Physiological Entomology (1986) **11**, 75–87

Effects of temperature and light on calling in the tiger moth *Holomelina lamae* (Freeman) (Lepidoptera: Arctiidae)

COBY SCHAL and RING T. CARDÉ Department of Entomology, University of Massachusetts, Amherst, Massachusetts, U.S.A.

ABSTRACT. In *Holomelina lamae* Freeman daily eclosion of adults is gated, with males emerging before females. By advancing the onset of photophase and by delaying the onset of scotophase, it was demonstrated that lights-on acts as the main phase-setting cue for calling. Few females call on the day they eclose. Calling is initiated *c.* 9 h after the onset of photophase in 2-day-old females, and shifts to earlier times in older females. The duration of calling also increases with age. That calling is controlled by an endogenous circadian clock is indicated by its persistence in continuous light (LL) and dark (DD). In LL calling is dampened rapidly, but a single scotophase re-entrains the rhythm. Decreases in temperature advance the onset of calling and the mean hour of calling, while increases in temperature delay both. However, the magnitudes of such phase-shifts depended upon hour of the photoperiod. Moreover, cooling and heating appears to exert both transient and long-term effects on the calling rhythm. An 8 h period at a reduced temperature in LL induces calling in females whose calling is dampened, and entrains the calling rhythm. Females maintained in DD from second instar larvae to the adult stage exhibit a circadian calling rhythm set by eclosion.

Key words. Lepidoptera, behaviour, chemical communication, calling rhythm, pheromone, circadian rhythm, temperature, *Holomelina*.

Introduction

The evolutionary advantages of gated developmental events and rhythmic behavioural activities mediated by exogenous cues have been widely noted (e.g. Brady, 1974; Cardé & Webster, 1981). Pheromone release in insects is of particular interest because it is expected that general activity, and emission and response in the two sexes will be temporally synchronized to reduce metabolic costs and predation (Cardé & Baker, 1984). Yet, several studies have documented differential responses by males and females to equal environmental shifts, resulting in asynchronous activities in the two sexes (e.g. Baker & Cardé, 1979). To determine the adaptive significance of such shifts and control mechanisms utilized by each sex, it is important to understand the coupling of the overt behavioural rhythm to both the circadian clock and environmental cues, and the interaction

Correspondence: Dr Coby Schal, Department of Entomology and Economic Zoology, P.O. Box 231, Cook College, Rutgers, The State University of New Jersey, New Brunswick, New Jersey 08903, U.S.A.

between the latter two. In this paper we focus on the endogenous and rhythmic nature of calling in *Holomelina lamae* Freeman and modulation of calling periodicity by temperature, photoperiod and time of eclosion.

H.lamae is a small diurnal arctiid moth which occurs in bogs in Wisconsin, Michigan, Maine, New Brunswick, Nova Scotia, and Prince Edward Island. As in *H.aurantiaca* and other sibling species (Roelofs & Cardé, 1971), the pheromone glands of *H.lamae* contain 2-methylheptadecane as a primary component (Schal & Cardé, 1986). Cardé & Roelofs (1973) showed that in *H.immaculata,* a nocturnal moth, the lights-off cue entrained the calling rhythm, calling was eliminated in continuous light at 24°C, and a decrease in temperature advanced calling to an earlier time in the scotophase.

The present paper examines: the entrained features of eclosion and calling rhythms in various light:dark conditions; the free-running nature of calling in constant light and dark; photoperiodic cues affecting entrainment of the circadian clock; the relationship between temperature changes and calling; the role of a gate in temperature sensitivity; whether a single temperature pulse can entrain the calling clock; and the role of eclosion in entraining subsequent calling.

Materials and Methods

Experimental Insects

H.lamae were taken from a laboratory colony which originated from five females collected on 10 August 1978 in Manistique, Michigan (R16W, T42N, sec 14) and maintained on pinto-bean diet. Larvae were reared in individual clear plastic cups at 27°C and LD 15:9 h regime.

Light:dark conditions and measurement of eclosion and calling

Early last instar larvae were transferred to environmental chambers (24°C, LD 16:8 h, 1400 lux) and checked daily for pupation. Visual checks of adult emergence were conducted every 2 h. Thus, the diel pattern of emergence and the time from pupation to adult eclosion could be assessed for each group.

For studies of calling in LD conditions, last instar larvae or young pupae were placed in growth chambers (as above) and allowed at least 8 days before observations of adults. For continuous light and dark (LL and DD) observations, individuals were placed in the experimental regime in the second instar. Except in the study of the effect of age on calling, all observations of calling were started with groups of twenty to fifty 2-day-old females (day 1 is day of emergence). A female was scored as calling if the pheromone gland was seen to rhythmically extend and retract, or if the wings were held in the typical roof-like calling position (Cardé & Roelofs, 1973).

A red light (Kodak Wratten filter No. 29 eliminating light below 680 nm) was used to view insects in scotophase. Observations were conducted hourly during calling periods and at least every 2 h during non-calling periods. Turgeon & McNeil (1982) pointed out significant disparities in various calling parameters obtained with different observation frequencies. These precautions are less important with *H.lamae* where females call continuously rather than in short discrete bouts. In addition, 'calling age' and chronological age are well matched.

To study the effects of temperature on calling periodicity, the temperature in the growth chamber was increased or decreased manually. In the range from 10 to 35°C, a 10°C change in temperature was accomplished in less than 10 min. Occasionally, insects were moved to an adjoining chamber which was held at the required temperature. Temperatures were monitored with a Bailey BAT-12 amplifying thermometer.

