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# Inter-species variation in unpalatability does not explain polymorphism in a mimetic species

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**Abstract**  Conspicuous colouration in unpalatable organisms acts as a warning signal of their unprofitability, a phenomenon known as aposematism. The protection conferred by such colouration can lead to evolutionary convergence in warning signals between aposematic species, because sharing warning signals reduces the per capita cost of predator learning. Consequently, most aposematic species display a single colour pattern and participate in a single mimetic community (i.e. mimicry ring) at any given locality. However, some, like the Amazonian butterfly *Heliconius numata*, are polymorphic and participate in several mimicry rings within the same locality. We tested whether the unexpected polymorphism of *H. numata* could be due to a weak defence against predators. Poorly defended species participating in a mimicry ring are subject to negative frequency dependent selection, because their presence weakens the protection provided by the shared signal. This could promote polymorphism and participation in multiple mimicry rings. Using wild caught great tits (*Parus major*), we compared the palatability of *H. numata* to one of its locally monomorphic co-mimics (*Mechanitis polymnia*) and to two other locally monomorphic *Heliconius* species (*H. melpomene* and *H. erato*). The tested birds strongly rejected the polymorphic species *H. numata*, as well as the two other *Heliconius* species. Unexpectedly, a significantly weaker rejection was found towards *M. polymnia*, which relies on different toxic compounds to *Heliconius*. Our study demonstrates that the origin of polymorphic mimicry in *H. numata* is unlikely to stem from low unpalatability and raises new questions on defence variation within mimetic communities.

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## Introduction

Many toxic species display conspicuous colouration which acts as a warning signal advertising their unpalatability to predators (Poulton 1890). Although some predators have evolved an innate avoidance of dangerous prey (Rubinoff and Kropach 1970; Lindström et al. 1999a), generally predators need to encounter a warning signal several times for them to learn to avoid it (Gittleman and Harvey 1980; Sillén-Tullberg 1985; Alatalo and Mappes 1996; Lindström et al. 1999c; Hauglund et al. 2006; Skelhorn and Rowe 2006a). Therefore, as the frequency of a warning signal in a population increases, the per capita cost of predator learning declines (Müller 1878), generating mutualistic relationships between chemically defended species displaying similar phenotypes ("Müllerian mimicry"). This positive selection for common, conspicuous signals often results in evolutionary convergence between distantly-related toxic species living in sympatry (Mallet and Gilbert 1995; Lindström et al. 2001; Rowland et al. 2007), which form mimicry rings of species with similar appearances. This phenomenon is observed in a large variety of organisms, including butterflies (Ritland 1991), millipedes (Marek and Bond 2009), and ladybird beetles (Brakefield 1985). An additional consequence of the positive frequency-dependent selection exerted by predators on warning signals is that species involved in Müllerian mimicry are expected to be locally monomorphic (Mallet and Gilbert 1995; Lindström et al. 2001; Rowland et al. 2007). However, some toxic species exhibit polymorphic warning signals and participate in several distinct mimicry rings within the same locality (Mallet and Singer 1987), for example Dendrobatid frogs (Wang and Shaffer 2008) and wood tiger moths (Hegna et al. 2015). The mechanisms driving these surprising polymorphisms are unclear. One hypothesis is that they stem from weaker chemical defences against predators ["quasi-Batesian mimicry" (Speed 1993, 1999)]. The involvement of poorly defended species within a mimicry ring is thought to weaken the protection provided by the shared signal, because the number of prey items that need to be sampled for predators to learn increases. This negative frequency dependent selection means that less toxic species can increase their fitness through polymorphism and participating at low frequencies in multiple well-defended mimicry rings (Speed 1993, 1999; Turner and Speed 1996; Speed and Turner 1999). Polymorphism is indeed often observed in Batesian mimics (Bates 1862). For instance, many palatable butterflies from the genus *Papilio* that mimic toxic genera such as *Battus* and *Parides* are polymorphic (Kunte 2009).

