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Wing color and mating preferences of *Heliconius sara*

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ABSTRACT

Convergence in aposematic coloration of chemically-protected butterflies, or Müllerian Mimicry, improves advertisement of unpalatability to predators. *Heliconius sara* (Heliconiinae) has two races in Costa Rica, each matching a different Müllerian model, one with and the other without a yellow stripe on the bottom edge of the hindwing. Color switching in *Heliconius* spp. is common but may involve a conflict between natural selection for convergence to models and sexual selection for maintenance of the original color pattern. Here, I manipulate the color pattern of *H. sara* to determine the degree to which sexual selection constrains color pattern switching. This study was conducted in the Monteverde Butterfly Garden in Monteverde, Costa Rica and looked at mating behaviors of 75 Atlantic race *H. sara*, which have uniformly black hind wings. Newly-emerged individuals were painted with a stripe of yellow or black on the bottom edge of the hind wing. Mating behavior was observed over 11 days. Males did not show a preference for female wing color for approach, chasing, courtship or rejection (Chi-squared test, $df = 3$, $p < 0.05$). However, females seemed to prefer black painted males for approach, chasing and courtship (Chi-squared test, $df = 3$, $p < 0.05$). If black is the ancestral condition, these results suggest that color pattern switching in *H. sara* is constrained by female mate choice.

RESUMEN

La convergencia en la coloración aposemática de mariposas químicamente protegidas, y el mimetismo de Müllerian, mejoran la advertencia de la letalidad a los depredadores. *Heliconius sara* (Heliconiinae) tiene dos razas en Costa Rica, cada una cópula con un diferente modelo de Müllerian, una con y la otra sin una raya amarilla en el borde inferior del ala. La conmutación del color en *Heliconius* spp. es común pero puede implicar un conflicto entre la selección natural para la convergencia a los modelos y la selección sexual para el mantenimiento del patrón original del color. Aquí, manipulo el patrón del color del *H. sara* para determinar el grado a el cual la selección sexual obliga el cambio del patrón del color. Este estudio fue conducido en el jardín en Monteverde, Costa Rica de la mariposa de Monteverde, observé los comportamientos de cópula del *sara* de la raza *H. sara* de 75 Atlántico, que tienen alas uniformemente negras. Se pintaron a los individuos emergentes con una raya de amarillo o de negro en el borde inferior del ala. El comportamiento de acoplamiento fue observado sobre 11 días. Los machos no demostraron una preferencia por el color femenino del ala para los

comportamientos de acercamiento, perseguir, cortejo o rechazamiento (Chi-squared test, $df = 3$, $p < 0.05$). Sin embargo, las hembras prefirieron preferir a machos pintados de negro para el acercamiento, perseguir y cortejo (Chi-squared test, $df = 3$, $p < 0.05$). Si el negro es la condición ancestral, estos resultados sugieren que el cambio del patrón del color en sara del *H. sara* obligada por escogencia femenina de la cópula.

INTRODUCTION

Wing color and pattern in butterflies may serve in predator avoidance, male-male interactions and social signals used during courtship (Silberglied 1989). Aposematic coloration warns predators of toxins or distastefulness (Gilbert 1983, Alcock 1984, Brower 1989). A type of mimicry produced when unpalatable species share the same aposematic coloring to protect themselves from predators is Müllerian mimicry (Gilbert 1982, Mallet et al. 1988, Turner 1989).

Heliconius spp. (Lepidoptera: Nymphalidae) are well known for their differentiation of wing color patterns and multiple mimicry complexes including exceptional Müllerian mimicry within their species (Mallet et al. 1989, Jiggins et al. 2001, Jiggins et al. 2004, Kronforst et al. 2006). *Heliconius melpomene* and *H. erato* are two well-recognized examples found in light gaps and secondary forests of the Neotropics that exhibit this complex (DeVries 1987, Davidson et al. 1999, Flanagan 2004). Within populations in Central and South America color switching has led to 11 variations in wing patterns and in the same geographical locations the wing patterns are almost perfect mimics (Turner 1981). Convergence of color pattern between *H. melpomene* and *H. erato* may not have been made without a cost. Butterfly fitness via mimicry is increased due to natural selection because they receive greater protection from predators, however it can decrease fecundity via sexual selection (Mallet et al. 1989).