Results

Eclosion periodicity

For any 'cohort' of *H.lamae* which pupated on the same day, females began to emerge 1 day before males. Yet, for any generation in synchronous culture, males emerged before females, indicating that many males pupated before females. Male eclosion peaked 6 h after lights-on (Fig. 1A). Female emergence began about 6 h after lights-on (time 0) 8 days after pupation; the main peak of female eclosion occurred 9.4 h after the start of photophase on the next day (Fig. 1B). Hence, both sexes of *H.lamae* exhibited a gated eclosion rhythm with

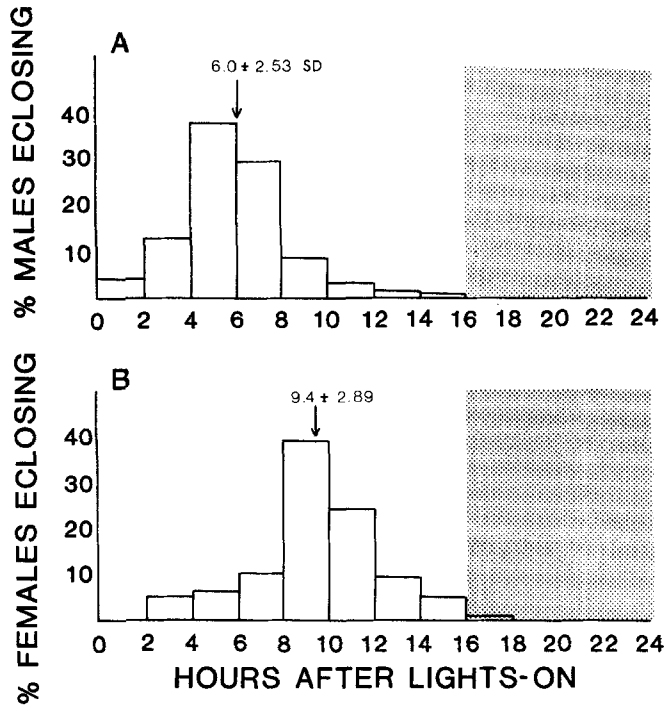

FIG. 1. Diel periodicities of eclosion for males (A) and females (B). Observations were conducted every 2 h. Numbers above arrows represent mean time of eclosion. Means for males and females are significantly different ($P<0.01$, Student's t-test). $n=338$ males, 369 females.

peaks offset by *c.* 3.5 h (Fig. 1). 68% of all males emerged between 4 and 8 h after lights-on, and 63% of females eclosed between 8 and 12 h after lights-on.

Effect of age on calling in LD 16:8 h

Only females emerging between 8 and 10 h after lights-on (peak eclosion Fig. 1B) were used in these observations. Only 33% of females called at peak calling on the day they eclosed (Fig. 2A, Table 1). On day 1, 16.5% of females called whereas 67.3% called on day 5 (linear regression, $Y=17.5+11.3X$, $P=0.05$). Calling was initiated approximately 9–11 h after lights-on at 24°C in 2–5-day-old females, and the peak of calling decreased progressively with age (Fig. 2A, Table 1). Usually, individual females continued to call for *c.* 8 h with rare interruptions of less than 5 min. At 24°C, most calling ceased within 2–3 h after lights-off.

Circadian rhythm of calling

Calling persisted in continuous dark (DD) in 2–5-day-old females which were reared in LD 16:8 h and had experienced one complete LD cycle as adults (Fig. 2B). Mean onset of calling and peak of calling were significantly delayed in the first calling period in DD (day 3) (Table 1). However, subsequent calling periods progressively advanced with onset of calling shifting from an average of 11 h on day 3 to 8.4 h after what would have been lights-on on day 5; peak calling advanced from 14.2 h in 3-day-old females to 10 h in females 5 days old. The calling period also narrowed progressively in DD females as indicated by the smaller standard deviation of mean peak hour of calling in 5-day-old-females (Table 1).

Three important features were exhibited by females transferred to LL after completing one calling cycle in LD (Fig. 2C, Table 1): progressively fewer females called, resulting in

