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# ENDOGENOUS AND EXOGENOUS FACTORS AFFECTING PERIODICITIES OF FEMALE CALLING AND MALE SEX PHEROMONE RESPONSE IN *GRAPHOLITHA MOLESTA* (BUSCK)

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**Abstract**—In *Grapholitha molesta* periodicities of both female calling and male response to sex pheromone by wing fanning while walking were determined in part by circadian rhythms. The lights-on photoperiodic cue was at least partly responsible for setting the phase of the female calling rhythm. Absolute temperature levels and not necessarily a decrease in temperature modified the timing of calling; there were both high and low thresholds of temperature and, at particular photoperiod times, a temperature range optimal for calling. When the previous performance of calling was prevented by subthreshold temperatures, calling during the next period commenced earlier if temperatures were favourable. Thus, the previous performance of calling may establish a refractory period, or temperature decrease may act as a cue resetting the phase of the calling rhythm. The ability to use both an endogenous clock and exogenous temperature cues to synchronize sexual activity appears adaptive for a temperate zone insect whose multiple generations are exposed to both long periods of favourable climatic conditions in summer and harsh, unpredictable conditions in spring or fall.

*Key Word Index*: *Grapholitha molesta*, Oriental fruit moth, circadian rhythm, response periodicity, calling periodicity, temperature-mediated behaviour, pheromone

## INTRODUCTION

KNOWLEDGE of diel sexual periodicity in moths is important from at least two standpoints. From an applied aspect, the duration of diel male sexual responsiveness to pheromone traps can determine the trap capture magnitude, which in turn influences estimates of population density. Pest management decisions in the future may be made using trap capture frequency to infer population size. Secondly, knowledge of the diel temporal activity pattern of a population is important to understanding intraspecific communication and its role in the temporal organization of a community.

Several reports have indicated that adult Oriental fruit moth *Grapholitha molesta* (Busck) activity in the field occurs in 2–3 hr preceding sunset (DUSTAN, 1961; ROTHSCHILD and MINKS, 1974; GENTRY *et al.*, 1975). Laboratory observations also showed mating behaviour to occur in the few hours before lights-off (DUSTAN, 1964; GEORGE, 1965). In the field ROTHSCHILD and MINKS (1974) found that cool spring temperatures were correlated with an advancement of male attraction to earlier, warmer hours of the day, implying that these exogenous temperature factors exerted some control over response time. However, there was some evidence of a circadian rhythm, because time of male attraction often remained advanced even on those spring days when the temperature remained high until late in the day. Also, laboratory mating periodicity persisted in continual light (GEORGE, 1965), further evidence for a circadian rhythm. There has been no direct experimental work to determine the factors influencing the observed periodicities of *G. molesta* female calling behaviour and male pheromone response. We report here laboratory experiments indicating that these periodicities are both endogenously and exogenously controlled.

## MATERIALS AND METHODS

### *Rearing*

*G. molesta* adults were from a laboratory colony originating from Michigan apples and maintained at Michigan State University since 1975. Larvae were reared on small green apples at 25–26°C, 70% r.h. and a 16:8, light:dark photoperiod regime. Photophase (daylight) light intensity was *ca.* 2100 lx and scotophase (night) intensity was *ca.* 0.3 lx. When available, feral adults were added to the mating stock. Adults were segregated by sex as pupae (GEORGE, 1965), and the subsequent adult males and females held in separate cages according to emergence date.

### *Female calling observations*

*Female age, circadian rhythm, photoperiod cues, and temperature effects.* Females more than one day old, except in those experiments measuring female age effects, were placed in individual clear plastic cups, 4 × 4 cm top diam., having plastic-lined cardboard lids. In the female age experiment, groups of females were 0–9 hr, and 1, 2, 3, 4, 5 and more than 6 days old.

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Each cup contained a 1 cm long dental wick soaked with distilled water to maintain *ca.* 100% r.h. Unless a special temperature effect was tested, calling observations were performed at the rearing temperature, 25–26°. A female was scored as calling if at least the ovipositor's anal papillae were extruded or, when the abdomen was turned away from the observer, if the female assumed the typical calling posture: wings elevated, legs extended, and abdomen raised above the substrate. The mean time of calling was calculated as the time at which 50% of the calling hours had occurred on a given day.