Sexual selection may constrain color convergence by favoring the original color pattern, even though natural selection through Müllerian mimicry causes convergence between models. Only rarely does mate choice evolve with mimicry, and when this is not the case, butterflies need to find a balance between natural and sexual selection (Jiggins et al. 2001). For these reasons sexual selection prevents new races from evolving, whereas natural selection allows the species to diverge into new color patterns.

Heliconius sara is distinguished from similar species by its small size and a basal forewing bar that crosses the wing well inside the cell. According to DeVries (1987), *Heliconius sara* is separated by the continental divide into two subspecies: *H. sara fulgidus* on the Atlantic slope and *H. sara theudela* on the Pacific slope. *Heliconius sara theudela* enters into a mimicry complex with *H. doris*, *H. pachinus* and *H. hewisoni* while *H. sara fulgidus* mimics *H. cydno*. Therefore, *Heliconius sara* on the Atlantic side has a hind wing that is uniformly black, while the Pacific race has a yellow stripe on the bottom edge of the hind wing (see Figure 1). The question I am looking at is “What if the Atlantic form were to evolve to the Pacific form?” Here, I examine the possible evolutionary conflict between natural selection for color convergence via Müllerian mimicry and mating disruption via sexual selection in *H. sara*.



Heliconius sara fulgidus

Heliconius sara theudela

Figure 1. The two *Heliconius* subspecies observed in my study are *Heliconius sara fulgidus* found on the Atlantic slope and *Heliconius sara theudela* found on the Pacific slope of Costa Rica.

MATERIALS AND METHODS

Study site

This study was conducted between April 24th and May 8th, 2007 in the Monteverde Butterfly Garden in Monteverde, Costa Rica. The garden area where observations were made was a 5 x 15 m area that contained the following *Heliconius* butterflies: *H. sara*, *H. erato*, and *Dryas julia*. The garden had a tin roof with two plastic panels along with thick plastic paneled walls to allow the filtration of ambient light. Screen panels are located on east and west side to allow air to circulate through the greenhouse. Dominant vegetation in the garden was *Lantana camara* (Verbenaceae), *Stachytarpheta jamaicensis* (Verbenaceae), *Asclepias curvassivica* (Asclepiadaceae) and *Gurania spp.* (Cucurbitaceae). There were also four hanging cups of *L. camara* cut stems with inflorescences infused with sugar water for food. The west end of the garden received full sun while the east end was mostly shaded.

Procedure

One hundred Atlantic *H. sara fulgidus* pupae were obtained by the Monteverde Butterfly Garden. The pupae were pinned in an enclosed chamber until enclosure, where they were then placed in two separate butterfly enclosures containing a hanging cup of *L. camara* inflorescences filled with sugar water as food. Doing so allowed their wings to dry and kept them from mating before I was able to paint their wings. Butterfly wings took approximately one day to dry, whereupon, I painted a yellow or black stripe on the bottom of the hind wing, corresponding to the wing color of *H. sara theudela* or *H. sara fulgidus*, respectively using Sharpie Paint Pens (see Figure 2). In an effort to keep male and female wing colors even, wing color was determined upon how many of each color and sex had already been released.



Figure 2. The two *Heliconius sara* races whom I painted the lower hindwing black or yellow in my experiment. *Heliconius sara fulgidus* (left) has a black stripe and *H. sara theudela* (right) has a yellow stripe.

Though *H. sara fulgidus* has uniformly black hind wings, half of the individuals were given a black stripe to rule out behavioral changes caused by painting the butterflies. All butterflies were painted to rule out paint as a variable. Immediately after painting, wings were held open for approximately eight to ten minutes to allow the paint to dry and avoid butterfly wings from sticking together, which would have killed them. Fifteen *H. sara* already living in the garden were left unaltered and interactions with them were noted but did not contribute to the study.

Over the course of four days 75 butterflies were released into the garden; 19 females and 20 males were painted black while 18 females and 18 males were painted yellow. Behavioral interactions between *H. sara* were observed and noted for approximately three hours for 11 sunny days during midday, when *H. sara* are most active (including the four days the butterflies were released).

Behavior was divided into five categories: approach, chasing, courtship, mating and rejection. Approach was considered a direct flight path of a male towards a female while chasing was defined as a male giving chase to a female. Courtship consisted of a string of interactions; the first being a male either giving chase to a female and forcing her to land on a leaf by the downbeat of his wings or by the male approaching a female already resting on a leaf; her wings may be open or closed. The second phase of courtship consisted of a male rapidly flapping his wings, creating a breeze towards the female (Crane 1955). A responsive female would also rapidly flap her wings in reply. Mating was defined to be when both sexes had wings closed in a typical daytime rest position facing opposite directions while in copula (Crane 1955). A mating pair was easy to observe since *Heliconius* stay in copula for one to three hours (Thompson 2006). When a male approached or attempted to court with a female who raised her abdomen or flew directly away it was defined as rejection.