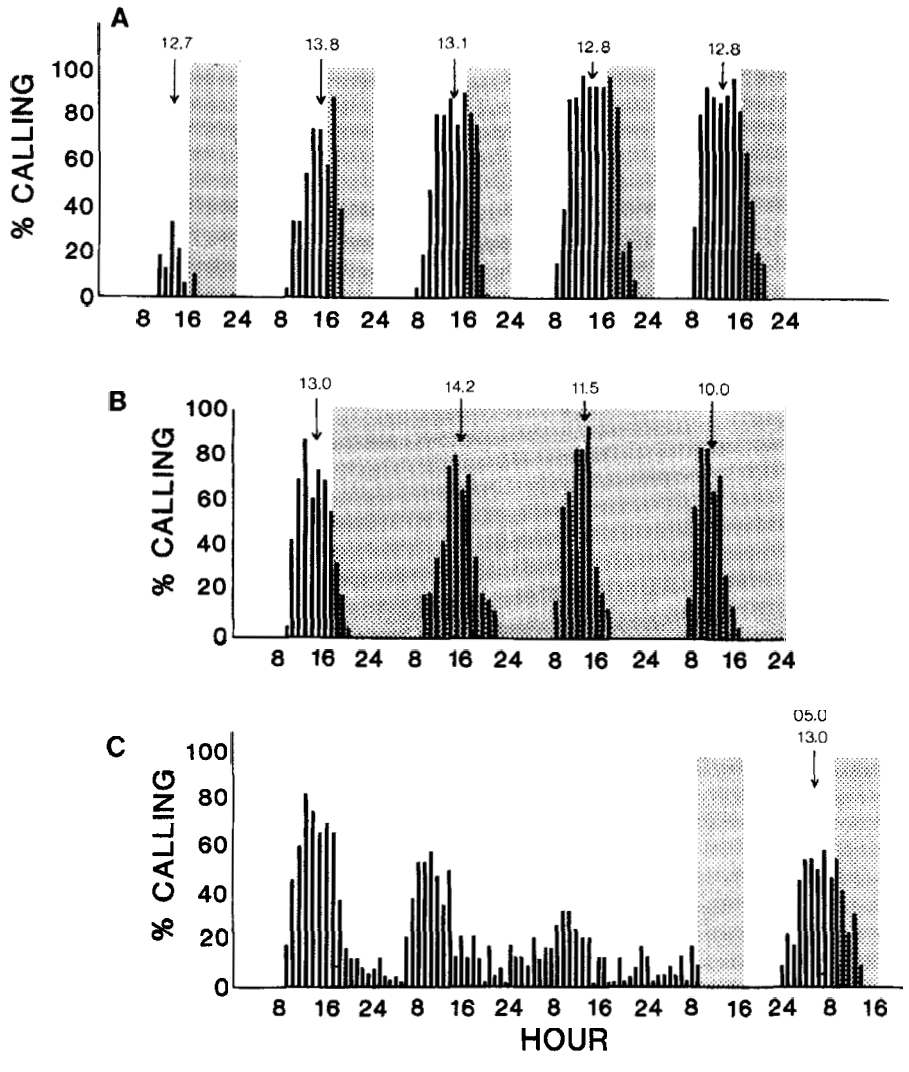

FIG. 2. Effect of age on calling in virgin females in LD (A) and calling periodicities in DD (B) and LL (C) starting with 2-day-old females at 24°C. Only females eclosing between 8 and 10 h after start of photophase were used. Linear regression of mean calling times (A), $Y=14.7-0.49X$, $P=0.03$). In (C) an 8 h scotophase was applied after dampening of the calling rhythm. Numbers above arrows denote mean calling times relative to lights-on or what would have been lights-on in LD. The top number in (C) is mean time of calling relative to the abscissa. $n=33, 30, 28, 26$ and 26 for 1, 2, 3, 4 and 5-day-old groups, respectively in (A). $n=27$ for DD, 24 for LL.

damping of calling periodicity; a significant advance in mean hour of calling occurred in the first calling period in LL (Table 1); and the declining parts of calling periods trailed off with some calling occurring at all times, whereas in LD and DD calling terminated and no females called for several hours (Figs. 2A, B). The occurrence of trailing on the first day suggests that scotophase or lights-off is important in turning calling off. Calculation of mean calling times for females in LL is tenuous after the second calling period because of the trailing and dampening of the calling function.

Light and phase setting

Setting of the circadian clock could be tied to lights-on, lights-off, or some interaction of the

TABLE 1. The influence of age, DD and LL on calling *Holomelina lamae* females at 24°C.

	Age (days)				
	1	2	3	4	5
LD 16:8					
Maximum % calling	33%	88%	89%	96%	96%
Mean onset of calling	12.2±1.6^{c}	9.0±0.9^{a}	8.8±2.0^{a}	11.4±2.0^{c}	10.2±1.4^{b}
Mean peak hour of calling	12.7±1.7^{a}	13.8±2.3^{b}	13.1±2.4^{ab}	12.8±2.6^{a}	12.8±3.1^{a}
n	33	30	28	26	26
LD 16:8 h for one adult cycle, then DD					
Maximum % calling		86%	78%	92%	83%
Mean onset of calling		9.2±1.0^{b}	11.0±2.0^{c*}	9.1±1.1^{b*}	8.4±1.4^{a*}
Mean peak hour of calling		13.0±1.8^{c*}	14.2±2.8^{d*}	11.5±2.1^{b*}	10.0±1.2^{a*}
n		27	27	27	24
LD 16:8 h for one adult cycle, then LL					
Maximum % calling		84%	58%		
Mean peak hour of calling		12.6±±3.2^{b*}	8.2±2.9^{a*}		
n		24	24		

Means (±SD) in the same row are not significantly different ($P>0.05$) if they are followed by the same letter (least-significant-difference test).

Means followed by * are significantly different ($P<0.05$) from controls of the same age (Student's *t*-test).

two. At 24°C under LD calling commenced *c.* 9 h after lights-on or 17 h after lights-off (Fig. 2A), and, as pointed out above, darkness under either LD or DD regime seemed to facilitate termination of calling (Fig. 2). In females in LL whose calling rhythmicity was dampened, an 8 h dark period out of phase with scotophase during the LD rearing phase (to 1 day post eclosion), reset

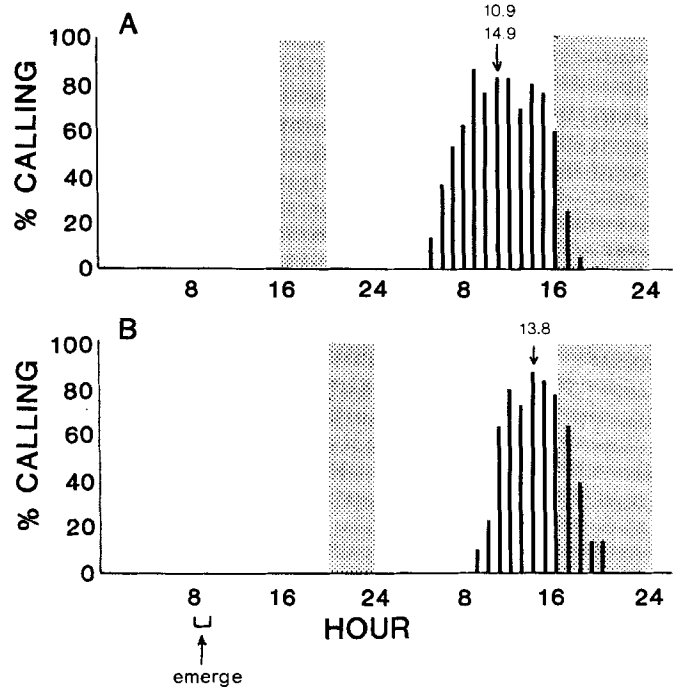

FIG. 3. Effect on female calling of varying the length and position of scotophase. Only newly emerged females eclosing between 8 and 10 h after lights-on were used. (A) A 4 h scotophase with lights-off as in LD, $n=30$. (B) A 4 h scotophase with lights-on as in LD, $n=30$. Numbers above arrows denote mean time of calling relative to lights-on; in (A) top number is relative to abscissa.

calling periodicity with a mean peak time of calling of 13 h after lights-on (Fig. 2C) which was not significantly different from day 5 females in LD ($P>0.05$, Student's t-test). Little or no resistance to a shift in the scotophase was evident. The decline in the proportion of calling females (58% of maximum) was attributable to initiation of oviposition in several of the 6-day-old females.

Lights-on acted as the major phase-setter. LD reared females were exposed upon emergence to a short (4 h) scotophase with either lights-on 4 h earlier than entrainment and lights-off in-phase with entrainment conditions (Fig. 3A), or lights-on synchronized with entrainment but lights-off delayed by 4 h (Fig. 3B). Advancing lights-on resulted in a mean peak calling time of 10.9 h which was 14.9 h after lights-on (Fig. 3A) representing a significant advance over controls ($P<0.05$, Student's t-test). A short scotophase with lights-on coinciding with that in entrainment resulted in mean time of calling of 13.8 h after lights-on (Fig. 3B) as in controls. The delay in calling in the former experiment probably represented resistance by the circadian clock to a shift in the time-setting 'lights-on' cue.

