Polymorphic mimicry, microhabitat use, and sex-specific behaviour

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Abstract

In order to assess the adaptive importance of microhabitat segregation for the maintenance of mimetic diversity, I explore how flight height varies between the sympatric forms of the polymorphic butterfly *Heliconius numata* and their respective models in the genus *Melinaea*. There is no evidence for vertical stratification of mimicry rings in these tiger-patterned butterflies, but males of *H. numata* tend to fly significantly higher than females and the *Melinaea* models. This difference in microhabitat preference likely results from females searching for host plants whereas males are patrolling for mates. I then present an extension of Müller's mimicry model for the case of partial behavioural or spatial segregation of sexes. The analysis suggests that sex-specific behaviours can make mimicry more beneficial, simply by reducing the effective population size participating in mimicry. The interaction between mimicry and sex-specific behaviours may therefore facilitate the evolution of polymorphism via enhanced, fine-scale local adaptation.

Introduction

Müllerian mimicry is the adaptive resemblance of several chemically defended prey that benefit by sending a unique warning signal to predators (Müller, 1879). Contrary to Batesian mimics (edible prey that parasitize the warning signals of nasty models), Müllerian mimics reinforce the efficiency of their own signal; the protection from predation is therefore maximal when all species converge on the same signal. Phenotypic deviants, not recognized as unpalatable by the predators, are selected against (Benson, 1972; Mallet & Barton, 1989; Lindström *et al.*, 1999; Kapan, 2001; but see Rowe *et al.*, 2004), which makes polymorphism and diversification theoretically unlikely (for a review see Joron & Mallet, 1998; see also Jeffords *et al.*, 1979 showing selection against deviant Batesian mimics).

However, there is pervasive diversity in warning signals in nature, found at all taxonomic and geographical scales. Examples include diverging geographic races

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in *Dendrobates* frogs (Symula *et al.*, 2001) and *Heliconius* butterflies (Turner, 1976; Brower, 1996), local coexistence of numerous mimicry rings (Mallet & Gilbert, 1995; Beccaloni, 1997a,b), and even local mimetic polymorphism (Owen *et al.*, 1994). In particular, the butterfly *Heliconius numata* Cramer (Nymphalidae: Heliconiinae) has up to seven different forms in some localities, each of which is involved in different mimicry rings with large ithomiine butterflies (Nymphalidae: Ithomiinae) (Brown & Benson, 1974; Joron *et al.*, 1999; Fig. 1).

Segregation by microhabitat is one likely cause for both the coexistence of Müllerian mimicry rings and the mimetic polymorphism of some species. If there is fine scale spatial heterogeneity in habitat, then any one predator may deal with only a limited set of prey types. Therefore, selection may not favour the resemblance between all prey species, and diversity could be maintained (Brown & Benson, 1974; M. Joron & Y. Iwasa, unpublished data). Empirical studies have provided data suggesting some level of horizontal segregation of butterfly mimicry rings in neotropical forest (Mallet & Gilbert, 1995; DeVries *et al.*, 1999; Joron *et al.*, 1999; Willmott & Mallet, 2004). Vertical segregation, a particular case of microhabitat segregation, has received attention in the study of tropical rainforests in the light

Fig. 1 Tiger-pattern mimicry rings found near Tarapoto, Eastern Peru. Top row: the five major colour-pattern forms of *Melinaea* species (Nymphalidae: Ithomiinae). Bottom row: their respective mimetic morphs in the polymorphic species *Heliconius numata* (Nymphalidae: Heliconiinae). The different forms are made of varying combinations of black, dark orange (grey areas on the figure) and bright yellow (white areas), which give them a strikingly different appearance in flight. By mimetic pair, from left to right: *M. marsaeus phasiana* and *H. n. arcuella*; *M. m. rileyi* and *H. n. aurora*; *M. m. mothone* and *H. n. bicoloratus*; *M. menophilus* ssp. nov. and *H. n. tarapotensis*; *M. ludovica ludovica* and *H. n. silvana*.

of resource partitioning theory. Some flight height stratification has been found within Neotropical butterfly guilds, albeit with much overlap (Papageorgis, 1975; Burd, 1994; Medina *et al.*, 1996; Beccaloni, 1997b). However, flight-height segregation of colour patterns can also be the consequence of ecological mechanisms other than mimicry, such as host-plant use at similar heights by sister taxa (Beccaloni, 1997b) or intraspecific signalling in different lighting environments (Endler, 1993).

