

Original Article

Geographically variable mate preferences shed light on the processes maintaining inversion polymorphism

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ABSTRACT

An important question in evolution is to understand the mechanisms that maintain phenotypic diversity, despite selection that should drive homogeneity. For example, selection by predators may promote the convergence of colour patterns among defended prey, resulting in the formation of mimetic communities. However, certain aposematic species, such as the Neotropical butterfly *Heliconius numata*, are polymorphic. In *H. numata*, wing pattern polymorphism, which is associated with chromosomal inversions, may be maintained via disassortative mating preferences, thought to favour the co-occurrence of individuals with different mimicry phenotypes in Peru. To test whether environmental variation due to geography influences mate choice, here we investigate the occurrence of disassortative mating among the two coexisting forms of *H. numata* in French Guiana, and its potential role in the maintenance of this polymorphism. Our experimental approach demonstrates that the two forms display weak and slightly asymmetrical disassortative mate preferences. Modelling and simulations suggest that this pattern of mate preference alone is not sufficient to maintain polymorphism, and predict the loss of the choosiest form, unless this form enjoys a survival advantage. In this aposematic species, such an advantage could arise from mimicry, but further studies into the benefits of mimicry and predator generalization are needed to test this hypothesis. More importantly, our results suggest that the balance between selective forces influencing polymorphism may vary across geographical and ecological contexts, and this warrants further study.

Keywords: aposematism; colour pattern; *Heliconius numata*; heterozygote advantage; Lepidoptera; mate choice; mimicry; modelling; Müllerian mimicry; phenotypic diversity

INTRODUCTION

Polymorphism is the occurrence of at least two forms of a trait within a population that is not due to recurrent mutation (Baird et al. 1852, Ford 1945, Hori 1993, Wang et al. 2013) and is an expression of biological variation found in a myriad of characters across the tree of life. Classical examples of traits showing discrete polymorphisms include the pin vs. thrum flower morphologies in *Primula*, favouring cross-pollination between flower types (Piper and Charlesworth 1986), or colours in the side-blotched lizard, *Uta stansburiana*, where male colour influences mating success via rock–paper–scissor interactions with other males and social/territory status (Sinervo and Lively 1996,

Hazard et al. 2019). Cases of polymorphism such as these are important in the study of evolutionary mechanisms, such as selection or drift, to explain the formation and stable coexistence of distinct characters.

Balancing selection has been found to maintain polymorphism in some cases (Charlesworth 2000, Llaurens et al. 2017) and one powerful mechanism responsible for this is negative frequency-dependent selection (NFDS). Batesian mimicry (e.g. Turner 1987) or disassortative mating (e.g. Hughes et al. 2013) are examples of interactions generating an advantage to rarer forms in polymorphic populations, i.e. NFDS. However, polymorphism is not predicted to occur when the fitness of a variant improves

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with its frequency, i.e. under positive frequency-dependent selection (PFDS). A classical example of PFDS is found with selection acting on warning signals and Müllerian mimicry. Indeed, prey with chemical defences benefit from predator avoidance if they display a warning signal familiar to local predators, so the protective value of a signal increases with their local abundance. According to Müller's theory (Müller 1879) and ample experimental evidence (e.g. Speed et al. 2000, Pinheiro 2003), unpalatable prey species are subject to reduced predation when they share the same warning signal, which produces uniformity in phenotype within and between species (Symula et al. 2001, Williams 2007, Boppré et al. 2017). Positive frequency-dependence operates locally and many aposematic species display geographical mosaics of warning signals, adapting locally via mutualistic mimicry (Müllerian mimicry) with local communities of signalling species. Despite local selection against variation in warning signals, exceptions exist; for example, strawberry poison frogs *Oophaga pumilio* (Richards-Zawacki et al. 2013), ladybird beetles *Harmonia axyridis* (Osawa and Nishida 1992), certain species of butterflies in the genus *Heliconius* (Brown and Benson 1974, Ogilvie et al. 2021), or male wood tiger moths *Arctia plantaginis* (Gordon et al. 2015, Henze et al. 2018) display multiple forms present simultaneously in a given locality.

In aposematic species, local adaptation is commonly associated with assortative mating and reproductive isolation (Schemske and Bradshaw 1999, Reynolds and Fitzpatrick 2007, Merrill et al. 2014), which does not explain the maintenance of variation within populations (but see, for example, McClure et al. 2019). By contrast, disassortative mating, which describes preferential mating between individuals with different phenotypes, promotes heterozygosity at the loci controlling mating cues and may contribute to the maintenance of polymorphism. Empirical examples linking the maintenance of polymorphism with disassortative mating preferences are not common but exist in a diversity of plant and animal systems (Thornycroft 1975, Llaurens et al. 2008, Stoeckel et al. 2008, Takahashi and Hori 2008). For example, the white-throated sparrow (*Zonotrichia albicollis*) presents a colour and behavioural polymorphism with two different forms (white and tan) forming mixed-phenotype pairs (Thornycroft 1975). Disassortative mating is expected to evolve in response to different types of selection acting on the ecological forms (Chouteau et al. 2017), when heterozygote advantage acts on the mating cue, or as a result of both a heterozygote advantage and other selection pressures (Maisonneuve et al. 2021a, 2021b).

Here we compare the patterns of mate choice in two populations of *Heliconius numata* to investigate whether disassortative mating operates in the same way in two different contexts and to ascertain how it contributes to the maintenance of phenotypic diversity in both populations. This chemically defended Neotropical nymphalid butterfly displays a number of discrete wing patterns linked to an inversion polymorphism on chromosome 15. Inverted and standard DNA segments at this locus do not recombine with each other, so captured genes are locked together, and segregate as a supergene. This supergene locus is present as three different allelic classes: a standard, ancestral noninverted allele, and two distinct derived inversion alleles (derived) (Joron et al. 2011, Jay et al. 2021). The number of aposematic wing-pattern phenotypes varies across the geographical

range of this species as a result of local adaptation to mimetic communities (Elias and Joron 2015). In the Peruvian Andes, where all three allelic classes are present, a large number of forms coexist at varying frequencies and the maintenance of polymorphism appears to result from an equilibrium between local mimicry benefits and disassortative mating (Chouteau et al. 2016, 2017). By contrast, in French Guiana only two forms of *H. numata* coexist in similar frequency (*H. n. numata*, associated with chromosomal inversions, and *H. n. silvana*, displaying the standard chromosomal form).

