polymorphic species. The network of interactions and the composition of mimicry rings appear driven for a large part by neutral processes of diversification.

Nevertheless, we find evidence that ecological saturation possibly linked to mimetic interactions did affect the dynamics of color pattern evolution and species diversification. This conclusion comes from several lines of evidences: the strong decrease in the rate of new color pattern appearance, the decrease in color pattern gains relative to losses in species-rich mimicry rings, the increasing rate of losses in species-rich mimicry rings. Ecological limitations to the overall diversity of color patterns and to the number of species interacting within a mimicry ring seem likely to change the dynamics and directionality of color pattern evolution, and thereby the species composition of mimicry rings.

Our results also have implications for the global biogeography of species interactions. Large networks of mimetic interactions seem to be primarily found at lower latitudes (although there has not been any formal validation of this observation). Our study shows that time is key to building a network of mimetic interactions, both in terms of color pattern diversification and species composition of mimicry rings. Thus, our results support the idea that complex networks of interactions are built over long geological times, such that specific conditions such as the age of biomes or their climatic stability (Fischer 1960) may be key to understanding latitudinal gradients in biotic interactions.

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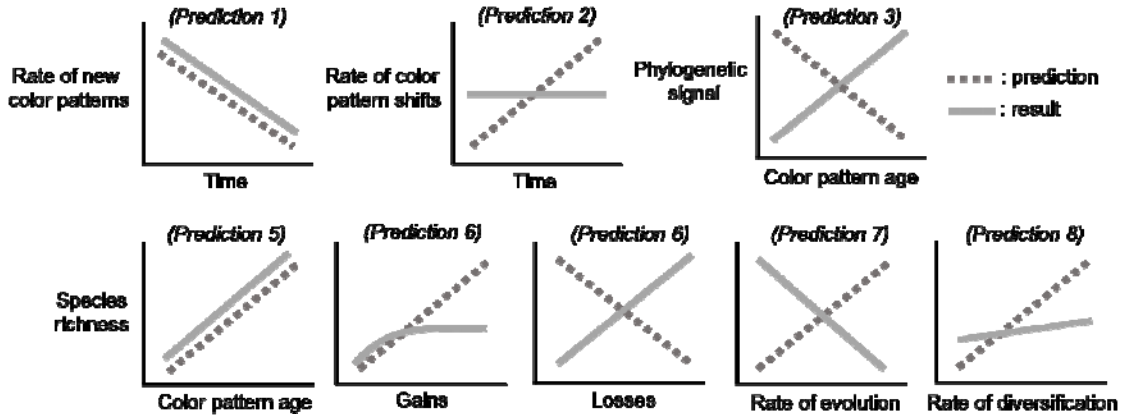

Figure 1. Graphical summary of seven out of eight predictions tested in this study and the results we obtained for Ithomiini butterflies.