If vertical segregation of predation is a major component of the maintenance of mimetic diversity in one place, taxa radiating in mimicry association should be found at different heights. Polymorphic, mimetic species should have forms flying at the heights of their respective models in the habitat. *Heliconius numata*, one of the most polymorphic Müllerian mimics with up to seven different morphs belonging to different coexisting tiger-patterned mimicry rings, is therefore an ideal system to test whether flight-height strata represent microecological niches important in the initial stages of mimetic diversification. Here I present data on flight height of the different forms of *H. numata* and their respective *Melinaea* co-models. Although I found no evidence for vertical segregation by mimetic pattern, there was a pattern of sex-specific flight height in *H. numata* probably linked to some aspects of male mate-location and female oviposition behaviours in this forest species. A simple density-dependent model suggests that such niche differences between sexes may facilitate the evolution of Müllerian mimicry and the maintenance of polymorphism by enhancing fine-scale local adaptation.

Materials and methods

Heliconius numata is a butterfly found in the lowland and submontane forests of the Amazon basin. It is polymorphic for so-called 'tiger patterns' of orange, yellow and black; each form is a very accurate Müllerian mimic of one or several species in the genus *Melinaea* (Nymphalidae: Ithomiinae) (Fig. 1). The corresponding mimicry rings also include other butterflies in the Ithomiinae, Danainae, Heliconiinae (Nymphalidae), and Riodininae (Lycaenidae), as well as day-flying tiger moths (Arctiidae: Pericopinae) (Brown & Benson, 1974). Because of the extreme resemblance of *H. numata* to *Melinaea*, the mimetic relationship is supposed to be strongest between these two taxa. Spatial variation in the frequency of morphs of these two taxa was shown to be correlated, suggesting strong local selection in *H. numata* for resemblance to *Melinaea* (Joron *et al.*, 1999). (For further illustrations and natural history of tiger-patterned mimicry rings involving *Heliconius* and Ithomiines see Brown & Benson, 1974; Brown, 1976; Beccaloni, 1997a; Joron, 2003.)

Ten sites were sampled in the Amazonian foothills of the Andes in the vicinity of Tarapoto, eastern Peru, in August and September 1997. The area is covered with mostly dry submontane forest (300–1000 m). Most collections were carried out in tall secondary forest with disturbed areas nearby (details in Joron *et al.*, 1999). Butterflies were caught using nets with a 2 m pole, allowing capture up to 4.5 m in height. Although some of the *H. numata* flying higher could be attracted to red/orange rags waved in the sun, these were excluded from the analysis as attraction may depend on form or sex. *Melinaea* fly slowly and are easily caught. Using previous measurements taken along the butterfly net (three 60 cm-long pole sections plus a 45 cm-diameter ring), I estimated for each butterfly the flight height before capture (to the nearest 10 cm below 2.5 m, to the nearest 50 cm above). Flight height varies according to rugged terrain and the presence of vegetation. In addition to sex, form, and flight height, two other variables likely to affect flight activity, time and weather, were recorded upon capture. Weather was measured using a subjective

estimate of cloud cover from 1 (low or no cloud cover) to 3 (heavy). Ithomiines were identified by Gerardo Lamas and vouchers were deposited in the Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Lima, Peru. *Heliconius numata* forms were identified using Brown (1976). For the analysis, only the five commonest forms (*H. n. aurora, silvana, tarapotensis, bicoloratus* and *arcuella* and their respective models; see Fig. 1) were considered.

Nonparametric Kruskall–Wallis tests were applied because the distribution of flight heights deviates from normality (Kolmogorov–Smirnov normality test, $P < 0.01$). To test for interactions, two-way nonparametric tests could not be applied because of the unbalanced structure of the dataset, so flight-height was square-root-transformed using the $\sqrt{(x + 0.5)}$ link to approximate a normal distribution (Kolmogorov–Smirnov test, $P > 0.15$). Generalized linear models were applied with sex and form as factors, and time or cloud cover as covariates.

Results

The data show that the different forms of *H. numata* do not fly at different heights (Kruskall–Wallis test, adjusted for ties: $H = 1.18$, d.f. $= 4$, n.s.), nor do they fly at different heights in the different sites ($H = 12.90$, d.f. $= 9$ n.s.). Despite the wide variation, however, there is a highly significant difference between sexes ($H = 31.56$, d.f. $= 1$, $P < 10^{-6}$): the 90 males were caught flying at a mean height of 2.30 m, whereas the 35 females were caught at a mean height of 1.09 m (see Table 1; Fig. 2), but no interaction was found between sex and form ($F_{4,112} = 0.75$, n.s.; Table 2; Fig. 3a). Time of capture had a significant effect in interaction with sex (Table 2; Fig. 3b) whereas the cloud cover has no significant effect (Table 2). Cloud cover is not independent of time of day, which could explain why the trend of increasing flight height with increasing cloud cover seen on Fig. 3c is not significant in the full model (Table 2).

