Evolutionary Ecology **13:** 721–754, 1999.

Research paper

Variable selection and the coexistence of multiple mimetic forms of the butterfly *Heliconius numata*

MATHIEU JORON[1]*, IAN R. WYNNE[2], GERARDO LAMAS[3] and JAMES MALLET[2]

[1]*Institut des Sciences de l'Evolution, Université de Montpellier II, Place E. Bataillon, F-34095 Montpellier, France;* [2]*Department of Biology, University College London, 4, Stephenson Way, London NW1 2HE, UK;* [3]*Departamento de Entomología, Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Apartado 14-0434, Lima-14, Peru*

*(*author for correspondence, fax: +31 (0) 71-527-4900; email: joron@rulsfb.leidenuniv.nl Inst. Evol. Ecol. Sciences, Univ. Leiden, PO BOX 9516, 2300 RA Leiden, The Netherlands)*

Received 20 June 2000; accepted 11 December 2000

Co-ordinating editor: C. Rowe

Abstract. Polymorphism in aposematic animals and coexistence of multiple mimicry rings within a habitat are not predicted by classical Müllerian mimicry. The butterfly *Heliconius numata* Cramer (Lepidoptera: Nymphalidae; Heliconiinae) is both polymorphic and aposematic. The polymorphism is due to variation at a single locus (or 'supergene') which determines colour patterns involved in Müllerian mimicry. We sampled 11 sites in a small area (approx. 60 × 30 km) of North-eastern Peru for *H. numata* and its co-mimics in the genus *Melinaea* and *Athyrtis* (Ithomiinae), and examined the role of temporal and spatial heterogeneity in the maintenance of polymorphism. Colour-patterns of *Melinaea* communities, which constitute the likely 'mimetic environment' for *H. numata*, are differentiated on a more local scale than morphs of *H. numata*, but the latter do show a strong and significant response to local selection for colour-pattern. In contrast, analysis of enzyme polymorphism in *H. numata* across the region revealed no spatial structure, which is consistent with a high mobility of this species. Differences in spatial variability in the two taxa may have caused *H. numata* to become polymorphic, while temporal variability, not significant in this study, probably has a lesser effect. The mimetic polymorphism is therefore explained by means of multiple selection-migration clines at a single locus, a similar process to that which explains narrow hybrid zones between geographic races of other *Heliconius* butterflies.

Key words: *Heliconius numata*, *Melinaea*, Ithomiinae, aposematism, Müllerian mimicry, polymorphism, spatial heterogeneity, frequency-dependent selection, population genetic structure

Introduction

Warning colour is a strategy used at both population and community levels. For populations of distasteful prey, conspicuousness has an initial disadvantage, but this is offset if predators, which associate prey appearance and prey toxicity, learn to avoid the prey more readily. The strength of this learned avoidance will depend on the rate of encounter with the warning pattern, and

the fitness of the different potential warning patterns is thus positively dependent on their numbers in the population, leading to monomorphism. But fitness will also depend on which warning pattern is most widely adopted by other distasteful species in the same habitat. This leads to different unpalatable species locally mimicking one another in Müllerian associations (Müller, 1879). Local uniformity of colour-pattern is therefore expected both within and among species.

Most aposematic insects are indeed monomorphic locally; polymorphism is usually limited to narrow contacts between divergent geographic races (Mallet *et al*., 1989, Mallet 2001). At the community level, Müllerian mimicry is also very widespread among different unpalatable insects, so the theory seems quite robust. However, several different mimicry rings usually coexist in any habitat (Mallet and Gilbert, 1995; Beccaloni, 1997a) and many unpalatable species occur in multiple mimetic forms that coexist over wide geographic areas (Cook and Brower, 1969; Brown and Benson, 1974; Clarke *et al*., 1995). Multiple warning-colour strategies within a community or within a species are difficult to explain since coexistence of self-reinforcing aposematic signals should be unstable. Therefore, mechanisms other than local warning-colour convergence need to be invoked to explain coexistence (Joron and Mallet, 1998).

Heliconius numata is a forest butterfly of the lowland and sub-montane parts of the Amazon basin. It is mimetic and polymorphic in most of its range: in any one locality, two to ten colour-pattern forms may be found flying together (Fig. 1), each of which participates in a separate 'tiger-pattern' mimicry ring, along with many other unpalatable butterflies and day-flying moths in the Ithomiinae, Danainae, Heliconiinae, Riodinidae and Pericopinae (Brown and Benson, 1974; Beccaloni, 1997a – the latter provides excellent photographs of Ecuadorian mimicry rings). These different mimicry rings are not always sharply distinct, but the various forms of *H. numata* are clearly discrete: most can readily be identified in flight (but see below) and, within each mimicry ring, are especially accurate co-mimics of ithomiine species in the genus *Melinaea*. Each form of *H. numata* is thus an almost perfect mimic of one or several sympatric *Melinaea* species (Fig. 1).

Brown and Benson (1974) argued that the ithomiine co-mimics of *H. numata* are unevenly distributed in space and their numbers fluctuate greatly in time. If this were true, then the mimetic environment will vary with respect to which pattern is best protected in a given place at a given time, and help maintain the polymorphism observed in *H. numata*. In this paper, we present results of a detailed survey *H. numata* and its co-mimics in a small area (approx. 60×30 km) of north-eastern Peru. The work was carried out to explore whether spatial and temporal variability in the mimetic environment can promote the co-existence of so many mimetic patterns.

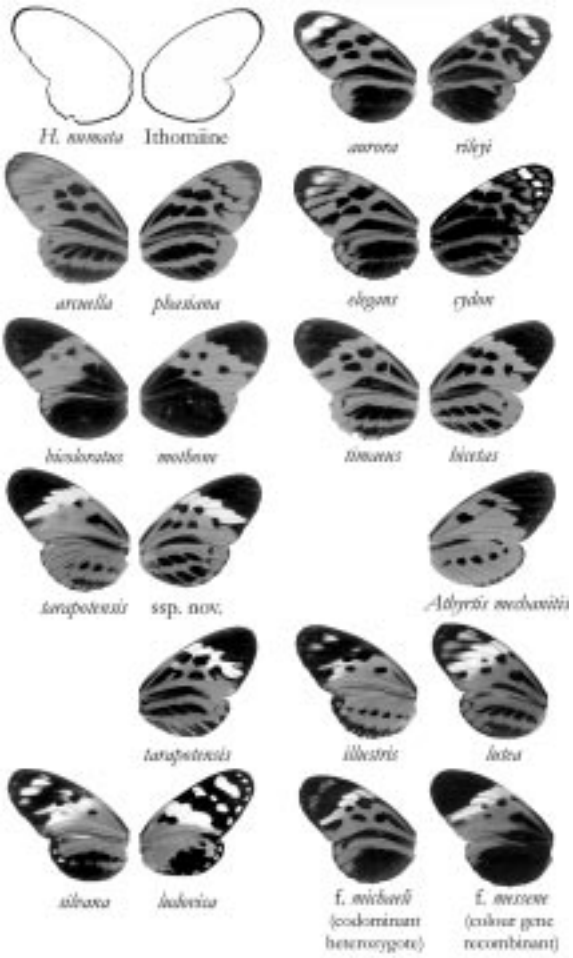

Figure 1. *Heliconius numata* forms (left wings) and their respective Ithomiine co-mimics (right wings) from the vicinity of Tarapoto. The colours are as follows: black for black, grey for orange or brown, white for yellow. Form names for *H*. *numata* and subspecies names for *Melinaea* are indicated directly on the figure. Left column: *Heliconius numata* f. *arcuella* and *Melinaea marsaeus phasiana*; *H*. *n*. f. *bicoloratus* and *M*. *marsaeus mothone*; *H*. *n*. f. *tarapotensis* and *M*. *menophilus* ssp. nov. + *M*. *satevis tarapotis*; *H*. *n*. f. *silvana* and *M*. *ludovica ludovica* (the small marginal dots are white). Right column: *H*. *n*. f. *aurora* and *M*. *marsaeus rileyi*; *H*. *n*. f. *elegans* and *M*. *satevis cydon*; *H*. *n*. f. *timaeus* and *M*. *menophilus hicetas* + *Athyrtis mechanitis mechanitis*; *H*. *n*. f. *illustris* (no co-mimic found); *H*. *n*. f. *lutea* (recombinant between the forms *arcuella* and *tarapotensis*); a heterozygous *H*. *numata* (similar to f. *michaeli*) from site no. 9; and a very rare *H*. *n*. f. *messene* that is a recombinant between the forms *bicoloratus* and *tarapotensis* in this area of Peru. All *H*. *numata* shown are males except f. *lutea*. All Ithomiines shown are females except *menophilus* ssp. nov., *ludovica*, and *rileyi*. Forewing length = 4 cm.

Materials and methods

Species studied

Heliconius numata lives in mature Amazonian forests. Male *H. numata* typically fly fast and conspicuously along sunny tracks and canopy valleys at 2–5 m above ground in 'promenading behaviour' (Brown & Benson, 1974), while females generally fly lower and more slowly in the understorey, typically looking for new shoots of their host plants, canopy *Passiflora* vines. Unlike most *Heliconius*, *H. numata* is a generalist feeder that utilises many species of *Passiflora* in several subgenera (mainly *Distephana* and *Granadilla*). It is usually scarcer than other *Heliconius*, but can become fairly common in selectively logged stands with cleared understorey, where host plant shoots are abundant. The biology and behaviour of *H. numata* has been described in detail by Brown and Benson (1974). *Melinaea* species have a flight and general behaviour very similar to that of female *H. numata*, but are often found resting in more humid parts of the forest.

Müllerian mimicry of Heliconius numata *forms with* Melinaea *species*

In any given locality, the different forms of *Melinaea* are 'good' species, with no intermediates. Between regions (see below) the *Melinaea* may often have different colour patterns, and many have been classified as parapatric subspecies (Brown, 1977). However, parapatry is sometimes so sharp and hybrids so rare (J. Mallet and G. Lamas, unpublished data) that assignment of races to species has been difficult. Indeed, subspecies have been switched frequently between nominal 'species', depending on the author (Brown, 1979). Several of Brown's (1977) subspecies may in fact be parapatric species in that intergradation in zones of contact is rare or absent. For example, we have observed sympatry or narrow parapatry of the *M. marsaeus* forms *mothone*, *phasiana*, and *rileyi*, so these act for the most part as separate species in the field. In view of the lack of known intergrades between the *M. satevis* forms *cydon* and *tarapotensis*, these two may also be separate species. The *M. menophilus* forms, on the other hand, seem frequently to intergrade with one another, but only rarely with *M. isocomma simulator*, which is here recognized as separate from *M. menophilus*. In this paper, we use nomenclature of the catalogue by G. Lamas (in preparation), which treats some of these geographic forms as subspecies within widely distributed 'super-species', rather than as the narrowly endemic true species which some may ultimately prove to be.

Many *H. numata* forms in the study area have more than one corresponding Müllerian co-mimic within *Melinaea* (Fig. 1): *H. n. bicoloratus* corresponds to *M. marsaeus mothone* and *M. isocomma simulator; H. n. tarapotensis* to *M.*

menophilus ssp. nov. and *M. satevis tarapotensis*; *H. n. aurora* and some of its forms to *M. marsaeus rileyi* and *M. satevis cydon*; *H. n. timaeus* to *M. menophilus hicetas* and *Athyrtis mechanitis*. *H. n. arcuella* corresponds to *M. marsaeus phasiana;* and *H. n. silvana* to *M. ludovica ludovica*. *Melinaea ludovica manuelito* was not seen during the study, even though mimicked by *H. n. illustris* which was seen. *H. numata aurora* is variable and intergrades phenotypically with some co-dominant heterozygotes; in this case, *Melinaea m. rileyi* and *M. s. cydon* (which do not intergrade, being separate species) represent two extreme phenotypes for the mimetic association, *H. n. aurora* being more similar to *M. m. rileyi*, and the form *elegans* very similar to *M. s. cydon*, but it is not easy to draw a line between the two. Given that these two colour-patterns are also difficult to identify in flight, we lump them here. In contrast, *Melinaea isocomma simulator* and *M. m. mothone* are so close morphologically that even pinned specimens are tricky to identify; *M. menophilus* ssp. nov. and *M. s. tarapotensis* are also very similar to one another (see Fig. 1). Perhaps surprisingly, all of the forms of *H. numata* are determined largely by a single locus, or 'supergene' (Brown & Benson, 1974; Joron, 2000). The forms *lutea, staudingeri*, and others (Fig. 1.) are probably recombinants within the supergene (see Brown, 1976; Joron, 2000), and are classified as non-mimetic for the purposes of this analysis.