We use this population to test the hypothesis that disassortative mating is a key factor explaining the maintenance of polymorphism, or to what extent other forms of selection are also needed. To address this, we combine experimental and modelling approaches. First, mate choice experiments were used to evaluate the strength and direction of mating patterns. In French Guiana, the *H. n. numata* form is associated with an inversion carrying deleterious mutations, so we expect this form to display disassortative mating, as in populations from Peru. By contrast, the recessive allele *silvana* is associated with the standard arrangement and should not have accumulated deleterious mutations. Our prediction is that disassortative preferences are expected only if this form is under negative selection by predation, as seen in Peru.

We then implement a modelling approach to explore the consequences of the observed mate choice pattern on polymorphism. In the event of asymmetry in mating patterns, the modelling provides a means to predict the asymmetry below which polymorphism is no longer maintained, and therefore enables us to test if the observed mate preferences alone should impact the maintenance of polymorphism. Our results show that the mating patterns differ markedly across the range of this species, and that the balance between the selective forces influencing polymorphism may vary across geographical and ecological contexts.

MATERIALS AND METHODS

Butterfly rearing

Butterflies were collected in French Guiana in 2011 and 2021 in eastern French Guiana. The main collection sites were Petit-Saut (5°08'57.8"N, 52°53'43.9"W), Montagne des Chevaux (4°42'06.0"N, 52°23'41.0"W), and Kaw (4°32'49.4"N, 52°08'38.8"W). Frequencies of each form in 2011 ($N = 21$) and 2021 ($N = 31$) for *H. n. numata* and *H. n. silvana* were 57% and 61%, and 43% and 39%, respectively. Collected individuals were kept in $8 \times 2 \times 2.5$ -m outdoor insectaries under ambient conditions at Petit-Saut (in 2011) and in Cayenne (in 2021), French Guiana, where all rearing was carried out. Butterflies were provided with sugar water solution and lantana flowers (Verbenaceae) as nourishment, and various plants of the genus *Passiflora* (*P. edulis*, *P. seratodigitata*, *P. laurifolia*, and *P. cirrhiflora*) for oviposition. Eggs and larvae were reared on these same host plants and newly emerged males and females were kept in separate cages so that females remained unmated.

Mate choice experiment

Captive-bred individuals were used for mate choice experiments (in 2011 $N = 30$ trials; in 2021 $N = 23$ trials); virgin females were

used within the first 10 days (mean = 5.6 days, SD = 4.3 days), and males were at least 15 days old (mean = 33.3 days, SD = 15.0 days) to ensure that they were mature and responsive. Two males and two females, one of each form, were introduced into a 4 × 2 × 2.5-m cage and were checked hourly for 24 h or until the first copulation occurred. Unmated females were checked for spermatophores at the end of the trials to ensure no mating went unnoticed. Unmated individuals were occasionally re-used once, with at least 48 h between trials. We obtained 53 mating events in two experimental seasons; 30 in 2011 and 23 in 2021. Results for both years were not found to be statistically different ($\chi^2 = 0.0817$, $P = .775$) and were therefore pooled (Table 1).

Table 1. The number of mating events obtained in the tetrad mating trials between the two forms of *Heliconius numata*, *H. n. silvana* and *H. n. numata*, from the population of French Guiana: the first value in parentheses was obtained in 2011 and the second in 2021.

	Male <i>silvana</i>	Male <i>numata</i>
Female <i>silvana</i>	17 (10 + 7)	15 (7 + 8)
Female <i>numata</i>	13 (8 + 5)	8 (5 + 3)

Statistical analysis

Overall mating preferences were analysed using both a goodness of fit test (chi-square analysis), and a maximum likelihood statistical significance test which can be more precise. This test investigates which theoretical model of mate choice best explains the observed mating. For each model, theoretical probabilities of mating for each possible pair were compared to observed probabilities of mating (as seen in Edwards 1972; McMillan et al. 1997, Jiggins et al. 2001, Naisbit et al. 2001, McClure et al. 2019) (Fig. 1). The theoretical probability of mating occurring can be calculated with the log-likelihood expression as per Edwards (1972):

$$\log L(P_{ij}) = \sum (m_{ij} * \log_e(\hat{P}_{ij}) + (N_{ij} - m_{ij}) * \log_e(1 - \hat{P}_{ij}))$$

where N and m are the total number of trials and the number of observed mating, respectively, for the pair ij . \hat{P}_{ij} is the estimated probability of mating for the pair ij under the tested hypothesis (be it assortative, disassortative, or random). The G-stat [$G = 2\Delta(\log L)$], which asymptotically follows a χ^2 distribution, enabled us to test for differences in the mating probability across trials by maximizing the log likelihood. Based on this distribution, a P -value can be calculated, representing the deviation between the null model (random mating in our case) and the tested model. A significant P -value means that the tested model explains the dataset better than random mating.