Effect of temperature decrease on calling

Groups of 2-day-old females were subjected to increases or decreases in temperature at three times during the photophase prior to the initia-

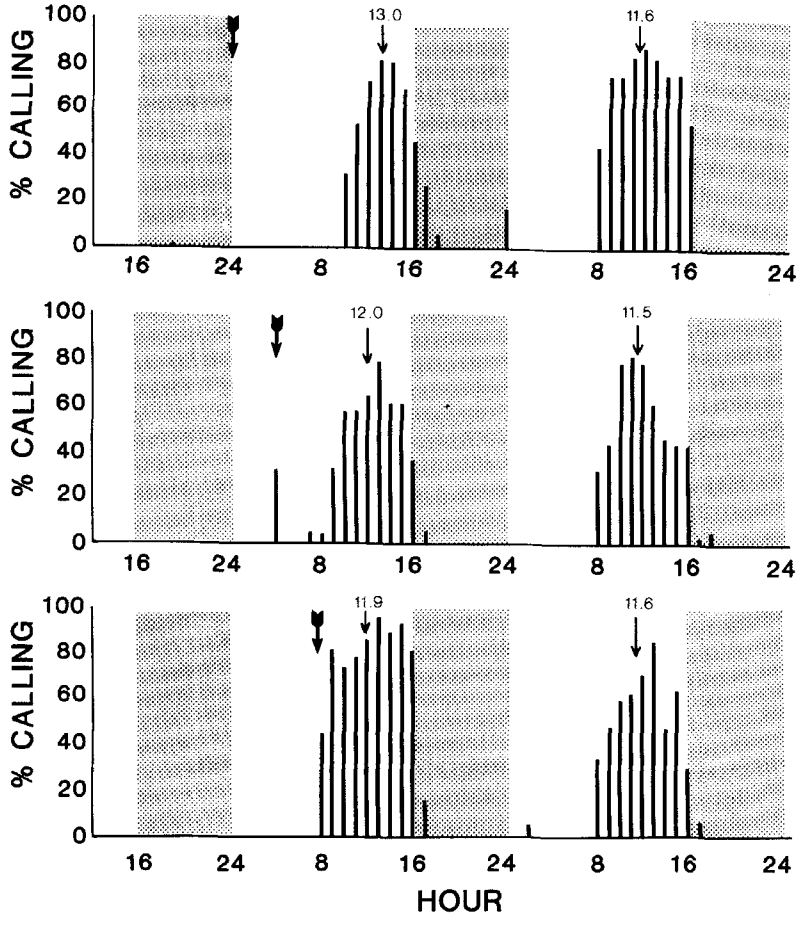

FIG. 4. Effect of temperature decreases at three parts of the photoperiod upon female calling. Temperatures were lowered to 14°C 0, 4 and 8 h after lights-on before the second calling cycle (n=26, 28 and 27, respectively), and maintained through two calling periods. Thick arrows denote time of temperature decrease. Numbers above arrows denote mean time of calling relative to lights-on.

TABLE 2. Influence of a decrease in temperature on periodicity of calling.

	Control 24°C	24°C to 14°C at L-on	24°C to 14°C 4 h after L-on	24° to 14°C 8 h after L-on
Day 2				
Maximum % calling	88%	82%	79%	96%
Mean onset of calling	9.0[b]	11.0[c]	9.7[b]	8.0[a]
Mean peak hour of calling	13.8[c]	13.0[b]	12.0[a]	11.9[a]
n	30	26	28	27
Day 3				
Maximum % calling	89%	88%	82%	85%
Mean onset of calling	8.8[a]	8.2[a]*	8.7[a]*	9.0[a]*
Mean peak hour of calling	13.1[b]*	11.6[a]*	11.5[a]*	11.6[a]
n	28	26	28	27

Means in the same row are not significantly different ($P>0.05$) if they are followed by the same letter (least-significance-difference test). Means for day 3 followed by a * are significantly different ($P<0.05$) from means for day 2 (Student's *t*-test).

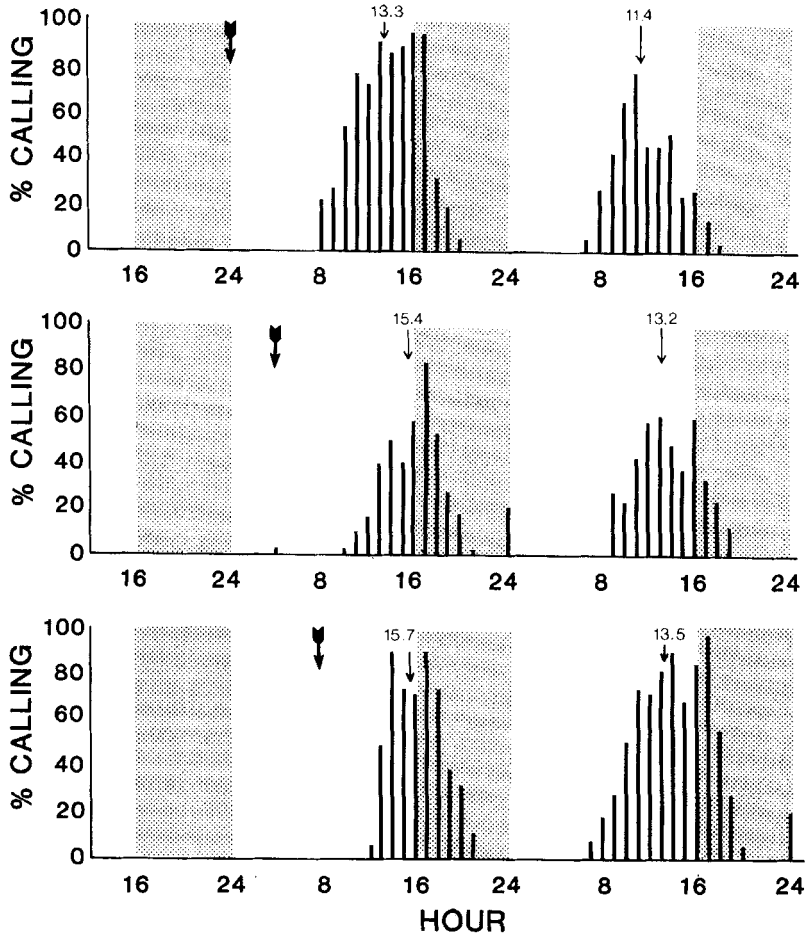

FIG. 5. Effect of temperature increases at three parts of the photoperiod upon female calling. Temperatures were raised to 34°C 0, 4 and 8 h after lights-on before the second calling cycle (n=37, 30 and 39, respectively), and maintained through two calling periods. Thick arrows denote time of temperature increase. Numbers above arrows denote mean time of calling relative to lights-on.