Photoperiod light intensities were identical to those used in rearing. During scotophase viewing was accomplished with a light having a Kodak Wratten filter eliminating light below 680 nm. Temperature changes were accomplished using two environmental cabinets and two large walk-in chambers. When an experimental group was transferred to a different chamber, the control group was sham-transferred back into the original chamber to control for temporary exposure to different temperatures and lower light intensity.

*Relative humidity.* The effects of relative humidity were investigated using 10 × 23 × 31 cm plastic boxes. Fifteen clear plastic cups described above, with bottoms removed, were glued over screen-covered holes in the box lids to create a continuous air space between the bar and cup interiors. The boxes contained varying-concentration KOH solutions resulting in relative humidities of 25, 50, 75, and 100% (pure $H_2O$) in airspaces over the solutions (SOLOMON, 1951). Each cup contained two 3–6-day-old females which were used once.

*Male wing fanning while walking response to pheromone.* Diel fluctuations of male sexual response were measured using an olfactometer similar to that described by SOWER *et al.* (1973). It comprised a series of glass orientation tubes 100 × 2 cm diameter, each connected to a 105°, 3-way connecting tube at the upwind end. Filtered laboratory air was blown into a glass manifold which distributed the air flow equally to all connecting tubes and thence to the orientation tubes at a velocity of 80 cm/sec.

Five males, 2 days or more old, were placed into each tube at least 1 hr before testing. Males were 0–9 hr, and 1, 2, 3, and 4 days old in experiments determining effects of age response to pheromone.

The synthetic pheromone mixture was comprised of 4 identified *G. molesta* components (CARDÉ *et al.*, 1979) in the amounts one μg (*Z*)-8-dodecenyl acetate, 0.07 μg (*E*)-8-dodecenyl acetate, 0.01 μg (*Z*)-8-dodecenyl alcohol, and 3 μg dodecanol applied in 10μl of hexane to a rubber septum dispenser (A. Thomas Comp.). The (*Z*)-8-dodecenyl acetate (Farchan Corp.) after purification contained no detectable quantities of either (*E*)-8-dodecenyl acetate or any 12 carbon alcohols as checked by gas–liquid chromatography. Other impurities were less than 0.1%. The (*E*)-8-12:Ac contained no detectable quantities of the (*Z*) isomer, less than 0.03% of any 12 carbon alcohols and no detectable quantities of other impurities. The (*Z*)-8-dodecenyl alcohol contained no detectable quantities of the (*E*) isomer, and no detectable quantities of any 12-carbon acetates or other impurities. Dodecanol contained no detectable amounts of any 12-carbon acetates or other 12-carbon alcohols and was greater than 98% free of other impurities. Before introduction of pheromone, background levels of wing fanning while walking were assessed. These background levels were subtracted from post-pheromone-introduction levels to result in percentage response to pheromone. Wing fanning while walking was chosen as the key response to observe because it was highly correlated with attraction (upwind flight) in a wind tunnel study of behavioural responses (BAKER and CARDÉ, 1979). In these tubes unrestricted upwind flight could not occur. After pheromone introduction, wing fanning while walking was recorded for 15 sec. The maximal number of males simultaneously displaying this behaviour during this period was the number scored. Usually males were used only once, but if used more than once, then only once per 24 hr period. Both assay and connecting tubes were rinsed thoroughly with acetone after each use. The septum was stored at −10°C in a glass vial. Photophase light intensity for all assays was 2100 lx. Scotophase intensity was 0.7 lx, the diffuse, low light level provided by an incandescent light-box immediately beneath the tubes.

## RESULTS

### *Female calling*

*Effect of age.* Newly-emerged females, 1–9 hr old, did not call (Fig. 1). Hour of calling onset and mean hour of calling were similar for all groups 1 day old or greater, with only some slight differences in percentage calling between groups at particular times. Calling periodicity appeared to coincide closely with the adult male attraction pattern, observed in the field to occur in the few hours prior to sunset in the summer (DUSTAN, 1961; GENTRY *et al.*, 1975; ROTHSCHILD and MINKS, 1974); at 25°C in the laboratory calling commenced about 3.5 hr before, and terminated by about 0.5 hr after lights-off. The calling rhythm also closely matched the oviposition pattern of females observed concurrently in the rearing box.

*Effect of photophase onset.* Advancing or retarding lights-on by 4 hr slightly accelerated and delayed, respectively, the subsequent mean calling time (Fig. 2). However, the magnitude of the shift in mean calling time was less than the shift in lights-on. This implies that either photophase onset is not the only phase-setting cue, or there was resistance to a shift by an underlying rhythm whose phase was not completely reset by the cue.