RESULTS

General Observations

Females tended to prefer black males in all five categories. Male choice was determined by male initiated behaviors like approach, chasing and courtship where female choice appeared to be end in mating and rejection. In the approach category, 96

yellow male and 201 black male approaches towards both colors of females, showed there is a significant trend of female preference towards black-painted males, as shown in figure 3a (Chi squared one-sample goodness of fit test, $\chi^2 = 33.5$, $df = 3$, $n = 237$, $p < 0.05$). Figure 3b indicates that black painted males most often chase black painted females (Chi squared one-sample goodness of fit test, $\chi^2 = 11.1$, $df = 3$, $n = 64$, $p < 0.05$). In 375 observed courtships, 295 were black males courting either color female and 159 were solely black females with black males. This result suggest in courtship, females prefer black males but males are not biased in their color preference of female wing color (Chi squared one-sample goodness of fit test, $\chi^2 = 119.3$, $df = 3$, $n = 375$, $p < 0.05$, Figure 3c). Black painted males are rejected more than yellow painted males by all females in this study. Yellow painted females rejected significantly more black painted males (23) than did black painted females (8) as seen in 3d (Chi squared one-sample goodness of fit test, $\chi^2 = 23.1$, $df = 3$, $n = 39$, $p < 0.05$). There was only one recorded mating therefore there was insufficient data for analysis. Overall interactions between both colored females and yellow males were substantially lower than those observed between black males (yellow = 206, black = 570). The only consistent overall trend is a female preference for black painted males which implies there is no male choice.

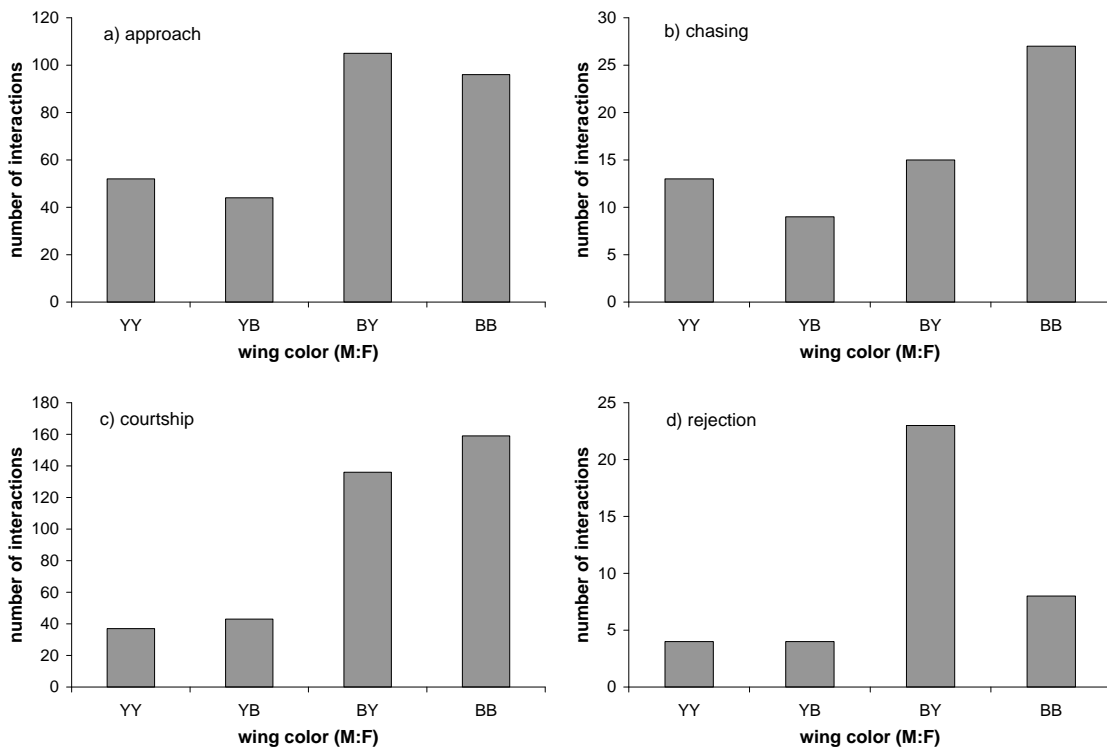


Figure 3. Behavioral observations and comparison of hind wing color preference in *Heliconius sara* including approach (a), chasing (b), courtship (c) and rejection (d) taken over 11 days at the Monteverde Butterfly Garden, Monteverde, Costa Rica. *Heliconius sara* sex and hindwing color are indicated by the x-axis by male: female order and yellow (Y) or black (B).