Naturally, the above associations assume that (avian) predators and humans perceive colour-patterns similarly. Although the patterns are similar in the visible light, they might differ in the degree to which they reflect UV, to which birds are known to be particularly sensitive. Beccaloni (1997a) provides UV photographs of the Ecuadorian races of some of these species, which show no special UV pattern.

Many other ithomiine species participate in these mimicry rings, and potentially affect the polymorphism in *H. numata*. Plates of similar mimicry rings can be found in Beccaloni (1997a; partially reproduced in Joron and Mallet, 1998), along with some hypotheses for their coexistence (see also Beccaloni, 1997b). In our area, the other prevalent mimetic species belong to the genera *Tithorea, Forbestra, Mechanitis, Hypothyris, Hyposcada and Callithomia* (Ithomiinae), *Lycorea* (Danainae) and *Dysschema* (Arctiidae: Pericopinae). However, to the human eye, these butterflies are poorer mimics of *Melinaea* than *H. numata*: most are smaller, they have a distinguishable pattern, different wing shape and size and, somewhat different behaviour (Beccaloni, 1997a, b). They can nearly always be identified in flight. Given that the mimicry is so accurate between *Melinaea* and *H. numata*, we hypothesise that their mimetic interaction is much stronger than with the other species, and therefore concentrated on these large-winged taxa. A colour representation of the tiger-patterned mimicry rings found in our study area is shown on our website: http://abacus.gene.ucl.ac.uk/jim/.

Study area and populations sampled

This study was conducted in the Amazonian foothills of the Andes, in the vicinity of Tarapoto, Departamento de San Martín, Peru (Fig. 2). This area was chosen because *H. numata* is particularly polymorphic in this area, with up to 10 forms, and the sites are accessible via well-maintained dirt roads. Most study sites (Table 1 and Fig. 2) were chosen along a road linking the cities of Tarapoto and Yurimaguas (100 km apart), in the eastern most Andean mountain range (locally called Cerros Escalera) and in the nearby Amazon lowland. The forest near Tarapoto is mostly old secondary growth of dry submontane forest (300–1000 m). Habitat in the Amazon lowland is less disturbed, moist, terra-firme lowland forest. The area lies at the boundary between three areas of butterfly subspecies endemism (Brown, 1979; Lamas, 1982; J. Mallet and G. Lamas, unpublished data), hereafter referred to as 'Upper Huallaga' (sites 1 and 2), 'Escalera' (sites 3 to 8) and 'Lower Huallaga' (sites 9–11). The Escalera sites were all at higher elevations in the mountain ridge separating the Upper and Lower Huallaga regions.

To estimate relative abundances, populations were sampled in August–September 1997, August–November 1998 and November–December 1999. Few data were gathered for *Melinaea* and *Athyrtis* in 1998, because we concentrated on an insectary experiment involving *H. numata* only (Joron, 2000). The species studied are present year-round, but seemed most abundant during the drier parts of the year, particularly August–December. Butterflies were caught using nets with a 2 m pole, allowing capture at over 4 m high. For butterflies flying higher, *H. numata* could be attracted to red rags waved rapidly in the sun, as described by Brown and Benson (1974). *Melinaea* are not attracted to red rags, but fly slowly and are easily approached. In either taxon, the different forms have a very similar behaviour (Joron, 2000), and we are confident that potential sampling biases towards any form are negligible.

Ithomiines were identified by GL 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), and were nearly always unequivocally ascribable to one mimicry ring; less than 5 percent of the individuals were non-mimetic intermediates.

Electrophoresis

Bodies were frozen immediately in liquid nitrogen, and wings were kept separately in glassine envelopes. Allozyme electrophoresis was performed on the *H. numata* specimens collected in 1999. The abdomen and thorax of

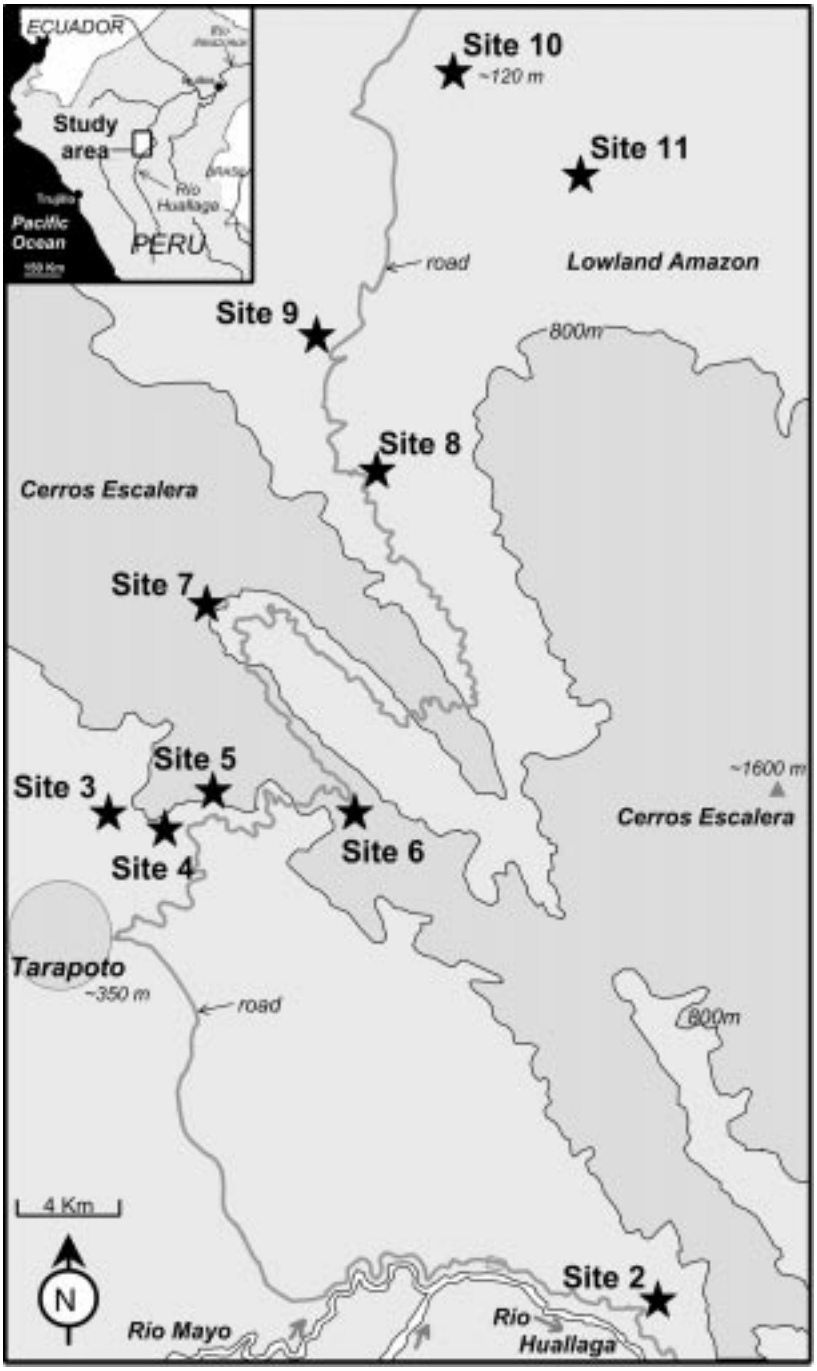

Figure 2. Map of the study area. Study site no. 1 is not shown (it is 15 km to the South). See Table 1 for the names and exact details of the sites.

Table 1. Study site details and sample sizes for populations of *H. numata* and *Melinaea* spp. Upper Huallaga: sites 1 and 2, Escalera: sites 3 to 7, Lower Huallaga: sites 10 and 11. Sites 8 and 9 are situated in a contact zone between Escalera and Lower Huallaga

Site No	Local name	Position			Vegetation	*Heliconius numata*			*Melinaea*		
		Latitude	Longitude	Altitude (m)		1997	1998	1999	1997	1998	1999
1.	Carretera a Sauce, km 7–11	6°43′30″S	76°14′45″W	450	2G	7	–	–	4	–	–
2.	Quebrada Pucayaquillo (near Shapaja)	6°35′10″S	76°13′05″W	300–450	2G	12	23	12	14	4	9
3.	Río Shilcayo	6°27′20″S	76°20′40″W	350–500	2G	32	22	17	17	3	7
4.	km 8–9. Sector Uruhuasha	6°28′00″S	76°20′05″W	700–850	2G	–	23	16	–	5	1
5.	km 10–14. Tarapoto-Yurimaguas	6°27′35″S	76°19′20″W	750–900	2G	28	11	22	6	1	19
6.	km 15–19. Ahuashiyacu-Túnel	6°27′25″S	76°18′00″W	800–1000	2G–1F	23	8	23	4	2	4
7.	km 28–30. Tarapoto-Yurimaguas	6°24′30″S	76°19′30″W	750–900	2G	11	4	–	2	1	–
8.	km 48–52. Tarapoto-Yurimaguas	6°22′10″S	76°16′45″W	450	2G	10	–	–	7	–	–
9.	km 60. Pongo de Cainarachi	6°20′20″S	76°18′40″W	180	2G	7	2	21	5	0	0
10.	km 72. Santa Rosa de Davidcillo	6°14′55″S	76°15′50″W	120	2G–1F	5	4	27	0	1	0
11.	km 4–8, Carretera Pongo-Barranquita	6°17′15″S	76°13′40″W	150	1F	16	3	7	0	1	20

2G = Second Growth, 1F = Primary Forest

each butterfly were homogenised separately on ice in 250 µl grinding buffer (recipe in Wynne *et al*., 1992). After centrifugation (2 min at 14,000 r.p.m.), homogenates were snap-frozen into many individual ~6 µl balls in liquid nitrogen (Wynne and Brookes, 1992). After storage, this technique allows access to a single aliquot for a set of runs without having to defrost the whole homogenate. Six of the 11 populations were analysed electrophoretically, with 12 to 26 individuals per population, totalling 116 butterflies. Electrophoreses were performed on Helena cellulose acetate plates on the following buffers: TrisGlycine pH 8.5, TrisCitrate pH 8.2, TrisCitrate pH 7.8 and Phosphate pH 6.3 (recipes in Richardson *et al*., 1986). Most systems were run for 30 min at 200 V. However, some were run up to 50 min to allow good separation of the alleles (given in Table 2). Gels were stained using recipes described elsewhere (Richardson *et al*., 1986; Mallet *et al*., 1993, for *Enol*, *PK* and 3-*P*). Line-up gels were run to ensure accurate scoring of heterozygotes.