*Demonstration of circadian rhythm.* Calling persisted with approximately the same 24 hr periodicity in continual light at 25°C (Fig. 2). Thus calling periodicity is at least partially determined by a circadian rhythm.

*Effect of relative humidity.* Calling proportion between 6 and 0.5 hr prior to scotophase was not obviously affected by relative humidities of 25, 50, 75, and 100% (Table 1). Neither onset of calling or percentage calling during peak hours differed significantly between humidities except for a slight distinction 3 hr before lights-off.

*Effect of temperature.* Calling was eliminated by temperatures above *ca.* 32°C or below *ca.* 15°C. However, the behavioural bases for suppression were

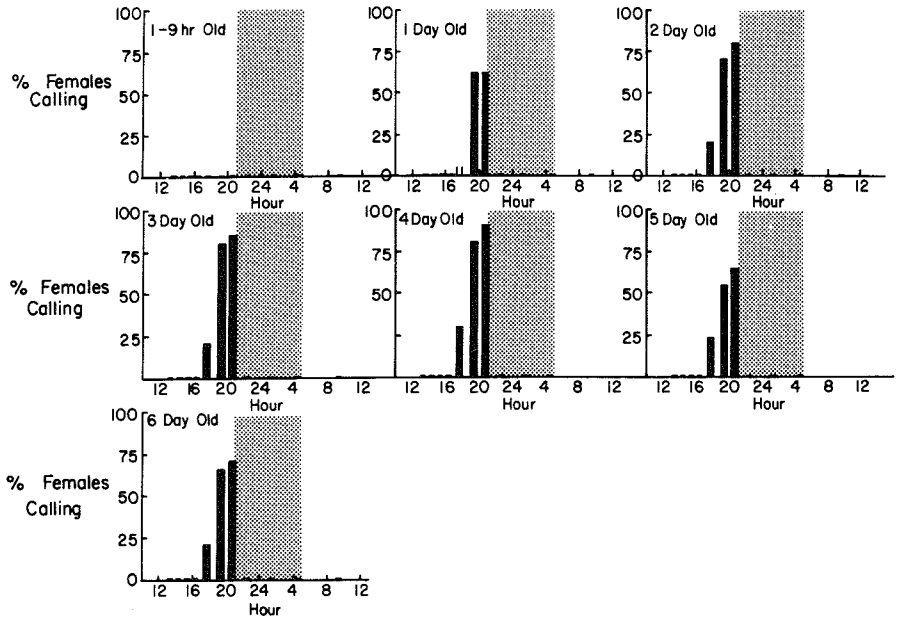


Fig. 1. Effect of age upon female calling. The different age groups were observed simultaneously on the same day. Shaded areas represent scotophase. $N = 50$ for 0–9-hr-old group; $N = 86$ for 5-day-old group; $N = 100$ for all other age groups. Temperature was 25°C

different at high versus low temperatures. At cold temperatures of *ca* 15°C or below, reduced calling was characterized by the absence of leg extension, body and wing elevation, and locomotor activity. At high temperatures of *ca* 32°C or above, however, lack of calling often resulted from females walking and flying, although there were also immobile, non-calling females.

Temperature changes within the 15°C to 32°C range appeared to alter the time at which calling occurred. Calling onset was advanced by as much as 4 hr when the temperature was decreased from 25°C to 20°C at various times during photophase (Table 2). This decrease did not advance the termination of calling, as evidenced by no reduction in calling in the few hours immediately preceeding scotophase.

Longer exposure to altered temperatures also changed the calling times. Mean hour of calling was