tion of calling. A 10°C drop at lights-on, from 24°C to 14°C, resulted in a significant 0.8 h advance in mean peak time of calling, but also a significant delay in the mean onset of calling (Fig. 4, Table 2). A temperature drop of the same magnitude, but 4 h after lights-on, resulted in a greater advance in mean peak time of calling ($P<0.05$, *t*-test; Table 2). A decrease in temperature 1 h prior to the normal initiation of calling at 24°C (8 h after lights-on) stimulated calling immediately. Here, mean peak time of calling was not advanced over the previous treatment (11.9 and 12 h after lights on, respectively: Fig. 4), but onset of calling advanced by 1.7 h over the previous experiment and 1 h over 24°C controls (Table 2).

Interestingly, a continued temperature of 14°C through the subsequent photophase (day 3) resulted in equal advances in mean peak calling times for all three groups; mean onset of calling was the same in the three groups as in controls (Fig. 4, Table 2).

Effect of temperature increase on calling

A 10°C rise at light-on, from 24 to 34°C, did not result in a significant advance in either mean onset of calling or mean peak time of calling in 2-day-old females (Fig. 5, Table 3). However, delaying the increase in temperature to 4 and 8 h after lights-on resulted in significant delays ($P<0.05$, *t*-test) in mean peak time of calling (1.6 and 1.9 h, respectively over 24°C controls) and in mean time of onset of calling (4.3 h and 4.2 h, respectively) (Figs. 6B, C, Table 3). The higher temperature also extended calling into the scotophase.

Mean peak time of calling of subsequent calling periods (day 3) at 34°C was significantly advanced (1.7 h) relative to controls only in females exposed to the high temperature at lights-on on day 2 (Table 3). In the other treatments mean peak calling times were not significantly different from controls.

The lower proportion of calling females at 34°C, particularly for 3-day-old females was due to some females ovipositing as early as day 2.

Temperature and entrainment

Starting with the second instar, larvae were reared in DD at 24°C. eclosion was random during any 24 h period and females pooled over a 12 h eclosion period exhibited no calling periodicity (Fig. 6A). However, groups of females which eclosed over a 3 h interval exhibited an endogenous calling rhythm (period *c.* 20 h) (Fig. 6B). It appeared that eclosion itself acted as a phase-setter in the absence of other environmental cues.

In DD, temperature also acted as a cue for the circadian clock (Fig. 6). Cooling 3-day-old females for 8 h at 14°C resulted in increased calling when the temperature was raised back to 24°C. An entrained rhythm cued to the temperature change was exhibited in subsequent calling with a period of *c* 24 h.

Females reared in LL which eclosed over a 3 h interval showed little calling behaviour with rarely more than 20% of the females calling at any one time. Of the females that called, a calling rhythm was not evident (Fig. 7). It appeared that, unlike calling in DD (Fig. 6), moths in LL did not entrain to eclosion time. However, in

TABLE 3. The influence of an increase in temperature on periodicity of calling.

	Control 24°C	24°C to 34°C at L-on	24°C to 34°C 4 h after L-on	24° to 34°C 8 h after L-on
Day 2				
Maximum % calling	88%	97%	83%	85%
Mean onset of calling	9.0[a]	9.7[a]	13.3[b]	13.2[b]
Mean peak hour of calling	13.8[a]	13.3[a]	15.4[b]	15.7[b]
n	30	37	30	39
Day 3				
Maximum % calling	89%	78%	60%	97%
Mean onset of calling	8.8[a]	8.8[a]*	10.3[b]*	9.5[ab]*
Mean peak hour of calling	13.1[b]*	11.4[a]*	13.2[b]*	13.5[b]*
n	28	37	29	37

Means in the same row are not significantly different ($P>0.05$) if they are followed by the same letter (least-significance-difference test). Means for day 3 followed by a * are significantly different ($P<0.05$) from means for day 2 (Student's *t*-test).

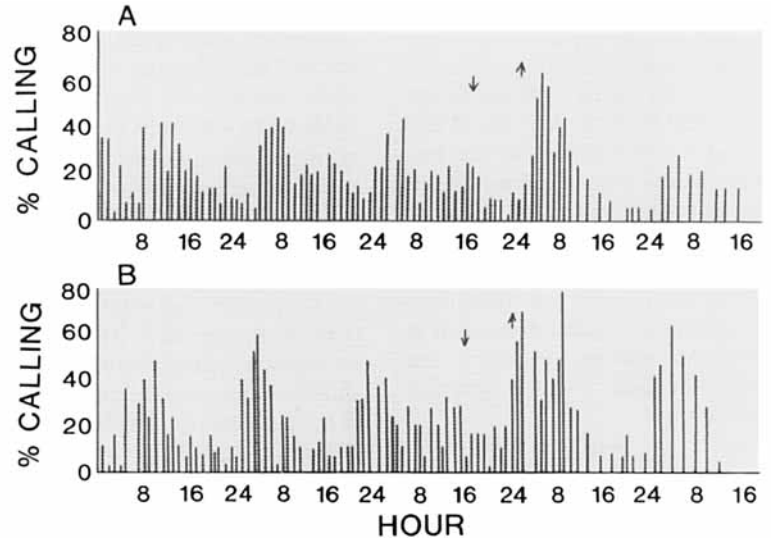

FIG. 6. Effect of rearing females in DD since the second instar upon calling in DD. Arrows denote drop to 14°C and return to 24°C, respectively. (A) Females eclosing within a 12 h interval, n=43. (B) Subset of (A): only females eclosing within a 3 h interval, n=24. Time is relative to eclosion of last females.