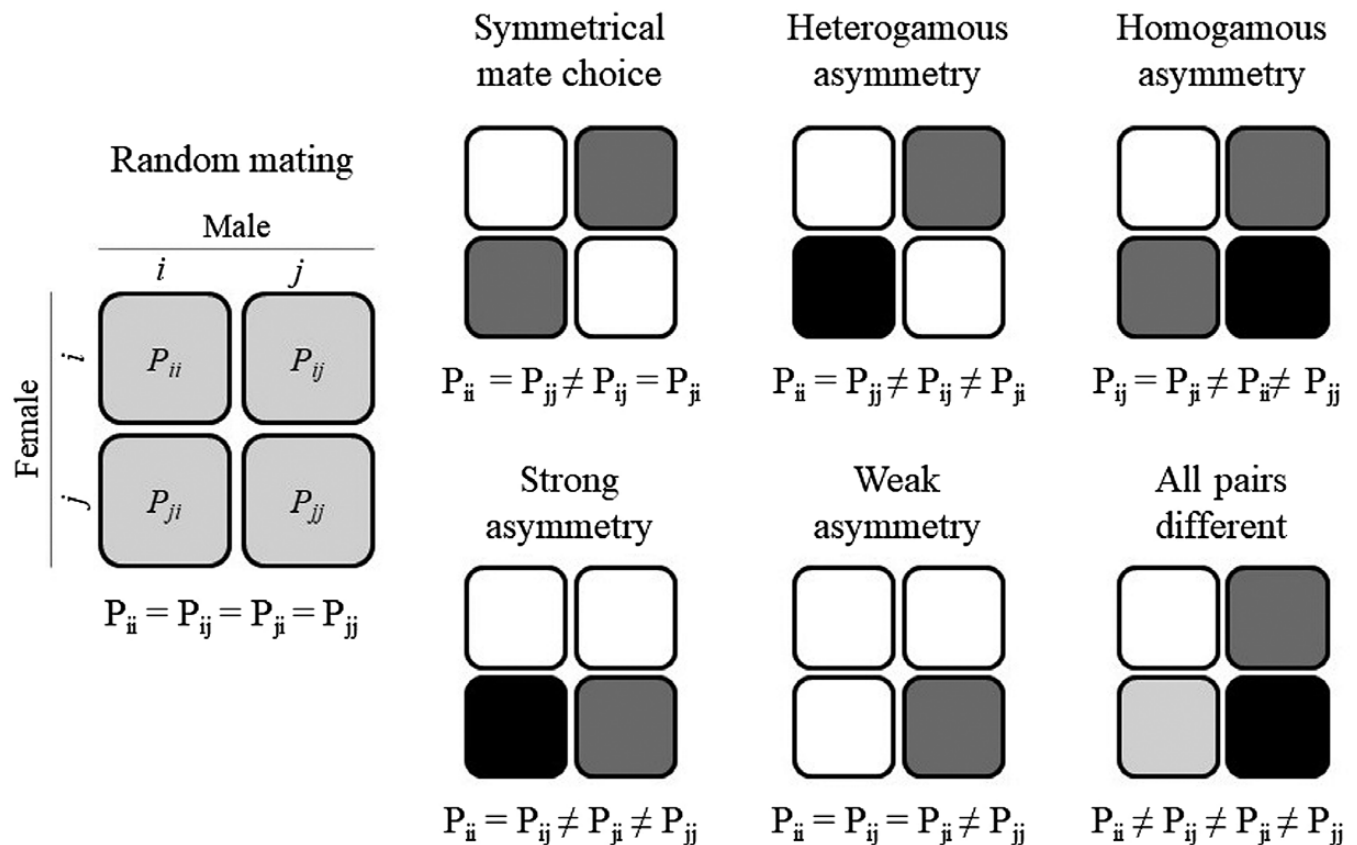


Figure 1. A graphical overview of possible mating preferences between two forms of *Heliconius numata*, *H. n. silvana* (i) and *H. n. numata* (j), in French Guiana, using likelihood analyses. Possible mating combinations within a type (homogamous) relative to between types (heterogamous) are shown. P_{ij} correspond to the probability of a female with the phenotype ' i ' mating with a male of phenotype ' j '; equations represent mathematically the relationship between the probability of mating of all possible pairs of forms in each model of mate choice.

Modelling

Agent-based and deterministic models were used to investigate the predicted effect of mate choice asymmetry on the maintenance of polymorphism within a population. By employing both modelling approaches, we were able to examine our hypothesis from two distinct perspectives. First, at the individual level, we simulated how behaviour affects phenotypic frequency using an agent-based model. This model simulated individuals with one diploid locus and two alleles, ancestral and inverted. Each individual began as a larva and became an adult after one time step (i.e. each time step corresponds to the time necessary for larval development from egg to adult (cf. Fig. 2)). Adults lived for multiple time steps, so generations were overlapping. Population size was limited by introducing a carrying capacity for the larvae. Each genotype was associated with a larval survival probability to simulate genetic disadvantage resulting from deleterious mutations trapped within inversions. Each adult phenotype was associated with a probability of survival, calculated as $S = 1 - P_{sil}/P_{num}$ where P_{sil} and P_{num} were the survival probabilities of adult *silvana* and *numata* forms respectively, dependent on local adaptation to the mimetic community. At each time step, all individuals that survived to adulthood could reproduce. Females mated only once. Each unmated female was paired with a randomly chosen male until mating occurred. Mating probability was based on phenotypes and was simulated for a preference gradient from assortative to disassortative. Asymmetry was calculated as: $\alpha = 1 - P_{num/num}/P_{sil/sil}$, where $P_{num/num}$ and $P_{sil/sil}$ were the probabilities of assortative mating. Females were tested with different males until mating to avoid introducing a direct cost of mate choice. All mated females could go on to produce offspring if they survived. The genotype of offspring was generated by combining one allele from each parent randomly chosen with a probability of 1/2. Seven different values of preference asymmetry were used ($\alpha = [0; 0.1; 0.2; 0.3; 0.4; 0.5; 0.6]$), and for each asymmetry value, six values of survival advantage were used ($S = [0; 0.2; 0.4; 0.6; 0.8; 1]$). Each combination of parameter was replicated 100 times. For all simulations, the initial state corresponded to a state where the recessive

allele (i.e. corresponding to the standard arrangement in the case of an inversion polymorphism) is fixed and 100 mutants were introduced at the first step of the simulation. Equilibrium was calculated as the mean frequency of the last 50 time steps. The model was implemented in Python 3 (v.3.10.9) and results were analysed with R (v.4.2.0).

Deterministic model

In addition to an agent-based model, a continuous-time deterministic model that tracks the population density of each genotype was implemented. Individuals were assumed to die independently of predation at a rate of d , and competition c for resources limits population size. Individuals can also die as a result of predation p , and predator learning generates positive dependent selection. Following [Joron and Iwasa \(2005\)](#), predator learning increased with an individual's unpalatability λ , assumed to be equal for all individuals. We considered the effect of disassortative mating on changes in phenotype frequency following [Maisonneuve et al. \(2021a\)](#). Based on the results obtained in the mating experiments, different levels of disassortative mating associated with each phenotype (ρ_{an} and ρ_{inv}) were used. We also considered the relative cost of choosiness c_r , i.e. the probability of a female remaining unmated after rejecting a male.