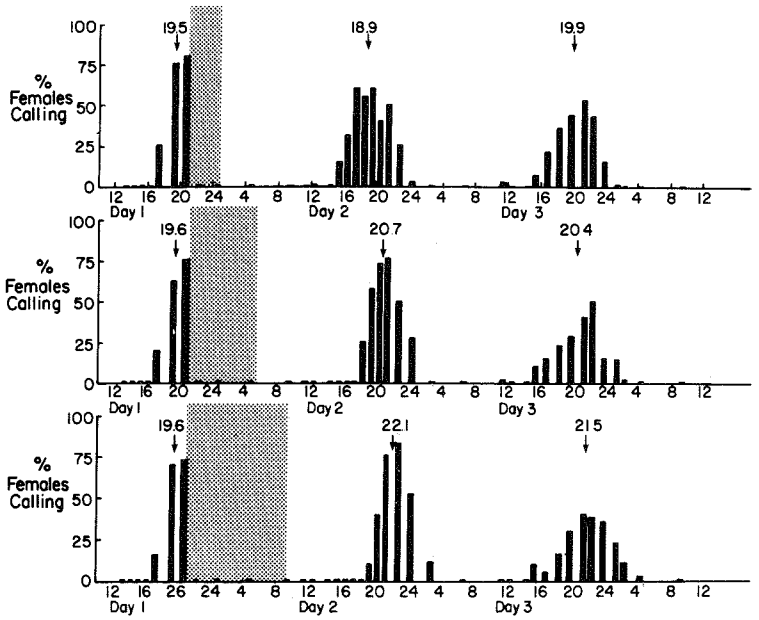


Fig. 2. Effect upon female calling of varying the photophase onset cut by 4 hr. Scotophase (shaded area) in the middle group was the normal, 8 hr duration. Numbers above arrows denote mean hours of calling (decimal hours). On day 3, calling periodicity persisted in continual light, indicating the presence of a circadian rhythm. $N = 200$ for each group. Temperature was 25°C.

Table 1. Effect of relative humidity upon female calling

| Hours before Scoto-phase | Percentage females calling (25°) | | | |
|---|---|---|---|---|
| | 25% r.h. | 50% r.h. | 75% r.h. | 100% r.h. |
| −6 | 0 a | 0 a | 0 a | 0 a |
| −5 | 0 a | 0 a | 0 a | 3.3 a |
| −4 | 6.7 a | 5.0 a | 5.0 a | 10.0 a |
| −3 | 23.3 a | 8.3 b | 11.7 ab | 26.7 a |
| −2 | 66.7 a | 63.3 a | 56.7 a | 65.0 a |
| −1 | 85.0 a | 76.7 a | 80.0 a | 81.7 a |
| −0.5 | 76.7 a | 78.3 a | 68.3 a | 73.3 a |

$N = 60$ for all groups. Percentages in the same row having no letters in common are significantly different according to a $\chi^2$ 2 × 2 test of independence with Yates' correction ($P < 0.05$).

advanced by a temperature decrease to 20°C and delayed by an increase to 31°C during the 12 hr before lights-off (Fig. 3). Temperatures of 15°C and 10°C appeared to suppress calling. Returning the temperature for the group held at 31°C to 25°C the next day resulted in a mean calling time 'normal' for 25°C. However, the 10°C-, 15°C-, and 20°C-exposed females returned to 25°C exhibited advanced mean calling times. Some females in the 10°C group called as soon as the temperature was raised (Fig. 3).

When the temperature was oscillated between 34°C and 10°C, maximal calling occurred only within a certain temperature range, approx 18°–24°C (Fig. 4). Moreover, increases in calling occurred during both rising and falling temperatures. Thus female calling was influenced by the absolute temperature level, not just relative changes such as a decrease in temperature. At a particular photophase hour, calling occurred when the temperature was within an absolute range, whereas low and high temperature thresholds eliminated calling. The range, and the thresholds themselves, however, varied with photoperiod hour. For instance, in the hours immediately before and after lights-off, high temperatures did not appear to reduce calling as much as during earlier photophase hours (Fig. 4).

Complicating the temperature modulation of calling time is the possibility that calling behaviour itself exerts some control over the timing of the next day's calling. This is evidenced in the experiment where the calling times of females exposed to cold on the previous day were advanced even after females were returned to warmer temperatures (Fig. 3). The fact that calling had not occurred or had occurred earlier on the cold-exposure day seemed to allow