Ithomiine models, in the genus *Melinaea*, were caught at a mean height of 1.26 m (Table 1) with no difference between sexes ($H = 2.77$, d.f. $= 1$, $P = 0.096$), colour-patterns ($H = 6.53$, d.f. $= 5$, n.s.), or time ($F_{1,49} = 0.01$, n.s.). *Melinaea* flight height was very significantly different from that of male *H. numata* ($H = 37.59$, d.f. $= 1$, $P < 10^{-6}$), but not different from that of female *H. numata* ($H = 1.65$, d.f. $= 1$, n.s.) (Fig. 2).

### An extension to Müller's model

In view of the consistent flight-height difference between sexes in *H. numata*, which may be viewed as a different microhabitat use by the two sexes, I implemented an extension of Müller's (1879) original model of mimicry that includes a measure of microhabitat segregation within the mimic species.

Table 1 Mean and median flight height (in metres) of *Heliconius numata* and *Melinaea* spp., as distributed by sex and by colour form.

	n	Mean height	SE	Median	Range
Heliconius numata	124	1.95	0.10	1.8	0.2–4.5
Females	34	1.09	0.11	1	0–2.5
Males	90	2.30	0.10	2	0.2–4.5
Colour forms*:					
silvana/illustris	16	1.99	0.24	1.65	0.3–3.5
bicoloratus	47	2.02	0.17	1.8	0.2–4.5
arcuella	9	1.88	0.42	1.9	0.3–4.0
aurora	16	1.67	0.24	1.55	0.2–3.5
tarapotensis	36	1.99	0.16	1.8	0.2–4.5
Melinaea spp.	54	1.26	0.08	1.25	0.3–3.0
Females	36	1.36	0.10	1.3	0.3–3.0
Males	18	1.05	0.12	0.95	0.4–2.0
Colour forms*:					
M. ludovica	1	0.40	–	–	–
M. m. mothone	4	1.55	0.32	1.55	0.8–2.3
M. m. phasiana	14	1.17	0.12	1.2	0.3–2
M. s. cydon	2	1.10	0.59	1.1	0.5–1.7
M. menophilus and *M. s. tarapotensis*	33	1.29	0.11	1.3	0.4–3.0

n, total number; SE, standard error of mean.
*Mimicry associations: *M. ludovica* is a model for *H. n. silvana*, *M. marsaeus mothone* for *H. n. bicoloratus*, *M. marsaeus phasiana* for *H. n. arcuella*, *M. satevis cydon* (and *M. marsaeus rileyi*, not found here) for *H. n. aurora* and variants, *M. menophilus* ssp. nov. and *M. satevis tarapotensis* for *H. n. tarapotensis* and variants.
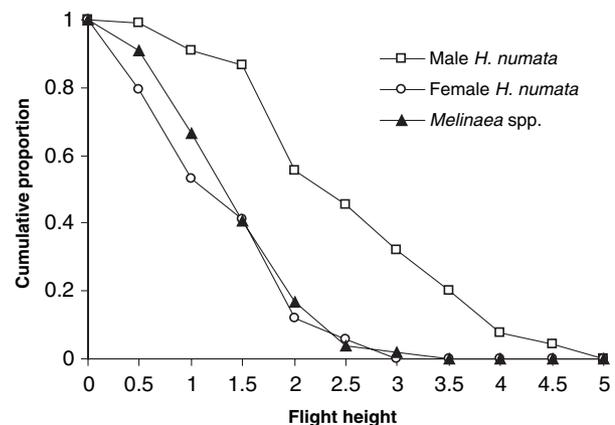

Fig. 2 Comparison of mean flight heights between *Melinaea* and *H. numata*. The *y*-axis gives the cumulative proportion of butterflies flying at or above the height given on the *x*-axis (in metres).

In Müller's (1879) model, n_k is the number of prey predators kill per unit time in a locality to learn the prey colour pattern; M is the population size of the model species and m the total population size of the focal (mimic) species. In the absence of mimicry, the models and the focal species are distinct and predators sample n_k individuals of each species. If the focal species is

Model	Source	d.f.	Type 3 SS	Mean square	F-value	P-value
Sex × form	Sex	1	3.93	3.93	42.84	0.000*
	form	4	0.06	0.02	0.16	0.956
	Sex × form	4	0.34	0.09	0.93	0.450
	Error	101	7.92	0.08		
Sex × time × clouds	sex	1	0.63	0.63	8.00	0.006*
	Time	1	0.04	0.04	0.54	0.464
	Clouds	1	0.01	0.01	0.09	0.761
	Sex × time	1	0.41	0.41	5.18	0.025*
	Sex × clouds	1	0.05	0.05	0.69	0.410
	Time × clouds	1	0.06	0.06	0.73	0.394
	Sex × time × clouds	1	0.14	0.14	1.81	0.182
	Error[a]	89	7.05	0.08		

Table 2 Results from generalized linear models testing for interactive effects of sex, mimetic form, time of capture, and cloud cover on *H. numata* flight height.

