J.Sci.Soc.Thailand, 18(1992) 93-103

RESEARCH ARTICLES

EFFECTS OF AGE ON CALLING AND MATING BEHAVIOR OF THE SAPODILLA FRUIT BORER, *NEPHOPTERIX* SP. (LEPIDOPTERA : PYRALIDAE)

BOONGEUA WITETHOM

Department of Biology, Prince of Songkla University, Hat Yai, Songkhla, Thailand.

(Received 2 May, 1992)

ABSTRACT

The calling and mating behavior of Nephopterix *moths was observed in the laboratory. The patterns of calling and mating behavior varied with age. Moths were most likely to call and mate when 2 to 5 days old. The effects of age on the onset of calling and the time spent calling were non-linear. The onset of mating also changed non-linearly with age, but time spent mating decreased linearly with age. It appears that the change of calling and mating patterns with age is correlated with increased reproductive maturity of females.*

INTRODUCTION

The sapodilla fruit borer, *Nephopterix* sp, is one of the most important pests of unripe sapodilla fruit[1]. It is difficult to control because the larvae feeds inside the fruit. Thus they are protected from insecticides. There are no effective control methods for this species. Therefore the use of sex pheromone, when identified and synthesized, would be a practical means of controlling or predicting population density[2]. Generally, females of many species of moths exhibit a calling behavior (pheromone-release behavior) just before mating during which the sex pheromone is released[3, 4]. Thus it is important to determine the time during which the calling and mating behavior of *Nephopterix* is likely to occur. I report here on the timing of the calling and mating behavior of *Nephopterix* moths as a function of age.

MATERIALS AND METHODS

The *Nephopterix* larvae were reared as described in Witethom and Silawatchananai[1] After pupation, individuals were sexed, placed in separated containers, and maintained at $25 \pm 1°C$, ca 70-80% RH, and under a reversed 15:9 (L:D) photocycle (8.00 AM light-

off and 5.00 P.M. 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 A.M.) 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 (46×41×41 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 contrasts[5]. The homogeneity of variances was checked by Cochran's C test[6].

RESULTS

Calling behavior

Female *Nephopterix* exhibited a sterotyped calling posture. During this time, a female arched the abdomen upward between the wings and extruded the normally hidden eighth and ninth abdominal segments. The arch of the abdomen and the extrusion of the terminal abdominal segments became more apparent as the calling period progressed. The female periodically contracted the terminal abdominal segments.

TABLE 1. The effects of age on onset of calling and time spent calling by female *Nephopterix*. Summary of ANOVA. Variances were homogenous (Cochran's C = 0.25 and 0.27, P>0.05).

		Onset of calling		Time spent calling	
Source of variance	**df**	**MS**	**P**	**MS**	**P**
Age	6	0.94	–	1773	–
Linear trend	1	2.58	0.00	4670	0.01
Quadratic trend	1	2.33	0.00	4904	0.01
Cubic trend	1	0.07	0.59	28	0.85
Error	95	0.23		749	

TABLE 2. The effects of age on onset of mating and time spent mating by *Nephopterix* moths. Summary of ANOVA. Variances were homogenous (Cochran's C = 0.20 and 0.23, P>0.05)

		Onset of mating		Time spent mating	
Source of variance	**df**	**MS**	**P**	**MS**	**P**
Age	6	1.99	–	1343	–
Linear trend	1	5.30	0.00	5752	0.00
Quadratic trend	1	4.41	0.00	923	0.18
Cubic trend	1	0.12	0.48	24	0.83
Error	95	0.25		512	