Genotyping

Genotyping of individuals at the wing pattern supergene was assessed for the French Guiana population by genotyping 31 wild-caught individuals captured in 2011 using the procedure described in [Chouteau et al. \(2017\)](#). Supergene genotypes were assessed by genotyping the *H. numata* orthologue of HM00025 (*cortex*) (GenBank accession no. FP236845.2), a major candidate gene influencing melanism and mimicry in this region ([Nadeau et al. 2016](#)). Genotypic differences at *cortex* are in linkage disequilibrium with genetic variation across the entire inversions and fully associated with wing pattern ([Joron et al. 2011](#), [Jay et al. 2021](#)). Genotypes were derived from indel and single-nucleotide polymorphisms. Briefly, PCR assays were carried out

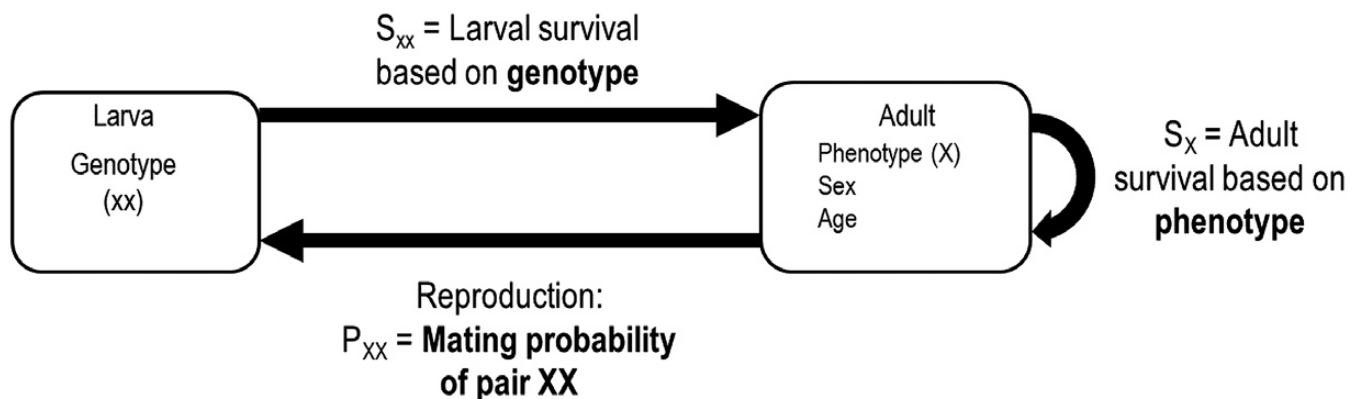


Figure 2. Overview of the individual-based simulation model. Squares represent the two different possible states for each individual and the information associated with each state. Arrows show the transition between states; each larva is associated with a survival probability of S_{xx} based on its genotype. If the larva survives, it becomes an adult with a survival probability S_x based on its phenotype. After transformation the adult can mate with a partner, and the pair of adults have a probability of mating P_{xx} based on the phenotype of both adults. Finally, a mated female can produce between 0 and R larva at each time step.

Table 2. Statistical analyses of the mating patterns observed using tetrad experiments both in French Guiana and in Peru (Chouteau *et al.* 2017), comparing the six models presented in Fig. 1 to random mating; for each pair, the best model is highlighted in bold.

Hypothesis comparison	<i>numata</i> and <i>silvana</i> (French Guiana)	<i>bicoloratus</i> and <i>silvana</i> (Peru)	<i>tarapotensis</i> and <i>silvana</i> (Peru)	<i>bicoloratus</i> and <i>tarapotensis</i> (Peru)
Random mating vs. Symmetrical mate choice	$P = .634$ (G-stat = 0.23)	$P = 5.313 \times 10^{-6}$ (G-stat = 20.72)	$P = 8.619 \times 10^{-5}$ (G-stat = 15.42)	$P = 8.619 \times 10^{-5}$ (G-stat = 15.42)
Random mating vs. Heterogamous asymmetry	$P = .810$ (G-stat = 0.42)	$P = 1.321 \times 10^{-5}$ (G-stat = 22.47)	$P = 2.140 \times 10^{-6}$ (G-stat = 26.11)	$P = 2.024 \times 10^{-5}$ (G-stat = 21.62)
Random mating vs. Homogamous asymmetry	$P = .103$ (G-stat = 4.54)	$P = 1.201 \times 10^{-5}$ (G-stat = 22.66)	$P = .000329$ (G-stat = 16.04)	$P = .000188$ (G-stat = 17.15)
Random mating vs. Strong asymmetry	$P = .102$ (G-stat = 4.56)	$P = .148$ (G-stat = 3.82)	$P = 2.000 \times 10^{-6}$ (G-stat = 26.24)	$P = 1.261 \times 10^{-5}$ (G-stat = 22.56)
Random mating vs. Weak asymmetry	$P = .046$ (G-stat = 3.99)	$P = .0759$ (G-stat = 3.15)	$P = .00354$ (G-stat = 8.50)	$P = .000911$ (G-stat = 11.00)
Random mating vs. All pairs different	$P = .192$ (G-stat = 4.74)	$P = 2.055 \times 10^{-5}$ (G-stat = 24.40)	$P = 6.709 \times 10^{-6}$ (G-stat = 26.73)	$P = 3.413 \times 10^{-5}$ (G-stat = 23.35)

