Two-headed butterfly vs. mantis: do false antennae matter? (#16517)

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Two-headed butterfly vs. mantis: do false antennae matter?

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The colour patterns and morphological peculiarities of the hindwings of several butterfly species result in the appearance of a head at the rear end of the insect's body. Although some experimental evidence supports the hypothesis that the "false head" deflects predator attacks towards the rear end of the butterfly, more research is needed to determine the role of the different components of the "false head". We explored the role of hindwing tails (presumably mimicking antennae) in predator deception in the "false head" butterfly Callophrys xami. We exposed butterflies with intact wings and with hindwing tails experimentally ablated to female mantises (Stagmomantis limbata). We found no differences in the number of butterflies being attacked and the number of butterflies escaping predation between both groups. However, our behavioural observations indicate that some aspects of the "false head" help C. xami survive some mantis attacks, supporting the notion that they are adaptations against predators.

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ABSTRACT

The colour patterns and morphological peculiarities of the hindwings of several butterfly species result in the appearance of a head at the rear end of the insect's body. Although some experimental evidence supports the hypothesis that the "false head" deflects predator attacks towards the rear end of the butterfly, more research is needed to determine the role of the different components of the "false head". We explored the role of hindwing tails (presumably mimicking antennae) in predator deception in the "false head" butterfly *Callophrys xami*. We exposed butterflies with intact wings and with hindwing tails experimentally ablated to female mantises (*Stagmomantis limbata*). We found no differences in the number of butterflies being attacked and the number of butterflies escaping predation between both groups. However, our behavioural observations indicate that some aspects of the "false head" help *C. xami* survive some mantis attacks, supporting the notion that they are adaptations against predators.

INTRODUCTION

Butterfly wings are canvases on which evolution designs solutions to the problems posed by thermoregulation, sexual selection and predation (*Monteiro & Prudic, 2010*; *Kemp & Rutowski, 2011*). These adaptations frequently involve compromises between selective pressures when optimal trait values differ between functions (*Ellers & Boggs, 2003*), although sometimes they coincide (*Finkbeiner et al., 2014*). Several butterfly species exhibit colour patterns and morphological peculiarities in their hindwings that suggest, at least to the human eye, that a butterfly resting with its wings closed possess a second head at the rear end of its body (*Robbins, 1980*; *Cordero, 2001*). This appearance is enhanced by peculiar behaviours, such as the back and forth movements of the closed hindwings that presumably permit the "false antennae"—the "tails" frequently present in the border of the anal angle of the hindwings (Fig. 1a)—mimic the movements of real antennae (*Robbins, 1980*; *López-Palafox et al., 2015*). False head butterflies are especially common among the subfamily Theclinae (Lycaenidae).

Although several specific hypotheses on the function of the "false head" have been advanced, all of them consider visually oriented predators as the main selective pressure, and avoidance or deflection of attacks as the main advantage (*Robbins, 1980*; *Cordero, 2001*). Although false head butterflies are textbook examples of anti-predator adaptations (e.g. *Wickler, 1968*; *Ruxton et al., 2004*), to the best of our knowledge, there are only two published experimental studies testing the effect of false heads on probability of predation in live butterflies. *Sourakov (2013)* exposed two *Calycopis cecrops* (Lycaenidae) butterflies, a species of with false head, and thirteen individuals from eleven species of butterflies and moths without false heads, to one individual predatory salticid spider (*Phidippus pulcherrimus*). The spider repeatedly failed to trap the lycaenid butterflies because directed all its attacks towards the false head, but captured all individuals from the other species, mostly (11 out of 13 cases) in the first or second attack. *Wourms & Wasserman (1985)* added artificial "false heads" to *Pieris rapae* (Pieridae) butterflies by attaching tails ("false antennae") and painting spots ("false eyes") on the anal angle of the hindwings, as well as by painting lines converging on the anal angle, three of the main components of the false heads identified by *Robbins (1980)*. *Wourms & Wasserman (1985)* compared predation rates by Blue Jays (*Cyanocitta cristata*) between intact butterflies and butterflies with false heads added. All control and experimental butterflies attacked were caught, but the percentage of butterflies escaping during handling was twice as large in the treatment with artificial false heads as in the control group (16 out of 60 *vs*. 10 out of 79, respectively). The authors mention that butterflies escaped due to "mishandlings" by the birds, i.e. due to errors resulting from misdirected strikes while handling captured prey (*Wourms & Wasserman, 1985*). Thus, the experimental research available supports the idea that false heads help butterflies to deflect attacks away from their less vulnerable end (*Wourms & Wasserman, 1985*; *Sourakov, 2013*). However, these studies have some limitations. *Sourakov´s (2013)* sample size was very small and the control group differed in a number of morphological and behavioural aspects besides the absence of a false head, while *Wourms & Wasserman (1985)* recognized that the wing shape of *P. rapae* is different from that of "false-head" Lycaenidae and that some of the behaviours associated with the functioning of false heads are absent in this species.