Table 2. Allele frequencies at 16 polymorphic loci in six populations of *H. numata*. Eleven other loci were monomorphic or had very weak activity: malic enzyme, fumarase, aconitase, alpha-glycerophosphate dehydrogenase, glutamic oxaloacetate transaminase (slow locus), Leu-Gly peptidase, Phe-Pro peptidase, hexokinase, sorbitol dehydrogenase, glucose dehydrogenase and alcohol dehydrogenase

Locus	Mobility	Site 2	Site 4	Site 5	Site 6	Site 9	Site 10
Phosphoglucomutase (PGM)							
(n)		12	14	20	23	21	26
	55	–	–	–	–	0.024	0.077
	77	0.042	0.071	0.075	0.043	0.095	0.154
	100	0.875	0.929	0.750	0.848	0.833	0.654
	122	0.083	–	0.125	0.087	0.048	0.077
	155	–	–	0.050	0.022	–	0.038
Glucose-6-phosphate isomerase (GPI)							
(n)		12	14	20	23	21	26
	−225	–	0.036	–	–	–	0.038
	−75	0.250	0.071	0.200	0.152	0.190	0.135
	100	0.625	0.750	0.600	0.804	0.643	0.615
	275	0.125	0.107	0.200	0.022	0.119	0.192
	450	–	0.036	–	0.022	0.048	0.019
Mannose-6-phosphate isomerase (MPI)							
(n)		12	14	20	23	21	26
	88	0.083	0.107	0.075	0.065	0.095	0.096
	92	0.292	0.143	0.275	0.261	0.286	0.423
	100	0.333	0.321	0.300	0.435	0.167	0.154
	109	0.292	0.429	0.325	0.239	0.405	0.288
	118	–	–	0.025	–	0.048	0.038

Table 2. (Continued)

Locus	Mobility	Site 2	Site 4	Site 5	Site 6	Site 9	Site 10
Malate dehydrogenase 1 (MDH-slow)							
(n)		12	14	20	23	21	26
	63	0.042	0.071	–	0.022	–	–
	100	0.958	0.929	1.000	0.891	1.000	1.000
	150	–	–	–	0.087	–	–
Malate dehydrogenase 2 (MDH-fast)							
(n)		12	14	20	23	21	26
	83	0.042	–	–	–	0.024	–
	88	0.042	–	–	0.022	0.048	0.019
	100	0.917	0.964	1.000	0.978	0.905	0.962
	120	–	0.036	–	–	0.024	0.019
Glutamic oxaloacetic transaminase (GOT-fast)							
(n)		12	14	20	23	21	26
	77	–	–	–	0.022	–	–
	92	–	–	0.050	0.043	–	0.019
	100	0.875	0.963	0.925	0.870	0.976	0.865
	105	0.083	–	–	0.022	–	0.019
	112	–	–	0.025	0.043	–	0.096
	118	0.042	0.037	–	–	0.024	–
Adenylate kinase (AK)							
(n)		12	14	20	22	19	26
	86	0.042	0.071	–	0.091	–	0.019
	100	0.958	0.929	1.000	0.909	1.000	0.962
	112	–	–	–	–	–	0.019
Pyruvate kinase (PK)							
(n)		12	14	20	22	19	26
	78	–	–	0.025	0.023	–	0.019
	100	1.000	1.000	0.975	0.977	1.000	0.962
	114	–	–	–	–	–	0.019
Enolase (ENOL)							
(n)		12	14	20	22	19	26
	81	–	–	–	0.023	–	–
	88	–	–	0.025	0.045	–	0.038
	100	1.000	1.000	0.975	0.909	1.000	0.923
	112	–	–	–	–	–	0.019
	125	–	–	–	–	–	0.019
	131	–	–	–	0.023	–	–
Isocitrate dehydrogenase (NAD) (IDH-slow)							
(n)		12	14	20	23	21	0
	93	–	–	–	–	0.024	–
	100	0.958	0.964	0.950	0.935	0.952	–
	107	–	–	–	0.022	–	–
	114	–	–	0.025	0.022	0.024	–
	121	0.042	0.036	–	0.022	–	–
	126	–	–	0.025	–	–	–

Table 2. (Continued)

Locus	Mobility	Site 2	Site 4	Site 5	Site 6	Site 9	Site 10
Isocitrate dehydrogenase (IDH-fast)							
(n)		12	14	20	23	21	25
	86	–	–	0.025	–	–	–
	100	0.917	1.000	0.975	0.977	1.000	1.000
	110	0.083	–	–	0.023	–	–
Leu-Ala peptidase (LA)							
(n)		12	14	20	23	21	26
	88	0.167	–	0.050	–	0.048	–
	100	0.833	1.000	0.950	0.955	0.929	1.000
	110	–	–	–	0.045	0.024	–
Glucose-6-phosphate dehydrogenase (G6PD)							
(n)		12	14	20	23	14	26
	92	–	0.036	0.050	0.043	0.036	–
	100	0.696	0.714	0.825	0.848	0.857	0.846
	104	0.087	0.036	0.025	0.087	–	0.019
	108	–	–	0.025	–	0.071	0.019
	115	0.130	0.143	0.075	0.022	0.036	0.096
	120	0.087	0.036	–	–	–	–
	124	–	0.036	–	–	–	0.019
3-Phosphoglycerate dehydrogenase (3-P)							
(n)		12	14	20	23	–	26
	81	–	–	0.100	0.022	–	–
	100	1.000	1.000	0.900	0.978	–	0.981
	131	–	–	–	–	–	0.019
6-Phosphogluconate dehydrogenase (6PGD)							
(n)		12	14	20	23	21	25
	78	0.083	–	0.025	–	–	–
	88	–	–	–	–	–	0.020
	100	0.917	0.926	0.875	0.955	0.929	0.920
	108	–	0.074	0.075	0.045	0.071	0.060
	122	–	–	0.025	–	–	–
β-Hydroxy-butyrate dehydrogenase (HBDH)							
(n)		12	14	20	23	19	26
	0	0.042	0.036	–	–	–	–
	50	–	0.036	0.025	–	0.053	0.019
	100	0.917	0.929	0.950	0.955	0.921	0.981
	200	0.042	–	0.025	0.045	0.026	–

Data analysis

Local colour-pattern diversity was calculated for each year and site in *H. numata* and in *Melinaea* spp. using Simpson's index ($H = 1 - \Sigma p_i^2$) where p_i is the frequency of the phenotype. Simpson's index is useful in this context because it is equivalent to the standard measure of allelic heterozygosity

in population genetics. Diversity differences were tested by means of a *t*-test (Sokal and Rohlf, 1995). Only sites with more than four individuals were used in the calculation. For *H. numata*, F_{ST} and Fisher's exact tests of population differentiation for allozyme and colour-pattern data were computed using Genepop 3.2 (Raymond and Rousset, 1995; 2000). Colour-pattern phenotypes were coded as haploid characters, and the same was performed for *Melinaea* colour-pattern frequencies. F_{ST} thus measures the proportion of total phenotypic diversity (or allelic heterozygosity) due to variation between as opposed to within populations. Overall temporal variability was tested in both species by log likelihood (=deviance) analysis using GLIM (generalised linear models), declaring *year*, *site* and *form* as categories, using a Poisson error for count data, and testing for significance of the three-way interaction. The change in deviance ($= -2 \times \log_e$ likelihood difference) between two nested models asymptotically follows a χ^2 distribution with d degrees of freedom, where d is the difference in numbers of parameters between the two models (Aitkin *et al.*, 1989).

Correlation between colour-pattern frequencies in the two species was tested by computing the product-moment correlation coefficient ρ (Sokal and Rohlf, 1995) on the data lumped for the 3 years (temporal variation was not significant – see below) in sites with large samples of *Melinaea* (sites 2, 3, 5, 6, 8, 11). The significance of ρ in each site can be estimated by a standard *t*-test approximation (Sokal and Rohlf, 1995). The significance levels P_i in the n different sites ($n = 6$ here) were combined using the Fisher method to get a overall test of the correlation: the quantity $-2\Sigma \log_e P_i$ follows a χ^2 distribution with $2n$ degrees of freedom (Sokal and Rohlf, 1995). A test of the average correlation over the whole data set was also performed by computing ρ between the whole matrix of *Melinaea* with that of *H. numata* (11 sites $\times$ 7 forms). In parallel, we computed a permutation test to calculate the probability of the observed form-frequency correlation in the range of possible data sets: we generated all the possible site permutations in one taxon (4.0×10^7 matrices), computed the correlation coefficient between taxa for each matrix generated, and extracted the one-tailed p value for the correlation coefficient associated with the actual data.

Results

Colour-pattern variation in H. numata

Colour pattern distribution among sites and years is given in Figure 3. Spatial differentiation of colour patterns in the 3 years for *H. numata* gave $F_{ST} = 0.20$ across all sites averaged over the 3 years, $p < 10^{-5}$ for each year; see Table 3).

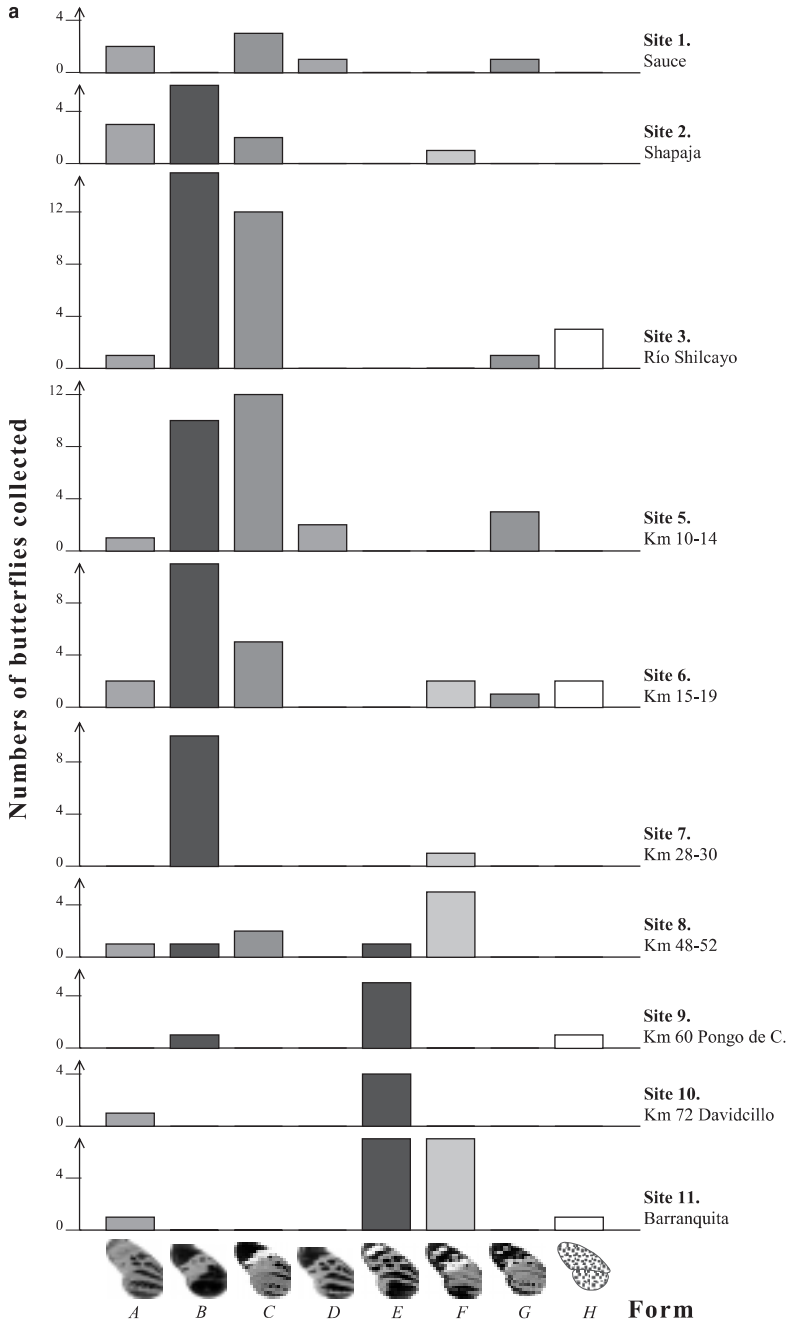

Figure 3. Frequency distribution of *Heliconius numata* forms at 11 sites around Tarapoto, Peru, in three years: (a) 1997, (b) 1998, (c) 1999. Colour-pattern classes: A: *H. n. arcuella*, B: *bicoloratus*, C: *tarapotensis*, D: *timaeus*, E: *aurora*, F: *silvana*, G: *illustris*, and H: non-mimetic forms.