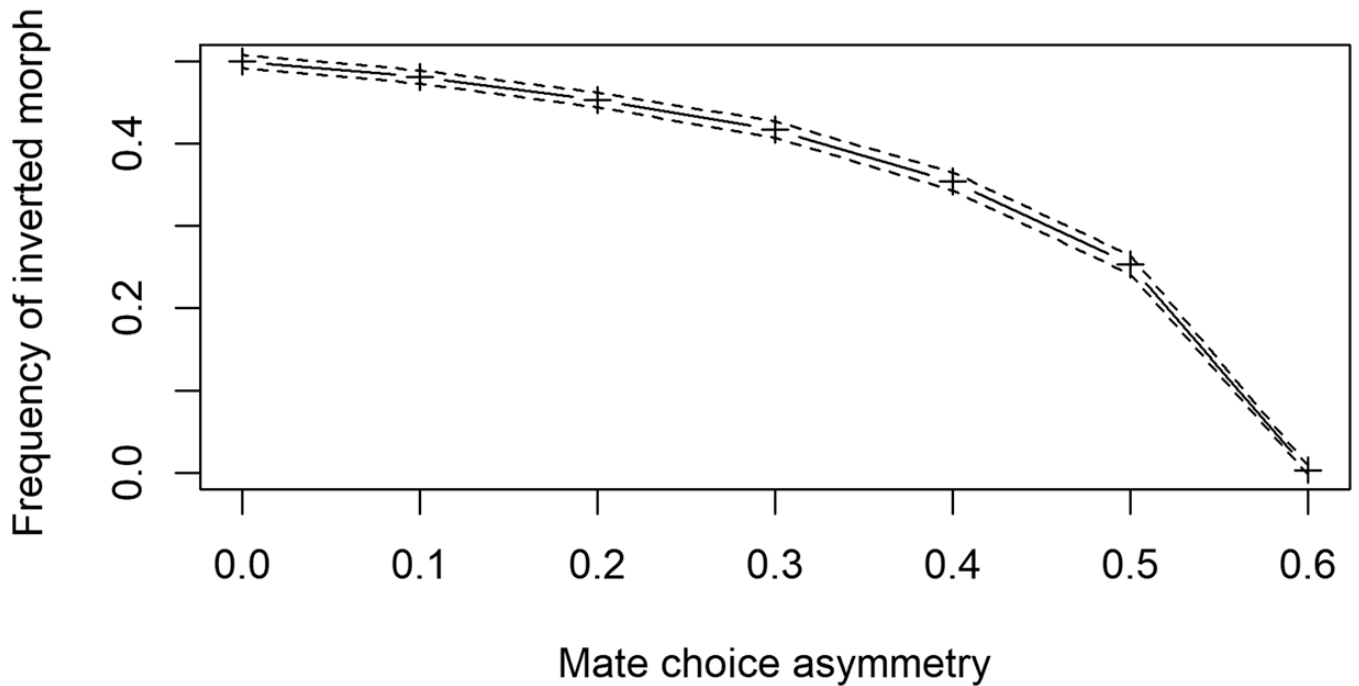


Figure 3. Mean (line) and standard deviation (dash line) of the frequency of the *numata* form at equilibrium for different values of asymmetry with 100 simulations each. Mate choice asymmetry is calculated as $\alpha = 1 - P_{num/num}/P_{sil/sil}$ with $P_{num/num}$ and $P_{sil/sil}$ the probability of mating of an homogamous pair respectively of the *numata* and *silvana* form. The final point, characterized by mate choice asymmetry $\alpha = 0.6$, represents the frequency at which equilibrium is reached with the observed probability of mating from the tetrad experiments.

in a 25- μ L final volume composed of 2 μ L of genomic DNA, 1 \times DreamTaq buffer, 200 μ M dNTP, 2.5 μ M forward primers (5'-CGCAACGTTATCGCTAGATAGGTTTCG3'), 2.5 μ M reverse primers-1 (5'-TAGTGTTAAAGCGAAAGCAC3'), and 2.5 μ M reverse primers-2 (5'-AANGCGAAASMACTGAYAACACG3'), and 0.25 units of DreamTaq DNA polymerase (ThermoScientific). Cycling temperatures were used as follows: 94°C for 2 min, followed by 20 cycles at 94°C for 30 s; a stepdown from 60 to 50°C for 30 s and 72°C for 90 s, followed by another 20 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 90 s; and a final elongation at 72°C for 15 min. Insertions/deletions in the HM00025 orthologue were scored by comparing

PCR product size on a 1.5% agarose gel, on which allele P_{sil} yielded an ~600-bp amplicon, and P_{num} an ~800-bp amplicon. Deviations from Hardy-Weinberg equilibrium were assessed using a chi-square test.

RESULTS

Mate choice experiment

Using a goodness of fit test, mating within and between *H. numata* forms was not found to be significantly different from random ($\chi^2 = 0.121, P = .728$), although this may be the result of a weak effect and/or low sampling size. As such, a log-likelihood