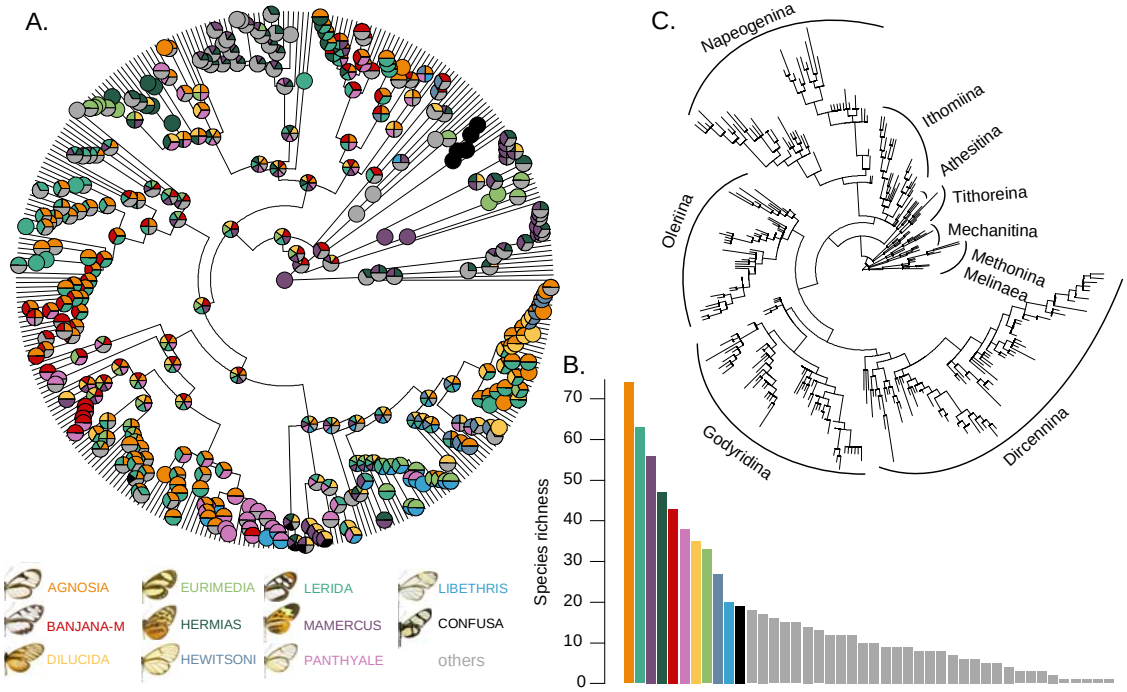

Figure 2. A. Example of a history of color pattern evolution drawn from the posterior distribution of the Bayesian inference. Only the 11 most species-rich mimicry rings are colored. Pie charts do not represent posterior probabilities, rather, they show all mimicry rings that were inferred at a given internal node at this given sample from the posterior distribution. B. Ranked species richness of mimicry rings. C. Phylogenetic tree of Ithomiini with branch lengths rescaled according to the average number of shifts estimated across 100 ancestral histories drawn from the posterior distribution.