[a]Because of the presence of some empty cells, the total number of observations in the sex × time × clouds model is a little reduced. *Significant effect, 0.05 level.
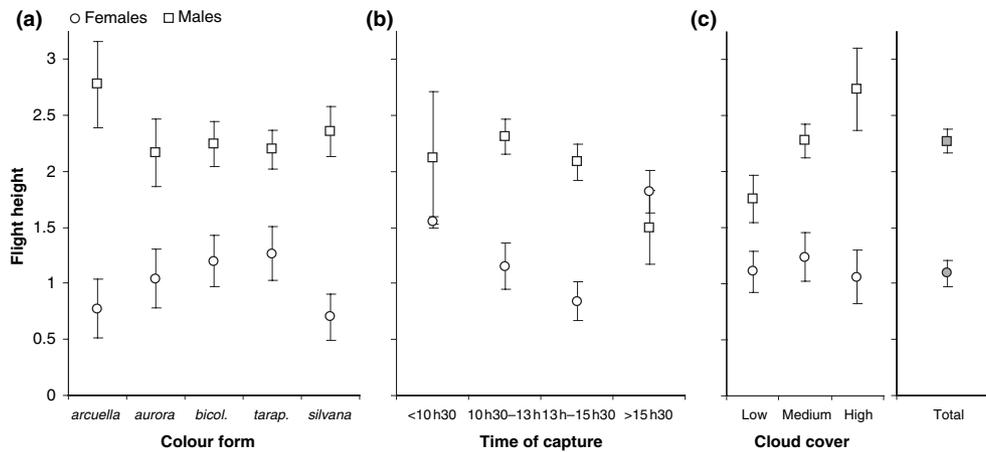
Fig. 3 Mean flight height of male and female *H. numata* as a function of mimetic form (a), time of capture (b), and cloud cover (c). Measurements are in metres ± standard error. Only time of capture has an effect in interaction with sex (see Table 2).

indistinguishable from the model, however, only $m_k = n_k[m/(m + M)]$ mimics are killed, and Müller (1879) showed that the ultimate benefits of mimicry, defined as the reduction in predation enjoyed by mimicking the models, are then $g = n_kM/[m(m + M)]$ (the demonstration is reprinted in Joron & Mallet, 1998).

Here I introduce the parameter d as the proportion of the focal species population that participates in the education of predators in the focal habitat; d can be viewed as a level of habitat segregation of sexes, or as a behavioural sexual diphenism, by which one sex is partly or completely independent of predation by the focal habitat's predators. d varies between 0.5 and 1 ($d = 1$ in Müller's equations). In the absence of mimicry, predators sample $n_{k,A}$ individuals of the focal species in the focal habitat (A), where $n_{k,A} = n_k$. With mimicry in the focal habitat, the learning predators sample $m_{k,A} = n_k \times [dm/(dm + M)]$ mimics per unit time in the focal habitat. The mortality of the proportion of the focal species' popula-

tion that has chosen another habitat (B) is $n_{k,B}$ whether there is mimicry or not in the focal habitat. The ultimate benefits of mimicry for the focal species are

$$g_d = \frac{(n_{k,A} + n_{k,B}) - (m_{k,A} + n_{k,B})}{m} = \frac{n_kM}{m(dm + M)}.$$

Note that the mortality $n_{k,B}$ outside the habitat cancels out in the calculation of g_d. On the diagram (Fig. 4a), g is the benefit of evolving from I to III, whereas g_d is the benefit of evolving from II to IV.

The ratio of g_d to g compares the mimetic benefits for a species with habitat segregation d, relative to that of a species without habitat segregation ($d = 1$). This ratio, G, has a simple expression:

$$G = \frac{g_d}{g} = \frac{m + M}{dm + M}$$

G is plotted on Fig. 4b as a function of the mimic-to-models ratio in the focal habitat, dm/M, for different

