Male age and color pattern predict female remating behavior in a polyandrous butterfly

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Abstract:

In polyandrous insects, females can mate with multiple males to improve their fitness. A female's decision to remate may be shaped not only by the phenotype of the available male, but also by the attributes of her previous mate. For example, females may become increasingly choosy and 'trade-up' male quality with successive matings. In this study, we investigated how male age, size, and wing color pattern affect female multiple mating decisions in *Heliconius numata*, a butterfly species with remarkable variation in wing coloration within populations. In *H. numata*, matings between individuals with the same wing coloration (assortative mating) produces fewer viable offspring than matings between individuals with different coloration patterns (disassortative mating). Despite this, we found no evidence that females preferred to remate with males bearing different color patterns, as predicted by the trade-up hypothesis. Instead, females of the two morphs (*H. n. bicoloratus* and *H. n. euphrasius*) showed a significant preference for remating with *H. n. euphrasius* males. In addition, the time between matings depended on the interaction between the morph of their first mate and the morph of the second male. Female remating behavior was influenced by the age of their first mate. Females that initially mated with older males remated sooner. Importantly, we found that remating frequency was globally low in both morphs. Our findings suggest that male wing color pattern and age influence female multiple mating behavior in *H. numata*, with females adjusting their sequential mating decisions based on traits of prior mates.

Keywords:

female choice, polyandry, sexual selection, color pattern, male age

Introduction

Mate choice is one of the main mechanisms of sexual selection (Andersson & Simmons, 2006; Rosenthal & Ryan, 2022). By discriminating and selecting among potential partners, females can increase their fitness via direct benefits such as nutritional components or material resources, as well as via indirect genetic benefits (Jennions & Petrie, 2000; Møller & Jennions, 2001). In the wild, females of polyandrous species can encounter potential mates either simultaneously or sequentially, and their decisions to mate and/or remate can impact their overall fitness and their offspring quality. Therefore, understanding the factors that influence mating decisions is essential for elucidating the mechanisms underlying female reproductive success, sexual selection and the evolution of mating systems.

Critical gaps still remain in our understanding of female mate choice. Several studies show that polyandrous females may assess a male against some attributes of the last male they encountered or mated with, and that their multiple mating decisions may maximize the genetic benefits to their offspring, termed the 'trade-up' hypothesis (Jennions & Petrie, 2000; Pitcher et al., 2003;Gabor & Halliday 1997). This hypothesis suggests that an unmated female should be relatively indiscriminate but become increasingly choosy with subsequent mating opportunities. Explicit tests of the 'trade-up' hypothesis in insects, fish, and reptiles show that females choose the second male based on traits such as body size or size and coloration of male ornaments, which are presumed to be indicators of male genetic quality (Gabor & Halliday 1997; Jennions & Petrie, 2000; Pitcher et al., 2003). In addition to phenotypic traits that indicate the intrinsic quality of males, females can also be sensitive to the complementarity between the genes of potential mates and their own, for instance to increase offspring heterozygosity and ultimately their fitness. To achieve this, females may prefer disassortative matings, where males have traits that are dissimilar to the female's traits (Bonneaud et al., 2006; Chouteau et al., 2017; Milinski et al., 2005). In vertebrates, disassortative mating and heterozygosity-based mate choice at the major histocompatibility complex have been shown to increase offspring survival following multiple matings (Laloi et al., 2011). However, much less is known about sequential disassortative mating decisions, in which females decide

whether to remate based on phenotypic cues traits that indicate both the male quality and offspring heterozygosity (Chouteau et al., 2017; Jay et al., 2021).

In the polyandrous butterfly *Heliconius numata*, populations often exhibit multiple color pattern morphs that coexist across the geographical range, and that are maintained by a series of inversions around the *ivory-cortex locus* (Fig. 1a). The number of morphs varies geographically; for example, up to seven morphs can coexist within a single population in Peru (Joron et al., 2011). Previous studies of mate choice in this species have shown that males exhibit relatively weak preferences, whereas females across all morphs display strong disassortative mate preferences for their first mate (Chouteau et al., 2017). Mating with a similar morph produces offspring with reduced survival (Fig. 2b). This cost arises because chromosomal inversions at the wing -pattern supergene accumulate recessive deleterious mutations, resulting in a high mutational load expressed in homozygous individuals (Jay et al., 2021). Therefore, females with the haplotype H1 and H123 incur a lower offspring survival rate when mated assortatively (Fig. 1b, Jay et al., 2021), which could make them more likely to remate and 'trade-up' genetically compared to other morphs.

