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Department of Biology, Kenyon College

ABSTRACT

Wing color is important to butterflies both for social signals and protection from predators. Butterflies gain protection from predators by having aposematically colored wings that warn the predator that the butterfly is distasteful. Some butterflies are also part of Mullerian mimicry complexes in which two distasteful species converge on the same color pattern to enhance their aposematic protection. *Heliconius erato* and *Heliconius melpomene* are Mullerian mimics that exhibit a wide variety of wing patterns throughout Central and South America. Their wing patterns have greatly diverged within each species, but between the species they are almost perfect mimics in each area. It has previously been found that *H. melpomene* prefer to court butterflies of their same color pattern race. This study examined a possible mechanism of this racial isolation by investigating the effect of wing color change on *H. melpomene* courting and mating behavior. To accomplish this, 26 female and 18 male *H. melpomene* had the red stripe on their forewing painted. Half of the individuals were painted white, and the other half were painted red as a control. Their mating and courting behavior was observed to see how they were affected by the color change. Over seven days, seven matings were observed – all between red individuals. White males also tried to court red females and were rejected on six different occasions. This demonstrates that decreased reproductive success is an immediate consequence of wing color change and wing color may be responsible for the racial isolation observed in *H. melpomene*. Because of this strong sexual selection to maintain existing color patterns, geographic isolation is most likely necessary for the evolution of any new mimicry patterns.

RESUMEN

El color de las alas de las mariposas se usa para señales sociales y para protección de los predadores. Las mariposas consiguen protección de predadores por tener alas con coloración aposemático que avisa a los predadores que la mariposa es venenosa. Algunas mariposas son parte de complejos de mímica Müllerian en que dos especies venenosas tienen alas muy parecidas para aumentar a su protección aposemático. *Heliconius erato* y *Heliconius melpomene* son mímicos Müllerian que demuestran una gran variedad de diseño de alas por todo Centro y Sur América. Sus diseños de ala son muy variadas dentro de cada especie, pero entre las dos especies son casi mímicos perfectos en cada lugar. Anteriormente fue descubierto que *H. melpomene* prefieren cortejar a mariposas de su mismo raza de diseño. Esta investigación examinó una posible razón por esta aislamiento racial. Se investigó el efecto de cambios en el color de las alas de *H. melpomene* en su apareando y cortejando. Para hacer esto, 26 hembra y 18 macho *H. melpomene* tuvieron la raya roja en su ala pintada. Un medio de los individuos fue pintado blanco y el otro medio fue pintado rojo como un control. Su apareando y cortejando fue observado para ver como fueron afectados por el cambio de color. En siete días, siete copulaciones fueron observados – todos entre individuos rojos. También habían seis veces cuando machos blancos trataron de cortejar a las hembras rojas y fueron rechazados. Esto demuestra que cambiando el color de las alas de mariposas causa una disminución en el éxito reproductivo de una mariposa y el color probablemente es responsable para el aislamiento racial que fue observado en *H. melpomene*. Por esta selección sexual muy fuerte que funciona para mantener el color de ala que existe, el aislamiento probablemente es necesario para la evolución de nuevos diseños mímica.