No segregation   **Segregation**

(a)
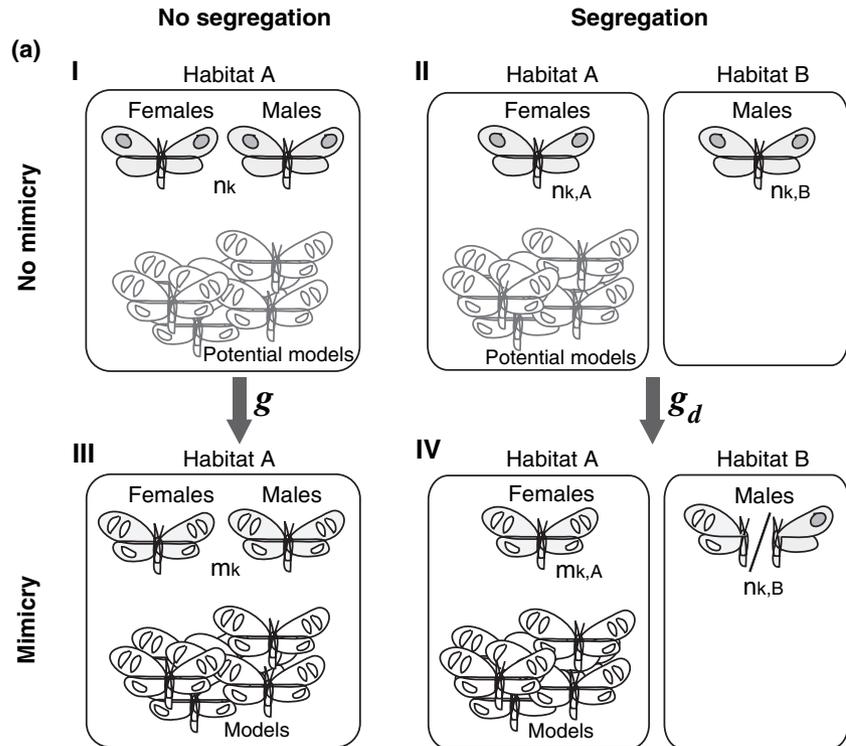
Fig. 4 The evolution of mimicry via micro-habitat segregation of sexes. (a) Diagram showing the four possible strategies, and the absolute mortalities suffered by the mimics. The calculation shows that the evolution from strategy II to strategy IV is easier than from I to III, because of Müllerian number-dependence. In IV, males may or may not evolve the same pattern as the mimetic females (see text). (b) Based on Müller's (1879) number-dependent model, the ratio of the benefits of mimicry when one sex participates more than the other to the teaching of predators in a habitat, to those when both sexes have identical ecology, G, is plotted against the mimic/model ratio in the focal habitat, dm/M. The parameter d varies from 0.5 (one sex not in microhabitat or not suffering predation) to 1 (sexes have identical ecology). Ecological segregation of the sexes always makes the evolution of mimicry more beneficial for the focal species.

(b)
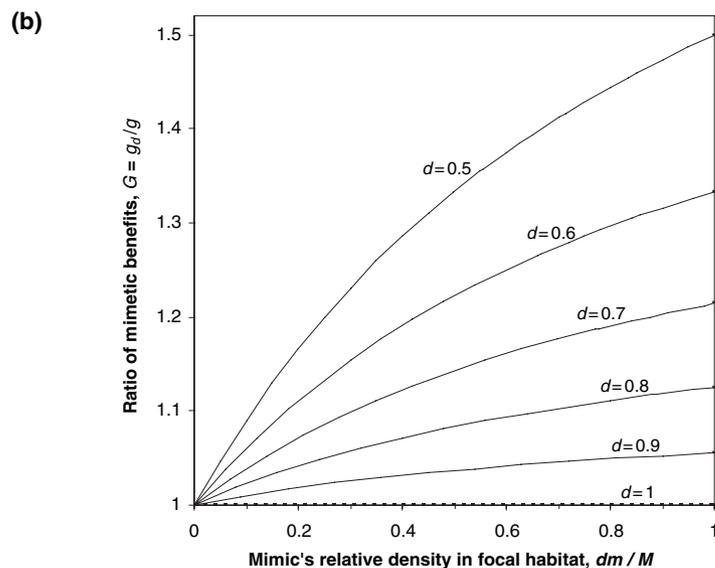
values of d. As $d < 1$, species with ecological segregation of the sexes always benefit more from mimicry than species whose sexes have similar ecology.

Sex segregation reduces the local population size of the mimic relative to models in the focal habitat (A). The benefits to the mimic are inversely proportional to the squared mimic-to-model ratio (Müller, 1879); therefore, as the mimic's population is reduced, the benefits of mimicry grow faster than the reduction in the number of

individuals that benefit from mimicry, resulting in mimetic benefits being an increasing function of the segregation. The effect can be limited if the sexes are only slightly divergent in ecology, or if the mimic is rare relative to the models, but it can be as high as a 30–50% difference in selection when the mimic is abundant and the sexes well segregated by microhabitat (Fig. 4b). Thus, habitat segregation should generally facilitate the evolution of local mimicry.