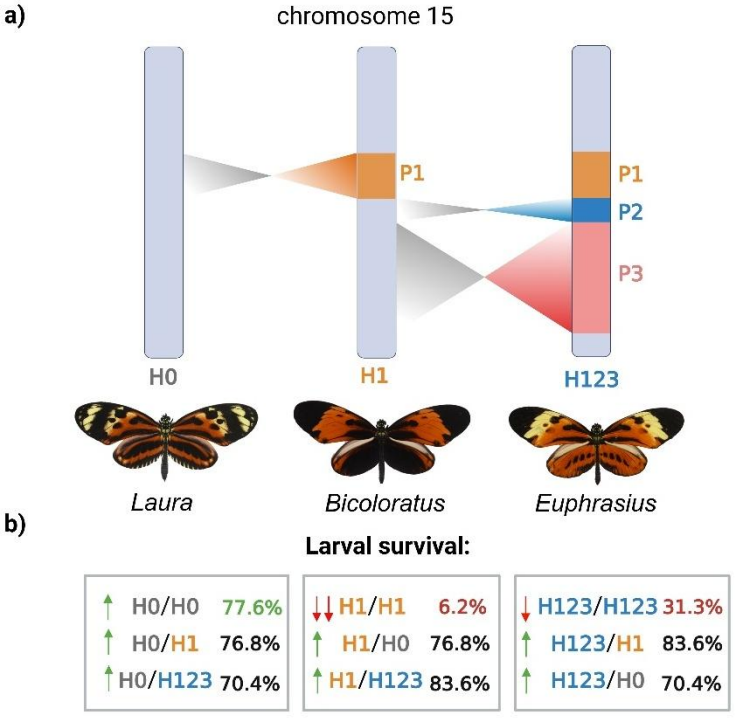


Figure 1. Chromosomal inversions at the mimicry supergene and their association with wing pattern diversity and offspring fitness in *Heliconius numata of Ecuador population*. **a)** Schematic representation of the supergene *P* in *Heliconius numata*, with its three chromosomal haplotypes: the ancestral gene order

(Hn0), associated with *H. n. laura*; inversion P1 (Hn1), associated with *H. n. bicoloratus*; and inversions P1, P2, and P3 (Hn123), associated with *H. n. euphrasius*. **b)** Comparison of survival rates among larval offspring with different supergene chromosomal types. Upward arrows denote high survival rate, while downward arrows represent low survival rate in homozygous and heterozygous individuals. Adapted fromJay & Joron, 2022.

Here, we examined female multiple mate choice in two morphs of *Heliconius numata* (*H. n. bicoloratus, H. n. euphrasius*). We tested if mate choice (likelihood and time to remating) is linked to attributes of the last male she mated with, such as whether male phenotypic traits, including age, size, and color pattern, influence multiple mate choice. We hypothesized that females would be more likely to remate and/or remate more quickly if they first mated assortatively, and potentially if the first male was older or smaller, as remating would be expected to increase both indirect benefits (offspring heterozygosity) and direct benefits (nutritional value of the spermatophore —a package containing sperm and accessory nutrients transferred by the male during copulation). Additionally, we also hypothesized that females from the *H. n. bicoloratus* morph, which show the lowest larvae survival as homozygotes (Fig.1b), would show a high frequency of rematings if first mated assortatively compared to females of *H. n. euphrasius*.

Methods

Butterfly collection and rearing

A total of 100 *Heliconius numata* pupae were purchased from a breeding farm located in Ecuador (*Quinta de Goulaine - Cría de Mariposas, Exposiciones, Eventos y Otros Productos*, n.d.) . During transport to the laboratory, the pupae were placed in cotton-lined containers to reduce the risk of physical damage. Stocks of butterflies were established and maintained in outdoor insectaries. Two stock populations were kept in mesh cages with the following dimensions: 3.5 m x 2.4m x 3m, each containing approximately 50 individuals. Newly emerged adults were marked with a unique code on the forewing using an ultra-fine black Sharpie marker. Individuals selected for mating experiments were kept in single-sex experimental cages (3.5m x 1.2m x 3m) to ensure they would remain unmated until the start of the experiment. The remaining adults were maintained in the stock cages to produce offspring and sustain the laboratory colony. Butterflies were maintained in Gamboa, Panama under natural lightning conditions, ambient temperature, and humidity, to reflect natural environmental conditions. Adults had free access to sugared water, bee pollen, and flowers of *Lantana camara, Psychotria poeppigiana,* and *Psiguria triphylla*. Plants

of *Passiflora vitifolia, Passiflora riparia, Passiflora quadrangularis,* and *Passiflora nitida* were provided for oviposition. Eggs were subsequently collected and kept in small containers at ambient conditions until hatching. Upon emergence, caterpillars were transferred onto *Passiflora riparia* plants, and the plants bearing the larvae were then placed inside a cage (3.5 m × 2.4 m × 3 m), where the caterpillars were reared under ambient conditions until pupation. The study population was mainly composed of three-color pattern morphs (*H. n. bicoloratus*, *H. n. euphrasius*, and *H. n. laura*) determined by three distinct alleles at the supergene *P* (Fig. 1a; Joron et al., 2006), but in this study we focus on two morphs (*H. n. bicoloratus*, *H. n. euphrasius*) to examine female remating behavior. Assuming that the genetic patterns in Ecuador are similar to those reported in Peru, most *bicoloratus* individuals were likely heterozygous(Cara et al., 2023), whereas *euphrasius* individuals could be either homozygous or heterozygous with the H0 haplotype associated with *H. n. laura* morph.