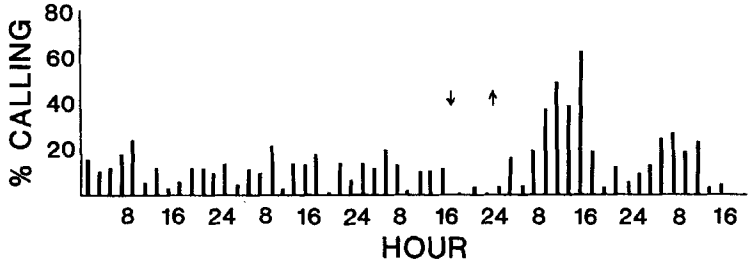

FIG. 7. Effect of rearing females in LL since the second instar upon calling in LL. Arrows denote drop to 14°C and return to 24°C, respectively. Females eclosing within a 3 h interval were used, n=24. Time is relative to eclosion of last females.

both situations a decrease in temperature, followed by return to 'normal' temperature, stimulated calling and served to set the circadian clock. As previously noted (Fig. 2C), a significant advance in calling occurred in LL.

Discussion

In *Holomelina lamae,* eclosion in both males and females and calling in females follow diel periodicities (Fig. 2). Moreover, calling is regulated by a circadian clock. At 24°C, the bulk of male eclosion occurs 4–8 h after lights-on, and *c.* 3.5 h before females. Verification of circadian and exogenous control of eclosion were beyond the scope of this work, but the gated pattern provides a good model system for such studies.

Endogenous control of calling

Age. Mean peak times of calling are gradually advanced and the durations of calling periods increase with age in LD 16:8 h. Few females call on the day they eclose. On subsequent days calling begins *c.* 9 h after lights-on and terminates within 3 h of lights-off. Also, a progressively greater proportion of older females call.

A similar relationship between onset of calling and age has been documented for several lepidopterous species (Hirano & Muramoto, 1976; Sweir *et al.,* 1977; Hendrikse, 1978;

Kanno, 1979; Szocs & Toth, 1979; Turgeon & McNeil, 1982; Webster & Cardé, 1982), but others do not follow this pattern (e.g. Baker & Cardé, 1986). However, such generalizations Sweir *et al.* (1977) and Webster & Cardé (1982) is that older females may increase their mating success by advancing the time of calling. This is consistent with our finding that female *H.lamae* release more pheromone in their first hour of calling than at any other time in the day (Schal & Cardé, 1986). However, such generalizations require closer examination, with factors such as demographics, thermal ecology, predation, and pheromone synthesis and release dynamics being taken into account.

Effects of light. In both LL and DD, LD-reared females exhibit a circadian rhythm of calling, but it appears that control of calling is differently affected by LL and DD. In DD the period shortens and mean peak times of calling are advanced 3 h over 4 days relative to females in LD. In LL, rapid dampening of the calling rhythm is exhibited, and few 4-day-old females call. Calling intervals in LL become broader with rhythmicity evident, but always overlaid on a background of random calling. The periods in LL and DD conform with Aschoff's (1979) rule that in day-active animals in constant conditions the period in LL is shorter than in DD.

Dampening, and arrhythmicity of behavioural and developmental patterns in LL have been documented in other insects. In the blowfly, *Lucilla cuprina,* flight activity persisted in LL, but rhythmicity rapidly disappeared above light intensities of 1 lux (Smith, 1983). Loss of rhythmicity was linked to stopping of the clock by photophases lasting more than 12 h, as previously documented for the eclosion rhythm in *Drosophila pseudoobscura* (Pittendrigh, 1966) and other insects. In the tiger moth, *H.immaculata,* Cardé & Roelofs (1973) have shown that LL eliminates calling in females. Whether this involves stopping of the circadian clock and/or inhibition by light is not known. It is therefore interesting that in congeneric *H.lamae* a free-running rhythm persists for two calling cycles in LL.

Exogenous factors affecting calling

Light. An 8 h scotophase after dampening of calling behaviour in LL induces calling *c.* 7 h after lights-on or 15 h after lights-off (Fig. 3C). That the former rather than the latter acts as the 'Zeltgeber' is indicated by the results of advancing and retarding the onset of the photophase and scotophase, respectively; advancing lights-on by 4 h results in a 2.9 h advance in mean calling relative to controls.

Retarding the onset of scotophase did not significantly affect the mean time of calling relative to controls. Baker & Cardé (1979) described a similar interaction between phase shifts in calling *Grapholitha molesta* females and the magnitude of the cue-shift. They suggested that the endogenous calling rhythm might resist the cue-shift resulting in only a partial phase-shift and that other factors might be involved in the phase-setting mechanism. It is likely that the calling pattern represents a transient condition and that prolonged exposure to the new photoperiod will result in shifts in calling time which match the cue shift.

In contrast to *H.lamae,* in *H.immaculata* lights-off acts as the cue that entrains the calling rhythm (Cardé & Roelofs, 1973). It appears that both *Holomelina* species conform to the general rule that nocturnal rhythms are set by dusk and that diurnal rhythms by dawn (Aschoff, 1965). Most importantly, these results highlight the danger in generalizing control mechanisms among closely related species.

Temperature. Calling persists in temperatures ranging from 10 (unpublished data) to 34°C with little change in the proportion of females calling. Temperature modifies the expression of calling behaviour in *H.lamae* as has been reported in several other Lepidoptera (Sower *et al.,* 1971; Sanders & Lucuik, 1972; Cardé & Roelofs, 1973; Cardé *et al.,* 1975; Castrovillo & Cardé 1979; Baker & Cardé, 1979; Alford & Hammond, 1982; Webster & Cardé, 1982). The effect of a 10°C drop in temperature depends on the time in the photoperiod at which it is applied. Early cooling at lights-on advances mean peak time of calling but retards the mean time of onset of calling. A temperature shift occurring 4 and 8 h after the start of photophase results in equal advances in mean peak times of calling over both controls and the previous experiment. Mean onset of calling is also advanced. Increasing the temperature from 24 to 34°C also influences calling as a function of the hour of the photoperiod. Heating at lights-on does not change the mean peak time of calling, but increases in temperature at 4 or 8 h after lights-on significantly delay

the onset of calling, its termination, and mean peak time of calling.