In this paper, we focus on the role of one component of the typical lycaenid butterfly false-head: the "tails" present in the border of the anal angle of the hindwings presumably resembling the antennae. These "false antennae" are present in most Lycaenidae species considered to have false heads (*Robbins, 1981*) and the peculiar behaviour consisting in the back and forth movement of the closed hindwings (HWM) observed in many perching Lycaenidae is thought to permit the false antennae to move like the real antennae (*Robbins, 1980*; *López-Palafox et al., 2015*). We present the results of an experiment in which we measured the effects of experimentally ablating the hindwing tails in the false head butterfly *Callophrys xami* (Lycaenidae: Techlinae) (Fig. 1) on the probabilities of exhibiting HWM and of being attacked and captured by female mantises (*Stagmomantis limbata*).

MATERIALS AND METHODS

Experimental butterflies were raised from eggs laid by three females collected in the Pedregal de San Ángel Ecological Reserve (PSAER) of the Universidad Nacional Autónoma de México (UNAM), located in the main campus of the UNAM in the South of Mexico City. Specimens were captured under a Scientific Collector permit granted to the second author by the Mexican Secretaría de Medio Ambiente y Recursos Naturales (FAUT-0237). *C. xami* is a multivoltine "false head" butterfly whose main food plant in the collection site is *Echeveria gibbiflora* DC (Crassulaceae). Rearing methods followed *Jiménez & Soberón (1988-1989)*. The predators used in the experiment were adult females (males did not attack butterflies in pilot tests) of the mantis *S. limbata*, a species living in the PSAER and, therefore, a potential natural predator of *C. xami*. Part of the females were the offspring of a female collected in the PSAER, whose nymphs were maintained individually in ½ L plastic containers until the fourth instar and afterwards in 1 L containers. Nymphs from instars 1 to 3 were fed *Drosophila nubin* ad libitum every other day, and afterwards with *Achaeta domesticus* crickets. The rest of the female mantis used were raised and donated by the Unidad de Manejo Ambiental Yolkatsin (México). All insects were maintained at ambient temperature under a 12 h dark–12 h light photoperiod in the insectary of the Instituto de Ecología (UNAM) located besides the PSAER.

The butterflies were randomly assigned to a treatment group: in the experimental group the hindwing tails were ablated (Fig. 1b), whereas in the control group the wings remained intact (Fig. 1a). Hindwing tails ablation was achieved by first introducing the butterflies in a -20°C freezer until they were immobile (between 2 and 5 min), then the tails were cut out with micro-scissors (Iris Scissors, Bioquip™). Manipulation of each butterfly lasted approximately 2 min. Control individuals were also introduced in the freezer and manipulated for a similar amount of time as experimental butterflies. A total of 26 butterflies of both sexes were attacked thus producing experimental data (14 males: 8 control, 6 experimental; 12 females: 6 control, 6 experimental; see Appendix). Twenty four female mantises were used, but five never attacked. Twelve mantises that attacked were used just once (six with experimental and six with control butterflies) and seven were used twice (four were used once with an experimental and once with a control butterfly, two only with control butterflies and one only with experimental butterflies). Mantises used twice had a time interval between trials of at least two weeks. To increase the probability of attack, mantis were starved three days before being exposed to a butterfly.

