MATERIALS AND METHODS

The Nephopterix larvae were reared as described in Witethom and Silawatchananai1. After pupation, individuals were sexed, placed in separated containers, and maintained at 25± 1°C, ca 70-80% RH, and under a reversed 15:9 (L:D) photocycle (8.00 AM light-off and 5.00 PM light-on). The light intensity during the photophase was 800-1,000 lux. After emergence, adults were kept under the same conditions till used in the experiments. Moths that emerged within 24-hr period (8.00-8.00 AM) were designated as having emerged on day 1 and those emerging subsequently as having emerged on day 2, 3 etc. Unless otherwise specified all observations were conducted during the scotophase. Observations were made under faint red light.

Observations of calling behavior

After eclosion, each virgin female was confined in a clear vented-plastic container (5 cm in diameter, 6 cm high). A piece of sterilized cotton soaked with 10 % honey solution was provided as food. The calling behavior (pheromone-release behavior) was observed at 15-min intervals throughout the 9-hr scotophase. The calling times during the first seven days were monitored. Calling proportions were calculated from numbers of calling females. The mean onset of calling and the mean time spent calling were computed using only females which actually called. Each female was randomly assigned to one day. The number of females examined varied between ages and the sample sizes are given with the results for each observation.

Observations of mating behavior

Eight to twelve pairs of 1-day-old moths were confined in a metallic mesh screen cage (46x41x41 cm). A piece of sterilized cotton pad moistened with 10% honey solution was supplied as food. The mating behavior and mating time was observed at 15-min interval during the 9-hr dark-period. Similar observation was repeated for 2-to 7-day-old moths. Each observation was replicated five times. Mating proportions were calculated from numbers of mating moths. The mean onset of mating and the mean time spent mating were computed using only moths which actually mated. The number of moths examined varied between ages and the sample sizes are given with the results for each observation.

Statistical analysis

All data were analyzed by using ANOVA. Hypothesis were tested using orthogonal contrasts⁵. The homogeneity of variances was checked by Cochran's C test⁶.