

5/27/17

Review of: Male sex pheromone components in *Heliconius* butterflies released by the androconia affect female choice (#18006)

PeerJ specifics:

1. Basic reporting:

This manuscript is well written with a solid foundation of literature to motivate the research conducted within; figures are legible and appropriately explained in captions. I have no major concerns with the analyses or writing, but please go through the references for consistency in formatting, especially italicizing Linnaean names.

2. Experimental design:

The authors do a thorough job of investigating male pheromone production in three distinct ways, strengthening the conclusions to be drawn from their findings. Reporting of protocols is explicit and clear from data collection to software used in statistical analyses.

3. Validity of findings

The authors present strong evidence for the existence of pheromones in mature males that enhance mating success. Results from the structural, chemical, and behavioral experiments work together to show that chemicals produced (or at least stored) in the hindwings of mature males contribute to reproductive success. I have only a few comments about the reporting of these results and their implications:

First, in L 333-337, the authors note that courting rates differed between control and treated males in one of the four species, yet they report percentages for successful matings pooling all tested species. What do the percentages of mating success look like when excluding the species in which control males courted more frequently than experimentally treated males?

Next, experimental addition of pheromones is the other side of the coin to experimental removal/blocking of such compounds. In L 422-424, the authors give the example of *H. cydno* males unsuccessfully courting heterospecific females. Assuming that the pheromone composition of *H. cydno* is made up of a handful of similar compounds to those studied here, how difficult would it be to synthesize and experimentally apply such compounds to these males to do a complimentary follow up experiment?

Finally, throughout this manuscript, the male butterflies are said to have both forewing and hindwing androconia. This is a reasonable assessment based on morphology alone, as Figure 1 shows sexual dimorphism in both wing pairs. However, the chemical analyses of different parts of the wings suggest that it is largely if not entirely the hindwing region that produces/stores pheromones. Furthermore, the behavioral studies only blocked regions of the hindwing manipulated males to demonstrate differential mating success. It seems that the dorsal hindwing is the key area for pheromone signals in these species.

In light of these results, is it less appropriate to continue calling the forewing regions androconia?
Perhaps a sentence or two could be added about if/how results presented here affect the classification of the regions on the forewing?

4. General comments to the author

This manuscript and the research within are both of high quality and definitely suitable for publication. As you can see, my corrections and suggestions are all minor. At most, a couple of points could be clarified with another line or two of explanation in the text, but no additional analyses will be required. I have mentioned some suggestions above with specific line references, but please see the attached file for the full list of line-by-line comments.

Specific (line-by-line) comments:

L 55-72 clarification: It is my understanding that pheromone calling is a largely female phenomenon in moths and male in butterflies. Your introduction implies as much through cited examples, but perhaps it would be worth stating outright whether this is the case or not.

L 87 Redundant author info: "Crane (Crane, 1955)"

L 111 -118: minor paragraph structure and flow: the sentence starting on 111 pivots to discuss female choice, then on line 114 males are the subject again, before returning to females as the subject in line 118.

L 235 minor clarification: you mention analyses with vegan, an R package, but don't clarify that analyses were carried out in R until mention of the GLMMs around line 280, perhaps clarification that the vegan analyses were R-based as well would be helpful.

L 333 "thepheromone" missing space between words

L 333-337 What do the percentages of mating success look like when excluding the species in which control males courted more frequently than experimentally treated males?

Edits to references, note I may have missed some formatting issues, so please double check all references:

L 460 italicize genus and species name in reference *Ostrinia nubilalis*

L468 italicize genus name in reference *Danaus*

L471 italicize genus name in reference *Heliconius*

L479 italicize genus and species name in reference *Bicyclus anynana*

L485 italicize genus name in reference *Glyptapanteles*

L495 italicize genus and species name in reference *Utetheisa ornatrix*

L505 italicize genus name *Heliconius*

L520 italicize genus name *Heliconius*

L522 italicize genus name *Heliconius*

L527 italicize genus name *Drosophila*

L531 italicize genus name *Drosophila*

L534 spacing issues, genus and species capitalization and italics: “butterflies *Colias eurytheme* and *C. philodice*”

L539 italicize genus and species name *Heliconius melpomene*

L547 italicize genus and species name *Heliconius erato*

L564 italicize genus name *Heliconius*

L567 italicize genus and species names *Drosophila santomea* and *Drosophila yakuba*

L577 italicize genus name *Heliconius*

L579 italicize genus name *Heliconius*

L586 italicize genus name *Heliconius*

L592 italicize genus name *Heliconius*

L612 italicize genus and species name in reference *Bicyclus anynana*

L634 italicize genus and species name *Nasonia vitripennis*

L640 italicize genus and species name *Spodoptera littoralis*

L641 italicize genus and species name *Spodoptera litura*

L643 italicize genus and species name *Heliconius melpomene*

L648 italicize genus name *Heliconius*

L658 italicize genus name *Heliconius*