Butterflies were individually exposed to one mantis in a glass chamber measuring 33.4 cm × 13.3 cm × 11.8 cm, with one of the two largest (33. 4 cm × 13.3 cm) sides covered with white Styrofoam. A Sony Handycam HDR-SR1was used to film most of the trials (23 out of 26). The mantis was introduced to the experimental chamber two hours before each trial. Afterwards, the butterfly was gently introduced in the chamber in a position as far as possible from the mantis. If after five minutes the mantis did not attacked, the trial was discarded. If the mantis attacked within five minutes after the introduction of the butterfly, we recorded the result (i.e. if the butterfly was captured or escaped) and finished the trial. We allowed just one attack with exception of one case in which the butterfly escaped the first attack (this was the result used in the analysis) but, since it was in one corner of the chamber, rapidly flew back close to the mantis and it was captured.

RESULTS

We staged 22 control and 22 experimental interactions between a mantis and a butterfly. Twenty six butterflies (59.1%) were attacked. The butterflies were attacked when they were walking (away or towards the mantis), perching after walking or after landing; in one case the butterfly was detected after stepping on one leg of the mantis. The number of butterflies attacked (Fig. 2a) was statistically independent of the presence of hindwing tails (Chi squared = 0.38, $P = 0.54$, *gl.* = 1). The number of attacked butterflies displaying hindwing movements (that presumably allow the hindwing tails to mimic the movement of antennae) during the whole interaction with a mantis (Fig. 2b) was statistically independent of the presence of hindwing tails (Chi squared = 1.47, $P = 0.22$, *gl.* = 1).

The number of butterflies surviving the attack (Fig. 2c) was statistically independent of the presence of hindwing tails (Chi squared = 0.25, $P = 0.62$, *gl.* = 1). In one of the 10 interactions in which the butterfly escaped, the mantis directed the attack towards the "false head" of a control butterfly despite the real head of the butterfly was closer to the head and front legs of the mantis. In other three interactions in which the butterfly escaped (two controls and one with hindwing tails ablated), the mantis directed the attack towards the rear side of the butterfly (which in two cases was closer to the mantis), grabbed part of the wings and the butterfly escaped after losing one or two pieces of wings; in one of these cases the mantis grabbed the distal tip of the forewings just with one leg and the butterfly escaped after some struggle, while in the other two cases the attack was directed to the "false head" area and the butterfly escaped rapidly. In other five cases (two controls and three with hindwing tails ablated), the butterfly escaped after being touched by the mantis. We do not have a video of the remaining case in which a control butterfly escaped.

DISCUSSION

Our results indicate that the presence of hindwing tails in perching *C. xami* butterflies does not affect the probability of surviving an attack from a mantis that is possibly a natural predator. At least three kinds of explanations are possible for this result. First, hindwing tails could perform no function in this species, but being present because they were inherited from their phylogenetic ancestors. We cannot discard this possibility, but phylogenetic inertia seems unlikely considering that in Theclinae (the diverse subfamily including *C. xami*) false head components evolve rapidly (*Robbins, 1981*). Second, hindwing tails could be involved in a different function, such as in courtship behaviour or flight manoeuvrability. This alternative deserves further study. Finally, hindwing tails could improve the deceiving effect of "false heads" (i.e. act as "false antennae") against predators different from mantises, such as birds that detect their prey and actively, and rapidly, approach it from a relatively long distance. In contrast, against a mantis, a predator that relies on crypsis and has a sit-and-wait strategy that allows more time to observe the prey at close range, hindwing tails could be useless. In fact, our observations suggest that *S. limbata* cryptic appearance and behaviour is quite successful against *C. xami* since in many cases the attacked butterflies approached the mantis (an extreme case was that of a butterfly that was detected because stepped over a mantis leg). Furthermore, the back and forth movements of the closed hindwings, that presumably permit the "false antennae" mimic the movements of real antennae (*Robbins, 1980*; *López-Palafox et al., 2015*), possibly have a negative effect because they appeared to attract the attention of the mantis in some cases (personal observations).