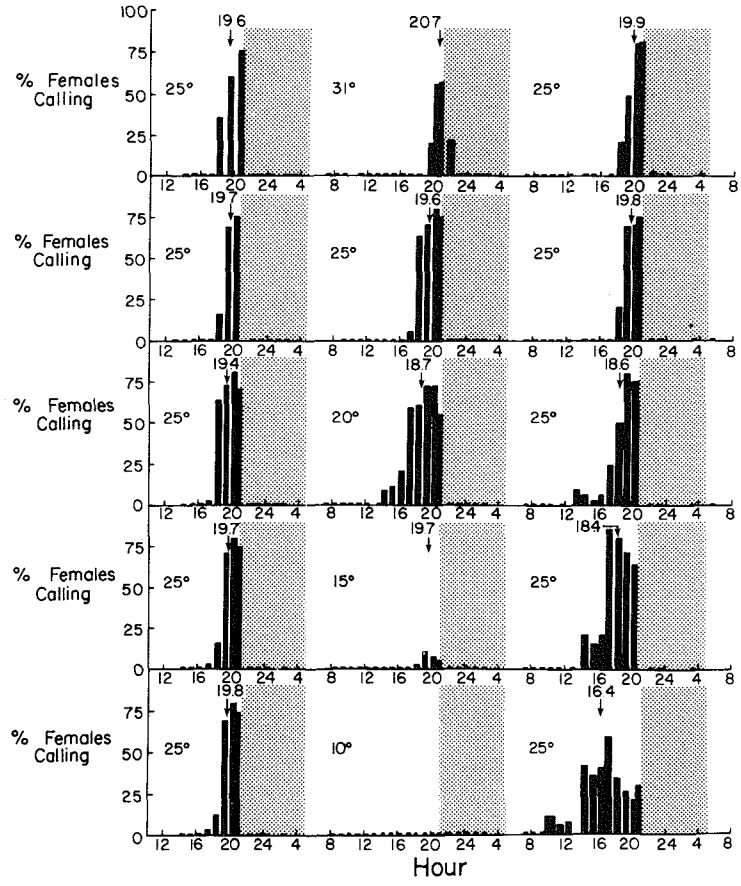


Fig. 3. Effect of a day-long temperature change upon mean hour of calling. After one full day at 25°C for all groups, the temperature was changed to the level indicated at 9.00 (decimal hours). At 9.00 the following day all temperatures were returned to 25°C. Numbers above arrows denote mean hours of calling. $N = 100$ for each group.

Table 2 Effect of a 5°C temperature decrease upon percentage of females calling at various hours before lights off

| Hours before scotophase | Percentage females calling Constant 25°C | Min at 20°C 20 | 60 |
|---|---|---|---|
| −9 | 0 | 2 NS | 2 NS |
| −8 | 0 | 0 NS | 0 NS |
| −7 | 0 | 22* | 36* |
| −6 | 0 | 40* | 58* |
| −5 | 0 | 44* | 78* |
| −4 | 0 | 72* | 76* |
| −3 | 25 | 76* | 83* |
| −2 | 72 | 86 NS | 84 NS |
| −1 | 70 | 66 NS | — |

$N$ = 50 for all groups. Females were used for one temperature decrease and discarded.

* = percentage significantly different from that of the constant 25°C group at the same hour according to a $\chi^2$ 2 × 2 test of independence with Yates' correction ($P<0.01$). NS = percentage not significantly different to that in the constant 25°C group at the same hour according to a $\chi^2$ 2 × 2 test of independence ($P\geq 0.05$)

Table 3 Effect of age upon percentage of males wing fanning while walking during the first 15 sec of exposure to synthetic pheromone

| Age of males | Percentage males wing fanning while walking |
|---|---|
| 0–9 hr old | 9 c ($n$ = 58) |
| 1 day old | 15 c ($n$ = 60) |
| 2 days old | 65 b ($n$ = 60) |
| 3 days old | 80 ab ($n$ = 58) |
| 4 days old | 84 a ($n$ = 58) |

Males were exposed to pheromone once and then discarded. Percentages having no letters in common are significantly different according to a $\chi^2$ 2 × 2 test of independence with Yates' correction ($P<0.05$).

earlier calling on the following, warmer day. A similar effect was observed in the experiment involving temperature oscillations (Fig. 5). Females prevented from calling by experiencing a decrease in temperature from 25°C to 10°C at 3.5 hr prior to scotophase, called immediately upon the first increase in temperature on the following morning (Fig. 5A). In contrast, females placed at 10°C immediately *after* the previous day's calling had ended (0.5 hr after lights-off), did not call with the first favourable temperatures (Fig. 5C). Their calling in favourable temperatures was quite similar to that of females held at 25°C (Fig. 5B). The effect of the previous day's calling may involve a refractory period during which calling cannot begin again. Such a refractory period would likely be related to the endogenous oscillator determining the calling rhythm. Alternatively, the previous day's temperature decreases themselves may have acted as exogenous cues that reset the rhythm's phase. This is less likely since one decrease to 10°C occurred at 9.00 (decimal hr, Fig. 3) and another at 17.5, yet calling commenced in both cases at about 10.0 the next morning soon after the temperature was increased (Figs. 3, 5A).