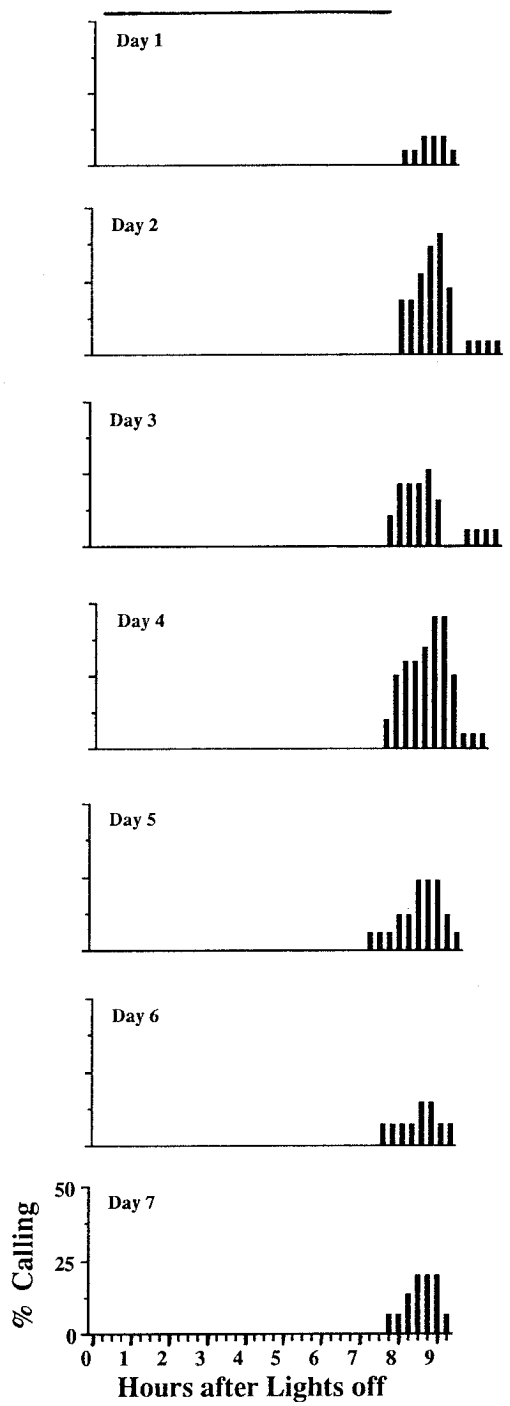

Fig. 1. Distribution of calling times for the first seven scotophase after eclosion in *Nephopterix* females. The line over the top histogram represents the duration of the scotophase. For ages 1 to 7 N=21, 22, 19, 20, 17, 14, 15.