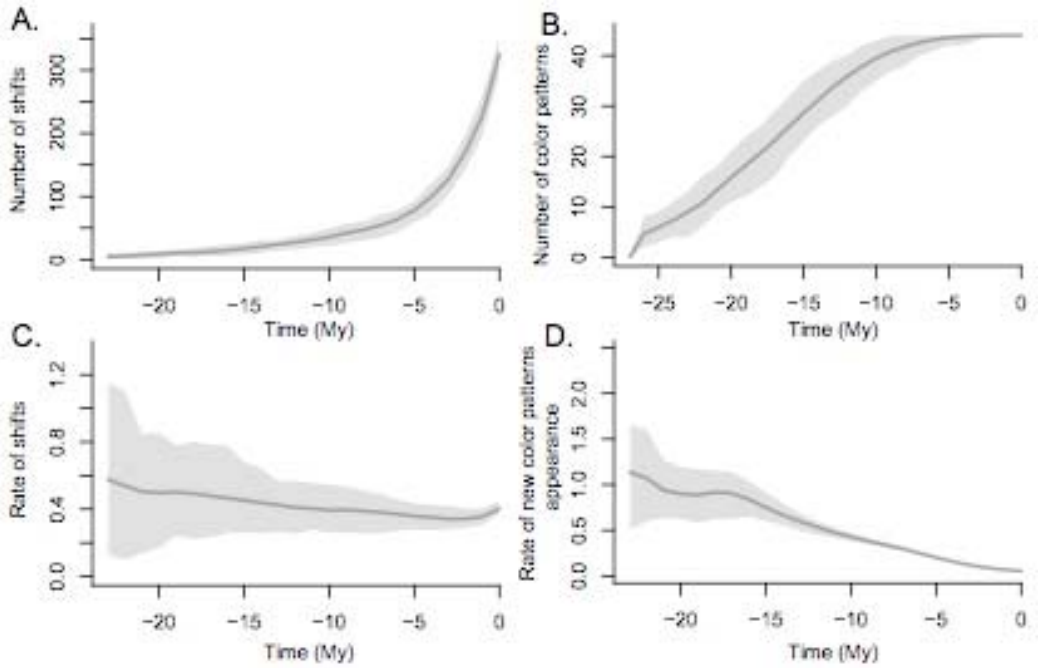

Figure 3. Number and rate of color pattern shifts through time. Cumulative number of new color patterns through time and rate of new color pattern appearance through time. Grey polygons show the 95% confidence interval from 100 posterior ancestral state estimations, line showing the median.

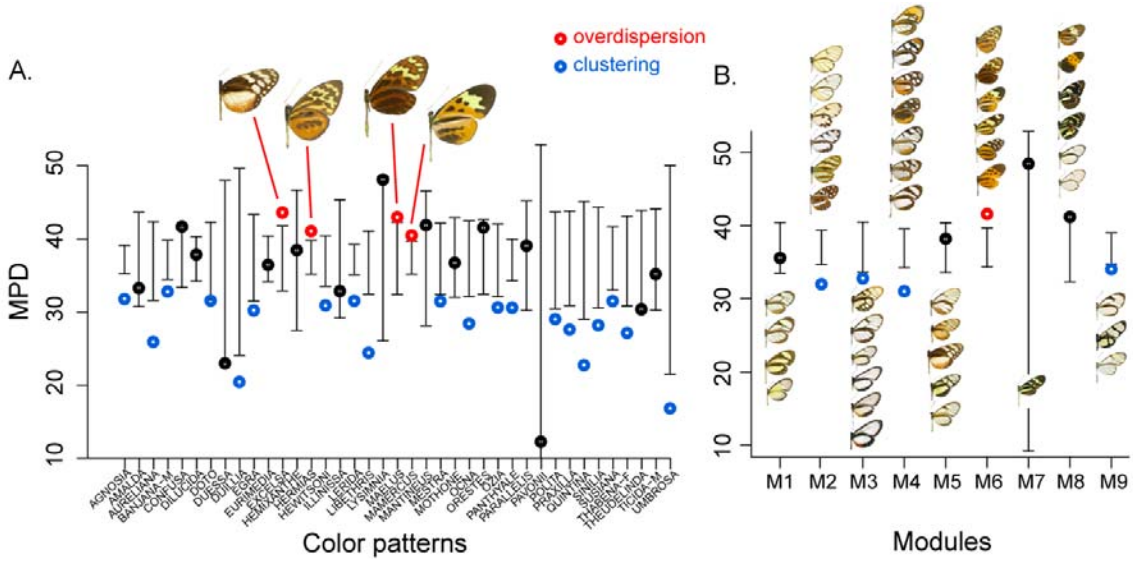

Figure 4. Phylogenetic diversity within modules (A.) and within mimicry rings (B.). Circles indicate the observed mean phylogenetic distance (MPD) score. Bars indicate the 95% confidence interval of MPD scores after 1000 permutations. Butterfly images show the color pattern associated with each mimicry ring or module. (C.) Relationship between phylogenetic signal for each color pattern (D score) and the time of first appearance.