Although our observations show that in many cases mantises did not direct their attacks towards the "false head" and that many attacks resulted in successful capture of butterflies (16 out of 26 in our experiment), our study also indicates that at least some aspects of the "false head" help *C. xami* survive some mantis attacks, supporting the notion that they are adaptations against predators (*Robbins, 1980*; *Cordero, 2001*; *Sourakov, 2013*). The three butterflies that we are sure were attacked in the "false head" zone were able to escape. In two of these cases (one control and one with hindwing tails ablated) the mantis teared small pieces of wing from the false head area, an observations consistent with the idea that the "false head" area breaks-off easily (*Robbins, 1980*). In the other case, the behaviour of the mantis suggested that she choose to attack the rear end of the butterfly because the attack was directed towards the "false head", even though the head and front legs of the mantis were closer (just a few millimetres away) to the real butterfly head; this case involved a control butterfly that escaped before having being gripped. Thus, our observations indicate that escaping from an attacking mantis also depends on other factors, such as the ability to take flight rapidly and the strength of the grip when the mantis grabs the wings.

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Figure 1 *Callophrys xami* (a) with hindwing tails intact (control) and (b) with hindwing tails experimentally ablated (dead experimental specimen with broken antennae). Photographs by Raúl Iván Martínez.

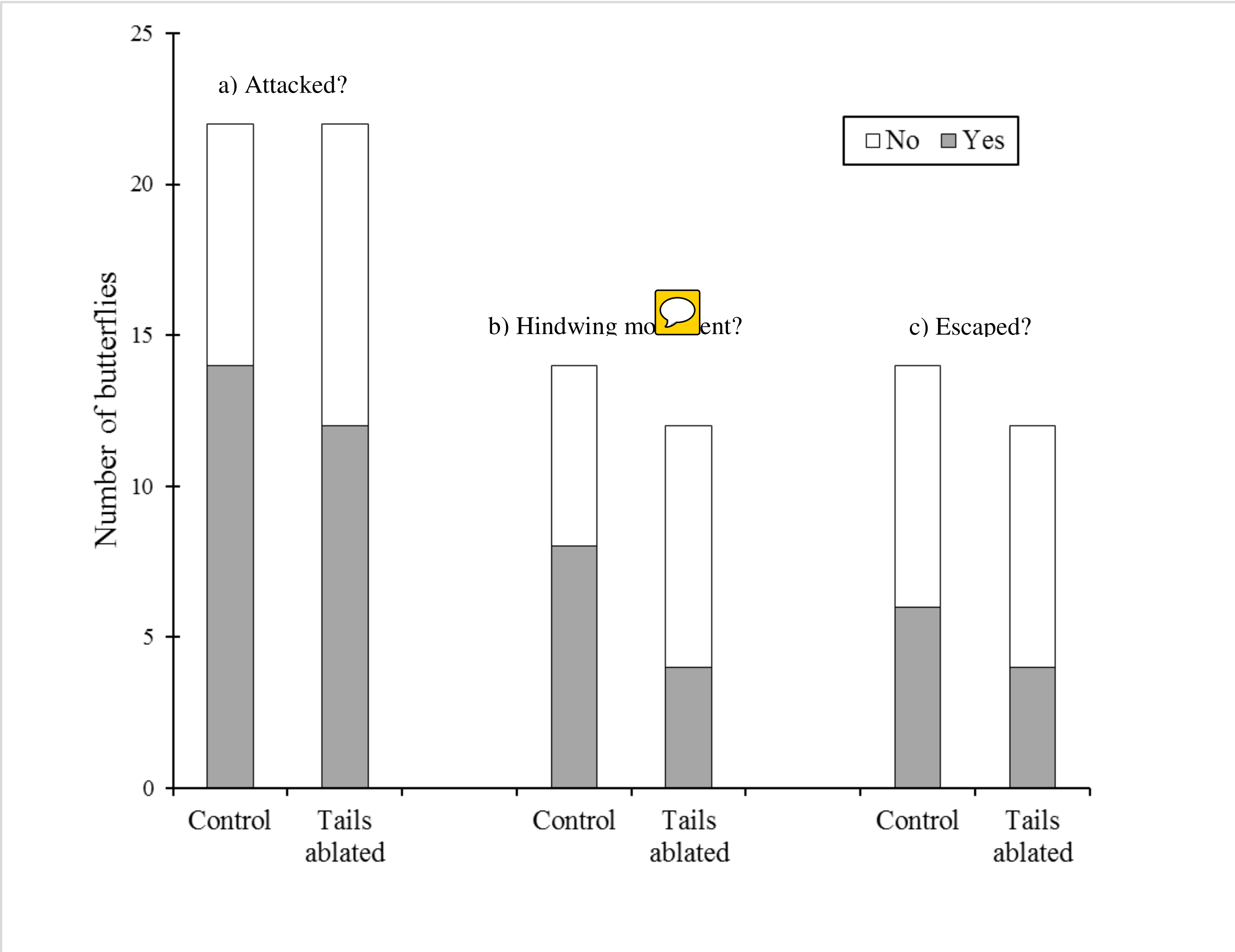