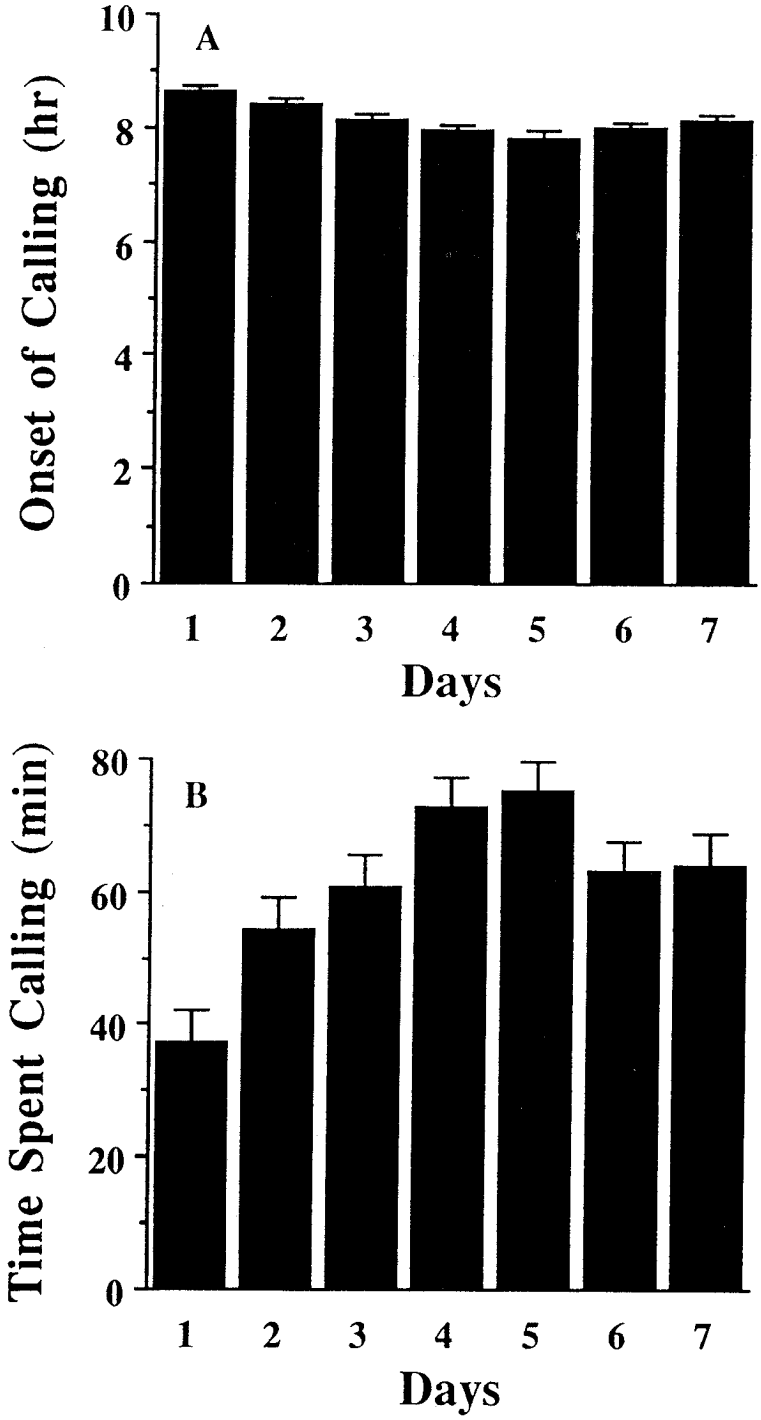

Fig. 2. Effects of age on (A) onset of calling and (B) time spent calling for *Nephopterix* females. Vertical bars represent ± SE. For ages 1 to 7, N=8, 21, 19, 19, 14, 10, 11.

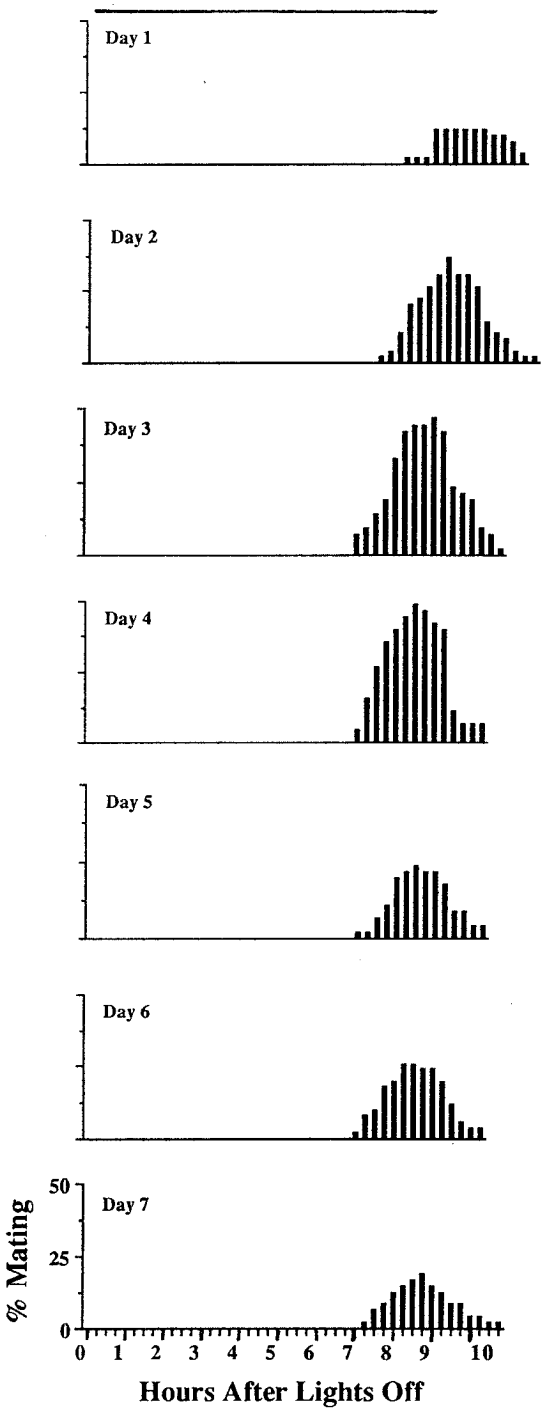

Fig. 3. Distribution of mating times for the first seven scotophase after eclosion in *Nephopterix* females. The line over the top histogram represents the duration of the scotophase. For ages 1 to 7, N=51, 49, 43, 45, 46, 50, 47.