A wide unpalatability spectrum has been documented in natural mimetic communities (Speed et al. 2012). Aposematic organisms often acquire toxins from their diet. For example, Dendrobatid frogs' toxicity relies on steroidal alkaloids such as the bathracotoxin, which is thought to be derived from intake of ants, beetles and small millipedes (Daly 1995; Saporito et al. 2012). Significant variations in unpalatability within species are therefore expected due to the availability of the different toxic sources (Tullberg et al. 2000), their chemical content level (Camara 1997) and inter-individual foraging decisions (Despland and Simpson 2005). A precise estimation of these variations in wild samples is needed to understand the dynamics of Müllerian mimicry (Speed 1993, 1999; Balogh et al. 2008).

Here, we test whether polymorphism of an aposematic species results from low unpalatability. *Heliconius* butterflies are a well-documented example of Müllerian mimicry

(Merrill et al. 2015). All *Heliconius* species are toxic because of caterpillars' ability to sequester cyanogenic glycosides from their *Passiflora* host plants (Cardoso and Gilbert 2007) and *de novo* synthesize cyanogenic compounds in adults and larvae (Engler-Chaouat and Gilbert 2007). Nonetheless, variations in toxicity levels have been documented within and among *Heliconius* species (Cardoso and Gilbert 2007; Engler-Chaouat and Gilbert 2007; Hay-Roe and Nation 2007). As expected for species involved in Müllerian mimicry, most *Heliconius* species display a single wing colour pattern within populations, although geographical variations in wing coloration are observed (Brown 1979; Supple et al. 2014). However, some species, like *H. doris* and *H. numata*, exhibit striking polymorphisms with several distinct colour patterns occurring within the same population (Brown and Benson 1974). In the case of *H. numata*, up to five highly distinct morphs can be observed within populations, each one mimicking a differently looking species (Brown and Benson 1974). These mimetic communities are spatially variable, and polymorphism in *H. numata* wing colour pattern is thought to be maintained by a tight equilibrium between migration and local selection (Joron et al. 1999; Joron and Iwasa 2005). However, the origin of this polymorphism remains controversial, especially because the genetic control of wing colour pattern variations in the species is typically associated with polymorphic Batesian mimics (Charlesworth and Charlesworth 2011). Colour pattern variations in *H. numata* are mainly controlled by a single genomic region (Joron et al. 2006), in contrast to the multilocus architecture detected in other *Heliconius* species, where different pattern elements are controlled by unlinked genomic regions (Baxter et al. 2010; Counterman et al. 2010; Huber et al. 2015). Single locus architecture is, however, observed in polymorphic palatable species involved in Batesian mimicry, such as *Papilio dardanus* (Clark et al. 2008) or *Papilio polytes* (Kunte et al. 2014). Indeed, by promoting polymorphism, the negative frequency-dependent selection acting on Batesian mimetic morphs is predicted to favour a supergene architecture of mimicry, with tight linkage between genes controlling mimicry (Charlesworth and Charlesworth 1975). As far as is known, *H. numata* is the only *Heliconius* species with supergene architecture for mimicry (Huber et al. 2015), raising the question of whether its local polymorphism and associated supergene architecture could stem from relaxed unpalatability. In the present paper, we use behavioural experiments with birds to assess whether the polymorphic species *H. numata* is less aversive than locally monomorphic species with multilocus genetic architecture (*H. erato* and *H. melpomene*).

