

Peer Review

Review of: "Partial Sex-Role Reversal of the Pink Bollworm Moth (*Pectinophora gossypiella*)"

Kota Sakagami¹

1. Museum of Nature and Human Activities Hyogo, Sandachō, Japan

The author investigated the mating behaviours of the pink bollworm moth in males and females under different rearing conditions. He found that both males and females have sexual selection mechanisms. These findings are novel and highly suggestive. However, there is room for further consideration in the interpretation of the data and insufficient data to ensure reproducibility, so I comment as follows.

Major comments

-You hypothesise "(1) small pink bollworm females initiate calling later at night to minimise competition with larger, more preferred females." But you recorded only the mating time in experiment 1. The time of mating is affected by the selection and the approach of males and the pre-mating behaviour of both sexes, as well as the female's calling.

Although experiment 1 shows changes in male selection due to the presence or absence of female information, natural preference for female body size is unknown. I assume that natural preference is the selectivity of males for female body size in each treatment under conditions in which both large and small females are calling. If males prefer the large female when they are calling, then early mating with the large female may be overestimated. Even if the large and small females were calling at similar times, if the males preferred the large female, the females would mate earlier overall. Alternatively, early mating with large females may be underestimated because of the higher rejection rate of large females (the results of experiment 2). Males rejected by the large females may reach the small females, resulting in an early mating time. Thus, I do not think this paper reveals any differences in calling time between large and small females under different circumstances.

If you have the data on calling timing for experiment 1, you need to include it; if not, you need to revise the introduction and discussion. I think you need to focus on whether changing the conditions changes the mating time.

-Why are paired samples t-tests used in the statistical analysis of Experiment 1? What is paired between treatments? I think unpaired t-tests or ANOVA would be appropriate for experiment 1.

Minor comments

-4th paragraph in Introduction: I cannot understand the meaning of the sentence “Male choosiness is expected in months.”

-Page 3, line 10: The “(” remains.

-Page 3, lines 13–14: Reference [41] is about bollworm moths (*Heliothis zea*) (not pink bollworm moth). You should revise this paragraph. If possible, it would be nice to have some basic information on how far each sex disperses and mates, and where on the plant they mate.

-Methods Experiment 1: The spatial scale of the experiment room is considered important for reproducibility because it affects the concentration of sex pheromones in the air. I would like you to add the size of the room used in the experiment and, if possible, how well it was ventilated.

-Page 4, line 20: cages (20X20X20 cm) → cages (20×20×20 cm)

-Page 4, lines 17–18: You define large and small body size by pupal weight, but what are the upper and lower percentages of the overall distribution, respectively? I would like to know the mean and standard deviation of pupal weights, the top rate of large females, and the bottom rate of small females.

-Page 4, line 24: How did you observe the behaviour of moths in the dark room? If you used any special methods, please describe them.

-Page 4, line 24: What time zone is the “sunrise” in, and what time range? Also, isn’t 3–4 hours before sunrise the time when the calling has already started?

-Page 5, line 3: Does “first” mean from the start of the observation?

-Results Experiment 1: The results section only shows comparisons based on statistical analysis of the data. Please provide statistical values such as mean, standard error, etc., so that we can see what kind of results were obtained from the data.

-In the caption of the table, it would be better to first write straightforwardly what kind of content is being shown. In this case, it would be better to write “Results of statistical analysis in Experiment 1” first.

-Figure 1: What do the error bars indicate?

-Table 2: The acceptance rate of the treatment of small females vs large is over 50%. Does this mean that there are female individuals that have mated more than once? I feel that females mating more than once could affect female selectivity. I would like you to mention that you have observed cases of the same female individual mating more than once. I would also appreciate it if you could describe how often this occurred, if possible.

-Figure 3: I think it would be easier to understand if the labels on the horizontal axis were reversed: large female/small female should be placed on top and large/small male on the bottom.

-2nd line in discussion: Both females and male moths → Both female and male moths

-4th paragraph in discussion: You mention the possibility that the high rejection rate of large females indicates an indirect strategy and give examples of indirect strategies in other animals. However, the results of Experiment 2 do not directly demonstrate an indirect strategy in females. It is unclear whether the high rejection rate in large females ultimately leads to mating with high-quality males and by what logic this is occurring. I think it would be better to write in this paragraph a perspective on how you would verify this possibility.

-Page 12, line 14, 15–16: “(personal observations, submitted)” remains in the main text. I think you should move these words to References.

Declarations

Potential competing interests: No potential competing interests to declare.