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Short-lived intertidal midge *Pontomyia oceana* have semilunar eclosion rhythm entrained by night light

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ABSTRACT: Semilunar or lunar rhythms are common in intertidal organisms. Some are controlled by endogenous oscillators that are entrained by environmental factors. This study investigates whether the semilunar eclosion rhythm of the short-lived (~1 mo) marine midge *Pontomyia oceana* may be controlled by a night-light entrained endogenous rhythm. Mating midges were collected in southern Taiwan. Their fertilized eggs and larvae were cultured following various treatments in the laboratory until eclosion. A cohort, i.e. those fertilized on the same evening, later exhibited, under different light conditions without lunar cues, 2 modes in eclosion days. The periods of the circasemilunar eclosion rhythms under the no-cue conditions were between 12 to 15 d. Four successive evenings of night-light synchronized the eclosion of midges into 2 concentrated peaks. Shifting the evenings of the entraining night-light resulted in a shift in the evenings of eclosion, although the degree of response was only ~50%. Night light in the first few days of life also had a concentration effect. Damping of the rhythm depended on the days in the life cycle during which the night-light was applied. The circasemilunar period of the rhythm was resistant to environmental changes as demonstrated by the low Q_{10} estimated from temperature treatments. This is the most short-lived species with demonstrated endogenous semilunar rhythm.

KEY WORDS: Circasemilunar rhythm · Biological clock · Intertidal insect · Emergence · Entrainment · Temperature compensation · Endogenous rhythm

INTRODUCTION

Lunar or semilunar cycles of biological phenomena are common in organisms that inhabit shallow seawaters (e.g. Bünning & Müller 1962, Christy 1978, Saigusa & Hidaka 1978, Neumann 1981, Palmer 2000, Hsueh 2002, Lüning et al. 2008, Oliveira et al. 2009). For example, the reproductive activities of many marine species, such as corals (Harrison et al. 1984), echinoderms (Kubota 2000, Morgan 2009), crustaceans (Morgan 1996), or fishes (Hsiao & Meier 1986, Susilo et al. 2009) all exhibit lunar or semilunar cycles. In these cases, reproductive activities such as the aggregation of adults, the spawning of gametes or the release of larvae are synchronized with lunar phases and/or semimonthly or monthly recurring tidal conditions.

Cyclic activities are either cued directly by external factors, or alternatively controlled by endogenous clock-like mechanisms, which in turn are entrained by periodic environmental changes. Relatively few cases of endogenous clock control of such activities have been established, and they are based on a wide range of evidence. Free-running, or the persistence of the cyclic activities after the cessation of environmental cues, is explainable only by the endogenous clock hypothesis. It is usually the first indication that cyclic activities are actually controlled by an endogenous oscillator. The trait is known in some marine and intertidal species (polychaete: Hauenschild 1960, Franke 1985; marine midge: Neumann 1966, 1988; terrestrial crab: Saigusa 1980; fish: Hsiao & Meier 1992; and macroalgae: Bünning & Müller 1962, Lüning et al. 2008).

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Entrainment of cyclic activities is a property of the clock mechanisms in which shifting the timing of the entraining factors causes corresponding changes of the associated activities (marine midge: Neumann & Heimbach 1985, Saigusa 1988, Neumann 1995; polychaete: Last et al. 2009). Unlike a simple direct cue in which responses are immediately released, entrainment, at least in circadian rhythms, commonly requires a few cycles before the phase of the endogenous rhythms is completely synchronized with the new external environment. Jet-lag in humans exemplifies such characteristics (Sack et al. 2007).

Temperature compensation is a feature of biological clocks, and it refers to mechanisms by which the periods of endogenous rhythms remain roughly constant across various temperatures (Pittendrigh 1954, Hastings & Sweeney 1957). The Q_{10}, or the proportion increase in metabolic rates for every 10°C increase in temperature, is generally between 2 and 3 for most other metabolic activities (see Rao & Bullock 1954), but may be close to unity in clock-controlled mechanisms. A few cases of temperature compensation in endogenous circasemilunar rhythms have been shown (such as in polychaete: Franke 1985; marine midge: Neumann 1988, Neumann & Spindler 1991; and fish: Hsiao & Meier 1992).