*Male wing fanning while walking response to pheromone*

*Age of males.* Percentage of males wing fanning while walking in response to the synthetic pheromone blend increased with age (Table 3). Males 1-day-old or less had comparatively lower response levels, 2-day-old intermediate response, and 3- to 4-day old the highest responses to pheromone.

*Demonstration of circadian rhythm.* In continual light at 25°C the *ca.* 24 hr periodicity of response persisted, indicating that male pheromone responsiveness is at least partially determined by an underlying circadian rhythm (Fig. 6). In continual light, mean hr of response was slightly delayed compared to males experiencing scotophase, apparently due to a slower rate of response decline rather than a delayed response onset.

*Effect of a decrease in temperature.* Diminishing the temperature from 25°C to 20°C at various periods before lights-off did not significantly increase wing

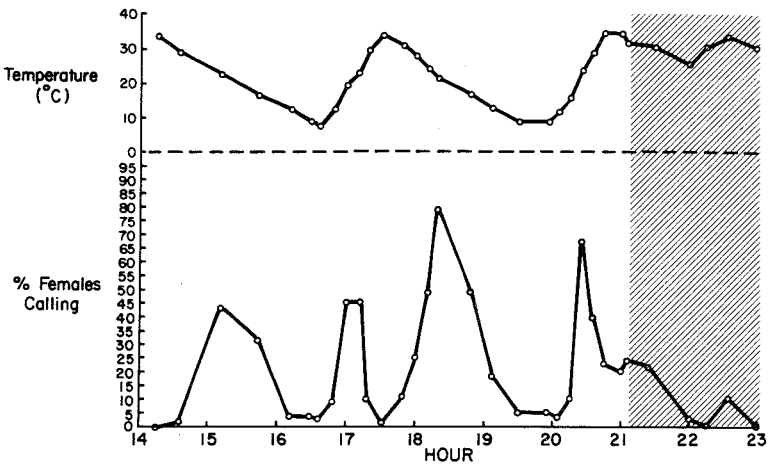


Fig. 4. Effect of an oscillating temperature upon percentage of females calling at various photoperiod times. Intermediate temperature levels appeared favourable for calling whether they were arrived at by an increase or decrease. Extreme high or low temperatures suppressed calling. $N$ = 125.

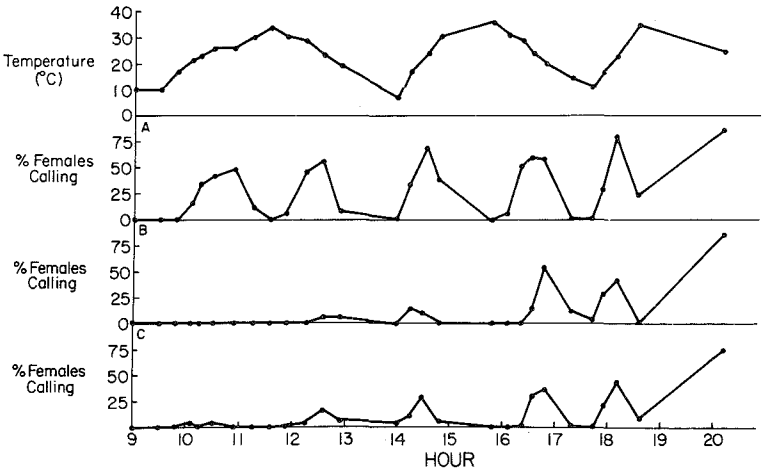


Fig. 5. Effect of previous day's calling upon subsequent response to oscillating temperature. A = females not calling during the previous day due to a temperature decrease to 10°C just before onset of calling; temperature was maintained at 10°C until 9.30 (decimal hours) on the day of testing. B = control group experiencing normal calling on previous day and held at continual 25°C until 10.30 on day of testing, whereupon the temperature oscillation was identical to the 2 other groups. C = control group placed in 10°C immediately *after* (21.30) experiencing normal calling on previous day and maintained at 10°C until 9.30 on the day of testing. $N = 50$ for each group.

fanning while walking response compared to males maintained at continual 25°C (Table 4). Thus, unlike females, the male sexual activity period could not be shifted by this 5°C decrease. In fact, at some hours of testing there were slight but significant decreases in response in the temperature-decreased group.