Remating experiment

To investigate the frequency of remating, the choice of male morph, and the duration of the delay between successive mating events (refractory period) in *Heliconius numata* butterflies, we performed remating choice trials with live female and male butterflies of the *H. n. bicoloratus* and *H. n. euphrasius* morphs at two time periods, February to April and October to December of 2024.

Heliconius males and females can mate multiple times; however, in this study we focus on female remating behavior (Cornish, 2001; Rather et al., 2022) . Trials were conducted with unmated females (2-4 days old). A total of 168 females (*H.n bicoloratus*: 79; *H. n. euphrasius*: 89) were used in this study. Females of each morph were randomly assigned to one of two different experimental groups for their first mating event: assortative mating (within-morph: *bicoloratus* female with *bicoloratus* male; *euphrasius* female with *euphrasius* male) or disassortative mating (between-morphs: *bicoloratus* female with *euphrasius* male; *euphrasius* female with *bicoloratus* male). One unmated female was released individually into a mating cage (1.2m x 1.2m x 3m), with sugar water solution, bee pollen, and a bouquet of nectar-bearing flowers) and given one hour to acclimatize. Then, three sexually-mature unmated males (> 6 days old) of the target morph, selected haphazardly from the experimental male stock cage, were released into the mating cage. We typically ran six to eight mating trials concurrently in different mating cages. Based on our observations, this species was most active between 9:00 and 17:00h. Before 9:00 h, individuals primarily feed or bask in the cage, whereas later in the afternoon they began searching for roosting sites to rest overnight. Therefore, mating cages were monitored every hour (9-17h) until copulation occurred, for a maximum observation period of 48 h. Copulation lasted approximately 1h30min on average. The

non-copulating males were removed from the cage to single-sex experimental cage to prevent interference with the mating pair and to confirm the identity of the successful male. After copulation or if no mating (n=34) was observed, the female was moved into the remating cage (3.5 m x 2.4m x 3m). Consequently, the remating cage contained both previously mated and unmated experimental females. The number of females in the remating cage increased gradually as individuals emerged and reached the required age for the experiment. In this 'remating-mating cage', butterflies had access to sugar water solution, bee pollen, and flowers of *Lantana camara, Psychotria poeppigiana,* and *Psiguria triphyllaI.* At least five unmated experimental males of each morph were maintained in the cage at all times, and additional unmated males were introduced weekly to ensure that females had continuous access to potential mates. This minimum number was chosen because males did not emerge simultaneously, and the relative abundance of *H. n. euphrasius* and *H. n. bicoloratus* varied over time.

Previously mated males were only introduced when sufficient numbers of unmated males were not availability throughout the experiment. These males were isolated for at least six days before being reused to allow for spermatophore replenishment. This design was intended to approximate semi-natural conditions in which both females and males with different mating histories coexist and interact, allowing females to encounter and be courted by both previously mated and unmated males from the same pool of potential mates. The 'remating cage' was also monitored every hour (9-17h) to detect mating events. Once copulation was observed, mating pairs were gently handled and transferred to individual pop-up cages. where we recorded the time, date, and ID of the female and male. After copulation ended the male was not reused and was move into stock cage. Females were then returned to the 'remating cage' again, where they were monitored for additional remating events. Every week, we introduced at least 8 new unmated males for each morph into the 'remating cage' to ensure the females had a wide range of males to choose from. After completion of the experiments, adults were kept in stock cages until their natural death.

Measurements of wing size

To examine if male wing size influenced female remating decisions, males were photographed 2 h after emergence, when their wings were dry but they were not yet capable of flight. Males were gently restrained using tweezers and placed on graph paper while maintaining a constant distance from the smartphone camera. We used Fiji (Schindelin et al., 2012) to quantify forewing length and width of experimental males. We also calculated the wing's aspect ratio, by dividing the forewing's length by width

(Flockhart et al., 2017). A subsample of 78 male wings were measured twice to examine measurement error and repeatability (**Figure S1 and Figure S2**).

Data analysis

All statistical analyses were conducted in R v. 4.3.1 (R Core Team, 2024).Wing measurement repeatability was estimated with Pearson's correlation coefficient, with the *cor.test* function (R Core Team, 2024).Generalized linear models (GLM) were implemented with the *lme4* package (Bates et al., 2015). Model terms were tested for significance using the *Anova* function of the *car* package(Fox & Weisberg, 2019). The model results and code are available in the Supplementary Material. Of the 168 females assayed in our trials, 7 individuals remated more than once. Therefore, we ran the analyses including and excluding female identity as a random intercept to account for the non-independence of multiple mating events contributed by the same female. The estimates and statistical conclusions were qualitatively unchanged, indicating that the results were robust when accounting for repeated observations from the same females. The model including female identity provided a substantially better fit than the corresponding model without the random effect ($\Delta AIC = 80.14$; $\chi^2 = 82.14$, $df = 1$, $P < 0.001$).