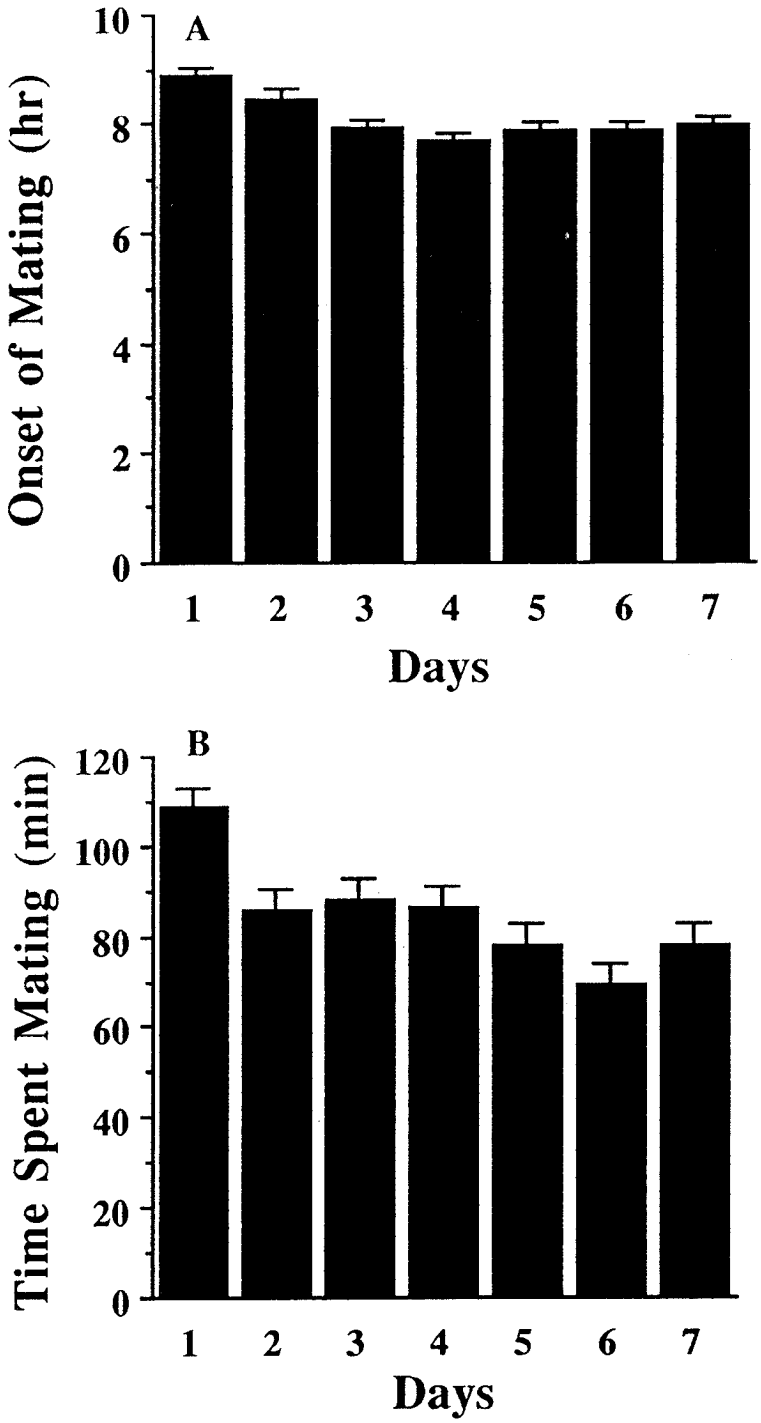

Fig. 4. Effects of age on (A) onset of mating and (B) time spent mating for *Nephopterix* moths. Vertical bars represent ± SE. For ages 1 to 7, N=6, 18, 21, 22, 11, 15, 9.