A critical period was evident, during which the expression of the rhythm was sensitive to exogenous perturbations. Cooling early in the photophase resulted in marked advances in the expression of the circadian rhythm, whereas later cooling modulated the calling pattern only slightly. In contrast, heating early in the photophase was ineffective in modulating calling, but later increases in temperature significantly delayed it. Thus, it appears that in *H.lamae,* temperature changes at various times exert non-linear advances or delays (calling latency) on calling as reported by Webster & Cardé (1982) for the omnivorous leafroller, *Platynota stultana.* Although our conclusions must remain tentative for lack of a complete phase–response curve, they do conform to the general notion (advanced mainly for light cues) that the inductive effect of an exogenous cue depends upon which portion of an underlying sensitivity rhythm is stimulated. That is, if the temperature change occurs when sensitivity is high, it will induce a phase shift in the expression of the rhythm. Various intensities of stimuli given at standard circadian times will also result in different phase–response curves, but this was beyond the scope of the present study.

These results are consistent with those of Baker & Cardé (1979), showing similar shifts when *G.molesta* females were heated from 25 to 31°C. Also concurring with their results and those of Webster & Cardé (1982) is the finding that absolute temperature, hour of the photoperiod, and the circadian rhythm determine when calling occurs. Hence, by continuously adjusting the temperature throughout the calling period, it is possible to significantly increase or decrease the duration of calling and disrupt calling into several distinct periods (R. P. Webster, personal communication).

It is noteworthy that temperature changes produced shifts in the expression of calling in the same direction in both nocturnally and diurnally active Lepidoptera. The opposite pattern occurs in the diurnal eclosion of *D.pseudoobscura,* where temperature decreases generated phase delays (Zimmerman *et al.,* 1968). Whether this supports the distinction between two types of clocks (Truman, 1972; see below) or represents different adaptations to different environments must await more studies on various insects.

Long-term exposure to high or low temperatures seems to induce different calling patterns. Ambient temperature of 14°C continued into the next calling cycle advances mean peak calling, but not its onset as compared to 24°C conditions. No significant differences occur among the three patterns representing cooling 24, 20 and 16 h before lights-on. After at least 20 h at 34°C, mean time of calling of *H.lamae* females tended to return to that expressed at 24°C.

Alford & Hammond (1982) found that in the noctuids *Trichoplusia ni* and *Pseudoplusia includens* temperature decreases at the start of photophase induced forward phase shifts in the time of calling. However, two light–dark cycles later (at the lower temperature) both moths showed an incomplete reversal to the original calling pattern exhibited at the rearing temperature. Truman (1973) reported that low temperatures experienced by pupae of *Antheraea pernyi* (Saturniidae) advance calling; temperature regimes experienced by adults do not influence calling periodicity. Conversely, Turgeon & McNeil (1983) showed that low pupal temperatures delay calling patterns in the noctuid *Pseudaletia unipuncta*; earlier calling occurs in females in low temperatures when pupa and adult temperature are the same. The axiom that a forward shift of calling at lower temperature confers selective advantage on females needs to be qualified and critically studied.

A temperature cycle can entrain the calling rhythm in otherwise constant conditions (review: Brady, 1974). in the present study a single 8 h decrease in temperature in LL or DD entrained the rhythm. To our knowledge, the only analogous example is the re-starting by 1 h increases or decreases in temperature of dampened-out locomotory rhythm in a beetle in LL (Thomas & Finlayson, 1970). Both in heating and cooling, and in Thomas & Finlayson's (1970) results, activity was induced *after* the temperature shift, indicating the *setting* of a circadian clock as opposed to simply *expressing* an underlying, covert rhythm. Similar results were obtained whether cooling occurred in LL or in DD, indicating that cooling did not act as 'night', as reported in temperature cycling experiments.

Duration of calling

Generally, duration of calling in groups of *H.lamae* seems to remain constant so that

advanced onset is accompanied by advanced termination of calling. However, age increases the duration of calling and DD appears to decrease it. Whereas groups of 2-day-old females call on average for 4.3 h, 5-day-old females average 7.7 h of calling. For females of the same age in LD, changes in calling periods for the population at different temperatures are generally attributable to greater variation among individuals; some 5-day females which oviposit early in the day begin calling *c.* 2–4 h before scotophase and do not terminate calling until 4 h after lights-off, thus contributing to the trailing of the calling period in scotophase.

Eclosion as Zeitgeber

Some newly eclosed females call on the day they emerge. Although eclosion in females reared in LL or DD is not gated and they emerge randomly throughout the 24 h clock, calling periods appear to be set by the time of emergence (Figs. 6 and 7). Thus, females which eclose at the same time exhibit synchronous calling on the day of emergence. Moreover, such females call approximately in-phase *c.* 22 h later, indicating setting of a circadian clock by eclosion which is then utilized to time calling events.

Truman (1972) grouped circadian clocks into two types: type I clocks control developmental events (e.g. eclosion), and type II control behavioural rhythms (e.g. calling). However, more recent reports indicate that differences between the two oscillators may be attributed to plasticity or dynamic range in a single clock (e.g. Peterson & Saunders, 1980). In the present report we have demonstrated interaction between developmental and behavioural rhythms, possibly suggesting a single control mechanism. Whether calling in *H. lamae* females follows type I or II clocks depends upon whether dampening of calling in LL results from stopping of the clock or from its uncoupling from the calling rhythm.

Acknowledgments

We thank N. Haver and D. Root for maintaining the insect colonies, N. Haver for preparing the figures, and R. Charlton, R. Collins, J. Tang and R. Webster for valuable discussions. Data analysis and writing of this paper were completed at Rutgers University.

References

Alford, A.R. & Hammond, A.M., Jr (1982) Temperature modification of female sex pheromone release in *Trichoplusia ni* (Hubner) and *Pseudoplusia includens* (Walker) (Lepidoptera: Noctuidae). *Environmental Entomology,* **11,** 889–892.