Among species that have been examined in relation to lunar rhythms, marine midges (with a short adulthood of only ~1 h) have a life cycle that may range from 6 wk to 1 yr, depending on season and latitude (Neumann 1976a, also see Garbary et al. 2009). The marine midge *Clunio* spp. has a lunar or semilunar rhythm of emergence. The larval stages of these midges may last for a few months. In extreme cases, only 1 generation may eclose per year at high latitudes (≥60° N) (Neumann 1986). However, at low latitudes, such as at 35° N in Japan, a generation of *C. tsushimensis* required only 6 wk to eclose at a high temperature (20 to 25°C) (Oka & Hashimoto 1959, Neumann 1986). Under invariant light conditions, such as 24 h period light–dark (LD) cycles, no lunar pattern of eclosion is observed in *Clunio* cultures of mixed generations. However, a lunar cue, such as 4 evenings of dim light (0.3 lux) simulating moonlight, causes cycles of synchronized semilunar eclosion in low-latitude northern European *C. marinus* (Neumann 1976b, 1985).

In contrast to *Clunio marinus*, the low-latitude (22° N) Taiwanese marine midge *Pontomyia oceana* has a life cycle of only ~1 to 1.5 mo (Soong et al. 1999, Soong & Leu 2005). Both species live on shallow protected shores and have similar life histories (Fig. 1). Short-lived adults (1 to 2 h) emerge in swarms, they lay sticky egg strings that attach to exposed hard substrata during low tides. The short life span of adults also requires stringent synchronization among individuals

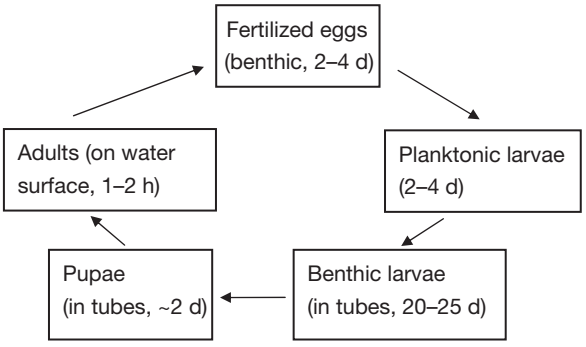

Fig. 1. *Pontomyia oceana.* Life history stages. Approximate duration of each stage indicated in parentheses based on laboratory cultures

(see Soong et al. 2006, Kao et al. 2010). In only a few days, fertilized eggs hatch, and planktonic larvae settle; then, benthic larvae build tubes out of debris (Fig. 1). Their lunar rhythmic life cycles appear to result from convergent evolution (see Neumann 1976a). *Pontomyia oceana* emerges synchronously in the evenings close to new and full moons in southern Taiwan (Soong et al. 1999). Owing to their short life cycles, they get only one chance to see consecutive evenings of night light. Moreover, those hatched half a month apart in nature experienced contrasting regimes of night-light intensity cycles in a month. It is unclear how they can manage to eclose synchronously 1 mo later in both situations, if indeed night-light is a cue or an entraining factor. Previous investigations have confirmed the importance of both endogenous circadian rhythms and temperature in determining daily eclosion times (Soong et al. 2006, Kao et al. 2010). The present investigation studies whether this short-lived marine midge relies on night-light entrained endogenous rhythms to synchronize its semilunar cycles of eclosion.

MATERIALS AND METHODS

Midge culture. In each experiment, mating pairs were collected on 1 evening of the spring tide from intertidal pools at Wanliton (21° 59′ 45″ N, 120° 42′ 19″ E), southern Taiwan, and returned to the laboratory. Fertilized eggs hatched in ~2 to 4 d, after which planktonic larvae were separated into containers. The photoperiods were maintained at 12:12 h light:dark (LD), unless otherwise specified. The light intensity was ~10 µmol photons m^{-2} s^{-1} at the water surface during the light period. The salinity was adjusted to 3.0% and the water temperature was maintained at 25 to 26°C, or under room temperature, except in the experiments on the effects of tempera-

ture. Larvae were fed algal powder that was prepared from dried *Ulva* and other macroalgae at a ration of ~0.4 g container^{-1} d^{-1}. The water was replaced as required, such as when mold grew on the bottom of the containers, or when copepods propagated excessively. The midges began eclosing as early as Day 24 after fertilization. The eclosed midges and sticky egg strings floating on the surface were removed and counted daily.