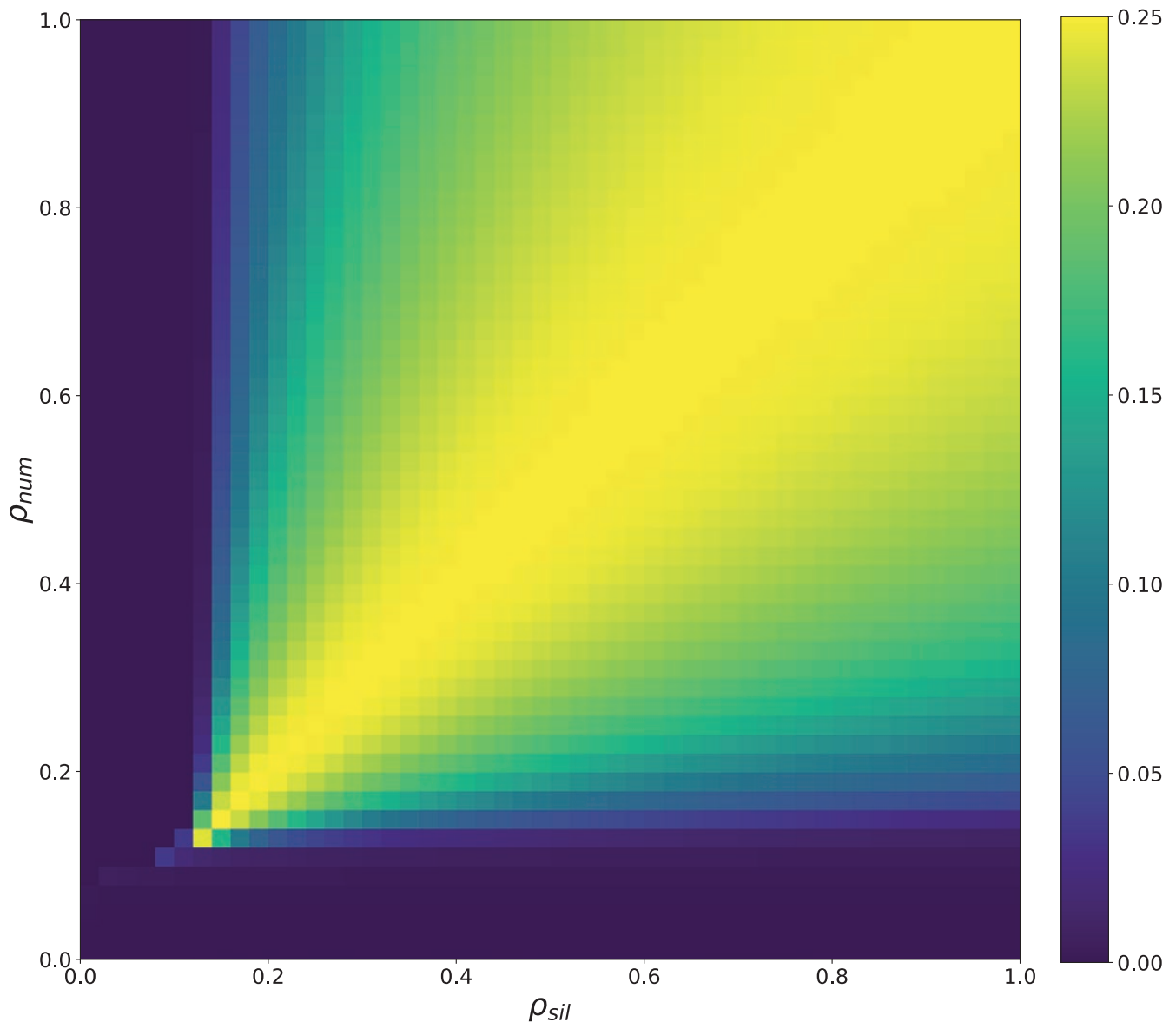


Figure 4. Phenotypic diversity at equilibrium as a function of mate choice of both forms (represented by ρ_{inv} for the mate choice of the form linked to an inversion and ρ_{an} for the mate choice of the form associated with the ancestral allele). The colour scale corresponds to the phenotypic diversity at equilibrium; it is the product of the phenotypic frequency of both forms. Higher values of phenotypic diversity ($P = 0.25$) correspond to an equilibrium whereby the two phenotypes are at equal frequency (0.5) and lower value ($P = 0$) to an equilibrium where one form has disappeared. Higher values occurred only when mate choice is similar for both phenotypes. Mate choice observed in the French Guiana population corresponds to $\rho_{inv} = 0.47$ and $\rho_{an} = 0$ (red cross).

analysis was also used to ascertain if some patterns of mate choice could explain the observed data better than random mating (Fig. 1; Table 2), and weak asymmetry was found to better explain the data ($G\text{-stat} = 3.99$, $P = .046$) and maximize the likelihood (Supporting Information Table S1). This suggests that the ancestral form *silvana* is probably not choosy, while the derived (inversion) form *numata* tends to prefer *silvana* (i.e. asymmetrical disassortative mating with lower likelihood of *numata* \times *numata* mating). Using the same analysis on the data obtained in Peru by Chouteau et al. (2017), we found that for one of the pairs, *silvana* \times *bicoloratus*, the best fitting model is a symmetrical mate choice ($G\text{-stat} = 20.72$, $P < .001$); both forms display the same mate preferences. For the two other pairs, *tarapotensis* \times *silvana*

and *tarapotensis* \times *bicoloratus*, the best fitting model is one with strong asymmetry ($P < .001$, $G\text{-stat} = 26.24$ and $P < .001$, $G\text{-stat} = 22.56$). In this particular model, one form, *tarapotensis*, does not show clear preferences, but the forms *silvana* and *bicoloratus* show strong disassortative preferences.

Agent-based and deterministic models

Both models show a decrease in the frequency of the *numata* form with increasing mate choice asymmetry (Fig. 3). In fact, the *numata* form disappears altogether when *silvana* shows no mate preference. As in the agent-based model, the deterministic model found that maintaining phenotypic diversity was possible only when mate choice was equal for both phenotypes.

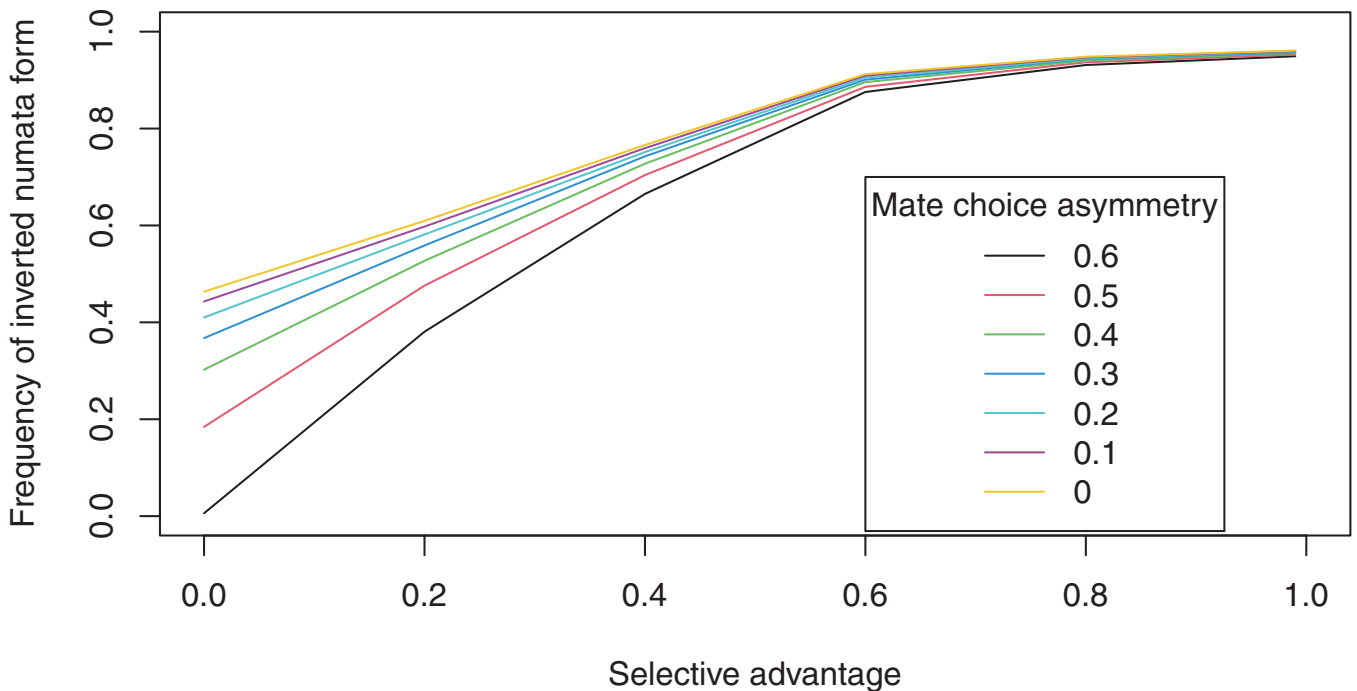


Figure 5. The effect of a selective advantage (calculated as $S = 1 - P_{sil}/P_{num}$, with P_{sil} and P_{num} the survival probability of the *silvana* and *numata* form) on the frequency of the *numata* form with different values of mate choice asymmetry using the agent-based model. In these simulations, we use the same values of asymmetry as in Fig. 3 but vary the value of selective advantage given to the choosy form.