Calling pattern

Most female commenced calling late ca. 7-8 hr after light-off. Each day the calling gradually increased and peaked at the end of the darkness. Some females continued calling till 1 to 2 hr after light-on. The calling percentage varied with age (Fig. 1). It was minimal in 1-day-old moths and rose drastically with age. The calling attained a maximum (45%) in 4-day-old females and thereafter declined in the older moths. The mean onset of calling was significantly influenced by a quadratic effect of age (Table 1). On average, 5-day-old females initiated calling at 7.8 hr after light-off which was 6 to 18 min earlier than the younger and the older (Fig. 2A). For mean time spent calling there was a quadratic age effect (Table 1). The calling time initially increased with age, peaking at 75 min for 5-day-old females, and thereafter declined in older moths (Fig. 2B).

Mating behavior

A male responded to a calling female by exhibiting vigorous wing fanning and antennal waving. When a calling female was located, the male anteriorly approached the female. While fanning, the male probed along the female body with exposed genitalia and arched the abdomen toward the female to promote the genital contact. If the female was receptive, the male immediately turned itself around, and copulation took place with the insects tail to tail. During mating the pair remained immobile.

Mating pattern

Mating occurred late ca. 7-8 hr after light-off during which the calling was frequently observed. The percentage of moths mating clearly varied with age (Fig. 3). It was low in 1-day-old moths and increased abruptly when they became older. The mating reached the maximal percentage (48.9%) in 4-day-old moths and gradually declined. There was a quadratic trend in the mean onset of mating (Table 2). The onset of mating of 4-day-old moths averaged 7.6 hours after light-off which shifted, by 12 to 78 min, earlier than that of the younger and the older (Fig. 4A). The mean time spent mating was decreased linearly with age (Table 2, Fig. 4B).

DISCUSSION

The calling and mating behavior of *Nephopterix* moths appears to be similar to the behavior of other moth species[7, 8, 9, 10, 11]. In many moth families, the sex pheromone gland is located in the terminal abdominal segments[3, 7, 12, 13, 14]. The calling behavior consists of exposing the gland by extruding it into the airstream[7, 15] along with related postural changes, i.e. wings extended away from the body and the abdomen eleveated into the air. The extrusion of the terminal segments of abdomen is an accepted criterior for pheromone release[16, 17, 18] However it is possible that the extrusion of the apical abdominal segments does not always result in pheromone release[14].

In several moth species, a male exhibits mating dance when a calling female is located[7, 8, 10, 11]. The mating dance is characterized by wing fluttering, antennal waving and exposing hair pencils. While probing along the female body, the male bent his abdomen toward the female to initiate genital contact. However Seol et al[9] was unable to observed the mating dance in the lesser mulberry pyralid, *Glyphodes pyloalis* Walker, since the male copulated almost immediately after landing near a calling female.

Both calling and mating activities of *Nephopterix* moths occur very late, ca 7-8 hr after the onset of the 9-hr scotophase, suggesting that in the field female *Nephopterix* probably call and mate at dawn. The present results show clearly that patterns of calling and mating varied with age. The percentages of individuals calling and mating was low on day 1, but increased rapidly on the next days, with a trend toward earlier onsets of calling and mating, Older females tended to spend more time on calling. However older moths spent less time on mating.

Effects of age on calling and mating have been studied in many moth species. In *G. pyloalis,* the frequency of calling was high in females up to 4 or 5 days old and thereafter decreased with age. By contrast the mating activity was low on day 1 and then increased with age[9]. A similar trend was observed in the rice stem borer, *Chilo suppressalis* (Wlk)[19] and the potato stem borer, *Hidraecia micacea* Esper[20]. Swier et al[21] established that ovarian development was highly correlated with calling behavior in the black cutworm, *Agrotis ipsilon* (Hufnagel), and that the percentage of 1-day-old black cutworm moths calling was lower than that of older moth[22]. In *Nephopterix,* the low percentages of 1-day-old individuals calling and mating suggests that females are not reproductively mature upon emergence.