In addition, we tested whether *H. numata* is a quasi-Batesian mimic. *H. numata* displays tiger-patterned colouration and participates in mimicry rings primarily composed of other *Heliconius* species and more distantly-related butterflies from the subfamily Ithomiinae (Joron et al. 1999). We compared the unpalatability of *H. numata* to one of its ithomiine co-mimics, *Mechanitis polymnia*. *Heliconius* and ithomiine butterflies rely on different chemical compounds for their toxicity. Ithomiines contain pyrrolizidine alkaloids (PAs), that are usually obtained as adults by feeding on flowers of Asteraceae and Boraginaceae (Pliske 1975; DeVries and Stiles 1990; Trigo and Brown 1990). Because of these differences in compounds, a direct analysis of chemical composition and concentrations would not give a reliable comparison of the unpalatability of *H. numata* and *M. polymnia* to predators. Rather, comparisons of predator's rejection responses to different butterflies seem the most appropriate way to compare unpalatability variation among mimetic species. Birds are thought to be important predators of butterflies, and several Passerine species such as tropical kingbirds [*Tyrannus melancholicus* (Pinheiro 1996, 2003)], cliff-flycatchers [*Hirundinea ferruginea* (Pinheiro 2003)] and rufous-tailed tanagers [*Ramphocelus carbo* (Brower et al. 1963)] have shown aversive responses to living *Heliconius*, as well as other unpalatable tropical butterflies. Indeed, the communities of predators

attacking *Heliconius* butterflies are probably highly diverse. It is therefore difficult to accurately estimate the reaction of such complex communities to variations in chemical defence of prey. Nevertheless, the use of model predators appears a reasonable proxy to compare the levels of aversion generated by these neotropical butterflies. Chickens, for instance, have proven useful when testing unpalatability variations in poison dart frogs, despite not being their natural predators (Darst and Cummings 2006; Stuckert et al. 2014). The passerine great tits (*Parus major*) have a wide diet that includes butterflies (Noke-lainen et al. 2014). Because they are bold and common birds, they have been successfully used to test several hypothesis about aposematism (Sillén-Tullberg et al. 1982; Alatalo and Mappes 1996; Exnerová et al. 2003) and mimicry evolution (e.g. Ihalainen et al. 2007; Lindström et al. 1999c; Lindström et al. 1997, 1999a, b; Rowland et al. 2007).

Behavioural experiments with birds attacking *Heliconius* butterflies have already been performed to test for interspecific differences in predators reactions towards different mimetic species of *Heliconius* (Brower et al. 1963), cryptic and mimetic tropical butterflies (Chai 1986) and local and exotic prey (Pinheiro 2003). However, these experiments did not disentangle the effects of visual signals and unpalatability on predators' behaviour, pre-venting conclusions regarding the birds' sensitivity to chemical defence. By removing visual cues, we tested birds' responses to taste only, thereby comparing the level of aversiveness produced by the polymorphic *H. numata* with the one produced by (1) closely related monomorphic species and (2) by distantly-related co-mimic species.

## Materials and methods

### Butterflies samples

Butterflies were collected in September 2014 in the vicinity of Tarapoto, Peru (San Martin department). We collected individuals from the polymorphic species *H. numata* ($n = 16$),
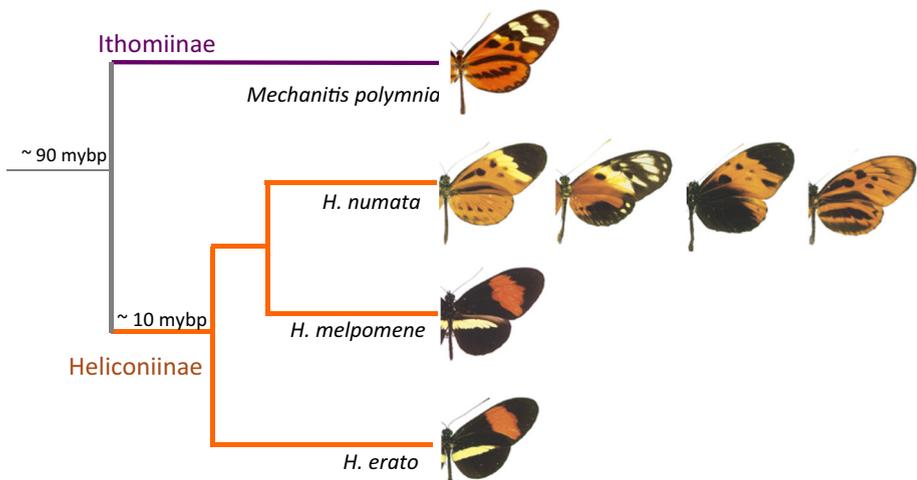