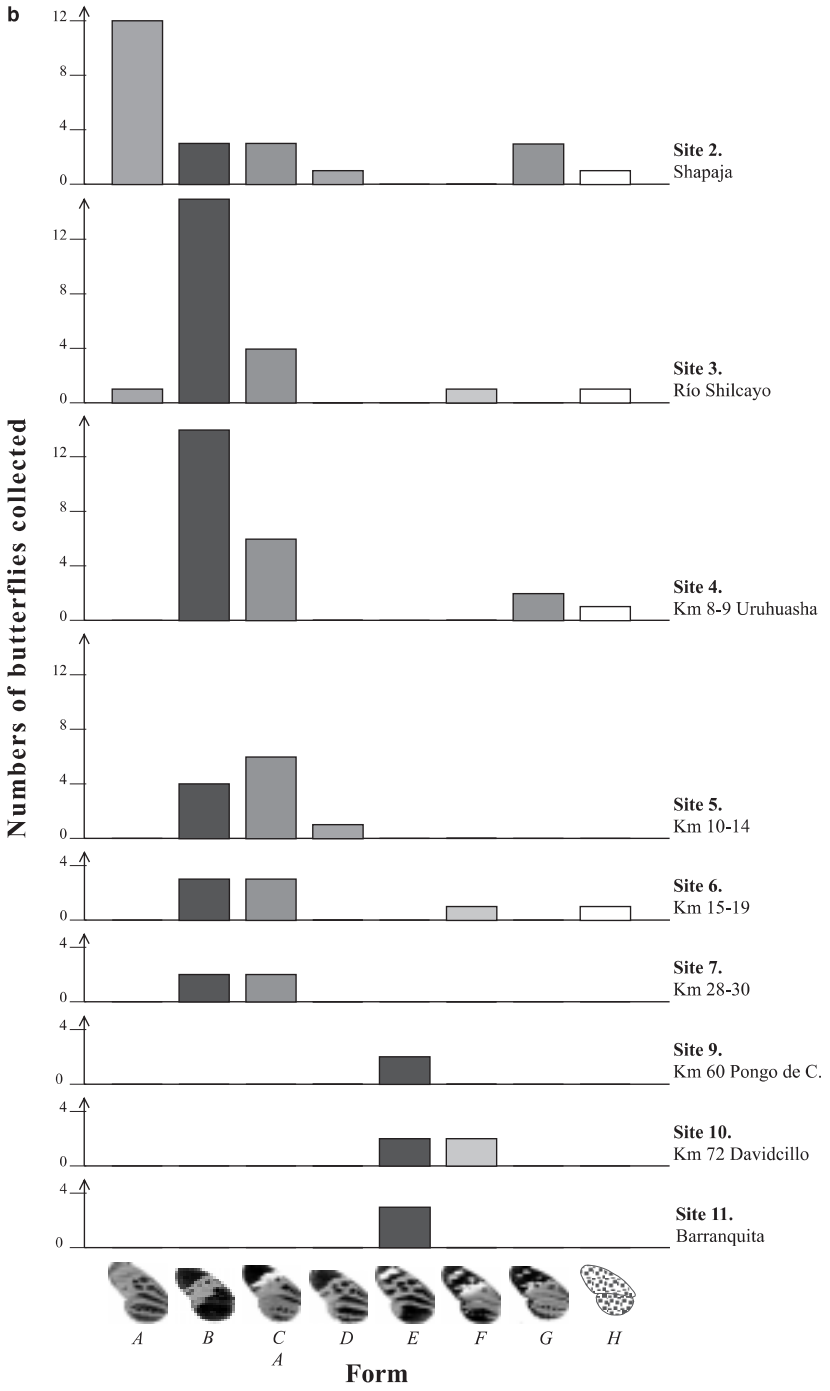

Figure 3. (Continued)

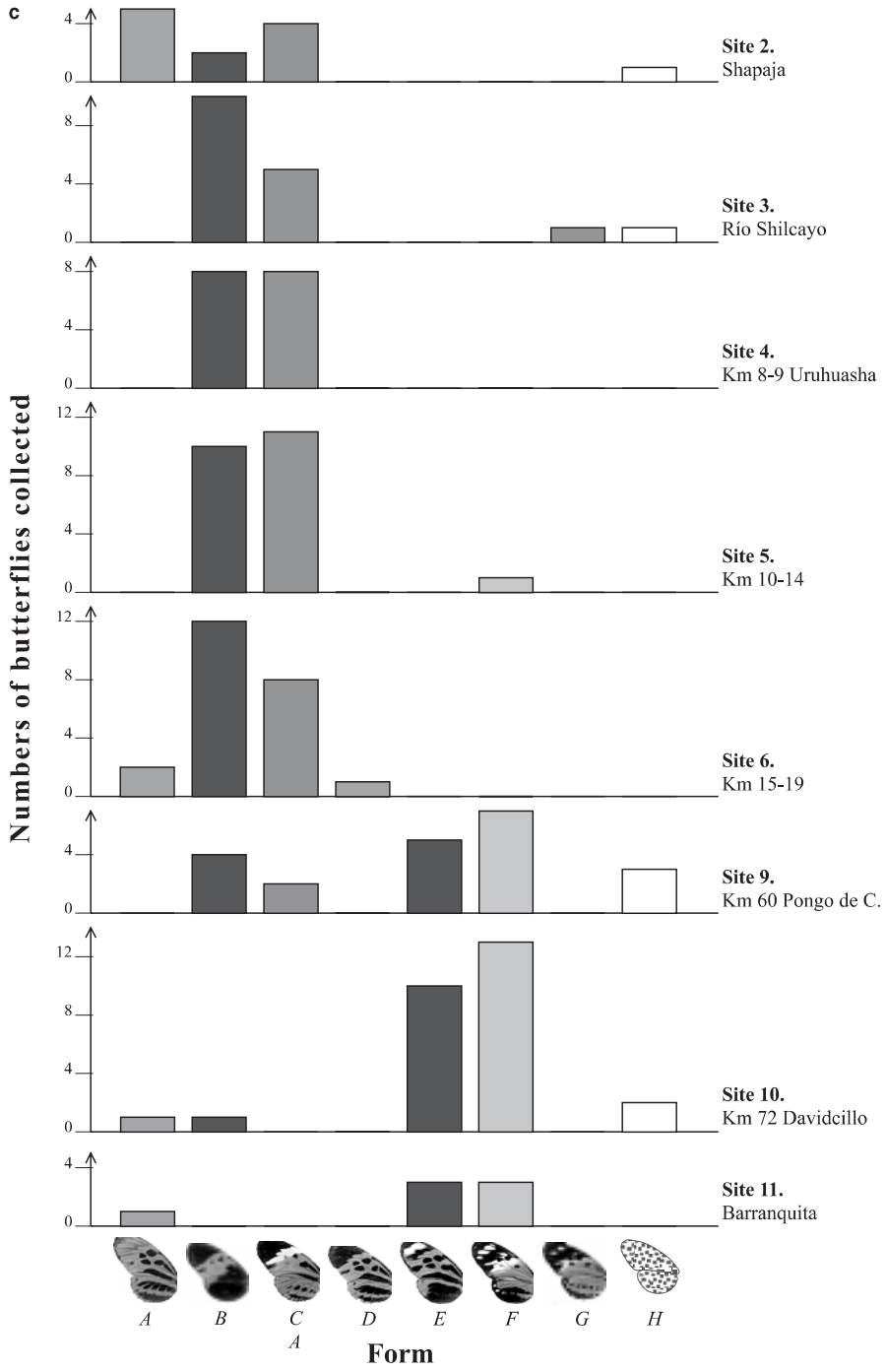

Figure 3. (Continued)

Table 3. F_{ST} estimates, and significance level (Fisher's exact test p value) calculated (a) from colour-pattern markers in *H. numata* and *Melinaea* in 1997, 1998, and 1999, and (b) from enzyme markers (16 polymorphic loci) in *H. numata* in 1999

	1997	1998	1999	Total
(a)				
H. numata	0.287***	0.175***	0.219***	0.202***
Melinaea spp.	0.505***	0.670[a]***	0.676***	0.608***
(b)				
H. numata				
Locus	F_{ST}			
PGM	0.019	NS		
GPI	0.006	NS		
MPI	0.011	NS		
MDH-*s*	0.036	**		
MDH-f	0.000	NS		
GOT-*f*	−0.001	NS		
AK	0.014	NS		
PK	−0.013	NS		
ENOL	0.007	NS		
IDH-*s*	−0.018	NS		
IDH-*f*	0.023	*		
L-A	0.040	**		
G6P	0.000	NS		
3-P	0.035	NS		
6PGD	−0.022	NS		
HBDH	−0.009	NS		
Total	0.008	**		

[a] Low total number of individuals.
*$p < 0.05$
**$p < 0.01$
***$p < 0.001$
NS = not significant

Pairwise comparison of populations showed that although the three areas of endemism are strongly differentiated for colour pattern in *H. numata*, local variation in morph frequencies within regions is weaker and mostly non-significant (Table 4a). Populations are typically composed of two or three common forms in roughly equal frequencies (see Fig. 3), plus several rare although apparently mimetic forms. The Lower Huallaga populations have a mixture of forms *silvana* and *aurora*; the Escalera populations have a mixture of forms *tarapotensis* and *bicoloratus*, with a tendency towards a higher frequency of the darker form *bicoloratus* at higher elevations (Fig. 3); the Upper Huallaga population is composed of the forms *tarapotensis*, *bicoloratus*, and *arcuella*. Intermediate forms may be found in the 'hybrid zones' between these divergent regions. For instance, the forms *lutea* and *staudingeri* are co-dominant heterozygotes and/or recombinants (Joron, 2000), and are especially frequent in

the Pongo de Cainarachi area, which is in the centre of racial hybrid zones described for many other butterflies, including other *Heliconius* species (Mallet, 1989; Mallet and Lamas, unpublished data).

Table 4. Pairwise comparisons of populations for colour-pattern and enzyme markers

Geographical area	Site No	1	2	3	4	5	6	7	8	9	10
(a) Comparisons of colour-pattern frequencies in *H. numata* in 1997, 1998 and 1999.											
1997											
Upper Huallaga	1										
	2	NS									
Escalera	3	**	NS								
	5	NS	NS	NS							
	6	*	NS	NS		NS					
	7	***	*	*		*	NS				
	8	NS	NS	***		***	*	***			
Lower Huallaga	9	*	***	***		***	***	***	*		
	10	*	**	***		***	***	***	NS	NS	
	11	***	***	***		***	***	***	NS	NS	NS
1998											
Upper Huallaga	2										
Escalera	3		***								
	4		***	NS							
	5		**	NS	NS						
	6		*	NS	NS	NS					
	7		NS	NS	NS	NS	NS				
Lower Huallaga	10		***	**	***	***	NS	NS			
	11		***	***	***	***	***	**		NS	
1999											
Upper Huallaga	2										
Escalera	3		**								
	4		**	NS							
	5		**	NS	NS						
	6		*	NS	NS	NS					
Lower Huallaga	9		***	***	***	***	***				
	10		***	***	***	***	***			NS	
	11		**	***	***	***	***			**	**
(b) Comparisons of allozyme allelic frequencies in *H. numata* populations in 1999. The significant difference between the most extreme sites (6 and 10) is the chief cause of structure across the whole sample; the other pairwise comparisons are non-significant.											
1999											
Upper Huallaga	2										
Escalera	3		NS								
	4		NS	NS							
	5		NS	NS	NS						
	6		NS	NS	NS	NS					
Lower Huallaga	9		NS	NS	NS	NS	NS				
	10		NS	NS	NS	NS	*			NS	

Table 4. (Continued)

(c) Comparisons of colour-pattern frequencies in *Melinaea* communities in 1997 and 1999. Comparisons in 1998 are not shown: all but two are non-significant, due to very low sample size.

1997										
Upper Huallaga	1									
	2	NS								
Escalera	3	NS	**							
	5	NS	*	NS						
	6	*	***	***		*				
	7	NS	NS	**		*	NS			
	8	NS	NS	**		*	**	NS		
Lower Huallaga	9	*	NS	**		**	*	NS	NS	
	10									
	11									
1999										
Upper Huallaga	2									
Escalera	3		**							
	4									
	5		***		NS					
	6		**		**	***				
Lower Huallaga	9									
	10									
	11		***		***	***				***

NS = not significant, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

In contrast to the strong spatial variation in morph frequency, overall temporal variability in colour-pattern frequencies was weak and insignificant ($\chi^2 = 66.7$, df $= 140$, $p \approx 1$).

Allozyme variation

Sixteen loci out of the 27 enzyme loci analysed were polymorphic, and show a very different spatial distribution from that of the colour patterns (Table 2). Although overall population differentiation is significant ($p < 0.01$ across all sites and loci), F_{ST} is very low ($F_{ST} = 0.007$). Pairwise comparisons of allele frequencies show that only two populations are significantly divergent from one another (sites 6 and 10, $p = 0.03$); all other pairwise comparisons are non-significant (Table 4b). No linkage disequilibrium was found, and there was no correlation between enzymes and colour pattern; the *Aconitase* locus, known to be linked to a colour gene in *Heliconius erato* (Jiggins *et al*., 1997), was monomorphic.