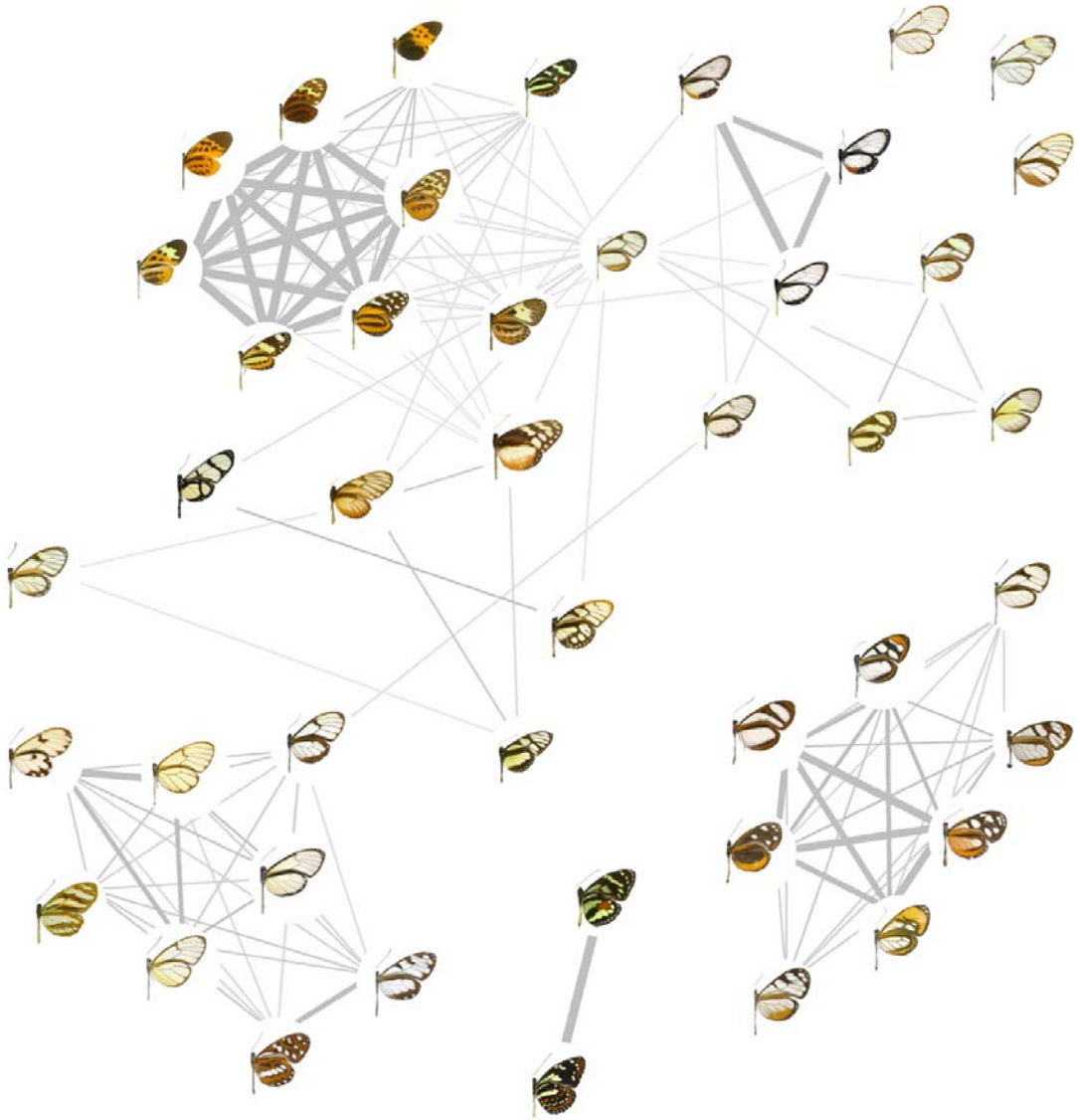

Figure 5. Graphical representation of the frequencies of pairwise associations of color patterns within polymorphic species calculated from 100 iterations of the Beckett (2016) algorithm. Only pairwise associations with a frequency higher than 0.25 are shown.