**Fig. 1** Phylogenetic relationships among the four species used in the experiments, which were collected in the same geographical region. *Mechanitis polymnia* (*first row*) belongs to the Ithominii tribe and belongs to the one of the mimicry rings of *H. numata* (*second row*). In the *last two rows* are the two locally monomorphic *Heliconius* butterflies tested, *H. melpomene* and *H. erato*

two locally monomorphic *Heliconius* species (*H. erato* and *H. melpomene, n = 11*) and an Ithominii species *Mechanitis polymnia*, displaying a tiger-pattern like *H. numata* (*n = 16*) (Fig. 1). Butterflies were snap-frozen alive to limit the degradation of biochemical compounds. To increase the experimental sample size, twenty samples were divided in half through the main body axis, and six uneaten samples were reused.

## Predators tested

The behavioural experiments were carried out in Konnevesi Research Station (Finland) under a license from the National Animal Experiment Board (ESAVI/9114/04.10.07/2014) and the Central Finland Regional Environment Centre (VARELY/294/2015). Sixty-eight wild-caught great tits (*Parus major*) were used for the experiment, comprising 15 juvenile females, 17 adult females, 9 juvenile males and 27 adult males. The birds were caught and tested in winter (November 2014) to avoid disturbing them during the breeding season. Birds were trapped in feeding boxes. All 68 birds used in the experiments were individually ringed and used only once. Each bird was kept separately in an indoor cage (65 × 65 × 80 cm), with a light period of 11.5 h. They were fed with peanuts, sunflower seeds, and fresh water ad libitum, except on training and experiment days, when they were deprived from food for 2 h before the training. Most of the birds were held in captivity for 2–3 days and were then released back in the place where they were caught.

## Training and experiment

In order to test butterflies' palatability, we designed the experiments to eliminate the confounding effect of visual information (Lyytinen et al. 1999). We first removed the butterflies' wings before presenting them to the birds. Butterfly bodies were then ground up in a small cup and dyed with dark (red + green + blue) Supercook food dye, to mask all possible phenotypic cues. Given that the butterflies often had a strong smell, we covered the cup with parafilm that prevented the birds from avoiding butterflies because of their smell.

Before starting the trial experiment on butterflies, each bird was trained to peck through the parafilm layer to get black-dyed and crushed palatable mealworms. After successful training, each bird was transferred to a 76 × 60 × 77 cm plywood experimental cage. The cage had a 64 cm high perch and was illuminated by a light bulb. The front wall was a one-way glass that allowed observation of birds during experiments. In the palatability assay, each bird was offered one crushed and dyed butterfly and one crushed and dyed mealworm, as a palatable control. Both items had approximately the same initial weight. As in the training phase, both cups were covered with the parafilm. Cups were weighed before and after the experiment, and the difference was used as a measure of the consumption of each prey. The positions of the two cups were randomized in each trial. Each experiment lasted 10 min and was considered valid only if the bird pecked through the parafilm of both cups. On average, both training and experiment were completed within 4 h.

## Disgust behaviours

All experimental trials were recorded using a digital camera, allowing us to score three behaviours thought to be associated with disgust: (1) presence/absence of head shaking after trying the butterfly (Guilford et al. 1987), (2) water drinking after tasting a butterfly, and (3) number of times that the bird came back to look for the butterfly.