Additional Observations

Unpainted *H. sara* (those already in the garden before painted individuals were added) interacted with the painted *H. sara* throughout the observation period. Overall 19 approaches, four chases, 64 courtships, six matings and two rejections were recorded. Besides matings, there were no major trends between the interactions. It is interesting to note that all of the matings occurred between unpainted males and painted females (yellow = 4, black = 2). On one occasion several painted males consistently attempted to pull a mating female of mating position with another male. On several instances female *H. sara* resting in the same area would be approached and courted by one or more males of different hind wing colors.

DISCUSSION

These results indicate that black is the preferred mating color for female *H. sara* while no mating color preference exists for male *H. sara*. Mating behavior in butterflies is generally initiated by the male while the final decision for mate choice is determined by the female (Gage et al. 2002). My study supports this by observations that all approaches, chases and courtships were initiated by males, while rejections were determined by females.

Perhaps females are soliciting the males by tactics that are undetectable by the untrained eye, which could attribute to disproportionate interactions with females between yellow and black painted males. According to Crane (1955) during courtship pheromones released by female butterflies can have a faint musky odor only detectable at very close range and difficult for an untrained person to detect. When at maximum excitement, the female can extrude two bulbous excrescences known as “stink clubs” near the junction of the penultimate and distal segments to solicit males. Chemical and behavioral selection would enhance fitness by allowing a new wing pattern to succeed while sexual selection changes to favor the new color.

A possibility is *H. sara theudela*, with a black hindwing, is the primitive color morph. Implying *H. sara fulgidus* has lost the yellow hindwing via evolution to mimic *H. cydno* who is the equivalent to *H. sara theudela* on the Pacific coast. It is possible the yellow females prefer yellow males, although this is unknown. A future study involving the same experiment but with the Pacific form instead where they are paint black and yellow over the yellow hind wing strip, could give insight. If females still prefer the black males it would support my hypothesis that the Pacific form evolved later in spite of sexual selection while females preferring yellow males would indicate sexual selection evolved to favor the new color because it is adaptive in light of Müllerian mimicry.

Another suggestion is that the dark hind wing pattern of *H. sara fulgidus*, in a mimicry complex with *H. cydno*, is the primitive wing color pattern and *H. sara theudela* has evolved by mimicking *H. doris*, *H. pachinus* and *H. hewisoni* (DeVries 1987) (see Figure 4). Previous studies suggest it is possible for sexual selection in *Heliconius* spp. to impede the evolution of new wing color patterns (Jiggins et al. 2001). Female mate choice of black hind wing would constrain the wing color and prevent sexual selection from diverging from the Atlantic wing pattern to the Pacific wing pattern. This in turn

may lead to the constraint of sex-limited mimicry in males. Sex-linked mimicry occurs when only one of the sexes in a species is mimetic (Gage et al. 2002, Thompson 2006),