To examine if remating decisions of *Heliconius numata* females varied by morph and/or depended on the previous mating event, we ran a binomial GLMM with remating success as response variable and the interaction between female morph (*H. n. bicoloratus*, *H. n. euphrasius*) and previous mating type (assortative, disassortative) as potential predictors. We also included age and size (forewing length and aspect ratio) of the previous male as predictors, to examine if female remating decisions further depended on certain traits of the previous mat. Wing size and male age were initially included in the global model but were removed from the final model because they did not explain variation in the response variable, and their removal reduced model complexity. l.

To evaluate which male morph females chose during remating, we analyzed only females that had remated. We used a chi-squared test to determine whether remating events were equally distributed between *H. n. bicoloratus* and *H. n. euphrasius* male. Subsequently, we conducted separate binomial GLMs for females that had previously mated assortatively and disassortatively, with remating male morph as the response variable and female morph as the predictor. We also included the age and size (forewing length and wing aspect ratio) of the previous male as predictors.

The mating status of males and females in the remating cages was recorded throughout the experiment. However, because male mating status was not part of our original experimental design and including it as an additional predictor would substantially increase model complexity relative to our sample size, we did not formally test its effect on mating outcomes. To examine if the refractory period (time taken to remate) of *Heliconius numata* females varied by morph and/or depended on the previous mating event, we ran a GLM with Poisson distribution with the number of days before remating as the response variable. We included as predictors the interaction between female morph (*H. n. bicoloratus*, *H. n. euphrasius*) and previous mating type (assortative, disassortative). We also examined if the refractory period depended on the age and size (forewing length and aspect ratio) of the previous male. However, we removed wing size from the final model because it was not significant, and its removal reduced model complexity. Afterwards, from the model results and since we visually identified one outlier in terms of previous male age, we removed the outlier from the dataset (see Figure S3) and re-ran the refractory period models to assess if the outlier was driving any of the results obtained. The results were qualitatively unchanged after its exclusion; therefore, this observation was retained in the final dataset and all reported results are based on the complete dataset.

Ethical Note

Ethical approval for this study was not required because it involved only *Heliconius* butterflies, which are not classified as endangered or protected species. Specimen collection and transport were conducted under permits issued by the Ministerio del Ambiente de Panamá (ARG-137-2025). Butterflies were handled only when necessary to either mark them or move between cages and this was done using established protocols to prevent wing damage. Butterflies used in this experiment were raised in outdoor insectaries, with free access to sugared water, bee pollen, and flowers during the course of the experiment. Plants of *Passiflora vitifolia, Passiflora riparia, Passiflora quadrancularis*, and *Passiflora nitida* were provided for oviposition, and larvae were reared on *Passiflora riparia* plants until pupation. After emergence, all adults were marked on the wing with a thin permanent marker. Females were allowed 1h to acclimate before males were introduced. No butterflies were sacrificed for these experiments. After completing the experiment, individuals were transferred to stock cage and provided with food for the remainder of their natural lifespan. All experimental procedures were conducted in accordance with the ASAB/ABS Guidelines for the treatment of animals.

Results

1. Patterns of remating

1.1 Number of remating events

We recorded a total of 176 mating events in our experiment, approximately equally split between the two female morphs (*H. n. bicoloratus* = 86; *H. n. euphrasius* = 90). Of these, 129 were first matings (Fig. 2a), while 47 were remating events, involving 39 females, some of which mated multiple times. Most of these females remated only once (N = 32), although one female mated four times over an average period of 10 days during the experimental period. The average number of matings per female was similar across morphs, with *H. n bicoloratus* females averaging 1.3 ± 0.65 matings (maximum = 4) and *H. n. euphrasius* females averaging 1.26 ± 0.47 matings (maximum = 3).

1.2 Probability of remating

Among the females that mated more than once, the number of remating events per female was similar across morphs (*H. n. bicoloratus* = 24; *H. n. euphrasius* = 23) and did not depend on whether the female's previous mating was assortative or disassortative (χ^2 = 2.823, df = 1, *P* = 0.093; Fig. 2), or female morph ((χ^2 =0.115, df = 1, *P* = 0.734). We also found that the probability of remating was independent of the age (χ^2 = 1.928, df = 1, *P* = 0.165) and size (χ^2 =0.589, df = 1, *P* = 0.443) of the male with whom they previously mated with. When examining the outcome of remating events, we found that females showed a significant overall bias regarding the morph of the second mate*s* (χ^2 = 7.681, df = 1, *P* < 0.01; Fig. 2). When we analyzed separately by previous mating type, females that had previously mated assortatively showed no evidence of a preference for either male morph (χ^2 = 0.18, df = 1, *P* = 0.67), whereas females that had previously mated disassortatively showed a marginal, non-significant tendency to remate with *H. n. euphrasius* males a (χ^2 = 3.50, df = 1, *P* = 0.07).