## Statistical analyses

To estimate the defence level against predators of each tested individual butterfly, we used the ratio $r = \frac{w_b}{w_b + w_c}$, where $w_b$ is the weight of butterfly consumed and $w_c$ is the weight of mealworm consumed. The distribution of this ratio in our dataset followed a normal distribution. We tested whether such ratio was different from random consumption of mealworm and butterfly (i.e. whether the mean ratio observed was significantly different from 0.5) by fitting a linear model including species as predictor variable. Then, we used a generalised linear mixed model assuming normal error distribution of the ratio to test for unpalatability differences among butterfly species and sexes. In addition, first tested item (butterfly or mealworm), bird sex, bird age and bird hunger level (estimated as number of visits to butterfly and mealworm) were included as control variables. Given that some butterflies were used in more than one experiment, butterfly ID was included as a random factor. The effect of butterfly sex and species on the relative consumption was tested by comparing AIC between models with and without each investigated effect, using a Chi-square test.

We followed a similar procedure for the behavioural variables. First, we tested whether birds react differently towards butterflies and mealworms fitting models for drinking after tasting a butterfly and head-shaking, specifying a binomial error distribution, and for the number of return visits to butterfly or mealworm, specifying a Poisson error distribution. Then, we applied models following the correspondent error distributions, to test for behaviour differences among butterfly species and sexes, including bird variables (as described above) and butterfly ID as a random factor. All the models were fitted using the package *lme4* (Bates et al. 2013). All statistics were carried out using R-3.1.2 (R Development Core Team 2014).

# Results

For all butterfly species tested, the relative butterfly consumption ($r$) was significantly lower than the expectations assuming a random consumption of butterflies and control (*H. numata*: $t = -8.28$; $p < 0.001$; *H. erato*: $t = -4.66$; $p < 0.001$; *H. melpomene*: $t = -3.09$; $p = 0.003$; *M. polymnia*: $t = -2.20$; $p = 0.031$), as expected given their described unpalatability. Similarly, birds shook their head more after tasting a butterfly than a mealworm ($z = -5.71$, $p < 0.001$). However, birds drank water similarly after tasting a mealworm or a butterfly ($z = -0.01$, $p = 0.993$) and similarly return visited mealworm and butterfly ($z = -1.05$, $p = 0.30$). Therefore, no further analyses were applied on these last two birds' behaviours.

## Relative butterfly consumption

A model including butterfly sex and species, and not necessarily its interaction, explained significantly more variation in relative butterfly consumption than one including only variation in predators' traits (bird sex, bird age, hungry level and first item tasted) (Table 1). Pairwise comparisons between models revealed that relative butterfly consumption significantly differed across species ($X^2 = 15.84$, $p = 0.001$), but did not differ between butterfly sexes ($X^2 = 2.93$, $p = 0.09$). *H. numata* was the least consumed, followed by *H. erato* and *H. melpomene*. *M. polymnia* was the most consumed butterfly (Fig. 2, S1). Bird sex ($X^2 = 1.67$, $p = 0.20$), bird age ($X^2 = 0.014$, $p = 0.90$) and first

**Table 1** Comparisons of models predicting relative butterfly consumption by birds

| Model number | Model description | Model df | AIC | df | $X^2$ | $p$ |
|---|---|---|---|---|---|---|
| 1 | Control variables | 7 | −76.40 | | | |
| 2 | ButterflySpecies + control variables | 10 | −83.32 | 3 | 12.92 | 0.005 |
| 3 | ButterflySpecies + ButterflySex + control variables | 11 | −84.25 | 1 | 2.93 | 0.09 |
| 4 | ButterflySpecies + ButterflySex + ButterflySp:ButterflySex + control variables | 14 | −82.62 | 3 | 4.37 | 0.224 |

All GLMMs included butterfly ID as a random factor and bird traits as control variables (bird sex + bird age + hunger level + first item tested). A Chi-square test was performed to compare each model to the one described on the previous row, and the associated $p$ value was reported

tested item (butterfly or mealworm) ($X^2 = 2.58$, $p = 0.11$) had no significant effect on relative butterfly consumption. Hungrier birds (those that made more return visits to the mealworm and butterfly) ate more butterfly ($X^2 = 8.60$, $p = 0.003$). However, a model including the interaction between species and hunger level did not better fit the data significantly ($X^2 = 3.71$, $p = 0.29$). Therefore, birds' hunger levels were similarly distributed across the experiments on the different butterfly species.