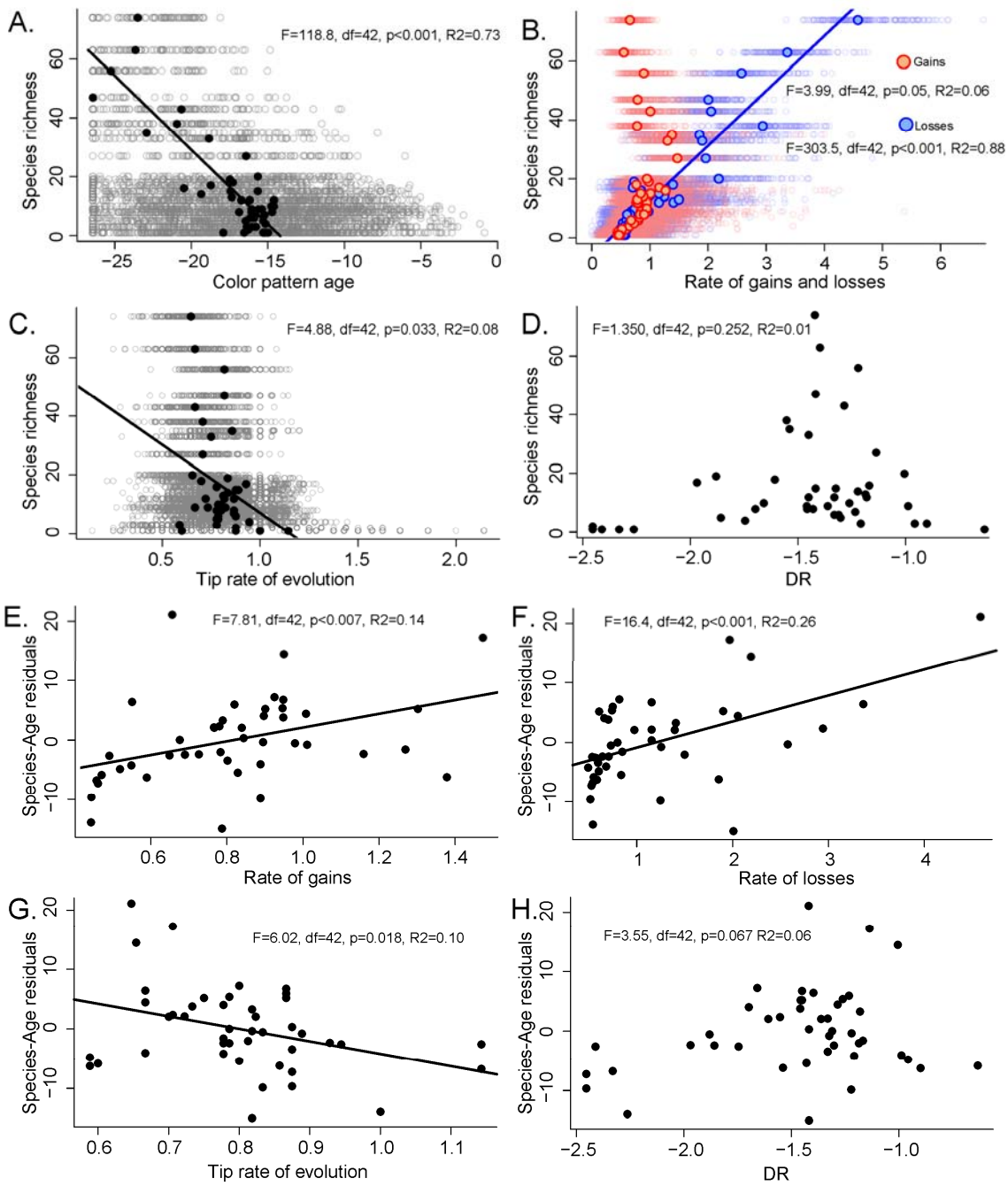

Figure 6. Relationships between mimicry species richness and different predictors. A - D: analyses performed on species richness; E - H: analyses performed on the residuals of species richness regressed against age. Predictors tested correspond to: A. age of color pattern, B. rate of gains and rate of losses, C. tip rate of color pattern evolution, D. diversification rate. Grey circles show the statistics calculated from 100 posterior ancestral state estimations, black dots show the median statistic for each color pattern. The results of the linear models fitted for each predictor individually are also shown. Results of the combined analyses are shown in Tables S2 and S3.