Laboratory Mating of Mutualistic Seed-Parasitic Moth Epicephala bipollenella (Lepidoptera: Gracillariidae)

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Source: The Journal of the Lepidopterists' Society, 68(4) : 285-286

Published By: The Lepidopterists' Society

URL: https://doi.org/10.18473/lepi.v68i4.a7

Journal of the Lepidopterists' Society
68(4), 2014, 285–286

LABORATORY MATING OF MUTUALISTIC SEED-PARASITIC MOTH *EPICEPHALA BIPOLLENELLA* (LEPIDOPTERA: GRACILLARIIDAE)

Additional key words: *Glochidion*, obligate pollination mutualism, Phyllanthaceae, pollinator

The techniques of laboratory mating are vital for the successful rearing of model animals and are essential tools in various experiments, stable behavioral tests, and genetic research (Ohshima 2012). Breeding pollinating insects in the laboratory that are obligatorily associated with plants, such as yucca moths and fig wasps, under laboratory conditions has been especially unsuccessful. In such obligate pollination mutualisms, the floral volatiles are extremely important signals for attracting the species-specific pollinators (Svensson et al. 2005; Okamoto et al. 2007; Chen et al. 2009), and in behavioral tests using sufficient individuals it is critical to clarify the ecological and evolutionary roles of floral volatiles. Thus, the establishing of a method for successfully rearing insect pollinators will be a breakthrough for ecological and evolutionary studies of mutualistic interactions. This study presents a first step toward that goal.

Epicephala bipollenella Li, Wang & Hu, 2012 is a small moth species belonging to the family Gracillariidae, which has been recorded from southern Japan, Taiwan, and China (Okamoto et al. 2013; Zhang et al. 2012). Female adults actively pollinate the flowers of *Glochidion zeylanicum* and lay eggs in the ovaries. The emerging larvae feed only on a subset of the developing seeds (Kato et al. 2003; Okamoto et al. 2013). Just before pupation, the larvae emerge from the host fruit and immediately pupate on the ground. Adult moths, especially females, can be found on the host plants during the night. Adult males are found only occasionally, because only females visit the male and female flowers to pollinate them actively and oviposit, while males do not visit flowers to forage. Nonetheless, the mating behaviors of *E. bipollenella* have never been observed in the field, suggesting that they may be mating during a specific time of day. This makes it difficult to establish a method of successfully rearing in *Epicephala* moths. Here, I present a method for laboratory mating experiments with *E. bipollenella*.

To obtain last-instar larvae of *E. bipollenella*, I collected the mature fruit of *G. zeylanicum* on 13–17 June 2013 on Amami-Oshima Island, Japan. The fruit were kept in soft plastic bags until the larvae emerged from them and completed their pupation. The pupae were moved into plastic containers without plants to prevent any negative effect of plant degradation on moth survival. During the pupal stage, the insects were kept at 28 ± 2 °C or 15 ± 1 °C. At 28 °C, the length of the pupal stage was ca. 10–14 days. At 15 °C, the emergence was delayed by approximately 10–20 days relative to that at 28 °C. These results suggest that *E. bipollenella* is a multiple-brooded species. After adult emergence, I paired the moths in two ways: in one, each male and female adult moth was paired more than two days after their emergence; in the other; the moths were paired within 24 h of emergence. Each pair of moths was placed into a single plastic centrifuge tube (118 mm long, 28 mm in diameter) with tissue paper soaked with 1% sucrose solution for nourishment, because a previous study indicated that the gracillariid moths can be reared on 1% sucrose solution (Ohshima 2005). Each pair was first put under a photophase (16 h light), followed by a scotophase (8 h dark; i.e., L:D=16:8). I monitored them every 30 minutes to confirm mating, including during the scotophase (under a red light to avoid interrupting the night period). These observations showed that *E. bipollenella* was not active during the photophase and mated only during two hours at the end

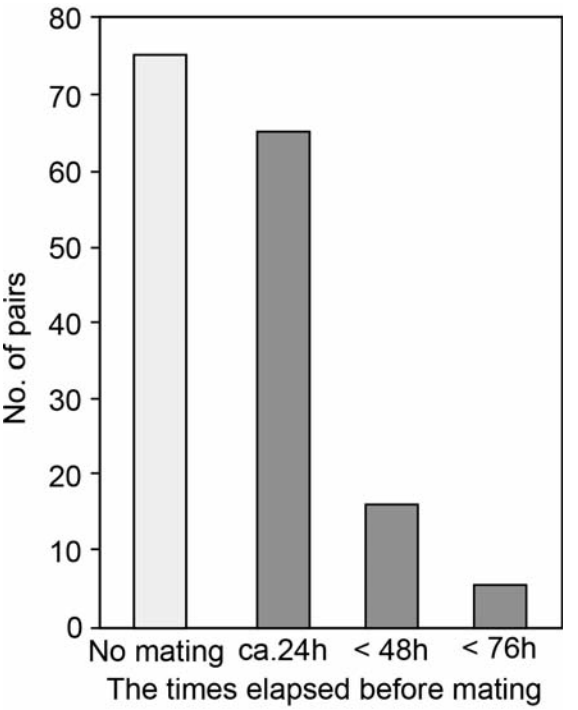

FIG. 1. The times elapsed before mating and the numbers of mated and unmated pairs.

of the scotophase. I confirmed the mating behavior of *E. bipollenella*, in which the male moth approaches the female and fans his wing, which is typical of various moth species (Parra-Pedrazzoli & Leal 2006). A total of 162 pairs of moths were used in the laboratory mating experiments: 65 mated at the end of their first scotophase; 16 pairs mated at the end of their second scotophase; five pairs mated at the end of a later scotophase, and the rest were not observed mating at all (Fig. 1). Thus, the copulation occurred in 53.1 % of all pairs and 1.3 ± 0.6 (SD) days after their emergence. These results strongly suggest that *E. bipollenella* mates during the first or second period of dusk after its emergence in the wild. Although multiple matings were observed in only one pair throughout the experiment, this indicates that these moths can mate multiple times at low frequencies. Further experiments are required using different combinations of moths to confirm whether multiple mating is a general phenomenon in *E. bipollenella.* The oviposition behavior of the female *E. bipollenella* on the host plant is frequently observed between evening and midnight in the wild (Kato et al. 2003), suggesting that the moths can be readily induced to oviposit on flowers. Therefore, cultivation of the host plant should permit the successive rearing of Epicephala moths under laboratory conditions.

ACKNOWLEDGMENTS

I thank I. Ohshima for technical advice on the maintenance of the moths; and H. Kitajima, T. Takanashi, M. Tokoro, and T. Urano for allowing me to use the laboratory. This work was supported by a Japan Society for the Promotion of Science Research Fellowships for Young Scientists grant.

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Submitted for publication 7 January 2014; revised and accepted 24 January 2014.