## DISCUSSION

### *Endogenous factors influencing sexual behaviour periodicity*

In *G. molesta,* periodicities of female calling and male pheromone response are determined in part by a circadian rhythm. The female rhythm's phase is directly influenced by the lights-on photoperiod cue. The magnitude of the phase shift does not correspond, however, to the cue-shift magnitude, and so the phase-setting mechanism may involve more than the simple lights-on signal. Alternatively, the phase-setting effect of lights-on may be partially obscured by the self-sustaining effect of the rhythm itself.

The circadian rhythm may persist by a type of interval-timer mechanism using the previous performance of calling (or pheromone response) to time the interval until the behaviour can next occur. Our results indicate that when the interval expires, calling is induced and may somewhat override exogenous temperature cues (Fig. 3). ROTHSCHILD and MINKS (1974) observed what perhaps may be the rhythm's similar influence upon *G. molesta* male attraction. In the spring, males fly earlier in the day and are "not influenced by the occasional warm day". A possible similar 'residual' effect of previous calling hour upon the following day's calling onset can be seen in the data of CARDÉ *et al.* (1975, Fig. A) for the

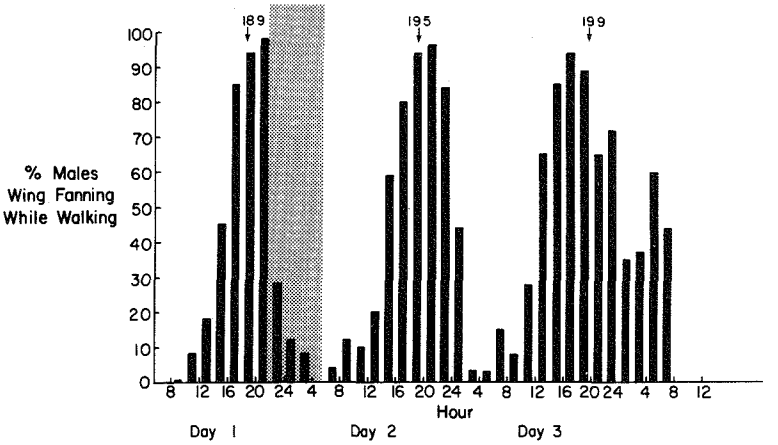


Fig. 6. Effect of continual light upon periodicity of male wing fanning while walking response to sex pheromone. Since response periodicity persisted, it was at least partly determined by a rhythm which appeared circadian. Numbers above arrows denote mean hours of response. Males were used for no more than one exposure to pheromone. $N = 60$ for most individual testing hours.

Table 4. Effect of a 5°C temperature decrease upon percentage of males wing fanning while walking during the first 15 sec of exposure to pheromone at various hours before lights off

| Hours before Scoto-phase | Percentage males wing fanning while walking | |
|---|---|---|
| | 25°C | 20°C |
| −8 | 0 | 15 NS |
| −7 | 32 | 43 NS |
| −6 | 18 | 18 NS |
| −5 | 63 | 55 NS |
| −4 | 76 | 53 NS |
| −3 | 97 | 74* |
| −2 | 96 | 96 NS |
| −1 | 92 | 71* |

* = percentage in same row significantly different according to a $\chi^2$ 2 × 2 test of independence with Yates' correction ($P<0.05$). $N = 40$ for all groups except those at 3, 2, and 1 hr before scotophase where $N = 34$, 25, and 35, respectively.

redbanded leafroller moth, *Argyrotaenia velutinana* (Walker). Perhaps the circadian rhythms of calling in these two insects are driven by a similar mechanism.

*Exogenous factors*

In addition to the endogenous influence of a circadian rhythm upon female calling periodicity, an exogenous factor, temperature, modified the rhythm's expression. Constant high temperature (31°C) delayed mean calling time by delaying calling onset and extending offset further into scotophase. Continual low temperature (20°C) advanced the mean calling hour mainly by advancing calling onset. At 15°C or lower, expression of the calling rhythm was almost completely repressed, a low temperature threshold consistent with the *ca.* 15°C adult flight activity threshold in the field reported by other authors (ROTHSCHILD and MINKS, 1974; ARMSTRONG, 1929; REICHART and BODOR, 1972). Rapid temperature decrease to 20°C resulted in an onset of calling advancement of up to 4 hr. However, as discussed below, the decrease itself may not actually induce calling but rather calling may result from an interaction of the current absolute temperature with both the hour of the photoperiod and the interval established by the previous performance of calling.