Entrainment. The first entrainment experiment (E1) tested the effect of 4 consecutive nights of dim lights, using no night light (i.e. under a dark cloth cover) as a control group. Night light, or ~0.1 µmol photons m^{-2} s^{-1} at water surface, was applied in the evenings of Days 10–13, 11–14 and 12–15 after fertilization of the egg. Only the first peaks of eclosion were recorded.

In the second entrainment experiment (E2), the effects of night lights on Days 7–10, 11–14 and 15–18 were compared. Moreover, a second bout of night light applied 30 d later was also used to test its effects on the second peak of eclosion, in 3 additional treatments. For example, one treatment involved night light on Days 7–10 and 37–40. Hence, a total of 7 entrainment treatments (including a control without any night light) were compared. The eclosion numbers were recorded to Day 51 to cover both peaks of eclosion.

In the third entrainment experiment (E3), the possible effects of night light on Days 0–3, i.e. from fertilization to embryo development, were tested. A total of 30 beakers under 12:12 h LD cycles and ambient temperature, separated into night light and control, were used.

In all entrainment experiments, subscripts were used to indicate the duration the night light was applied, e.g. between Day 10 to 13 as M_{10-13}. The control group denoted M_c has no night light in any days.

Temperature compensation. The photoperiod was maintained at 12:12 LD cycles throughout this experiment for 3 treatments at temperatures of 24, 27 and 30°C, respectively; the entraining night light was applied on Days 11 to 14. Two peaks of eclosion were recorded in this experiment.

Potential temperature compensation was evaluated by calculating the Q_{10} of eclosion results at different temperatures using the Van't Hoff's formula: $Q_{10} = (X_2/X_1)^{10/(t_2-t_1)}$, where, for treatments 1 and 2 respectively, $1/X_1$ and $1/X_2$ are the intervals and t_1 and t_2 the temperatures.

The actual calculations were based on 24 (t_1) and 30°C (t_2) predictions (R_1, R_2) from the linear regression formula (x: temperature, y: eclosion days), which was based on eclosion data from the experiment. At each temperature, the intervals between the means of the first and the second peaks of eclosion were used as the estimated period of the endogenous rhythm. The estimation of Q_{10} was based on a regression formula between the temperature and the periods of the 3 temperature treatments.

Lunar or semilunar rhythm. In a previously published experiment (designated R1 herein), fixed 24 h LD cycles (3 photoperiod treatments, 10:14, 12:12 and 14:10 h) without lunar cues were used to test the effect of the photoperiod on the distribution of eclosion numbers in the 2 eclosion peaks (Soong & Leu 2005). The same data set was used here to determine the periods of the circasemilunar eclosion rhythm.

In the second rhythm experiment, midges were cultured under continuous light (LL) and total darkness (DD) without lunar cues, and their numbers of daily eclosions were recorded (R2). Under DD, the midges were counted and removed using flash lights covered with red film that had been found not to affect eclosion time (Kao et al. 2010). In a third experiment (R3), LD, DD and a control LDN (with night light applied from Day 11 to 14) were compared.

Cosinor analysis (Nelson et al. 1979) was used to assess the statistical significance of the rhythms in eclosion numbers in these experiments. The method approximates summation of least squares of time series data from a cosine function of assumed period; it does not require many data cycles that a cohort of midges does not have. Computations were made using the available software in the Circadian Rhythm Laboratory, University of South Carolina's website (www.circadian.org/main.html). At 1 d intervals, various periods of ~15 d were tested for possible fitting of the eclosion data to a cosine-shaped rhythm; p-values were calculated after multiple test corrections. To compare the extents of dispersion of eclosion days between treatments with and without the entraining night light, the variances of the eclosion peaks were compared using *F*-tests.