Community structure in Melinaea *spp.*

We analysed the differentiation of communities with respect to their colour patterns. Overall spatial differentiation in *Melinaea* colour patterns was very high and highly significant ($F_{ST} = 0.60$ averaged over all years, $p < 10^{-5}$; Table 3, and note that one pattern may include two *Melinaea* species). Pairwise comparisons give a picture that is less clear than for *H. numata* (Table 4c): nearby populations may be highly differentiated (e.g. sites 5 and 6: no form in common in the 2 years), while distant ones may be quite similar (e.g. site 2 and sites 7–8). Spatial variability is thus about three times as great in *Melinaea* communities as in *H. numata*. A regional biogeographic trend is present (see Fig. 4), with *M. m. rileyi* and *M. s. cydon* found only in the Lower Huallaga. Sites even within the Escalera area are significantly different from one another: for example, the populations were strongly differentiated between dark-form dominated sites (at high elevations, site 6) and yellow-barred form dominated sites (at low elevation, sites 3–5); this pattern was far less marked in *H. numata*. Overall, more dark forms were caught at higher elevation than pale forms. As with *H. numata*, colour pattern frequencies within sites gave no evidence of temporal fluctuation ($\chi^2 = 1.89$, df = 50, $p \approx 1$).

Local colour-pattern diversity in H. numata and Melinaea spp.

In the 3 years, local colour pattern diversity was higher in *H. numata* populations than in *Melinaea* communities, as shown in Figure 5, but only in 1999 was the difference significant (*t*-tests of comparisons of means: 1997, $t_s = 1.65$, df = 14, *n.s.*; 1998, $t_s = 1.76$, df = 8, *n.s.*; 1999, $t_s = 4.91$, df = 11, $p < 0.001$). Sample sizes are small in *Melinaea*, particularly in 1998, but, because the picture is consistent between years in the two taxa (see above), we lumped the counts for the 3 years; the difference in mean local diversity between the taxa remained highly significant (over all years: $t_s = 3.53$, df = 18, $p < 0.01$). In the Ithomiines, colour-pattern diversity was stronger between than within sites ($F_{ST} > 0.5$), whereas in *H. numata* the reverse is true ($F_{ST} < 0.5$).

Correlation of colour patterns between the two taxa

In most sites the commonest colour pattern in *Melinaea* also tended to be commonest in *H. numata* (see Fig. 6). Four of the six sites with substantial sample sizes show a significant correlation of the colour-pattern frequencies in the two taxa (Table 5 and Fig. 6). The overall correlation is highly significant on these six sites ($\chi^2 = 22.94$, df = 12, $p = 0.002$). The global product-moment

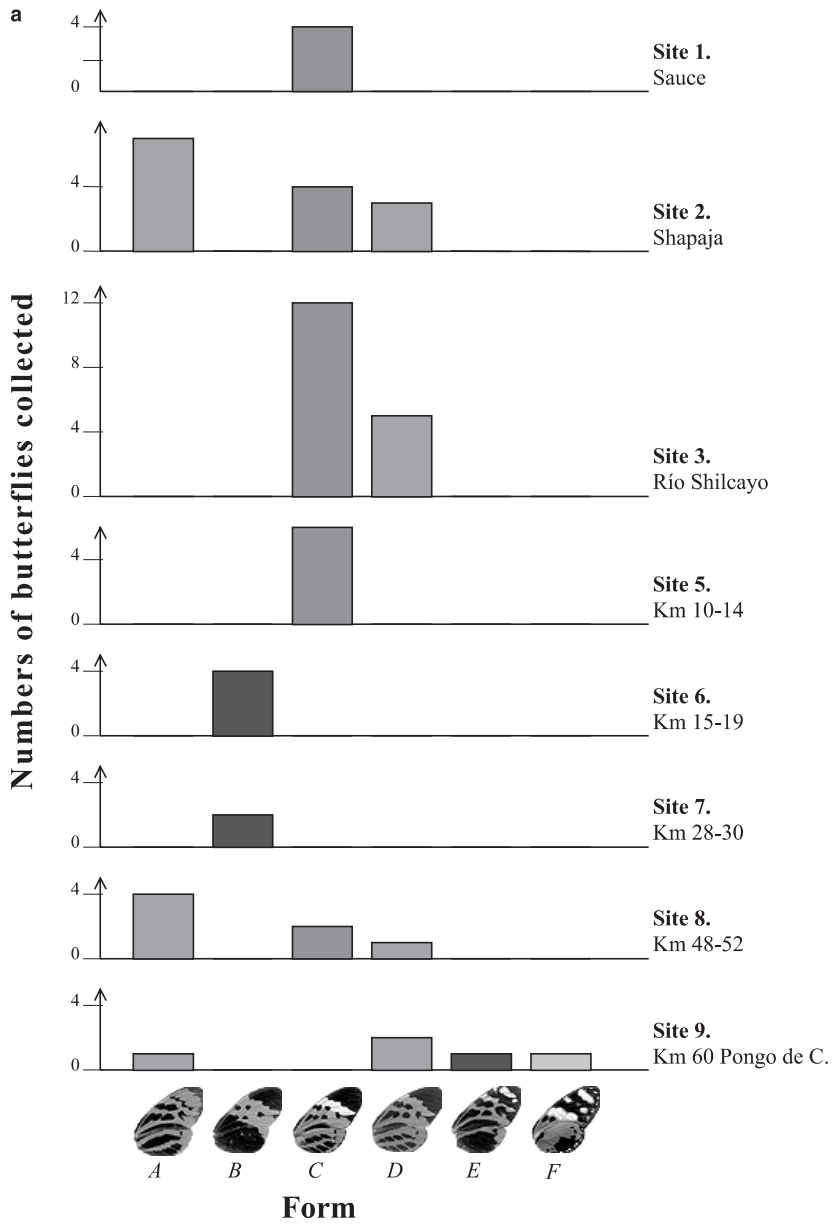

Figure 4. Frequency distribution of *Melinaea* and *Athyrtis* colour-patterns at the same 11 sites as in Figure 3, in the three years: (a) 1997, (b) 1998, (c) 1999. Colour-pattern classes follow those in Figure 3: A: *M. m. phasiana*, B: *M. m. mothone* and *M. i. simulator*, C: *M. m.* ssp. nov and *M. s. tarapotensis*, D: *M. m. hicetas* and *Athyrtis mechanitis*, E: *M. m. rileyi* and *M. s. cydon*, F: *M. l. ludovica*.

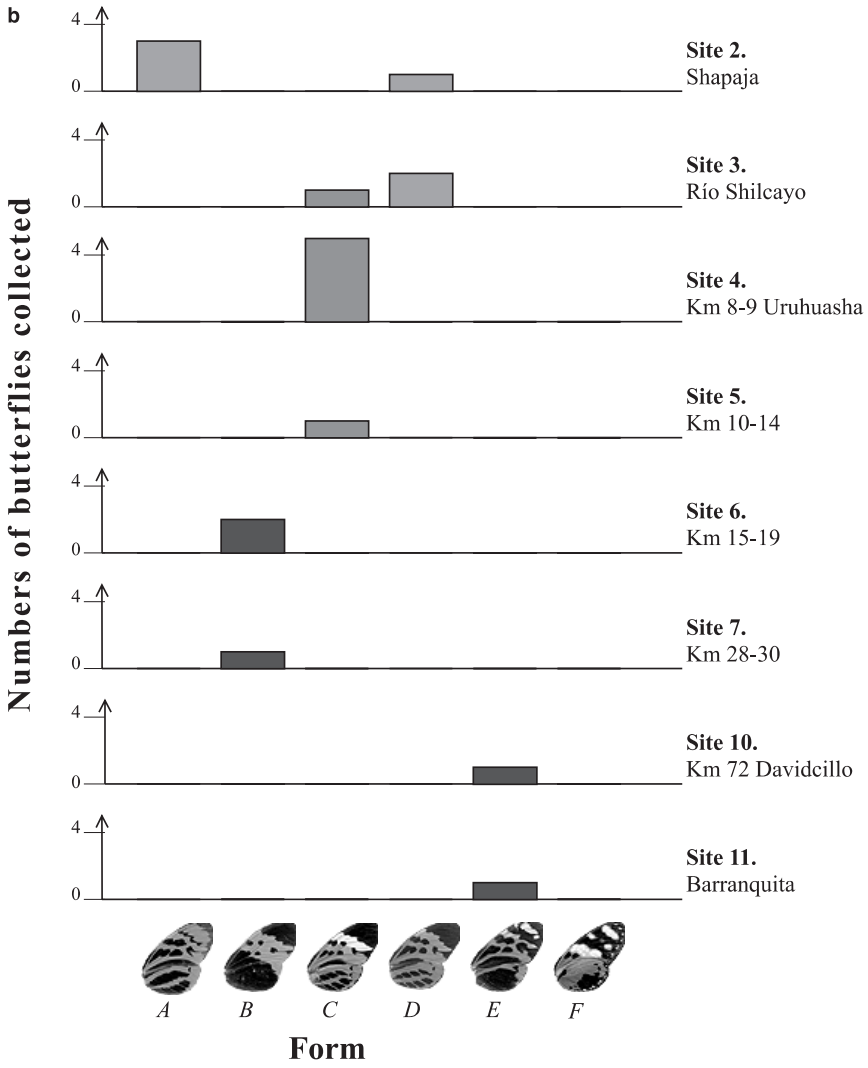

Figure 4. (Continued)

correlation ρ on all 11 sites was 0.524, giving a t-test significance $p = 10^{-4}$. Finally, the permutation test shows this correlation is very highly significant ($p = 4 \times 10^{-4}$). In summary, the correlation is in effect not perfect in all sites, particularly in site no. 8 which happens to be the most diverse (Table 5), but there is a very significant association between the two taxa overall: the *H. numata* colour forms tend to match the locally abundant *Melinaea* colour-patterns (Fig. 6).

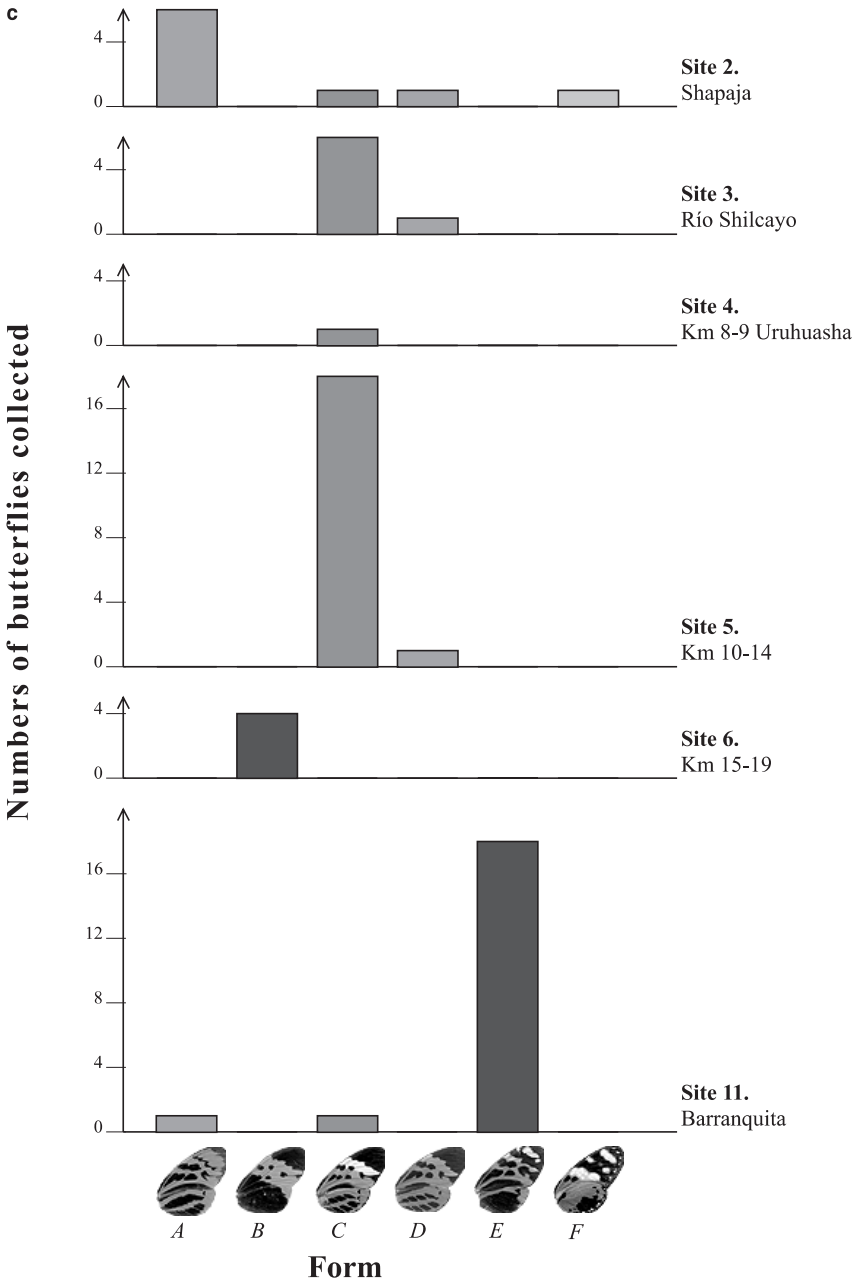

Figure 4. (Continued)