Figure 2 Experimental ablation of hindwing tails ("false antennae") in the "false head" butterfly *Callophrys xami* and its effect on interactions with female mantis (*Stagmomantis limbata*). Control butterflies were manipulated in the same way as experimental butterflies but their hindwing tails were not ablated. (a) Number of butterflies attacked (gray) or ignored (white). (b) Number of butterflies that performed hindwing movements (gray) or not (white) before being attacked. (c) Number of butterflies escaping (gray) or being captured (white). None of the differences between control and experimental groups were statistically significant (see text).

APPENDIX

Raw data from the experiment on the effect of ablation of butterfly (*Callophrys xami*) hindwing tails ("false antennae") on hindwing movement (HWM) and capture by female mantis (*Stagmomantis limbata*). Control butterflies were manipulated in the same way as experimental butterflies but their hindwing tails were not ablated.

Treatment	Mantis	Butterfly [a]	HWM	Result
Control	4	37-F	No	Captured
Control	11	14-M	No	Captured
Control	TRONCO	106-F	No	Captured
Control	XOXO	117-F	No	Captured
Control	1	1-M	Yes	Captured
Control	11	126-M	Yes	Captured
Control	15	90-F	Yes	Captured
Control	Z	78-F	Yes	Captured
Control	5	39-M	No	Escaped
Control	13	94-M	No	Escaped
Control	1	38-M	Yes	Escaped
Control	12	86-M	Yes	Escaped
Control	16	92-M	Yes	Escaped
Control	17	127-F	Yes	Escaped
Tails ablated	14	70-M	No	Captured
Tails ablated	14	102-F	No	Captured
Tails ablated	1E	68-M	No	Captured
Tails ablated	2E	60-M	No	Captured
Tails ablated	AGRESIVA	101-F	No	Captured
Tails ablated	TRONCO	128-F	No	Captured
Tails ablated	7	33-F	Yes	Captured
Tails ablated	15	119-M	Yes	Captured
Tails ablated	10	8-M	No	Escaped
Tails ablated	6	42-F	No	Escaped
Tails ablated	5	18-M	Yes	Escaped
Tails ablated	XOXO	129-F	Yes	Escaped

[a] M: male, F: female.