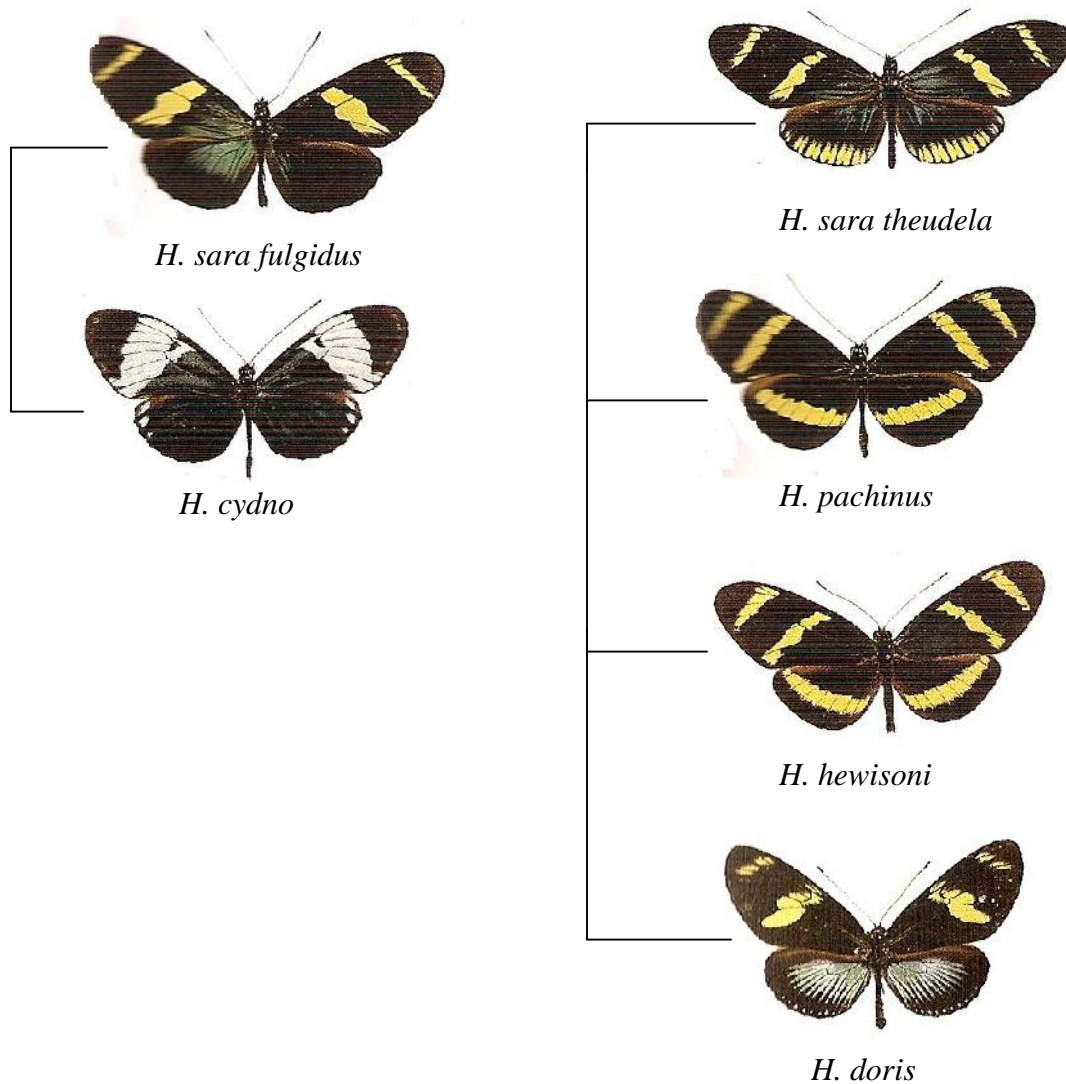


Figure 4. The phylogeny of two *Heliconius sara* races with *H. sara fulgidus* (left) and *H. sara theudela* (right) with their comimics (DeVries 1987).

which is an example of extreme sexual selection such as *Papilio polyxenes asterius* who models *Battus philenor* in a North American mimicry complex or *Spereyia diana* where female-limited mimicry is seen (Krebs 1988, Codella 1989). Genetically between *H. melpomene* and *H. erato* there is a presence or absence of a mark on one of about eight single loci (Futuyma 1986). Due to the male contribution of wing color to their daughters, if the color allele is not on the sex chromosome then female selection of black males will result in black female offspring.

Although females preferred to be courted by black males, observations showed they were willing to be courted by yellow males as well. This indicates there is a chance for the Atlantic form to converge to the Pacific form in which case natural selection would have to be stronger and more beneficial than female selection in mate choice. In future generations the most adaptive response to convergence of natural selection would be for sexual selection to evolve and “catch up” for maximum fitness and fecundity.

Jiggins (2001) proposed that races do not just arise from geographical isolation, but rather the butterflies mimic the most abundant or unpalatable species in the area due to reproductive selective pressures. A classic example is the mimicry complex *H. melpomene* and *H. erato* where evolution has actually caused mimetic shifts leaving the butterflies in reproductive isolation. Wing patterns have evolved a mimicry complex according to geographical range exhibiting sometimes the benefits of mimicry outweigh pressures from sexual and natural selection. It appears the evolution of mimetic patterns is a delicate balance between natural and sexual selection where the benefits of mimicry sometimes outweigh pressures from selection.

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