### Head shaking

When butterfly species and butterfly sex were included as predictor variables, no better model fit was obtained (Table 2), suggesting similar reaction towards all butterflies disregarding their species or their sex. Bird sex ($X^2 = 0.018$, $p = 0.89$), bird age ($X^2 = 0.97$, $p = 0.32$), first tested item (butterfly or mealworm, $X^2 = 0.09$, $p = 0.76$) had no significant effect on head shaking behaviour. However, hungrier birds (that return to visit the mealworm and the butterflies more times) shook their head less ($X^2 = 3.87$, $p = 0.05$).

## Discussion

Low level of chemical defence has been suggested to explain the paradoxical polymorphism of warning signal observed in some aposematic species (Fisher 1958; Sheppard 1975; Lindström et al. 2001). When a mimetic species is poorly defended (i.e. a quasi-Batesian mimic), its protection mainly stems from its better-protected co-mimics.



Fig. 2 Differences in the eaten proportion of body weight among *Heliconius numata* (*H.n*), *H. erato* (*H.e*), *H. melpomene* (*H.m*) and *Mechanitis polymnia* (*M.p*). The line represents the median eaten weight proportion for control prey (palatable mealworms)
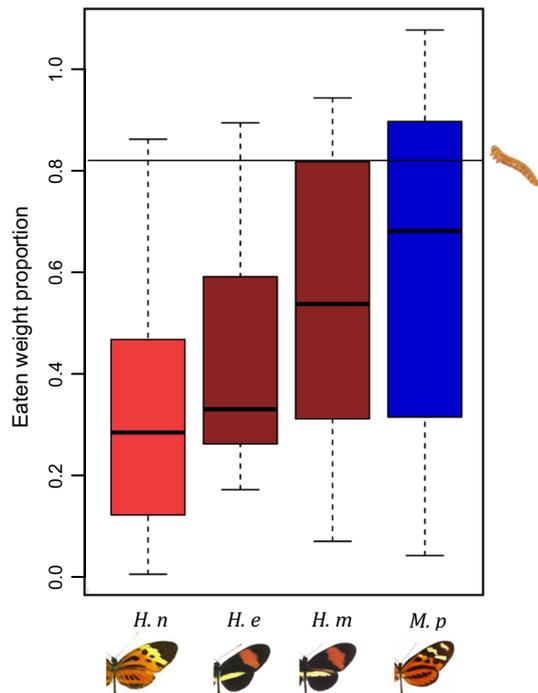
**Table 2** Comparisons of models predicting birds' shaking head after testing a butterfly

| Model number | Model description | Model df | AIC | df | $X^2$ | $p$ |
|---|---|---|---|---|---|---|
| 1 | Control variables | 6 | 90.59 | | | |
| 2 | ButterflySpecies + control variables | 9 | 94.15 | 3 | 1.90 | 0.59 |
| 3 | ButterflySpecies + ButterflySex + control variables | 10 | 96.10 | 1 | 0.05 | 0.82 |
| 4 | ButterflySpecies + ButterflySex + ButterflySp:ButterflySex + control variables | 13 | 97.82 | 3 | 4.28 | 0.23 |

All GLMM included butterfly ID as a random factor and bird traits as control variables (bird sex + bird age + hunger level + first item tested). A Chi-square test was performed to compare each model to the one described on the previous row, and the associated $p$ value was reported

However, as the abundance of the poorly defended species increases, the protection conferred by its mimics declines. Selection by predators is thus expected to favour any new colour pattern arising in poorly defended species which mimics another community of better defended species. This negative frequency dependent selection is thus hypothesised to promote polymorphism within weakly defended species (Turner and Speed 1996; Speed and Turner 1999). Although quasi-Batesian mimicry has been extensively described in theoretical models, empirical data documenting the levels of unpalatability in polymorphic aposematic species are scarce. Here, we compared the aversiveness of the locally polymorphic species *Heliconius numata* to two locally monomorphic co-mimics, *H. erato* and *H. melpomene*, and an ithomiine species, *Mechanitis polymnia*, which displays a tiger pattern similar to *H. numata*. Our experiments did not support the hypothesis that polymorphism in chemically protected species stems from weaker levels of defence, but in fact suggest a greater defence level for the polymorphic species.