Therefore, in the absence of constraints and/or conflicts on colour pattern evolution, we expect species with some degree of habitat segregation to show enhanced local adaptation. Local adaptation results in adaptive spatial variation. In mimicry this is measured, for instance, by a correlation between model and mimic frequencies across localities in a geographic area (see e.g. Joron *et al.*, 1999; Kapan, 2001; Symula *et al.*, 2001).

Discussion

Absence of stratification of tiger mimicry rings

The absence of flight height differences between the different forms of *H. numata* and its models makes vertical stratification an unlikely explanation for colour-pattern divergence in this species, and for the maintenance of diversity of these mimicry rings. The range of flight-height variation in *H. numata* is wide compared with the flight height of their *Melinaea* model, and spans most of the ithomiine flight-height strata described by Beccaloni (1997b). *Heliconius numata* therefore appears as a flight-height generalist within a habitat.

Previous studies on Neotropical butterflies have shown some stratification in flight height (Burd, 1994; Medina *et al.*, 1996; Beccaloni, 1997b) or in roosting height (Mallet & Gilbert, 1995), but we do not know whether this level of segregation causes a selection strong enough to allow for the divergence of mimicry rings. Here, the accurate mimicry of the different forms of *H. numata* to the different *Melinaea* species does suggest that predators can discriminate between these patterns, but it is possible that some generalization between all tiger patterns allows several forms to coexist as a result of a somewhat relaxed selection (Mallet & Joron, 1999; Rowe *et al.*, 2004). Nevertheless, variation in form frequency appears to stem from a strong horizontal heterogeneity of mimetic communities (Joron *et al.*, 1999) rather than a vertical segregation of the models (this study). Rather than a strong stratification of mimicry rings, the lack of spatial movement of predators, along with some possible level of microecological specialization in the butterflies across the habitat (Srygley & Chai, 1990b; DeVries *et al.*, 1999; Willmott & Mallet, 2004; Merchán *et al.*, 2005), may thus allow the landscape-scale coexistence of mimicry rings. Therefore, a mosaic of microhabitats is likely to enhance the level of local diversity in mimicry (Mallet & Gilbert, 1995; M. Joron & Y. Iwasa, unpublished data).

It is possible that our measurements omit some aspects of the *H. numata* life history, such as roosting behaviour. It is indeed suggested that relatives of *H. numata* roost high in the canopy, despite a very wide range of roosting height measurements (Turner, 1975; Mallet & Gilbert, 1995). Part of the predation might take place at or around such roosting sites where butterflies are rarely observed (Mallet & Gilbert, 1995). However, a scenario for divergence based on roosting segregation requires that colour forms that all fly at the same heights and with considerable variation during the day roost at sharply distinct heights in the canopy during the night. Although this hypothesis is difficult to evaluate in the absence of data for nocturnal behaviour of these butterflies and their predators in the canopy, it does not seem to fit the available data for the tiger-patterned mimicry rings (Turner, 1975; Mallet & Gilbert, 1995). Horizontal heterogeneity of mimicry and gene flow remain more likely causes for the polymorphism in *H. numata*.

Sex-specific flight height in *H. numata*

The difference in flight height between male and female *H. numata* is one of the striking results of this study. Sex-specific flight height has rarely been mentioned in the mimicry literature, although this behavioural dimorphism is easily observed in the field for *H. numata*. When not feeding at flowers, females are usually found looking for host-plants low in the understory, with a weak wing beat, quite like their *Melinaea* models. During sunny hours, male *H. numata* are usually found patrolling their home range (Mallet, 1986; Mallet *et al.*, 1987), searching for mates as well as pollen sources, following a nearly constant route. At any one spot, males may be observed cruising vigorously at mid-elevation, alternating between gliding and flapping sequences, a flight pattern named 'promenade' by Brown & Benson (1974). Other *Heliconius* species such as *H. heurippa* and *H. cydno* seem to have a similar behaviour (M. Joron, personal observations; C. Jiggins, J. Mallet, personal communications; see also Gilbert, 1991; Merchán *et al.*, 2005).

Male *Heliconius* are known to use visual cues to locate potential mates (McMillan *et al.*, 1997; Jiggins *et al.*, 2001; Sweeney *et al.*, 2003), and male *H. numata* are readily attracted to red or orange rags waved in the sun (Brown & Benson, 1974; M. Joron, personal observations). Besides, female *H. numata* can mate multiply, and may accept males even when a few months old (Brown & Benson, 1974; M. Joron, personal observations). Therefore, the higher flight of *H. numata* males may be a mate-locating strategy that allows locating females visually on a wide radius without having to enter the dense lower vegetation. *Heliconius numata* are found in a wide diversity of habitats, but are especially common in tall secondary forest, where males are seen flying above the shrub layer (>2 m). It may be significant that in *H. cydno*, also found in tall forest with similar vegetation as *H. numata*, males also tend to fly high whereas females are found in the understory (Estrada & Jiggins, 2002; Merchán *et al.*, 2005; M. Joron, personal observations).