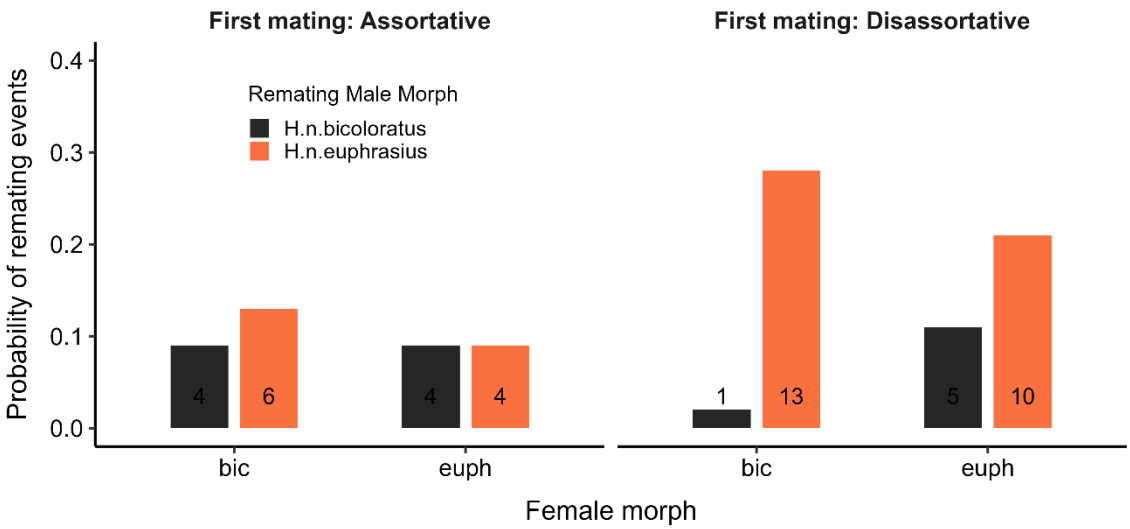


Figure 2. The proportion of remating events was pronounced after dissassortive first matings, females that had previously mated disassortatively showed a tendency to remate with *H. n. euphrasius* males rather than *H. n. bicoloratus* males. Sample sizes for each treatment are indicated on the bars.

Refractory period

The refractory period, i.e. number of days taken to remate, was similar between the two female morphs (*H. n. bicoloratus*: 14.1 ± 10.5 days; *H. n. euphrasius*: 15.3 ± 12.1 days). We found that refractory period was independent of the wing size (χ^2 = 0.595, df = 1, *P* =0.082). Additionally, the refractory period was independed of female morphs (χ^2 = 0.013, df = 1, *P* = 0.91), the previous mating type (assortative vs. disassortative; χ^2 = 0.416, df = 1, *P* = 0.519) and their interaction (χ^2 = 0.241, df = 1, *P*=0.624; Fig. 3a). However, females that had previously mated with an older male remate sooner compared to females that mated with a younger male χ^2 = 21.786, df = 1, *P* < 0.001; Fig. 3b).