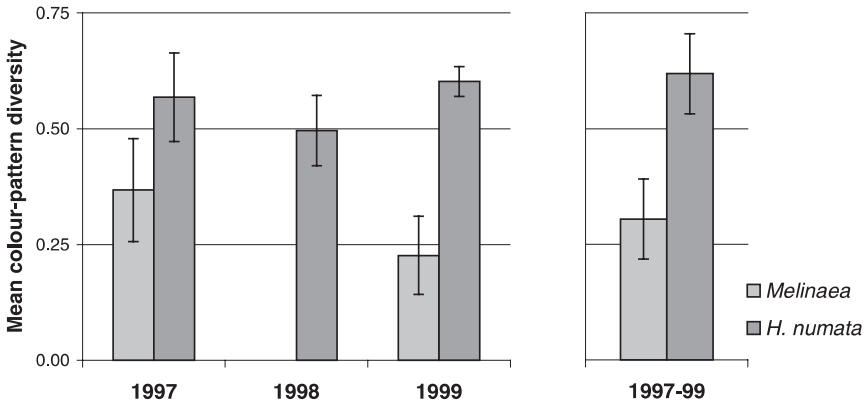

Figure 5. Mean local colour-pattern diversity (Simpson's index). Error bars are standard errors. Significance levels for comparisons: ** $P < 0.01$, *** $P < 0.001$.

Discussion

Population differentiation in H. numata *and* Melinaea *spp*.

On the one hand, colour-patterns in *Melinaea* species are strongly spatially differentiated ($F_{ST} = 0.60$), while local communities have low diversity ($H = 0.28$). Morph frequencies in the Müllerian mimic *H. numata* are correlated with those of *Melinaea*, but, on the other hand are only a third as differentiated between populations ($F_{ST} = 0.20$), and are twice as diverse locally ($H = 0.61$). Presumably, the lower degree of inter-population differentiation in *H. numata* is due to substantial gene flow, because spatial differentiation at putatively neutral molecular markers is negligible over the same sites ($F_{ST} = 0.007$).

This very low population differentiation at molecular markers in *H. numata* may seem surprising considering that this species displays a typical *Heliconius* home range behaviour (Brown and Benson, 1974). Individuals fly within their home ranges predictably every day, as described for other species (Mallet, 1986a, b), and probably do not live in more than one 'mimetic environment' during most of their lifetime. Mark-recapture studies on *H. numata* will be necessary to verify this, but our observations support this explanation. For instance, during an experiment involving insectary breeding of *H. numata*, marked individuals that had escaped the cages regularly returned over the following days to the flowers near the cages. Home range behaviour is also obvious in the field, where known individuals patrol the same path repeatedly. This may seem to contradict the very low degree of population differentiation. However, freshly emerged *Heliconius erato* disperse widely before establishing a home-range, after which they remain for the rest of their life within a few

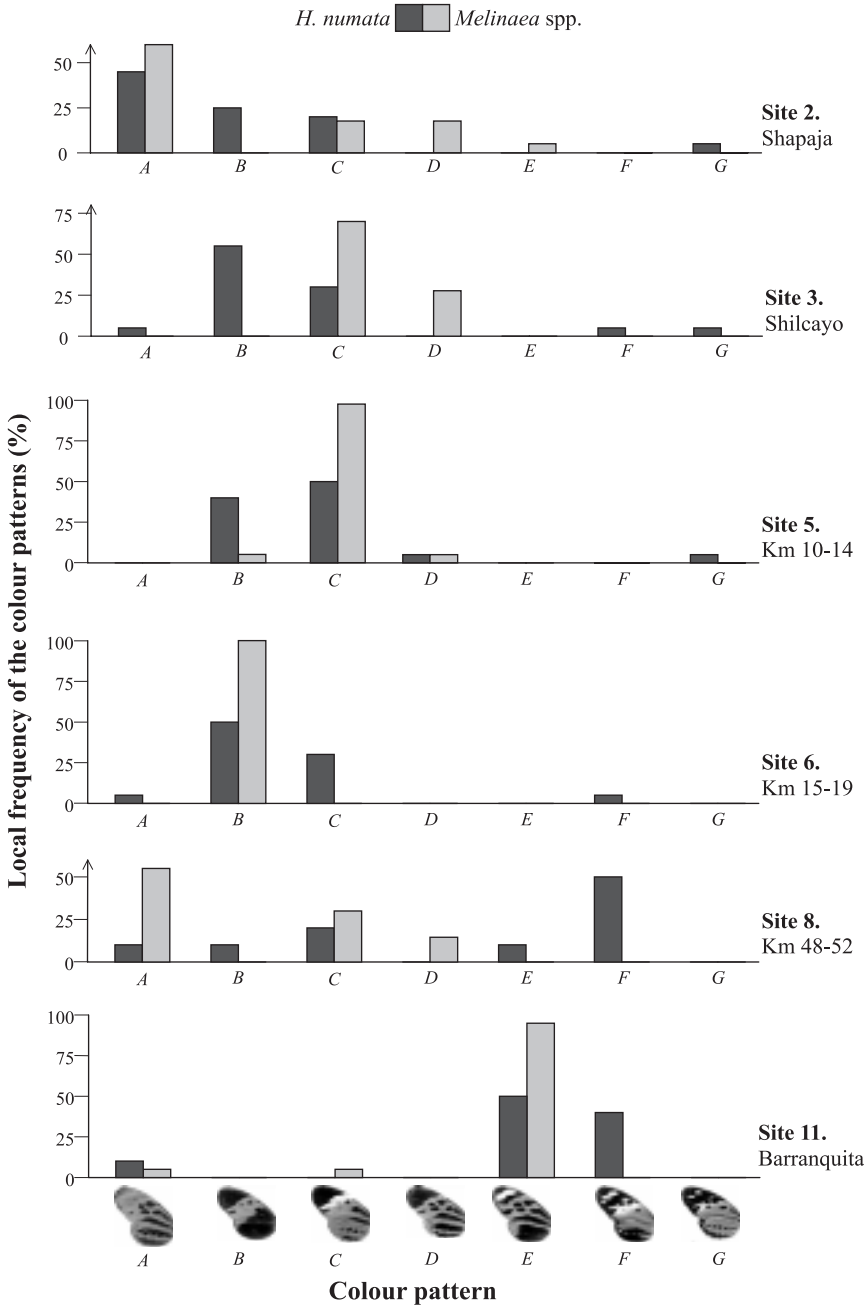

Figure 6. Relative frequencies of the colour patterns summed over the three years in *H. numata* (dark grey) and *Melinaea* (light grey), for the six sites used in the correlation analysis. Colour-pattern classes are as in Figures 3 and 4.

Table 5. Product-moment correlation coefficient (ρ) of colour-pattern frequencies in the two taxa, for six of the populations sampled

Site No	ρ	p-level
2	0.782	0.027
3	0.194	0.644
5	0.756	0.035
6	0.837	0.014
8	−0.044	0.910
11	0.748	0.038
Combined probability		0.002

hundred metres (Mallet, 1986a). High rates of gene flow were confirmed in Peruvian races of *H. erato* and *H. melpomene* by measurements of linkage disequilibrium in inter-racial hybrid zones (Mallet *et al*., 1990, 1998). As in *H. numata*, both *H. erato* and its sister species *H. himera* have very low population differentiation over hundreds of kilometres ($F_{ST} < 0.006$; Jiggins *et al*., 1997). It is likely that *H. numata*, which is larger and can fly faster, has similar or more extensive dispersal. Thus, *H. numata* individuals may rarely live in the immediate area in which they were born, thereby increasing local phenotypic variation (H) and decreasing phenotypic variance between populations (F_{ST}) compared with the selective optimum set by the local mimetic environment (i.e. *Melinaea* and other Ithomiines).

Is the mimetic environment variable?

In support of their hypothesis that spatio-temporal environmental heterogeneity promotes polymorphism in *H. numata*, Brown and Benson (1974) showed that Ithomiine communities were temporally variable in the understorey of an Atlantic forest site in Southern Brazil. However, their data were obtained from a seasonal habitat and did not include *Melinaea*, probably the primary models for *H. numata*, which happened to be rare at this site. Our data are for natural or partially disturbed forest, and concentrate on *Melinaea* and *H. numata*. In addition, although *H. numata* and *Melinaea* spp. clearly co-vary in colour pattern across the neotropics (Brown and Benson, 1974; Brown 1976, 1977, 1979), ours is the first geographic study at the regional level where spatially varying selection and gene flow are expected to maintain polymorphisms.

Our data suggest that mimetic pattern frequencies in *Melinaea* and other Ithomiines can be highly variable within a region, providing a strong selection pressure for local differentiation in *H. numata*. Although the precise reasons for this spatially heterogeneous community structure in *Melinaea* are unknown, it is almost certainly due to ecological factors such as competitive exclusion or

narrow ecological requirements, since there is no reason to suspect that *Melinaea* disperse much less than *Heliconius*. Strong selection due to Müllerian mimicry could in theory destabilise local communities that otherwise might be stable due to niche partitioning, leading to exclusion of rare species within some areas. Spatial variability in habitat may select for particular species of *Melinaea*, which, if abundant enough, may purify the local area of species having different colour-patterns, leading to the patchy distribution of ithomiine mimicry rings we now observe.

Temporal variation in the frequencies of *Melinaea* mimicry rings was not evident in this study. However, if the spatial scale of variation in *Melinaea* is small compared to the dispersal abilities of its co-mimic *H. numata*, the temporal variation in mimetic pressures suggested by Brown and Benson (1974) may not be necessary for the maintenance of polymorphism. Instead, it seems likely that *H. numata* is selected towards different Müllerian models in different sites which are close enough for gene flow to maintain local polymorphism. Indeed, this is exactly the principle explaining hybrid zones of other mimetic species, such as those between geographic races of *H. erato*, where clines are maintained by a selection-migration balance (Mallet *et al.*, 1990). What now needs to be understood is why species of *Melinaea* are spatially segregated, and how this is related to their wing-pattern divergence and speciation.

While temporal fluctuations of selection might aid the maintenance of several forms in *H. numata*, the outcome would depend enormously on the scale of variation. Rapid fluctuations, like those described by Brown and Benson (1974) from south-eastern Brazil, are unlikely to be consistent enough for selection to be effective on these co-mimics; selection would simply be averaged geometrically over time, and inevitably cause a loss of the less-often protected forms of *H. numata*. On the other hand, if fluctuations are on a longer timescale, one would expect one pattern to eventually predominate for long enough to cause the loss of local polymorphism (Dempster, 1955). It is likely that spatial structure and dispersal are the important key factors allowing the polymorphism to be maintained, even if temporal heterogeneity exists. Especially in this largely non-seasonal area, temporal fluctuations therefore do not seem likely to maintain polymorphism in the absence of strong spatial variation in selection pressure (see also Roughgarden, 1979 p. 259; Muko and Iwasa, 2000).