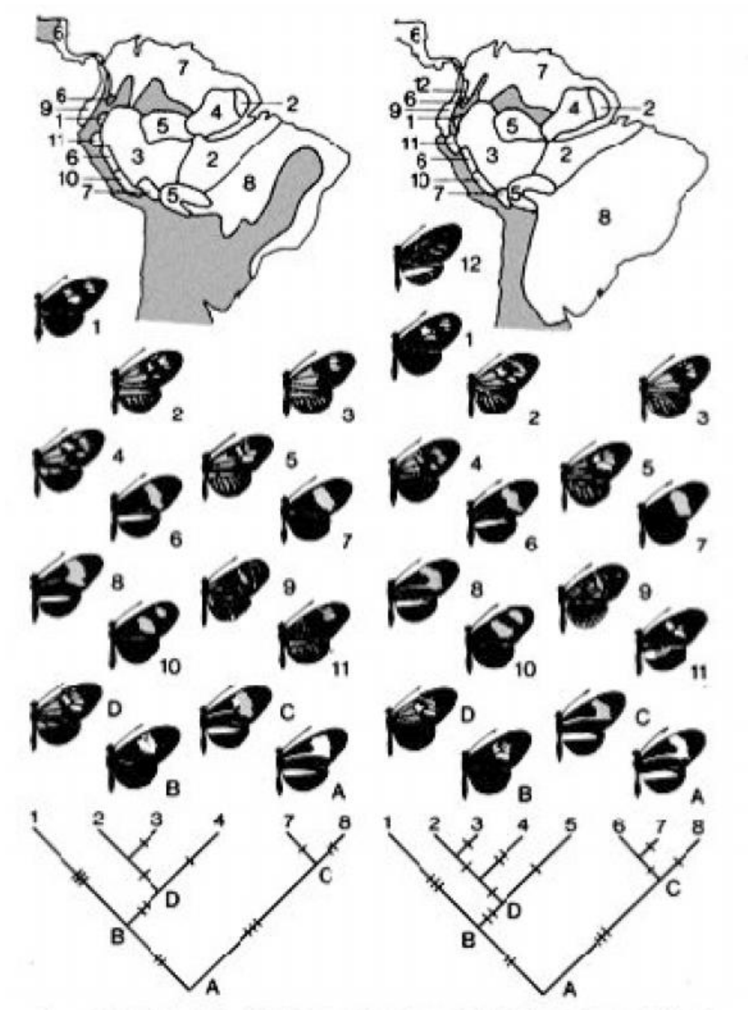
INTRODUCTION

Color patterns of butterflies' wings serve two very important functions: social signals used during courtship and male-male interactions, as well as protection from predators (Silberglied 1977, Poulton 1908, Cott 1940 in Silberglied 1989). Many butterflies have aposematic coloring to warn predators that they are distasteful, and some butterflies form mimicry complexes to further enhance aposematic protection (Turner 1989). One type of mimicry is Mullerian mimicry in which unpalatable species mimic each other (Turner 1989). An especially interesting Mullerian mimicry complex occurs between *Heliconius erato* and *Heliconius melpomene*. Both species have evolved many different wing pattern races that exist throughout Central and South America. Although there is a major divergence of wing pattern within each species, there is almost perfect mimicry between the species in each area (Turner 1981) (Figure 1).

The evolution of mimicry in a species of butterfly results from a balance of sexual and natural selection. The fitness of the mimetic butterflies is increased via natural selection because they have greater protection from predators, however sometimes this can decrease fitness via sexual selection (Benson 1972, Mallet and Barton 1989). Butterflies that evolve a new mimetic pattern may have lowered reproductive success since it can be more difficult for them to attract a mate due to their new coloration (Burns 1966).

Taking this into account, a variety of research has been conducted on the race phenomenon of *H. melpomene* and *H. erato.* In one study researchers investigated whether there was assortative mating between the various *H. melpomene* races. They found that male *H. melpomene* are more likely to approach and court their own color patterns as compared to those *H. melpomene* of other color pattern races (Jiggins et al. 2004). These authors also found that some races of *H. melpomene* had more attractive patterns than others and red was found to be especially attractive across all races (Jiggins 2001; Jiggins et al. 2004). For example, they found that almost all races were attracted to the red stripe on the forewing of the "postman" race and there was a higher probability that males would court a race other than their own if the "postman" race was the other option (Figure 2, see Tarapoto race). Crane (1955 in Silberglied 1989) also found that red was important for *H. erato*. Crane painted the red stripe on the forewing of both sexes and found that the farther the color was from the natural red, the less the painted butterfly was recognized by other *H. erato.* Crane's study did not measure recognition in a quantifiable way, and so this raises the question of exactly what the consequences of wing pattern change are for butterflies and how these consequences may play into sexual selection and the evolution of mimetic patterns. It is interesting to consider whether it is really visual cues involving wing color, or other factors such as chemical signaling, that may be causing the sexual selection that isolates *H. melpomene* races. This study aims to examine this question by looking at the effects of color change on the mating and courting behavior of *H. melpomene*.

a)



b)

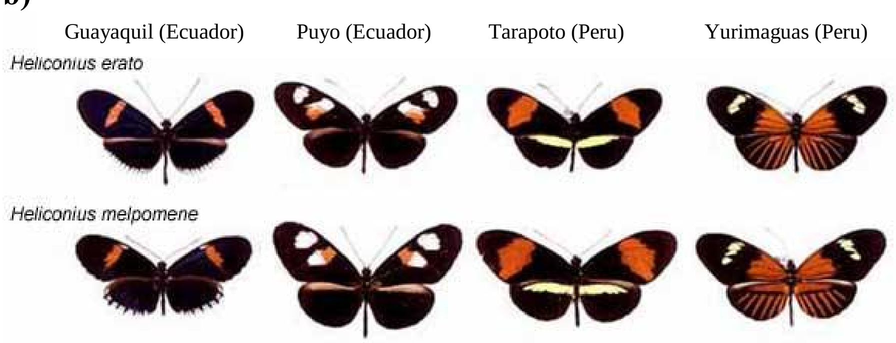


Figure 1a. The distribution and phylogeny of the various races of *H. melpomene* (left) and *H. erato* (right). Numbers denote existing forms and letters denote hypothetical ancestors (Turner 1981).
1b. A few of the races of *H. melpomene* and *H. erato* in color (www.cnrs.fr, November 26, 2006).