When mate choice is strongly asymmetrical, as observed in our study system, phenotypic diversity decreases and the choosy phenotype disappears (i.e. a single phenotype remains, corresponding to the blue area in Fig. 4). Similarly, if mate choice were to be completely absent (Fig. 4, ρ between 0 and 0.15), polymorphism is not a stable state and one form was found to eventually dominate as a result of genetic drift. However, when mate choice is similar for the two phenotypes, phenotypic diversity is at its maximum (in yellow in Fig. 4); this state of equilibrium, whereby the two phenotypes are present at similar frequencies, is what is observed in French Guiana despite an asymmetrical mate preference. To explain the continued presence of polymorphism despite asymmetrical mate preferences, we hypothesize that another factor may be compensating for its effect.

Survival advantage

When a survival advantage was added and allowed to vary for the choosy form, this compensated for the effect of asymmetry in mate preference; as a result, polymorphism was maintained at equilibrium (Figs 5 and 6). The continued presence of polymorphism in the population of French Guiana, where only one of two forms is choosy, suggests that some as yet unmeasured advantage for this phenotype is probably operating.

Deviation from Hardy–Weinberg equilibrium

Two supergene alleles are present in French Guiana: P^{sil} , bearing the ancestral arrangement, and P^{num} , displaying inversions. Allelic frequency in the 31 individuals sampled are respectively 0.435 and 0.565. Genotype frequencies do not deviate from Hardy–Weinberg equilibrium ($\chi^2 = 6$, $P = .1991$), suggesting that the

Guianese populations do not exhibit an excess of heterozygotes, in contrast to the Peruvian populations.

DISCUSSION

Polymorphism, the coexistence of multiple phenotypes within a population, requires some form of balancing selection to be maintained. In traits under positive frequency-dependent selection, such as warning coloration, polymorphism is unexpected but sometimes observed. This suggests mechanisms acting on polymorphism other than PFDS alone, and characterizing these is invaluable in improving our understanding of the processes shaping diversity. Here we test whether individuals of a polymorphic butterfly in the genus *Heliconius* display disassortative mating and whether this is sufficient to maintain polymorphism. In *H. numata*, wing pattern polymorphism is controlled genetically by a locus on chromosome 15 (Joron et al. 2011), with three polymorphic chromosomal inversions forming three main allelic classes. Inverted and standard segments do not recombine with each other, so captured genes are physically linked, and segregate as a supergene. Under suppressed recombination, deleterious mutations occurring on the segment that is nearly always heterozygous are not purged and accumulate in the inversion (Jay et al. 2021), forming a genetic load with a strong deleterious effect (65% reduction in larval survival) in homozygotes for the inversion (Jay et al. 2021). Selection against individuals homozygous for the inversion could therefore explain the evolution of disassortative mate preferences (Maisonneuve et al. 2021a).

In Peru, where a large number of different forms and three chromosomal arrangements coexist within populations, female mate preferences were previously found to be disassortative for all forms tested, including the *silvana* form associated with the