Although we found no significant temporal variability in the 3-year timescale of our study, evidence for longer-term fluctuations in some Ithomiines exists. For example, specimens of *Melinaea s. tarapotensis* were collected during a brief trip to this area in the 1970s (Brown, 1977). A decade later, however, not a single *M. s. tarapotensis* was found by Mallet and Lamas during a continuous period of residence in Tarapoto in 1986–1987. Then in 1997–1999 the form was again caught in reasonable numbers at many of the same

localities visited in the 1980s. Similarly, *Athyrtis mechanitis* was never found in 1986–1987, although it was fairly common in many sites in 1997–1999 and is recorded from Tarapoto earlier this century. Large population fluctuations of *A. mechanitis* also occur in Tambopata and Madre de Dios in southeastern Peru (G. Lamas, personal observations). This temporal variability of large Ithomiines could have very important effects on colour pattern evolution by causing sudden local 'mimetic switches'. However, in this case no mimetic diversity was lost in the process of turnover: *M. menophilus* ssp. nov is a common co-mimic of *H. numata tarapotensis*, and was abundant throughout the 1970s–1990s. Turnover in *A. mechanitis* and *M. s. tarapotensis* therefore probably had a rather restricted effect on the maintenance of the polymorphism in *H. numata* in this case. Nonetheless, these density changes suggest that ithomiine population fluctuations in some areas might have more important effects on mimicry than found here.

Response to selection

The polymorphism in this part of the range of *H. numata* can therefore be interpreted as a mosaic of adjacent populations between which selection is acting in opposing directions. *H. numata* shows a clear response to local selection: in most sites, the first or second most frequent colour-pattern coincides in the two taxa (see Fig. 6). Selection at the local scale is therefore sufficient to pull the *H. numata* morph frequencies towards the local *Melinaea* pattern. For example, there is a higher frequency of *numata* dark forms in areas where dark *Melinaea* species are present (Fig. 6). The lack of spatial structure at allozyme loci suggests that selection acts directly on the colour-pattern supergene, and has little effect on unlinked loci. A similar pattern of variation was found in *H. erato* and *H. melpomene* in the same area: little allozyme structure was found in spite of strong selection on a narrow hybrid zone separating colour-pattern races (Mallet and Barton, 1989; Mallet *et al.*, 1990).

At the scale of biogeographic sub-region, colour-patterns in these taxa also match one another (Figs. 3 and 4). Selection is thus sufficiently strong and consistent over time to create fine-scale biogeographical patterns. This biogeographical pattern of selection is again similar (although with totally different colour patterns) to that in the mimetic pair *H. erato* and *H. melpomene* in this area (Mallet *et al.*, 1990), whose hybrid zones correspond with one of the biogeographic boundaries we find in *H. numata* and *Melinaea*. The *H. numata* and *Melinaea* clines seem less steep than the *erato* and *melpomene* clines (Mallet *et al.*, 1990), but more populations near the centre of the contact zone must be sampled to test this adequately.

The data on Ithomiines collected by Brown and Benson (1974) from southeastern Brazil suggest a similar spatial structure to that in our area. Our data

also show that *H. numata* is found at some sites where no *Melinaea* were found in the 3 years. In sites where both taxa were collected, *Melinaea* was generally found in the humid understorey, while *H. numata* flies in a variety of microhabitats. It appears that *H. numata* is more of a microhabitat generalist than its co-models, which may in part explain why populations of *H. numata* are less spatially structured than communities of *Melinaea*.

Is Heliconius numata *a Batesian (or quasi-Batesian) mimic?*

Some authors have argued that mildly unpalatable mimics can cause an increase in predation on other members of their mimicry ring, so that they would be most fit when rare. It has been suggested that this might select them towards mimetic polymorphism as in Batesian mimicry, even though the species on its own might cause sufficient negative reinforcement to be considered unpalatable and aposematic (Huheey, 1976; Owen and Owen, 1984; Speed, 1993). Speed (1993) and Turner (1995) suggest, for example, that the polymorphic heliconiine *Laparus doris* could fit into this intermediate category, for which Speed coined the term 'quasi-Batesian mimicry'. The existence of this intermediate form of mimicry is, however, under discussion (Turner *et al*., 1984; Mallet and Joron, 1999; Mallet, 2001; Speed, 2001) and depends strongly on untested assumptions about learning and forgetting by predators (Speed and Turner, 1999; Mallet and Joron, 1999).

Ithomiine and danaine butterflies are highly unpalatable, while Heliconiines are generally considered less well defended (Beccaloni, 1997a) in accordance with one aspect of the quasi-Batesian mimicry hypothesis. *Heliconius numata* has several other characteristics similar to those of typical Batesian mimics: it is wary, has a fast escape flight, is solitary, is not particularly tough (by ithomiine standards), and is a polymorphic mimic. It would be tempting to consider this species a quasi-Batesian or classic Batesian mimic, were it not in a genus whose members are so notoriously unpalatable that they act as models in several mimicry rings (as noted by Bates, 1862: 550). The palatability of *H. numata* has not been assessed directly, but its sister species *H. ismenius* was tested in a feeding experiment using wild-caught jacamars (*Galbula* spp.) as predators (Chai, 1996): the young birds learnt very quickly and subsequently rejected the butterfly on sight. Similarly, Srygley and Chai (1990) showed that, although Heliconiines as a group had only intermediate levels of unpalatability, *Heliconius* fell into the same 'unpalatable' category as most of the Ithomiines: in particular, the silvaniforms were almost never attacked (only 0–5% of *H. hecale* and *H. ismenius* were attacked after predators learnt), and even *L. doris*, supposedly a quasi-Batesian candidate (Turner, 1995), was always ignored. Jacamars, which were seen in this study and during previous work in our area (Mallet and Barton, 1989), are among the few birds that regularly catch

butterflies and other flying insects from perches in light gaps in the forest. Because light gaps are also much frequented by *H. numata*, jacamars are among the more important potential predators of *Heliconius*. Yet they clearly consider silvaniform *Heliconius* to be highly distasteful.

In addition, because spatial variability in selection is enough, quasi-Batesian mimicry is not necessary to maintain polymorphism, and polymorphism on its own is poor evidence for the existence of quasi-Batesian mimicry. Mathematical models show that, in a spatially structured landscape with a high variance in selection pressure, polymorphisms can be stable on a local scale (Joron and Iwasa, in preparation).

Scarce models and abundant mimics: is this the true picture?

Heliconius numata seems more common in our collections than its *Melinaea* co-mimics (Table 1), so one might wonder who is mimicking whom. Although less defended than *Melinaea*, the more numerous *H. numata* could well be the model because the effect of number-dependence is very powerful (Müller, 1879; see Joron and Mallet, 1998, for a reprint of Müller's original theory; also Mallet, 2001). Yet *H. numata* is a polymorphic species, while the colour pattern diversity in *Melinaea* is largely at the community level this leaves little doubt that *H. numata* has converged (or 'adverged', *sensu* Turner, 1995; Mallet, 2001) towards the Ithomiines rather than vice-versa. So the problem remains: why has *H. numata* become polymorphic when it is more common than its models?

The relative scarcity of *Melinaea* may be illusory due to the ecology and habits of the two genera. *Melinaea* butterflies spend much of the day idly in the understorey, typically perching on a leaf ≤1 m from the ground (Joron, 2000) and flying weakly when disturbed; *H. numata*, in contrast, are very active and more conspicuous in open areas where they can be caught easily. At other times of day, *Melinaea* females lay eggs in the canopy on epiphytes such as *Markea* and *Juanulloa* (Brown, 1987). Male *Melinaea* forage for pyrrolizidine alkaloids on *Tournefortia* (Boraginaceae) vines and Eupatoriae (Compositae), and congregate in the mornings around these plants in the canopy (K. Brown, J. Mallet, personal observations). This activity in the canopy is rarely witnessed or sampled by ground-dwelling vertebrates such as ourselves, but is likely to be very important in the education of birds that frequent the canopy, particularly in the early mornings when birds are most active (Mallet, 1986b; Mallet and Gilbert, 1995). Most of the interesting *Melinaea* activity therefore probably occurs high in the canopy in the morning and late evening (possibly because the humidity is high enough to for them to venture into the open without dehydrating). Perhaps most importantly, the majority of our collections were made in partially disturbed habitats easily accessible from roads. Here, the tall canopy

emergent trees typically harbouring *Juanulloa* and *Markea* have largely been felled, while the sun-loving *Passiflora* hosts of *Heliconius* become common. Almost certainly, therefore, our sampling underestimates the abundance of *Melinaea* relative to *H. numata*.

The presence of other ithomiine genera and co-mimics might have additional effects on selection due to the spatial variation in mimicry. Although easily distinguished from the larger *Melinaea*, *Mechanitis* and several *Hypothyris* species can be very common locally, and, due to number dependent selection, may themselves play an important role in educating predators. For these species, it is again true that some sites in the Escalera area are dominated by yellow-banded colour patterns somewhat like smaller versions of *Melinaea menophilus* ssp. nov. (e.g. *Mechanitis polymnia*, *Tithorea harmonia*; sites 2–5); high-altitude sites are dominated by melanic colour-patterns very much like that of *Melinaea m. mothone* (e.g. *Mechanitis mazaeus deceptus*, *Hypothyris meterus meterus*; sites 6 and 7); while Amazonian sites have yellow-apex colour-patterns such as that of *Melinaea s. cydon* (e.g. *Hypothyris anastasia anastasina*; sites 10–11; Joron, Mallet and Lamas, unpublished data; see also Figure 1). The response observed in *H. numata* to mimetic selection of the most common *Melinaea* pattern is therefore probably augmented by the correlated colour patterns of many other Ithomiines, even though the mimicry of these other species is less exact than that in Figure 1.

Comparison with another polymorphic Müllerian system

The best-known case of polymorphic Müllerian mimicry is probably the *Danaus*/*Acraea* system in tropical East Africa: *Danaus chrysippus* and *Acraea encedana* mimic one another and are polymorphic over an area the size of Europe (this area is, however, small compared with the entire range of *D. chrysippus*). The area of polymorphism may represent a wide and possibly ancient hybrid zone (Smith *et al.*, 1997, 1998) for this highly mobile butterfly, in which long-distance gene flow is balanced by only weak selection in the vast area of overlap (Mallet and Joron, 1999). Despite this, it was shown that *A. encedana* and *D. chrysippus* forms tend to fluctuate in concert in the Kampala region of Uganda (Owen *et al.,* 1994), suggesting, again, that selection for mimicry is still acting; however, it is not clear whether this correlation is always detectable locally; given its migratory habits, *D. chrysippus* is anyway not expected to show much fine-scale spatial structure. In the *Melinaea*/*H. numata* system, several species of *Melinaea* are involved, and mimetic selection should be much stronger on these rarer species. Gene flow is probably an order of magnitude less ($\sim$10 km per generation vs. $\sim$100 km for *Danaus*). The dynamics of the different colour-patterns could therefore be much more constrained by local micro-habitat adaptation than the forms of *Danaus* and *Acraea*.

Conclusions and perspectives

Our study clearly shows that the colour patterns of *H. numata* are mimetic, and correlate locally with strong spatial variation in the genera *Melinaea* and *Athyrtis*. The degree of spatial variability is perhaps surprising across such a small region (approx. 60×30 km, Fig. 2). Given the species or near-species status of the *Melinaea* forms, and the clear intraspecific polymorphism of the *numata* forms, it is most likely that *H. numata* is a mimic of *Melinaea* rather than the reverse (see also Mallet, 2001). The wide dispersal and lower spatial differentiation of the *H. numata* forms, when compared with the strong differences between sites for *Melinaea* forms, suggest that *H. numata* frequencies evolve towards those of *Melinaea*, albeit imperfectly due to high rates of gene flow in *H. numata*. We found no evidence for temporal variability in mimicry rings over a 3-year period, but there were marked changes over a period of decades in two large Ithomiines, *Athyrtis mechanitis* and *Melinaea satevis tarapotensis*. Temporal variability in model species, while not causing important changes in mimetic environment in this study, is therefore a potential selective force at other times and places.