## Variations of defence across aposematic species

The communities of predators attacking *Heliconius* living in the Amazonian forest are probably highly diverse, including for instance, different Passerine (Brower et al. 1963; Chai 1986; Chai and Srygley 1990; Pinheiro 2003) and Piciform (Pinheiro 2013) birds. Behavioural experiments using neotropical predators co-existing with unpalatable butterflies have shown that *Heliconius* and its co-mimics are frequently avoided after visual recognition (Brower et al. 1963; Chai 1986; Chai and Srygley 1990) although it can differ among bird species from neo-tropical communities. Similarly, bird species from the Amazonian forest might behave differently from great tits used in this experiment. Hence, the palatability measure reported here may not be guaranteed to fully correspond to the strength of selection in the wild. However, when neotropical predators tasted unprofitable prey, they released it almost immediately (Pinheiro 2013), in a similar fashion to the high rejection behaviour observed in our experiments using great tits. Furthermore, all butterfly species tested in our experiment were significantly less eaten and produced more head-shake behaviours than the control prey, suggesting that a bad taste caused rejection by great tits. Our experimental design thus allowed us to test palatability variations among wild butterflies, while considering predators' sensitivities that might resemble actual predators' behaviours and allowed us to exclude the effects of their previous experiences and visual unprofitability cues of the prey.

Ithomiine and Heliconiine species rely on different toxic compounds [pyrrolizidine alkaloids (PA) and cyanogenic glycosides (CG), respectively] whose effects on predators can differ. In our experiment, the tested ithomiine species (*M. polymnia*) was consumed more than *Heliconius* samples, suggesting a lower repulsiveness. The high consumption of *M. polymnia* might be due to low quantities of PAs in their bodies, and/or because great tits are less sensitive to such substances. A study comparing different species of ithomiine butterflies showed a high variation in PA content of different species, with the highest content of PAs found in *Mechanitis* butterflies (Trigo and Brown 1990). Studies with domestic chicks have shown that Diptera treated with PAs were initially eaten, but were progressively avoided by experienced predators (Silva 2000). Insects containing PAs are rare in Central-Finland but experiments using PA-rich *Oreina* beetles and blue tits showed that the beetles were highly unpalatable for birds (Waldron et al. in review). Experienced tropical predators sight-reject *Mechanitis* as much as other unpalatable butterfly species (Chai 1986; Pinheiro 1996). We therefore hypothesize that cyanogenic compounds from *Heliconius* sp. have a stronger smell and/or taste than PAs, and produce a quicker

repulsion. This suggests that to develop an aversion birds may need to attack more PA defended butterflies than CG defended butterflies.

## Origin of polymorphism in unprofitable species

Using tropical omnivorous birds [silver-beaked tanagers, (*Ramphocelus carbo*)], Brower et al. (1963) showed that the polymorphic species *H. doris* was the least protected butterfly against predators out of the five *Heliconius* species tested. In contrast, we found that the polymorphic species *H. numata* was as rejected as the monomorphic species *H. melpomene* and *H. erato*. Therefore, the origin of polymorphism in Müllerian mimics does not necessarily stem from quasi-Batesian mimicry dynamics (Speed and Turner 1999). The level of predators' rejection produced by *H. melpomene* and *H. erato* is high enough for them to be protected, and for participating in Müllerian mimicry, while their warning coloration is under positive frequency-dependent selection. Therefore, the similar level of aversiveness observed in *H. numata* is probably not causing the appearance and persistence of its polymorphic warning pattern.