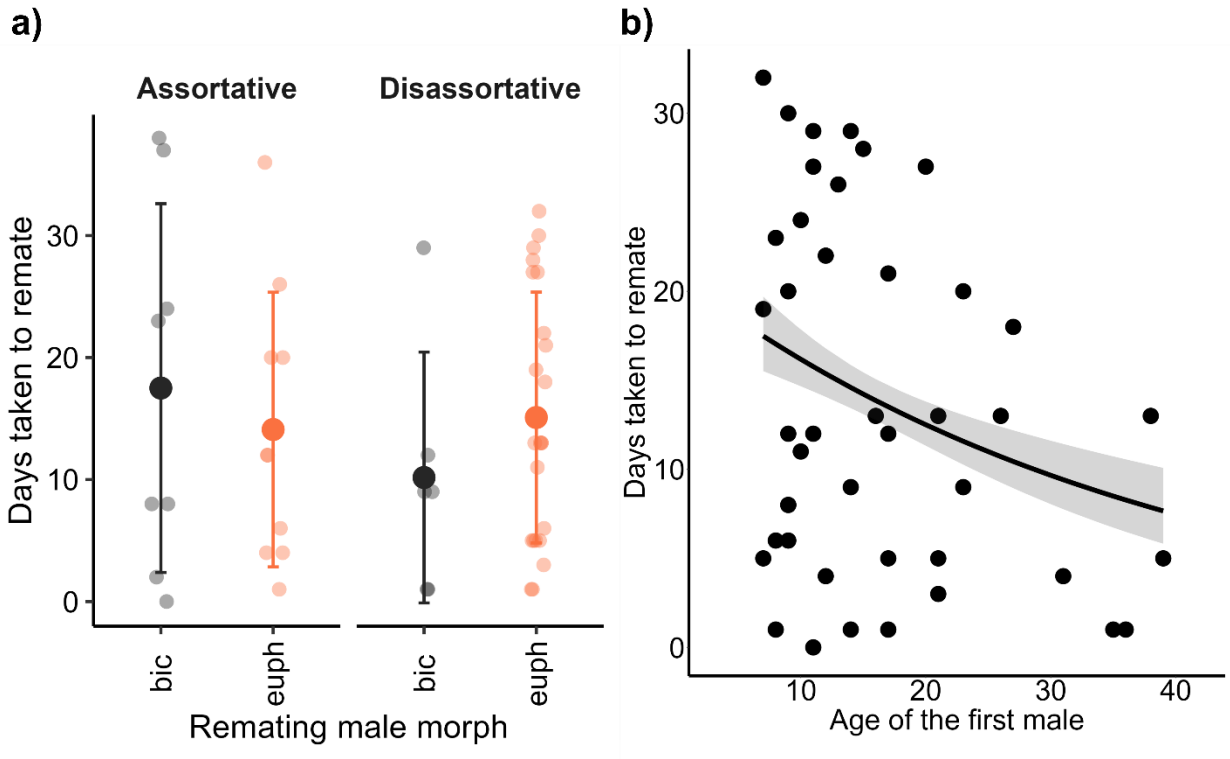


***Figure 3*. a)** The refractory period, i.e., the number of days taken to remate, depended on the interaction between the previous mating type (assortative vs. disassortative) and the morph of the remated male. **b)** The number of days taken to remate was negatively correlated with the age of the male with whom the female previously mated.

Discussion

The 'trade-up' hypothesis proposes that, in polyandrous species, females will adjust their remating behavior to obtain a higher-quality mates, thereby increasing reproductive benefits. To test this in *H. numata*, we examined female remating behavior in two color pattern morphs (*bicoloratus and euphrasius)*, assessing how often and how quickly females remate and whether these decisions depended on the phenotype, wing size and age of their first mate. In *H. numata*, there is a cost to assortative mating in *H.n. bicoloratus* and *H. n. euphrasius*, as it incurs a significant reduction in larval survival relative to dissassortative matings (Fig 1b, Chouteau et al., 2017; Jay et al., 2021). Surprisingly, we found little support for the 'trade-up' hypothesis in our *H. numata* populations. In both morphs, the probability of remating was low (1.3), but nearly identical to remating frequencies (1.2) observed in the wild (Walters et al., 2012).

However, there were no differences in the probability of remating between females that mated assortatively relative to those that mated disassortatively (Fig 2).

Likewise, time to remating provided only limited support for the 'trade-up' hypothesis. Females tended to remate sooner when their previous mate was an older male (Fig 3b). However, there was no difference in time to remating between females that mated assortatively and those that mated disassortatively. Nevertheless, postcopulatory processes, such as sperm competition, cryptic female choice, or sperm precedence, may influence reproductive success. Future work should investigate offspring production, paternity, and sperm precedence following sequential matings. Together, these data will provide a more comprehensive understanding of the fitness consequences of remating decisions in *H. numata*.

It is possible that the cost of assortative mating could differ between *H. numata* morphs in Peru (Chouteau et al. 2017) and the Ecuadorian populations that we used in our experiments. Thus, the lack of evidence for a 'trade-up' behavior in this study may reflect lower or different mutation load in the Ecuadorian population compared to the previously studied Peruvian population. Alternatively, the mutational load may be similar in wild Ecuadorian and Peruvian populations of *H. numata* but deleterious mutations may have been gradually purged from it under captive breeding conditions (Hedrick & Garcia-Dorado, 2016). In this light, preliminary and ongoing pedigree analysis seems to indicate that assortative matings among *bicoloratus* and *euphrasius* morphs do not obviously suffer the decreased larval survival that was observed in wild populations of *H. numata* in Peru (unpublished data). Our sample sizes are small and further studies are needed in both wild and captive Ecuadorian populations to determine whether homozygosity consequences vary geographically in *H. numata*, which would then influence mating patterns and genetic load dynamics differently across populations.

Although our data was not consistent with the 'trade-up' hypothesis, the remating pattern we observed were also not random. Instead, females that initially mated with an older unmated male had a shorter delay before remating than those that mated with younger unmated males, regardless of the male morph. Studies on other butterflies have reported that females mated with older males showed reduced hatching success and shorter refractory periods, which may reflect age-related changes in ejaculate effects. This may lead to sexual conflict because males may benefit from females delaying remating, whereas females may benefit from remating sooner, as known from other studies (Díaz et al., 2024; Kehl et al., 2015; Sanghvi et al., 2025). However, these studies focused on insects with short adult lifespans. In contrast, *Heliconius* butterflies can live for more than six months and there is little evidence for

reproductive senescence (Foley et al., 2025). Thus, the age difference among males used in our study, which ranged from 6 to 61 days (Fig. 3b) would seem unlikely to lead to major declines in sperm quality. In addition, all males used in our study were unmated and it seems unlikely that there would be differences in the mass of the spermatophores of old versus young males. Indeed, given that it takes time and resources for males to assemble a spermatophore (Cardoso & Silva, 2015), it is likely that older unmated males have larger spermatophores than younger males. Nonetheless, this possibility remains to be tested and, if shown to be the case, then these results would be consistent with a potential trade-up strategy.