Previous studies showed that the onsets of calling and mating shifted earlier with age[14, 19, 22]. Swier et al[22] noted that the time of starting calling in *A. ipsilon* was correlated with increased reproductive maturity, and it seems that by calling earlier, older females increase their chances of mating by being the first to attract males. The advance in the onset of calling and mating in response to age was also reported in the tobacco hornworm, *Manduca sexta* (L.) by Itagaki and Conner[14]. They also mentioned that mating times could not be equated calling times because calling preceeded mating, and mating resulted in the cessation of calling.

The present results show that the mean onset of mating are slightly earlier than that of calling especially in 3-day-old moths and the older. This implies that some female *Nephopterix* probably secret pheromone without assuming a typical calling posture. However some species do not display this age correlate change in calling and mating initiation[9, 20].

Most female moths lengthen their calling time when they get older[9, 19]. The prolonged calling in older moths was also reported in *H. micacea,* suggesting that by extending calling duration, older females increase their probability of competing for males[20]. In the case of *Nephopterix,* older females probably enhance their chances of mating by both calling earlier and broadening the calling time.

CONCLUSSION

Female *Nephopterix* exhibit a calling behavior just before mating during which the sex pheromone is presumably released. The production and release of pheromone in *Nephopterix* is under investigation. It seems that the sex pheromone should be extracted from 2-to 5-day old virgin females because they are most likely to call. According to their high mating activity, the bioassay of the pheromone extract should be performed using 2-to 5-day-old males. It appears that the optimal time for the extraction and bioassay is ca. 7-8 hr after the onset of the 9-hr scotophase during the hours when they observed maximum sexual behavior.

ACKNOWLEDGEMENTS

I thank Dr. L. Lebel for his statistical advice and review of the manucript, Dr. M. Horak and Mr. M. Shaffer for identifying *Nephopterix*. I also gratefully acknowledge financial support from the Faculty of Science, Prince of Songkla University.

REFERENCES

1. Witethom, B. and Silawatchananai, P. (1990). Biology and life table of *Mussidia pectinicornella* Hampson (Lepidoptera: Pyralidae), on unripe sapodilla fruits. Songklanakarin *J. Sci. Technol.* **12,** 361-367.
2. Mitchell, E.R. (1981). Management of Insect Pests with Semiochemicals-Concepts and Practice. Plenum Press, New York.
3. Jefferson, R.H, Shorey, H.H., and Rubin, R.E. (1968). Sex pheromones of noctuid moths. XVI. The morphology of the female sex pheromone glands of eight species. *Ann. Entomol. Soc. Am.* **61,** 861-865.
4. Brady, U.E. and Smithwick, E.B. (1968). Production and release of sex attractant by the female Indian meal moth, *Plodia interpunctella*. *Ann. Entomol. Soc. Am.* **61,** 1260-1265.
5. Rosenthal, R. and Rosnow, R.L. (1985). Contrast Analysis: Focused Comparisons in the Analysis of Variance. Cambridge University Press, Cambridge.
6. Sachs, L. (1984). Applied Statistics. Springer-Verlag. New York.
7. Weatherston, J. and Percy, J.E. (1968). Studies of physiologically active arthropod secretions. I. Evidence for a sex pheromone in female *Vitula edmandsae* (Lepidoptera: Phycitidae). *Can. Entomol.* **100,** 1065-1070.
8. Werner, R.A. (1977). Behavioral responses of the spear-marked black moth, *Rheumaptera hastata,* to a female-produced sex pheromone. *Ann. Entomol. Soc. Am.* **70,** 84-86.
9. Seol, K.Y., Honda, H. and Matsumoto, Y. (1986). Mating behavior and the sex pheromone of the lesser mulberry pyralid, *Glyphodes pyloalis* Walker (Lepidoptera: Pyralidae). *Appl. Entomol. Zool.* **21,** 228-235.
10. Brown, H.E., Wood, L.T., Shaver, T.N., and Worley, J. (1988). Behavioral responses of male *Eoreuma loftini* (Lepidoptera: Pyralidae) to ovipositor extracts. *J. Econ. Entomol.* **81,** 184-188.
11. Swaby, J.A., Daterman, G.E., and Sower, L.L. (1987). Mating behavior of Douglas-fir Tussock moth, *Orgyia pseudotsugata* (Lepidoptera: Lymantridae), with special reference to effects of female age. *Ann. Entomol. Soc. Am.* **80,** 47-50.
12. Werner, R.A. (1977). Morphology and histology of the sex pheromone gland of a geometrid, *Rheumaptera hastata*. *Ann. Entomol. Soc. Am.* **70,** 264-266.
13. Attygalle, A.B., Cai-hong, W., Schwarz, J., Vostrowsky, O., Hasenfuss, I., and Bestman, H.J. (1988). Sex pheromone of female *Myelois cribrella* Hübner (Lepidoptera: Pyralidae): Chemical identification, electrophysiological evaluation, and field attractancy tests. *J. Chem. Ecol.* **14,** 485-494.