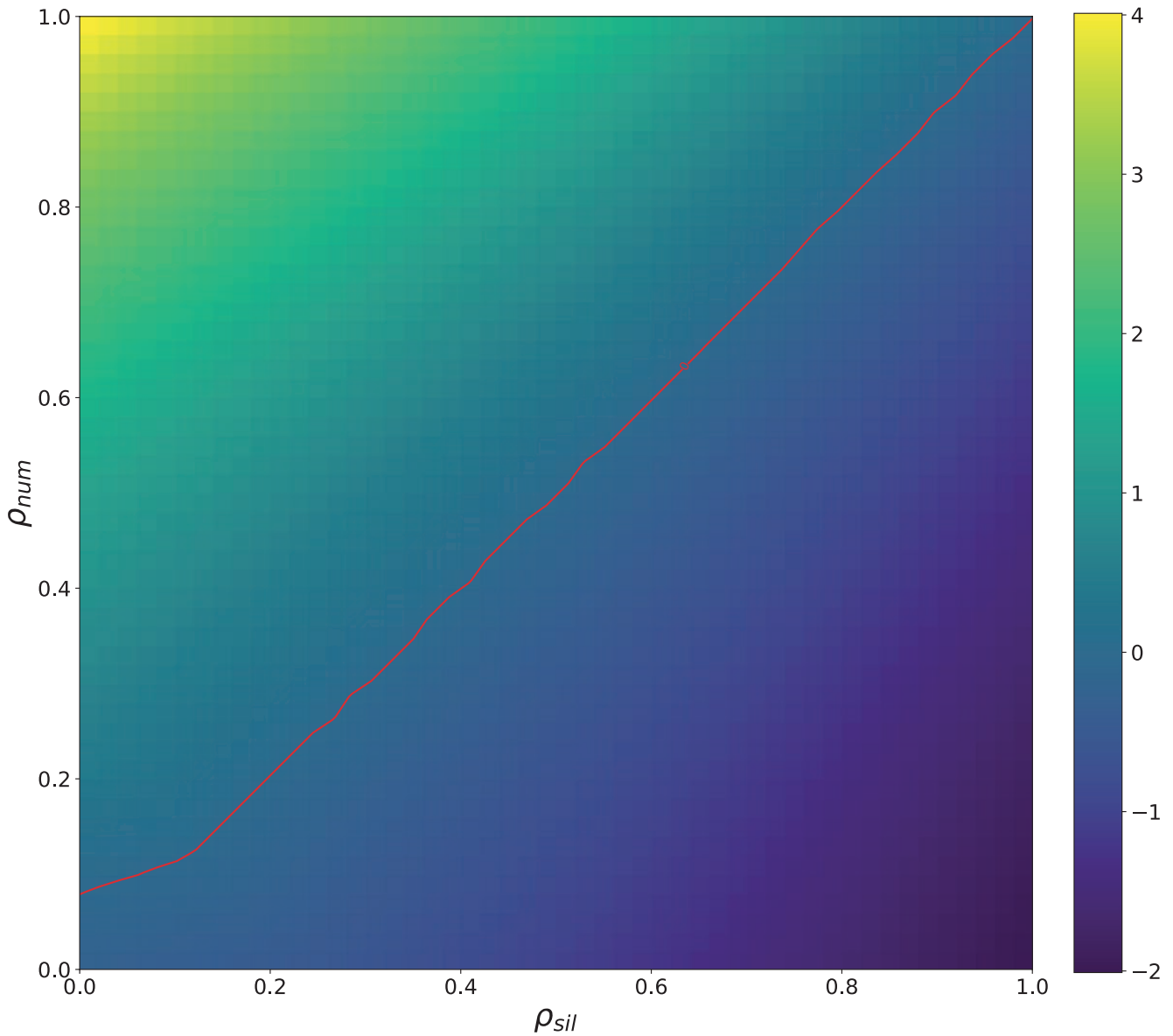


Figure 6. The selective advantage necessary to maintain the two phenotypic morphs at an equal frequency as a function of mate choice (as in Fig. 4, ρ_{inv} represents the mate choice of the form linked to an inversion and ρ_{an} the mate choice of the form associated with the ancestral allele). The red curve indicates when there is no selective advantage, below which the advantage is negative and above which the advantage is positive for the *numata* form. To maintain an equal frequency of each form despite the observed mating preferences measured in our study system, the survival advantage needed would be between 1 and 2.

standard arrangement (Chouteau et al. 2017). Despite these strong female preferences, an analysis of the data from Peru using our log-likelihood method demonstrates that only the ancestral form and the form linked to the first inversion show equal disassortative mating preferences (Supporting Information Table S2). The other form, *tarapotensis*, which is linked to the same inverted allele as the *numata* form used here, appears to have a weaker preference resulting in a less marked disassortative mating pattern (Table S2). As such, for the forms produced by this allele, it seems that mate preferences are not strong. The genetic load carried by this allele is weaker compared to the other allele with an inversion (Jay et al. 2021). As a result, the selective pressure on mate choice associated with this allele is not strong but seems to be similar in both populations. This observation

reinforces the hypothesis of a link between selection of mate preferences and inversion as hypothesized by Maisonneuve et al. (2021a).

Unlike what was found in Peru, the *silvana* tested in French Guiana did not show a disassortative mate preference. The standard, noninverted arrangement is devoid of deleterious effects when homozygous (Jay et al. 2021), so the expectation is for *silvana* butterflies not to display disassortative preferences, as seen in French Guiana. One explanation for the preferences displayed by Peruvian *silvana* is the very low abundance of its comimic *Melinaea ludovica* (Joron et al. 1999, Chouteau et al. 2016), and Chouteau et al (2017) suggested that the lower protection against predators that this form enjoys via mimicry might generate selection on mating preference of this form

for other forms displaying allelic dominance (inversions). In French Guiana, however, the comimic *M. ludovica* is abundant and we expect *silvana* to be as well protected against predation as the *numata* form; selection should therefore not favour disassortative preferences in *silvana*. The weak mating preferences shown by the *numata* form provide further support to the inference of a link between disassortative preferences and inversions (i.e. genetic load), and the absence of preference in the *silvana* form supports the hypothesis that mating preferences in this noninverted form, when present, may be the result of the local mimicry environment. Altogether, this suggest that mate preferences are not strongly selected in French Guiana and the absence of heterozygote excess observed in this population further reinforces this assumption.

However, mating preferences can contribute to maintaining polymorphism only if they are symmetrical because rare forms enjoy a mating advantage generating negative frequency-dependent selection (Thornycroft 1975, Sinervo and Lively 1996, Chouteau et al. 2017). By contrast, disassortative mating alone cannot lead to the maintenance of polymorphism when preferences are asymmetrical. Both our agent-based and deterministic models show that polymorphism decreases with increasing mate choice asymmetry. Symmetrical mate choice parameters maximize phenotypic diversity (Fig. 2: yellow area). When mate choice is strongly asymmetrical, the choosy allele rarely forms homozygotes and disappears from the population unless other selective forces balance this effect. This means that in a population where the *numata* forms make up 50% of the population, 56% of mating should be mixed pairings, compared to 50% if mating occurs at random. This pattern of disassortative mating is likely to lead to a 12% reduction of homozygosity at the locus controlling wing pattern polymorphism. In French Guiana, asymmetrical mate choice should result in the loss of the *numata* allele, suggesting that other sources of selection may be acting on the frequency of this form. In the Andean populations of Peru, polymorphism is interpreted to be the result of an equilibrium between a strong mimetic advantage of the inverted forms, heterozygote advantage due to a genetic load associated with inversions, and disassortative mating preferences. Based on our results and models, we posit that in French Guiana, the *numata* form may also have a directional selective advantage, presumably owing to increased adult survival via mimicry. This survival advantage should be strong enough to compensate for the effect of mate preferences and possibly the effect of genetic load carried by the inversion, but not so strong as to trigger the evolution of mate preference in the *silvana* form.

CONCLUSION

Our study shows that the population of *H. numata* in French Guiana does not display disassortative mating as expected from studies done on the Peruvian population. Instead, the population in French Guiana appears to have a weak asymmetric pattern of disassortative mating. The ancestral form shows no preference, while the derived form exhibits mild preferences for the ancestral form. Based on our models, this asymmetry, no matter how mild, should result in the extinction of the choosier form rather than polymorphism in French Guiana. To explain

the maintenance of polymorphism in this population, models suggest that a selective advantage is present in the choosy form; a probable advantage would be mimetic and further studies of this are warranted.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website.

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CREDIT STATEMENT

Pierre Lacoste (conceptualization, experiments, modelling, analysis, manuscript draft), Mathieu Chouteau (conceptualization, experiments), Ludovic Maisonneuve (modelling, manuscript draft), Rémi Mauxion (field work and experiments), Mathieu Joron (conceptualization, design, and supervision of experiments, modelling and analyses, manuscript draft) and Melanie McClure (conceptualization, design and supervision of experiments and analyses, manuscript draft). All authors contributed to the discussion of the results and provided their contribution to the manuscript.

CONFLICT OF INTEREST.

The authors declare no conflict of interest.

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DATA AVAILABILITY

All data and code are available on the following Git-Lab directory: https://gitlab.com/Pierre_lacoste/variation-in-mate-preferences.

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