Alternatively, it is possible that there are subtle behavioral differences between older and younger males and that these differences may influence female remating decisions. For example, if younger males were more vigorous in their courtship or possessed stronger chemical signals, females might preferentially respond to these cues. In *Heliconius,* pheromone production and complexity increase as males reach sexual maturity, with the richest blends occurring around 4–8 days after eclosion (Cama et al., 2024). These chemical signals are released through the androconia-specialized wing scales that are critical for successful courtship and female attraction (Darragh et al., 2017). However, androconia scales are fragile and susceptible to wear as male age, potentially declining the efficiency of pheromone release and weaking the female attraction (de Oliveira Borges et al., 2020; Dell'Aglio et al., 2017). Given that males in our study ranged from 6 to 61 days old (Fig 3b), age-related degradation of chemical signals may increase the relative attractiveness of younger males as subsequent mates. However, in polyandrous systems, unmated females are expected to show lower choosiness for their first mating, regardless of male age or signal quality. By contrast, if age-based preferences manifest more strongly or exclusively during re-mating, this would support a compensatory mechanism associated with upgrading mate quality. Evaluating preferences in both unmated and experienced females thus allow us to test whether age discrimination is context-dependent.

In addition, we observed differences in remating patterns related to male morph preferences. Females shown a tendency to select *H. n. euphrasius* males as second mates, after they mated dissasortatively. Surprisingly, and inconsistent with our original hypothesis, this result was driven by females whose first mating was disassortative. In this case, most of the rematings (23 of 29) were with an *H. n. euphrasius* male. This result was not related to size, as male size was not associated increased remating probabilities neither varies among the morphs. One explanation for these results may be an innate preference for certain color signals. For instance, in guppies *Poecilia reticulata*, females prefer males with larger and brighter orange patches, a preference that arises from a sensory bias but that also indicates

male health and quality (Eakley & Houde, 2004). Similarly, in psyllids *Diaphorina citri,* females have been shown to prefer orange males in subsequent matings based on prior experience, as such choices are associated with increased fecundity (Stockton et al., 2017). Comparable mechanisms may occur in *Heliconius*, where visual signals play a key role in mate recognition. A previous study has demonstrated female discrimination based on male appearance (Southcott & Kronforst, 2018). In *H. numata*, the *H. n. bicoloratus* morph displays black and orange wing patches, whereas *H. n. euphrasius* exhibits a similar pattern but with the addition of yellow elements (Fig. 1a). This additional coloration could enhance visual contrast and signal salience, suggesting that the wing pattern of *H. n. euphrasius* males may contribute to greater female attraction. Additionally, it is possible that *H. n. euphrasius* males may differ in behavioral traits such as courtship persistence, or pheromone composition, or spermatophore productions, although these possibilities remain to be tested. Such could further enhance their attractiveness and contribute to the observed mating bias (González-Rojas et al., 2020; Mérot et al., 2017).

In summary, our study revealed that female *Heliconius numata* exhibit that remating dynamics shaped by their first mating (assortative vs disassortative) and male traits. We also found that remating probabilities were not influenced by male age or size. Instead, females of both morphs (*H. n. bicoloratus* and *H. n. euphrasius)* showed a marginal non-significant preference for *H. n. euphrasius* males after they mated dissasortatively. However, the remating delay was influenced by the interaction between previous mating and male morph or matings with older males, providing limited support for 'trade-up' hypothesis in our population. This behavior may be mediated by visual or chemicals signals that influence subsequent mating decisions. These findings highlight the complex interplay between mating history, male age, and reproductive strategy in a polymorphic butterfly, and warrant further investigation on the physiological and genetic mechanism of postcopulatory female choice.

Data availability

https://figshare.com/s/1c9756c8cac26812cef2

Supplementary information

Measurements of wing size

Wing length and width were measured on the right wing of each individual. Length was defined as the linear distance between the proximal and distal edges of the wing, and width as the maximum perpendicular distance across the wing (see Figure S1).

To assess measurement error and repeatability, a subsample of 78 male wings was measured twice. Repeatability was evaluated using Pearson's correlation coefficient between repeated measurements (Figure S2).