Contrary to mate-searching males, females may be searching the denser shrub layer, meticulously looking for oviposition plants. Most *Heliconius* species, including *H. ismenius* (the sister species to *H. numata*), oviposit on Passifloraceous plants found in light gaps or in

second-growth understory, and rarely in the canopy (Mallet & Gilbert, 1995, and references therein; J. Longino, personal communication). In the present study, ovipositing *H. numata* females were indeed found around low shoots (mean height 1.10 m, SD = 0.70 m, range 0.2–2.5 m, $n = 11$). The generally low flight height of female *H. numata* must therefore reflect the preferred height for larval host plants, and a finer-scale, more systematic use of their habitat.

The flight-height variation over the course of the day seems in accordance with the mate search vs. host search diphenism. The sexual difference in flight height is nonsignificant in the early morning and late afternoon, and peaks in the middle of the day (Fig. 3b). Both sexes typically start the day by looking for pollen and nectar at *Psiguria*, *Palicourea*, and other flowers, and males and females seem to have the same floral resources (M. Joron, personal observations; J. Mallet, personal communication; see also Gilbert, 1991; Mallet & Gilbert, 1995, although these studies do not differentiate by sex). By mid-morning males start looking for mates, whereas females start searching for oviposition plants. This appears to be reflected in the general differences in flight height observed in this study. We may hypothesize that the male mate-location cruise requires higher levels of energy, encountered during the hottest hours of the day, as has been found in *Pieris rapae* (Hirota & Obara, 2000) and in speckled woods *Pararge aegeria* (van Dyck *et al.*, 1997). By the end of the afternoon, prior to roosting, sexes tend to fly at similar heights again, so the sex-specific flight height most likely reflects the conspicuously different activities of the two sexes during the day.

Sex-specific flight height was apparently not taken into account in previous studies on mimetic stratification. A prediction would be that other forest interior *Heliconius* species, such as *H. pachinus* studied by Mallet & Gilbert (1995), should show a sex-specific flight height during the day.

Sex-specific behaviour and mimicry

From the correspondence in flight height between female *H. numata* and *Melinaea* spp. it is tempting to conclude that the mimetic relationship with *Melinaea* is stronger in female than in male *H. numata*, even though *H. numata* is not sexually dimorphic. As behaviour and flight patterns may be used as a recognition cues by predators (Srygley & Ellington, 1999), male and female *H. numata* may not rely equally on mimicry to avoid predation. Ovipositing females tend to have predictable flight-patterns around host-plants and a less agile flight, so they are thought to be subject to overall higher predation rates (Srygley & Chai, 1990a; Ohsaki, 1995; Srygley & Kingsolver, 2000). Mark-recapture studies in various butterfly species consistently show lower recapture rates for females, which may in part be a result of a higher risk of predation (Ehrlich & Gilbert, 1973; Kingsolver, 1996, 1999; Srygley

& Kingsolver, 2000). In contrast, males may rely more on escape, agility, and higher activity levels to avoid predation (Turner, 1978; Mallet & Singer, 1987; Turner, 1995; Merchán *et al.*, 2005), which may compensate for the loss in mimetic protection that results from specific mate-location behaviours (as in Batesian mimicry, e.g. Burns, 1966; Ohsaki, 1995). Again, male butterflies are likely to enjoy best agility and reactivity during the hot hours of the middle of the day (Hardy, 1998; Stutt & Willmer, 1998; Hirota & Obara, 2000), which is also when the sexes have the largest difference in flight heights in the present study (Fig. 3b). Stronger reliance on anti-predator signals in one sex leads one to expect a higher stability of mimetic wing patterns in this sex, such as higher pattern symmetry (Forsman & Merilaita, 1999), canalization (M. Linares, personal communication), or stronger dominance relationships between the colour-pattern alleles, which could be relatively easily tested.

Flight height differences between sexes represent most of the variation in the present data, with a scale of segregation comparable with that of previous studies for whole butterfly guilds (e.g. Beccaloni, 1997b). My extension of Müller's (1879) number-dependent model, to account for partial segregation of the sexes in micro-habitat, suggests that mate search behaviour and different microhabitat use by sexes can interfere with aposematic colours and mimicry (see also, e.g. Forsman & Appelqvist, 1999 for sex-by-colour pattern interactions in grasshopper survival). Indeed, as mimetic benefits are inversely proportional to the relative abundance of the prey species in the local mimetic community (Müller, 1879; Kapan, 2001), the benefit of joining a local mimicry ring is enhanced when predation is uneven between sexes, as opposed to a situation where both sexes have identical ecologies.