14. Itagaki, H., and Conner, W.E. (1988). Calling behavior of *Manduca sexta* (L.) (Lepidoptera: Sphingidae) with notes on the morphology of the female sex pheromone gland. *Ann. Entomol. Soc. Am.* **81,** 798-807.
15. Sanders, C.J. (1969). Extrusion of the female sex pheromone gland in the eastern spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Torticidae). *Can. Entomol.* **101,** 760-762.
16. Baker, T.C. and Carde, R.T. (1979). Endogenous and exogenous factors affecting periodicities of female calling and male sex pheromone response in *Grapholitha molesta* (Busck). *J. Insect Physiol.* **25,** 943-950.
17. Conner, W.E., Eisner, T., VanderMeer, R.K., Guerrero, A., Ghiringelli, D. and Meinwald, J. (1980). Sex attractant of an Arctiid moth *(Utethesia ornatrix):* A pulsed chemical signal. *Behav. Ecol. Sociobiol.* **7,** 55-63.
18. Webster, R. P. and Cardé, R.T. (1982). Relationships among the pheromone titre, calling and age in the omnivorous leafroller moth *(Platynota stultana). J. Insect Physiol.* **28,** 925-933.
19. Kanno, H. (1979). Effects of age on calling behavior of the rice stem borer, *Chilo suppressalis* (Walker) (Lepidoptera: Pyralidae). *Bull. Entomol. Res.* **69,** 331-335.
20. West, R.J., Teal, P.E.A., Laing J.E., and Grant, G.H. (1984). Calling behavior of the patato stem borer, *Hydraecia micacea* Esper (Lepidoptera: Noctuidae) in the laboratory and the field. *Environ. Entomol.* **13,** 1399-1404.
21. Swier, S.R., Rings, R.W., Musick, G.J. (1976). Reproductive behavior of the black cutworm, *Agrotis ipsilon. Ann. Entomol. Soc. Am.* **70,** 919-924.
22. Anonymous. (1977). Age-related calling behavior of the black cutworm, *Agrotis ipsilon. Ann. Entomol. Soc. Am.* **70,** 919-924.

บทคัดย่อ

จากการศึกษาพฤติกรรมการเรียกหาและพฤติกรรมการผสมพันธุ์ของผีเสื้อกลางคืนชนิด *Nephopterix* พบว่าแบบแผนของพฤติกรรมดังกล่าวมีการเปลี่ยนแปลงตามอายุ ส่วนใหญ่ผีเสื้อกลางคืนชนิดนี้จะแสดงพฤติกรรมการเรียกหาและพฤติกรรมการผสมพันธุ์เมื่อมีอายุ 2-5 วัน เวลาในการเริ่มและระยะเวลาที่ใช้ในการแสดงพฤติกรรมนี้ขึ้นอยู่กับอายุของเพศเมียและมีลักษณะเป็นแบบ non-linear เวลาในการเริ่มผสมพันธุ์ก็เปลี่ยนแปลงตามอายุแบบ non-linear เช่นกัน แต่ระยะเวลาที่ใช้ในการผสมพันธุ์จะลดลงเมื่อผีเสื้อกลางคืนมีอายุมากขึ้น จากผลการทดลองแสดงว่าพฤติกรรมการเรียกหาและพฤติกรรมการผสมพันธุ์ซึ่งขึ้นอยู่กับอายุนี้มีความสัมพันธ์กับการเจริญของระบบสืบพันธุ์ของเพศเมีย