Figure S1. Measurements of wing length and width

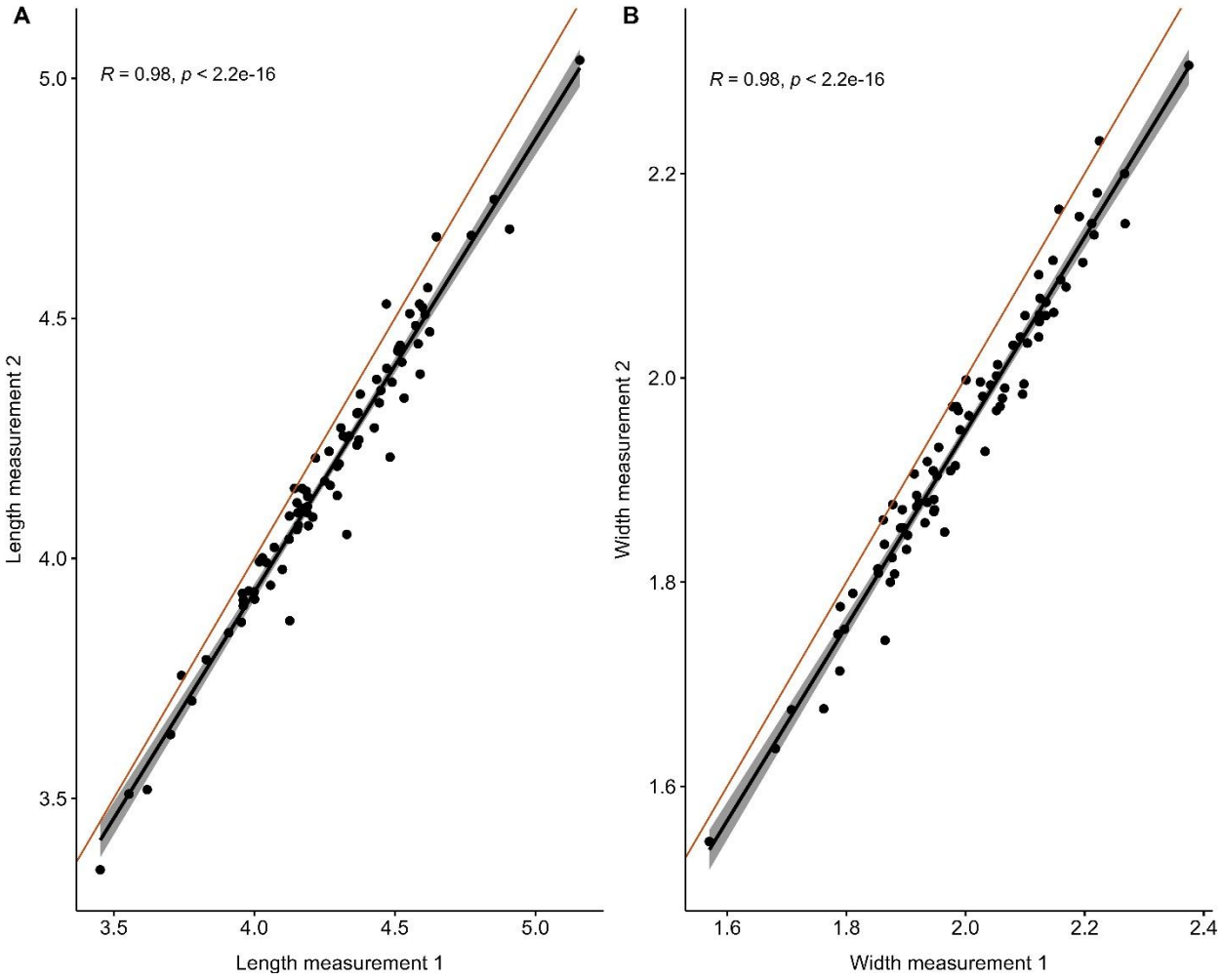


Figure S2. Assessment of measurement error and repeatability in wing measurements using Pearson's correlation.

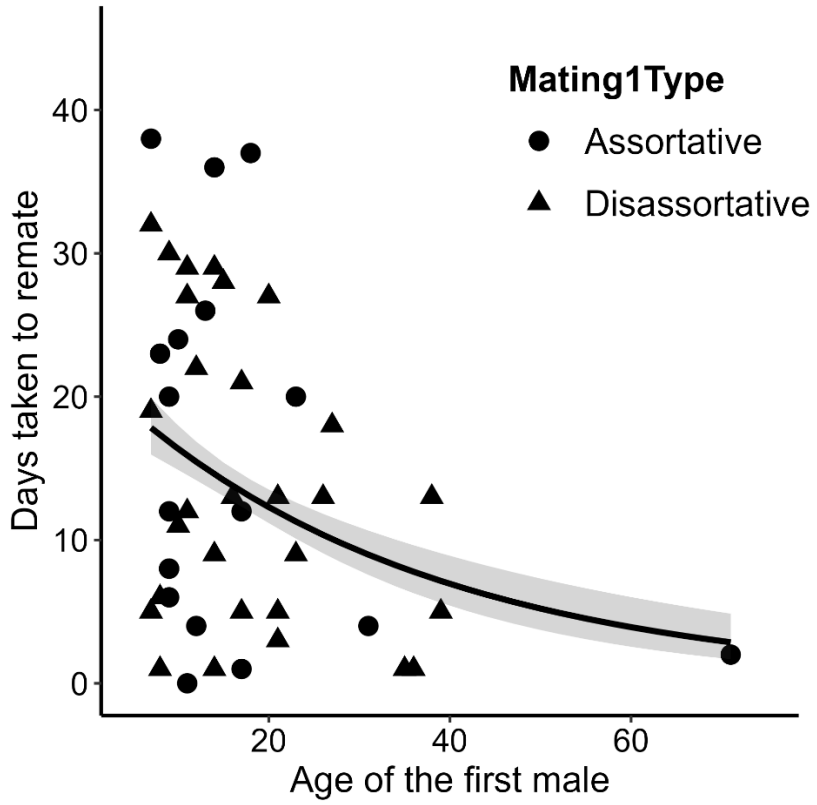


Figure S3. The number of days taken to remate was negatively correlated with the age of the male with whom the female previously mated (assortative vs disassortative mating; outlier included)

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