Overall, our data strongly support Brown and Benson's (1974) hypothesis that spatio-temporal variability in the mimetic environment (*Melinaea*) promotes polymorphism in *H. numata*. Brown and Benson (1974) documented substantial temporal variability in the abundance of a variety of Ithomiines, but did not document much change in *Melinaea* themselves. Brown and Benson did document broad spatial variability in the mimicry rings across South America; our study has now demonstrated that the mimetic environment provided by ithomiine models is highly spatially variable, even on a 'micro-biogeographic' scale. Given the single locus inheritance of the different forms of *H. numata*, the mimetic polymorphism is explained by means of multiple selection-migration clines at a single locus. These are the same factors, in fact, as those which explain narrow hybrid zones between races of *H. erato* and *H. melpomene*. Similar factors may explain other puzzling Müllerian mimetic polymorphisms, for instance in *Danaus chrysippus* and *Acraea encedon* in Africa (Smith *et al.*, 1993, 1997, 1998) and in Heliconiines such as *Heliconius cydno* and *L. doris* in South America (Linares, 1997; Kapan, 2001; Mallet, 2001).

Acknowledgements

We are grateful to Moisés Abanto, Gorky Valencia and Andrew Brown who helped collecting butterflies, and to Rainer Schulte who improved our local mobility. John Allen and an anonymous reviewer provided useful comments

on the manuscript. INRENA granted permission to export biological material. The work was funded by financial aid from the Institut Klorane and from the Centre National de la Recherche Scientifique to MJ and by a collaborative grant from the French Ministry of Foreign Affairs and the British Council to JM and MJ. This is publication ISEM-2000-82 of the Institut des Sciences de l'Evolution, Montpellier.

References

Aitkin, M., Anderson, D., Francis, B. and Hinde, J. (1989) *Statistical Modelling in GLIM*. Oxford University Press, Oxford, UK.

Bates, H.W. (1862) Contributions to an insect fauna of the Amazon valley. Lepidoptera: Heliconidae. *Trans. Linn. Soc. Lond.* **23**, 495–566.

Beccaloni, G.W. (1997a) Ecology, natural history and behaviour of ithomiine butterflies and their mimics in Ecuador (Lepidoptera: Nymphalidae: Ithomiinae). *Trop. Lepid.* **8**, 103–124.

Beccaloni, G.W. (1997b) Vertical stratification of ithomiine butterfly (Nymphalidae: Ithomiinae) mimicry complexes: the relationship between adult flight height and larval host-plant height. *Biol. J. Linn. Soc.* **62**, 313–341.

Brown, K.S. (1976) An illustrated key to the silvaniform *Heliconius* (Lepidoptera: Nymphalidae) with descriptions of new subspecies. *Trans. Am. Entom. Soc.* **102**, 373–484.

Brown, K.S. (1977) Geographical patterns of evolution in Neotropical Lepidoptera: differentiation of the species of *Melinaea* and *Mechanitis* (Nymphalidae, Ithomiinae). *Syst. Entomol.* **2**, 161–197.

Brown, K.S. (1979) *Ecologia Geográfica e Evolução nas Florestas Neotropicais*. Universidade Estadual de Campinas, Campinas, Brazil.

Brown, K.S. (1987) Chemistry at the Ithomiinae-Solanaceae interface. *Ann. Miss. Bot. Gard.* **74**, 359–397.

Brown K.S. and Benson, W.W. (1974) Adaptive polymorphism associated with multiple Müllerian mimicry in *Heliconius numata*. *Biotropica* **6**, 205–228.

Chai, P. (1996) Butterfly visual characteristics and ontogeny of responses to butterflies by a specialized tropical bird. *Biol. J. Linn. Soc.* **59**, 37–67.

Clarke, C.A., Clarke, F.M.M. and Gordon, I.J. (1995) Mimicry and other controversial topics in East African Lepidoptera. *J. East Afr. Nat. Hist.* **84**, 3–18.

Cook, L.M. and Brower, L.P. (1969) Observations of polymorphism in two species of heliconiine butterflies from Trinidad, West Indies. *Entomologist* **102**, 125–128.

Dempster, E.R. (1955) Maintenance of genetic heterogeneity. *Cold Spring Harb. Symp. Quant. Biol.* **70**, 25–32.

Huheey, J.E. (1976) Studies of warning coloration and mimicry. VII. Evolutionary consequences of a Batesian–Müllerian spectrum: a model for Müllerian mimicry. *Evolution* **30**, 86–93.

Jiggins, C.D., McMillan, W.O., King, P. and Mallet, J. (1997) The maintenance of species differences across a *Heliconius* hybrid zone. *Heredity* **79**, 495–505.

Joron, M. (2000) Warning colour and Müllerian mimicry: the diversification puzzle. *Doctoral Thesis*, Département Biologie, Evolution, Environnement, Université de Montpellier II, Montpellier, France, pp. 225.

Joron, M. and Mallet, J. (1998) Diversity in mimicry: paradox or paradigm? *Trends Ecol. Evol.* **13**, 461–466.

Kapan, D. (2001) Three-butterfly system provides first field test of Müllerian mimicry. *Nature* **409**, 338–340.

Lamas, G. (1982) A preliminary zoogeographical division of Peru, based on butterfly distributions (Lepidoptera, Papilionoidea). In G.T. Prance (ed.) *Biological Diversification in the Tropics*. Columbia University Press, New York, USA, pp. 336–357.

Linares, M. (1997) Origin of neotropical mimetic diversity from a three-way hybrid zone of *Heliconius cydno* butterflies. In H. Ulrich (ed.) *Tropical Diversity and Systematics. Proceedings of the International Symposium on Biodiversity and Systematics in Tropical Ecosystems, Bonn, 1994*. Zoologisches Forschunginstitut und Museum Alexander Koenig, Bonn, Germany, pp. 93–108.

Mallet, J. (1986a) Dispersal and gene flow in a butterfly with home range behaviour: *Heliconius erato* (Lepidoptera: Nymphalidae). *Oecologia* **68**, 210–217.

Mallet, J. (1986b) Gregarious roosting and home range in *Heliconius* butterflies. *Natl. Geogr. Res.* **2**, 198–215.

Mallet, J. (1989) The genetics of warning colour in Peruvian hybrid zones of *Heliconius erato* and *H. melpomene*. *Proc. R. Soc. London B Biol. Sci.* **236**, 163–185.

Mallet, J. (2001) Causes and consequences of a lack of coevolution in Müllerian mimicry. *Evol. Ecol.* **13**, 777–806.

Mallet, J. and Barton, N.H. (1989) Strong natural selection in a warning colour hybrid zone. *Evolution* **43**, 421–431.

Mallet, J., Barton, N.H., Lamas, G., Santisteban C.J., Muedas M.M. and Eeley, H. (1990) Estimates of selection and gene flow from measures of cline width and linkage disequilibrium in Heliconius hybrid zones. *Genetics* **124**, 921–936.

Mallet, J. and Gilbert, L.E. (1995) Why are there so many mimicry rings? Correlations between habitat, behaviour and mimicry in *Heliconius* butterflies. *Biol. J. Linn. Soc.* **55**, 159–180.

Mallet, J. and Joron, M. (1999) Evolution of diversity in warning color and mimicry: polymorphisms, shifting balance and speciation. *Annu. Rev. Ecol. Syst.* **30**, 201–233.

Mallet, J., Korman, A., Heckel, D.G. and King, P. (1993) Biochemical genetics of *Heliothis* and *Helicoverpa* (Lepidoptera: Noctuidae) and evidence for a founder event in *Helicoverpa zea*. *Ann. Entom. Soc. Am.* **86**, 189–197.

Mallet, J., Longino, J.T., Murawski, D., Murawski, A. and Simpson de Gamboa, A. (1987) Handling effect in *Heliconius*: where do all the butterflies go? *J. Anim. Ecol.* **56**, 377–386.

Mallet, J., McMillan, W.O. and Jiggins, C.D. (1998) Mimicry and warning color at the boundary between races and species. In D. Howard and S. Berlöcher (eds) *Endless Forms: Species and Speciation*. Oxford University Press, New York, USA, pp. 390–403.

Muko S. and Iwasa, Y. (2000) Species coexistence by permanent spatial heterogeneity in a lottery model. *Theor. Popul. Biol.* **57**, 273–284.

Müller, F. (1879) *Ithuna* and *Thyridia*: a remarkable case of mimicry in butterflies. *Trans. Entomol. Soc. Lond.* **1879**, xx-xxix.

Owen, D.F., Smith, D.A.S., Gordon, I.J. and Owiny, A.M. (1994) Polymorphic Müllerian mimicry in a group of African butterflies: a re-assessment of the relationship between *Danaus chrysippus, Acraea encedon* and *Acraea encedana* (Lepidoptera: Nymphalidae). *J. Zool.* **232**, 93–108.

Owen, R.E. and Owen, A.R.G. (1984) Mathematical paradigms for mimicry: recurrent sampling. *J. Theor. Biol.* **109**, 217–247.

Raymond, M. and Rousset, F. (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenism. *J. Hered.* **86**, 248–249.

Raymond, M. and Rousset, F. (2000) GENEPOP 3.2. Updated version: ftp://ftp.cefe.cnrs-mop.fr/genepop/(March 2000).

Richardson, B.J. Baverstock, P.R. and Adams, M. (1986) *Allozyme Electrophoresis. A Handbook for Animal Systematics and Population Studies*. Academic Press, San Diego, USA.

Roughgarden, J. (1979) *Theory of Population Genetics and Evolutionary Ecology: an introduction*. MacMillan Publishers, New York, USA.

Smith, D.A.S., Owen, D.F., Gordon, I.J. and Owiny, A.M. (1993) Polymorphism and evolution in the butterfly *Danaus chrysippus* L. (Lepidoptera: Danainae). *Heredity* **71**, 242–251.

Smith, D.A.S., Gordon, I.J., Depew, L.A. and Owen, D.F. (1998) Genetics of the butterfly *Danaus chrysippus* (L.) in a broad hybrid zone, with special reference to sex ratio, polymorphism and intragenomic conflict. *Biol. J. Linn. Soc.* **65**, 1–40.

Smith, D.A.S., Owen, D.F., Gordon, I.J. and Lowis, N.K. (1997) The butterfly *Danaus chrysippus* (L.) in East Africa: polymorphism and morph-ratio clines within a complex, extensive and dynamic hybrid zone. *Zool. J. Linn. Soc.* **120**, 51–78.

Sokal, R.R. and Rohlf, F.J. (1995) *Biometry. The Principles and Practice of Statistics in Biological Research*. W.H. Freeman & Co., New York, USA.

Speed, M.P. (1993) Müllerian mimicry and the psychology of predation. *Anim. Behav*. **45**, 571–580.

Speed, M.P. and Turner, J.R.G. (1999) Learning and memory in mimicry: II. Do we understand the mimicry spectrum?. *Biol. J. Linn. Soc*. **67**, 281–312.

Speed, M.P. (2001) Batesian, quasi-Batesian or Müllerian mimicry? Theory and data in mimicry research. *Evol. Ecol*. **13**, 755–776.

Srygley, R.B. and Chai, P. (1990) Predation and the elevation of thoracic temperature in brightly colored Neotropical butterflies. *Am. Nat*. **135**, 766–787.

Turner, J.R.G. (1995) Mimicry as a model for coevolution. In R. Arai, M. Kato and Y. Doi (eds) *Biodiversity and Evolution*. The National Science Museum Foundation, Tokyo, Japan, pp. 131–150.

Turner, J.R.G., Kearney, E.P. and Exton, L.S. (1984) Mimicry and the Monte Carlo predator: the palatability spectrum and the origins of mimicry. *Biol. J. Linn. Soc*. **23**, 247–268.

Wynne, I.R., Loxdale, H.D. and Brookes, C.P. (1992) Use of cellulose acetate system for allozyme electrophoresis. In R.J. Berry, T.J. Crawford and G.M. Hewitt (eds) *Genes in Ecology*. Blackwell, Oxford, UK, pp. 494–499.

Wynne, I.R., and Brookes, C.P. (1992) A device for producing multiple deep-frozen samples for allozyme electrophoresis. In R.J. Berry, T.J. Crawford and G. M. Hewitt (eds) *Genes in Ecology*. Blackwell, Oxford, UK, pp. 500–502.