Aschoff, J. (1965) The phase angle difference in circadian periodicity. *Circadian Clocks* (ed. by J. Aschoff), pp. 262–276. North Holland Publishing Company, Amsterdam.

Aschoff, J. (1979) Circadian rhythms – influence of internal and external factors on the period measured in constant conditions. *Zeitschrift für Tierpsychologie,* **49,** 225–249.

Baker, T.C. & Cardé, R.T. (1979) Endogenous and exogenous factors affecting periodicities of female calling and male pheromone response in *Grapholitha molesta* (Busck). *Journal of Insect Physiology,* **25,** 943–950.

Brady, J. (1974) The physiology of insect circadian rhythms. *Advances in Insect Physiology,* **10,** 1–115.

Cardé, R.T. & Baker, T.C. (1984). Sexual communication with pheromones. *Chemical Ecology of Insects* (ed. by W. J. Bell and R. T. Cardé), pp. 355–386. Chapman & Hall, New York.

Cardé, R.T., Comeau, A., Baker, T.C. & Roelofs, W.L. (1975) Moth mating periodicity: temperature regulates the circadian gate. *Experientia,* **31,** 46–48.

Cardé, R.T. & Roelofs, W.L. (1973) Temperature modification of male sex pheromone response and factors affecting female calling in *Holomelina immaculata* (Lepidoptera: Arctiidae). *Canadian Entomologist,* **105,** 1505–1512.

Cardé, R.T. & Webster, R.P. (1981) Endogenous and exogenous factors controlling insect sex pheromone production and responsiveness, particularly among the Lepidoptera. *Scientific Papers of the Institute of Organic and Physical Chemistry of Wroclaw Technical University,* **22,** 977–991.

Castrovillo, P.J. & Cardé, R.T. (1979) Environmental regulation of female calling and male pheromone response periodicities in the codling moth (*Laspeyresia pomonella*). *Journal of Insect Physiology,* **25,** 659–667.

Hendrikse, A. (1978) Factors influencing the courtship behaviour of *Yponomeuta vigintipunctatus* (Retz.) (Lepidoptera: Yponomeutidae). *Netherlands Journal of Zoology,* **28,** 139–149.

Hirano, C. & Muramato, H. (1976) Effect of age on mating activity of the sweet potato leaf roller, *Brachmia macroscopa* (Lepidoptera: Gelechiidae). *Applied Entomology and Zoology,* **11,** 154–159.

Kanno, H. (1979) Effects of age on calling behaviours of the rice stem borer, *Chilo suppressalis* (Walker)

(Lepidoptera: Pyralidae). *Bulletin of Entomological Research,* **69,** 331–335.

Peterson, E.L. & Saunders, D.S. (1980) The circadian eclosion rhythm in *Sarcophaga argyrostoma*: a limit cycle representation of the pacemaker. *Journal of Theoretical Biology,* **86,** 265–277.

Pittendrigh, C.S. (1966) The circadian oscillation in *Drosophila pseudoobscura* pupae: a model for the photoperiodic clock. *Zeitschrift für Pflanzenphysiologie,* **54,** 275–307.

Roelofs, W.L. & Cardé, R.T. (1971) Hydrocarbon sex pheromone in tiger moths (Arctiidae). *Science,* **171,** 684–686.

Sanders, C.J. & Lucuik, G.S. (1972) Factors affecting calling by eastern spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae). *Canadian Entomologist,* **104,** 1751–1762.

Schal, C. & Cardé, R.T. (1986) Rhythmic extrusion of pheromone gland elevates pheromone release rate. *Experientia* (in press).

Smith, P.H. (1983) Circadian control of spontaneous flight activity in the blowfly, *Lucilia cuprina. Physiological Entomology,* **8,** 73–82.

Sower, L.L., Shorey, H.H. & Gaston, L.K. (1971) Sex pheromones of noctuid moths. XXV. Effects of temperature and photoperiod on circadian rhythms of sex pheromone release by females of *Trichoplusia ni. Annals of the Entomological Society of America,* **64,** 488–492.

Sweir, S.R., Rings, R.W. & Musick, G.J. (1977) Age-related calling behavior of the black cutworm, *Agrotis ipsilon. Annals of the Entomological Society of America,* **70,** 919–924.

Szocs, G. & Toth, M. (1979) Daily rhythm and age dependence of female calling behaviour and male responsiveness to sex pheromone in the gamma moth, *Autographa gamma* (L.) (Lepidoptera: Noctuidae). *Acta Phytopathologica Academiae Scientiarum Hungaricae,* **14,** 453–459.

Thomas, R. & Finlayson, L.H. (1970) Initiation of circadian rhythms in arrhythmic churchyard beetles (*Blaps mucronata*). *Nature,* **228,** 577–578.

Truman, J.W. (1972) Circadian rhythms and physiology with special reference to neuroendocrine processes in insects. *Proceedings of the International Symposium on Circadian Rhythmicity, Wageningen, 1971,* pp. 111–135. Pudoc, Wageningen.

Truman, J.W. (1973) Temperature sensitive programming of the silk moth flight clock: a mechanism for adapting to the seasons. *Science,* **182,** 727–729.

Turgeon, J. & McNeil, K. (1982) Calling behaviour of the armyworm, *Pseudaletia unipuncta. Entomologia Experimentalis et Applicata,* **31,** 402–408.

Turgeon, J. & McNeil, J. (1983) Modification in the calling behaviour of *Pseudaletia unipuncta* (Lepidoptera: Noctuidae), induced by temperature conditions during pupal and adult development. *Canadian Entomologist,* **115,** 1015–1022.

Webster, R.P. & Cardé, R.T. (1982) Relationships among pheromone titre, calling and age in the omnivorous leafroller moth (*Platynota sultana*). *Journal of Insect Physiology,* **28,** 925–933.

Zimmerman, W.F., Pittendrigh, C.S. & Pavlidis, T. (1968) Temperature compensation of the circadian oscillation in *Drosophila pseudoobscura* and its entrainment by temperature cycles. *Journal of Insect Physiology,* **14,** 669–684.

Accepted 21 August 1985