Müller's model therefore suggests that microhabitat differences between sexes will result in higher benefits for close local mimicry in (at least) one sex. In a heterogeneous landscape, any mechanism that makes local adaptation more likely will also make polymorphism more likely if different alleles are beneficial in different places (see Mitter *et al.*, 1979; Hedrick, 1986), and this also holds in the face of positive frequency-dependence as is typical of Müllerian mimicry (Molofsky *et al.*, 2001; M. Joron & Y. Iwasa, unpublished data). *Heliconius numata* show both sexual differences in microhabitat (this study) and heterogeneity in selection at a very small scale (5–10 km; Joron *et al.*, 1999), which is precisely the combination of factors that should favour local adaptation and, with selection-migration balance between neighbouring sites, polymorphism (Mallet & Barton, 1989; Joron *et al.*, 1999; M. Joron & Y. Iwasa, unpublished data). Therefore, the coincidence of sex-specific behaviours and a spatially variable selection may have enhanced local mimicry adaptation and facilitated the evolution of a spectacular mimetic polymorphism in *H. numata*.

More detailed data on the ecology of mimicry is therefore needed to assess the extent of microhabitat segregation within and between mimicry rings (Willmott & Mallet, 2004). Further theoretical investigation could also predict which level of microhabitat selection is necessary to cause a switch in the outcome of local density dependence resulting in local adaptation. Note that, interestingly, the evolution of mimicry is facilitated in species with spatial segregation (Fig. 4a, evolution from II to IV), but this does not mean the evolution of mimicry enhances the ecological divergence of sexes (evolution from III to IV), unless males benefit from a relaxed predation in the alternative microhabitat. In other words, evolving habitat (or behavioural) preferences between sexes may facilitate the subsequent evolution of mimicry, but evolving mimicry first may prevent the evolution of habitat preferences.

The segregation of sexes with respect to predation is found, for instance, if predation on one sex is partly independent of wing colour. This may be the case for species with vigorous male flight such as *H. numata*. In that case, and in the absence of conflicts between the selection of male and female traits, male colour will follow the selection for mimicry in females, and differences in predation pressures between sexes (Ohsaki, 1995) will not necessarily result in sexual dimorphism; in contrast, microhabitat selection will help local adaptation. This suggests that sexually dimorphic behaviours may foster the evolution of nonsex-limited genetic polymorphism, which has only been investigated theoretically in a handful of studies (Hedrick, 1993, on sex-dependent habitat selection; see also earlier work by Li, 1963; Kidwell *et al.*, 1977), and has received little attention by empiricists despite the wealth of data on sex-dependent habitat selection and behaviour (see Merilaita & Jormalainen, 1997; Forsman & Appelqvist, 1999, and references therein).

The segregation of sexes with respect to predation may also be caused by their partial spatial separation, possibly linked to behavioural differences, as is found for example in lekking species, or if males use specific or localized resources such as mud puddles (e.g. *Battus* swallowtails) or pyrrolizidine alkaloid plants (Ithomiines). If colour is also a warning signal for the males in their microhabitat, there could be a frequency-dependent barrier against a change of colour in males. Microhabitat segregation might then result in a sexual dimorphism where the local (mimetic) colour only evolves in females, whereas the other sex either retains the ancestral colour, or evolves mimicry to a different model.

Some sexually dimorphic Müllerian mimics have sexes in different mimicry rings: examples include, in the Neotropics, *Parides* species (Papilionidae) (West, 1994), *Godyris zavaleta*, several *Pteronymia* and *Hyalyris* species (Nymphalidae: Ithomiinae) (D'Abrera, 1984; Willmott & Mallet, 2004), or *Heliconius demeter* (Brown & Benson, 1975). Such dimorphisms could result from different

microhabitat use by the sexes, or from strong behavioural differences. Fine-scale microhabitat segregation of sexes such as that caused by the concentration of male at hill-tops leks in *Papilio* (e.g. Lederhouse & Scriber, 1996), can also be a scenario for sex-limited Batesian mimicry evolution involving lower levels of stabilizing sexual selection on male colours (Turner, 1978; Herrel & Hazel, 1995). Further studies on mate-location strategies may reveal comparable interactions between sex-specific behaviours and colour mimicry, with potential implications ranging from female transvestism (Sherratt, 2001) to speciation (Jiggins *et al.*, 2001; Naisbit *et al.*, 2001).

Acknowledgments

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