The three *Heliconius* species tested here were collected in the same geographical areas, and are likely threatened by the same community of predators. Differences in predator community composition are therefore unlikely to result in *H. numata* being subject to different selection regimes. Furthermore, aversiveness was considerably higher for *H. numata* than for the other tiger-patterned species (*Mechanitis polymnia*) tested in our study. Although *M. polymnia* is not a perfect mimic of *H. numata*, this suggests that quasi-Batesian interactions do operate within mimicry rings in natural populations, but in the opposite direction of what was expected. Although supergene architecture for mimicry, is definitely favoured by balancing selection regime (Charlesworth and Charlesworth 2011), the genetic configuration observed within *H. numata* species is unlikely to stem from quasi-Batesian mimicry.

Alternative hypotheses that allow mimetic phenotypes to drift between local adaptive patterns through reductions in predation pressure are therefore more likely to account for the origin of the polymorphism observed in *H. numata*. For instance, polymorphism in protected species can be predicted by an evolutionary model based on an optimal foraging behaviour, in which predators can learn based on previous experiences, and estimate the benefits and the costs of attacking certain prey (Sherratt 2011; Aubier and Sherratt 2015). When aposematic species are relatively rare, they can experience disruptive selection and produce different phenotypes if predators are hesitant to attack novel prey (Marples and Kelly 1999; Sherratt 2011). However, the high spatial heterogeneity in abundance and presence of the different mimicry rings (Joron et al. 1999), combined with a migration-selection equilibrium (Joron and Iwasa 2005) in which this species is involved still stands as the most plausible factor promoting the persistence of polymorphism in *H. numata*.

## Variability in aversiveness within mimicry rings

Differences in unpalatability within a mimicry ring may interfere with the positive frequency-dependent selection exerted by predators on warning patterns. Our experiment showed that *H. erato* and *H. melpomene,* which mimic each other, are similarly toxic, as expected by Müllerian mimicry (Sherratt 2008). However, within the "tiger pattern" mimicry ring, we found contrasting levels of unpalatability, with *H. numata* producing more aversive reactions than *M. polymnia*. Our study only included two tiger-patterned species, whereas many more species typically comprise the tiger pattern mimicry ring in

natural mimetic communities. Nonetheless, it still raises questions about the consequences on unpalatability variability among coexisting mimicry rings and how multispecies assemblages comprising distantly related species with different defences can affect the evolutionary convergence of mimetic warning signals.

The presence of less protected species within a mimicry ring can have negative consequences for the more unpalatable species (Speed et al. 2000; Skelhorn and Rowe 2006b). However, when a warning signal is very common, even low levels of unpalatability are sufficient to generate avoidance by predators (Ihalainen et al. 2008). Indeed, the presence of less protected co-mimics is not necessarily associated with parasitic dynamics (Rowland et al. 2007). Furthermore, the presence of a few strongly aversive individuals in a mimicry community has been demonstrated to provide sufficient protection against predation for the whole mimicry ring, because birds are particularly risk-averse (Barnett et al. 2014). Despite its mild unprofitability, *M. polymnia* can therefore still participate in protection against predators within its mimicry ring, suggesting that abundant mimicry rings can exhibit differences in defence levels across individuals and/or species. Additionally, a high density of alternative prey can relax the selection for unpalatability similarity (Kokko et al. 2003; Lindström et al. 2004; Ihalainen et al. 2012).

## Conclusions

We demonstrated that a lower unpalatability does not necessarily explain the polymorphic mimicry of *H. numata*. Other factors, such as variations in abundance or spatial heterogeneity of mimetic communities, might promote polymorphism in species involved in Müllerian mimicry. In addition, we found evidence for species differences in aversiveness within a mimicry ring, suggesting that large variations of unpalatability levels among Müllerian mimics are present in nature. This study therefore stresses the need to investigate variations in unpalatability and chemical defences levels together with the relative densities of species within mimicry rings in the wild, in order to better understand how selection shapes the evolution of colour pattern polymorphism in chemically protected species.

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