METHODS

Study Organism

H. melpomene are a useful model organism for the study of color change and its effect on mating behavior. Their mating behavior usually involves males searching for females using visual cues, and then once they locate them, courtship behaviors usually incorporate tactile and chemical communication (Jiggins et al. 2004). *H. melpomene* is an ideal *Heliconius* subject to study mating behavior because unlike other *Heliconius* that mate at female pupation, *H. melpomene* mate as adults. The adults are polygamous, which gives the opportunity to observe multiple mating events of one individual (Jiggins et al. 2004).

Study Site

This study was conducted at the Monteverde Butterfly Garden in Monteverde, Costa Rica in a 5 x 15 m area that that contained *Heliconius* butterflies of the following species: *H. sara, H. charitonius, H. hecale,* and *Dryas julia.* The most dominant vegetation in the garden was plants of the species: *Lantana camara* (F. Verbenaceae), *Stachytarpheta jamaicensis* (F. Verbenaceae), *Asclepias curassivica* (F. Asclepiadaceae), and *Gurania sp.* (F. Cucurbitaceae). There were also hanging cups of *L. camara* injected with sugar water for the butterflies to feed from. One end of the garden received full sun while the other tended to be slightly shadier.

Procedure

H. melpomene pupae were obtained from Finca de Mariposas la Guácima and pinned to an eclosion chamber at the Monteverde Butterfly Garden. Upon eclosing, the sexes were placed into separate net cages that were about one m tall and a half m in diameter. Once the butterflies were a day old and their wings were strong and dry, half of each sex had the red stripe on their forewing painted white using a Sharpie Paint Pen, and the other half had the same stripe painted red as a control.

A total of 26 female and 18 male butterflies were released over the course of three days; half of each sex was painted red and the other half was painted white. No more than 10 *H. melpomene* already lived in the garden and were left unaltered. The mating and courting behavior of the butterflies was observed in the morning on seven days (including the three days butterflies were released) for about five hours at a time. Courting behavior was defined as a male butterfly hovering over a perched female.

RESULTS

Only red individuals mated and all of the mating occurred between unaltered or red-painted males and red-painted females: three times with red-painted males and four times with unaltered males (Table 1). Almost all of the mating behavior occurred in the same area of the garden where abiotic conditions were breezier and slightly brighter. Four of the matings took place on the same *L. camara* plant. Matings were easy to observe because the butterflies remained in copula for one to three hours.

TABLE 1. *Courting and mating behavior of painted and unaltered* H. melpomene *butterflies as observed on seven days at the Monteverde Butterfly Garden*

	Red-Painted Female (n=13)	White-Painted Female (n=13)
Red-Painted Male (n=9)	3 matings	1 male courted the female and lost interest
White-Painted Male (n=9)	6 males courted and were rejected	
Unaltered Male (n<10)	4 matings	

In addition to never mating, the white males were observed to be rejected when they tried to court red-painted females (Table 1). The white males hovered over females who usually kept their wings completely closed while the male tried to court them and if the male still did not stop, they usually flew away from him. One white male even attempted to grab onto a female and mate with her, but she managed to escape.

Finally, there was one occasion when a red-painted male began courting a white-painted female and shortly thereafter moved away from her (Table 1). In this case, the female seemed interested in mating with the male since she was flapping her wings, too, but the male lost interest and so they did not mate. There were very few other interactions between the *H. melpomene* individuals and there were no obvious male-male chases or other noteworthy behaviors.

DISCUSSION

These results demonstrate that wing color changes in *H. melpomene* lead to decreased reproductive success. This was shown since none of the white butterflies mated. This study reinforces the idea that pattern and visual cues are important in mate selection for *Heliconius* butterflies and it suggests that wing color most likely plays a role in *H. melpomene* preference to court and mate with butterflies of their own color pattern (Jiggins et al. 2001, Jiggins 2004). This study did not find any interactions between two white individuals, which is most likely due to the fact that since the butterflies were painted they still remained "mentally" red. Overall, this study demonstrates that sexual selection will work to maintain the current color pattern since new patterns tend to reproductively isolate butterflies.