Male response to pheromone was not advanced by a temperature decrease from 25°C to 20°C. It is not clear why no advancement was observed even in this limited experiment. A field study of *G. molesta* male attraction periodicity to synthetic pheromone indicated that the quite discrete attraction period of only a few hours was advanced during cooler spring periods compared to summer (ROTHSCHILD and MINKS, 1974). In New York the hour of 50% male trap capture in the field also appeared to be earlier at lower temperatures (BAKER and CARDÉ, unpublished), although it is unclear whether this advancement was caused by an immediate or long-term exposure to cooler temperatures.

It seems inconsistent that females should exhibit a temperature-sensitive response for calling behaviour while males, for pheromone response, do not. Possibly our method of measuring male periodicity of responsiveness was insufficient, and a more discriminating assay would reveal male temperature-sensitivity. Using an identical olfactometer, male *Laspeyresia pomonella* (L.), another olethreutine, also failed to exhibit a temperature-modulated response shift whereas females at lower temperatures clearly advanced their calling time (CASTROVILLO and CARDÉ, 1979). However, males of *Argyrotaenia velutinana*, a tortricine, in colder temperatures did exhibit an advanced response period of the same magnitude as female calling advancement. Clearly additional experimentation with *G. molesta* would be necessary before it can be concluded that temperature cues affect only female calling and not male responsiveness.

*Optimal temperature range of calling*

Alteration of calling time by short-term exposure to lower temperature has now been reported for the cabbage looper, *Trichoplusia ni* (Hübner) (SOWER *et al.*, 1971); the spruce budworm, *Choristoneura fumiferana* (Clemens) (SANDERS and LUCUIK, 1972); *A. velutinana* (CARDÉ *et al.*, 1975); *L. pomonella* (CASTROVILLO and CARDÉ, 1979); *Holomelina immaculata* (Reakirt) (CARDÉ and ROELOFS, 1973); *Synanthedon pictipes* (Grote and Robinson) (GORSUCH *et al.*, 1975); and *G. molesta*. Many of the authors have noted the adaptive value of early activity on cold spring days, especially for relatively small moths such as torticids whose large surface area to volume ratio facilitates heat loss (CARDÉ *et al.*, 1975). Although a decrease in temperature alone evokes an advancement in calling time, evidence for *G. molesta* indicates that the temperature level has a major effect regardless of whether this level is reached by a temperature decrease or increase. There appears to be a temperature range during which calling can be expressed optimally for a particular photoperiod time and state of the underlying rhythm. The optimal range may increase under the influence of the rhythm; during late photophase and early scotophase, temperatures of 30°–33°C did not reduce the calling proportion to the levels seen earlier in photophase at the same temperatures. In *G. molesta*, and perhaps other species of moths which exhibit temperature-modulated calling advancement, the temperature decrease may act to increase early calling by lifting inhibition rather than inducement. The lower temperature level may remove calling suppression caused by higher temperatures, allowing expression of the calling rhythm earlier in the photoperiod.

*The function of the rhythm*

In *G. molesta* the periodicity of female calling is endogenously controlled, modified by exogenous factors. Male pheromone response periodicity is also endogenously controlled and probably also modulated by exogenous factors, although we were not able to define such an interaction in the laboratory. CORBET (1966) hypothesized that field periodicities observed at the lowest latitudes are most likely the expression of a rhythm alone, and at the highest latitudes responses to only exogenous factors. Behavioural periodicities of temperate insects should be determined by both endogenous and exogenous

factors (CORBET, 1966). For *G. molesta* calling behaviour, this combination is indeed the case. During its many generations a year, *G. molesta* may be exposed to both the harsh, unpredictable conditions of early spring (or fall), placing energetic and physiological constraints upon diel activity periods, in contrast to the relatively stable summer conditions. The combination of rapid response to exogenous cues modifying an underlying stability-lending rhythm appears to allow the moth to function under differing seasonal conditions. Temperate region insect species packing is less dense than in the tropics (PRICE, 1975). For the adult stage this is especially true in early spring. Thus, in the spring there is greater temporal flexibility to respond to often fleeting favourable conditions without incurring detrimental levels of interspecific competition. In the rather unvarying conditions of midsummer, *G. molesta* may achieve optimal temporal synchrony with its potential mates and the rest of the more tightly packed community of insect adults mainly by its circadian rhythm. For a number of moth species, such diel temporal isolation has likely been important as a reproductive isolating mechanism (ROELOFS and CARDÉ, 1974).

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