If there is strong sexual selection for the maintenance of an existing color pattern, then how is it possible that so many different races of *H. melpomene* and *H. erato* could have evolved? Much effort has gone into investigating this phenomenon and it has been suggested that the many races are the result of island refuges created when rain forests in South America were reduced due to cool, dry conditions during the Pleistocene era (Simpson and Haffer 1978). The clear regional patterns of the different races are well correlated to the areas that most likely contained continuous forest during the last glacial maximum (Brown 1981). During these isolating events it may have been easy for mutations to cause variations in color pattern in each population since the presence or absence of each mark on *H. melpomene* and *H. erato* is controlled by one of about eight single loci (Futuyma 1986). It is also proposed that the races did not simply arise from geographic isolation, but rather the resulting selective pressure that caused the butterflies to mimic the most abundant or distasteful species in the area (Brown 1974). Further study has also suggested that refuges are not necessary and it is possible for races to form parapatrically if there are changes in butterfly abundance within a continuous forest. The new race forms when the butterflies starts to mimic that newly abundant species (Turner 1981). This seems unlikely because converging on the abundant pattern will still ultimately lead to a decrease in fitness; fitness may be increased slightly if converging on the abundant pattern actually provides greater protection from predators, however this will be outweighed by the dramatic decrease in reproductive success. This provides an interesting example of the way that the evolution of mimetic patterns is really a balance of natural and sexual selection and in this case sexual selection is clearly playing a larger role.

Another mimetic phenomenon that demonstrates an interesting balance between natural and sexual selection is sex-limited mimicry. Sex-limited mimicry occurs when just one of the sexes, generally females, in a species is mimetic. This shows extreme sexual selection. Males are very constrained and are not able to adopt the more protected aposematic coloring because females strongly preferentially mate with males of the ancestral color pattern. It is also interesting to consider the opposing selective pressures placed on females in sex-limited species. There is pressure via sexual selection for the females to retain their original color because males may preferentially mate with females with male-like color patterns (Burns 1966). However, an opposite pressure exists through natural selection which favors females with mimetic patterns since they are less susceptible to predation. Unlike the other cases discussed, in this situation the sexual selection to maintain the ancestral pattern is not as strong and natural selection selects for some butterflies to evolve the mimetic pattern.

Overall, this study shows that color is a very important element in mating success and strong sexual selection to maintain the original color pattern can constrain the evolution of new mimetic patterns in *H. melpomene*. New races are likely to evolve only during large isolating events when new *H. melpomene* races escape the presence of another race that would otherwise be maintained through sexual selection. Natural selection also plays a role in maintaining the original race since it is the one most recognized as distasteful by predators. Thus sexual selection and natural selection both play an important part in maintaining the existing color pattern of an area and so the evolution of mimetic patterns is unlikely: the costs of developing a new mimetic pattern generally outweigh the benefits.

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LITERATURE CITED

Benson, W. W. 1972. Natural selection for Mullerian Mimicry in Heliconius erato in Costa Rica. Science 176: 936-939.

Brown, K. S. 1981. The Biology of Heliconius and Related Genera. Annual Review of Entomology 20: 427-56.

Brown, K. S., P. M. Sheppard, and J. R. G. Turner. 1974. Proceedings of the Royal Society of London 18: 369-378.

Burns, J. M. 1966. Preferential Mating versus Mimicry: Disruptive Selection and Sex-limited Dimorphism in Papilio glaucus. Science 153: 551-553.

Futuyma, D. J. 1986. The Evolution of Interactions Among Species. *In* D. J. Futuyma. Evolutionary Biology, pp. 482-504. Sinauer Associates, Inc., Sunderland, Massachusetts.

Jiggins, C. D., R. E. Naisbit, R. L. Coe, and J. Mallet. 2001. Reproductive isolation caused by colour pattern mimicry. Nature. 411: 302–305.

Jiggins, C. D., C. Estrada, and A. Rodrigues. 2004. Mimicry and the evolution of premating isolation in Heliconius melpomene Linnaeus. J. Evol. Biol. 17: 680-691.

Joron, M. and I. Olivieri. 2000. La selection naturelle. www.cnrs.fr Accessed on 2006 November 26.

Mallet, J. and N. H. Barton. 1989. Strong Natural Selection in a Warning-Color Hybrid Zone. Evolution 43: 421-431.

Silberglied, R. E. 1989. Visual Communication and Sexual Selection Among Butterflies. *In* R. I. Vane-Wright and P. R. Ackery (Eds.). The Biology of Butterflies, pp. 207-223. Princeton University Press, Princeton, New Jersey.

Simpson, B. B. and J. Haffer. 1978. Speciation patterns in the Amazonian forest biota. Annual Review of Ecology and Systematics 9: 497-518.

Turner, J. R. G. 1981. Adaptation and Evolution in Heliconius: A Defense of NewDarwinism. Annual Review of Ecology and Systematics, 12: 99-121.

Turner, J. R. G. 1989. Mimicry: The Palatability Spectrum and its Consequences. *In* R. I. Vane-Wright and P. R. Ackery (Eds.). The Biology of Butterflies, pp. 207-223. Princeton University Press, Princeton, New Jersey.