RESULTS

Entrainment

For the E1-entrainment experiment, the numbers of eclosions in the 4 treatments were between 76 and 122. The 3 treatments with night light yielded a much more synchronized distribution of eclosion days, between Days 25 and 30, than the control conditions without night light, which yielded eclosion days from Day 24 to 37 (Fig. 2). The lack of a night light was associated with a much larger spread of eclosion days (variance: V = 12.5) than were night light entrainments (V = 1.0 to 1.4, $p < 0.01$, in all 3 cases, *F*-tests). Applying the entraining night light at later dates made the mean eclosion days come later. However, the delay in the mean eclosion

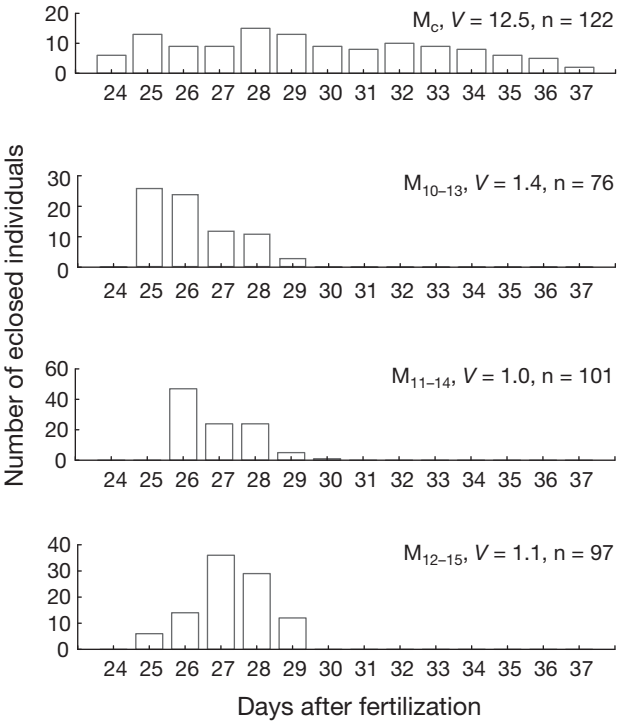

Fig. 2. *Pontomyia oceana.* Frequency distributions of eclosion days under different night-light treatments in Entrainment Experiment E(1). M_{10-13}, M_{11-14}, M_{12-15}: subscript refers to ranges of nights with artificial moon light; M_c: control group; *V*: variance. The relationship between the first evening of night light (*x*) and the mean eclosion day (*y*) is: $y = 0.52x + 21.1$, $p < 0.01$, $R^2 = 0.13$

day did not completely reflect the shift in the entraining cues. The slope of the regression between the mean eclosion day (*y*) and the first day of the use of the night light (*x*) ($y = 0.52x + 21.1$, $R^2 = 0.13$, $p < 0.01$) was 0.52. In other words, for every 1-d delay (or advance) in the entrainment, the delay (or advance) in eclosion was 0.52 d, on average.

For E2, the numbers of eclosions were between 81 and 281. The control group without night light was associated with a wide range of eclosion days, from Day 28 to 37. In contrast, for most of the entrained cohorts, the ranges of eclosion days were no more than 5 d, in the first peak. The control was also characterized by a significantly greater variance than all other treatments in the first eclosion peaks ($p < 0.01$, in all 6 cases, *F*-tests).

The second eclosion peak, however, was more complicated. With respect to the treatment with the intermediate entraining night light, i.e. on Days 11 to 14, just the first round of exposure to night light sufficed to synchronize both peaks of eclosion (Fig. 3). In other words, the variance of the eclosion days was significantly smaller than that of the corresponding peak in the control ($p < 0.01$). A second round of exposure to night light, as in the entrainment treatment $M_{11–14, 41–44}$, was not necessary to synchronize the second peak. In $M_{7–10}$, only the first peak of eclosion was more concentrated than that of M_c ($p < 0.01$). The second peak of eclosion, however, remained unsynchronized as determined by comparison with that of the control group M_c ($p > 0.05$). After a second exposure to night light, as in $M_{7–10, 37–40}$, the second peak of eclosion was also concentrated ($p < 0.01$; by comparison with that of M_c; Fig. 3). Similarly, in $M_{15–18}$, only the first peak of eclosion was concentrated upon 1 round of night light treatment. However, the second round of exposure to night light was administered in $M_{15–18, 45–48}$ when the second round of eclosion had started for ~5 d. Almost no eclosion occurred thereafter (Fig. 3).

The timing of the night light in E2 also affected the phases of the eclosion peaks. The first peaks in $M_{11–14}$ and $M_{15–18}$ occurred within prediction from the E1 experiment ($p > 0.05$ for both slopes and intercepts, ANCOVA). However, the first peak in $M_{7–10}$ (and $M_{7–10, 37–40}$) was much later than that expected from the regression formula based on experiment E1 (Fig. 3).

For E3, 3 to 133 midges emerged in individual beakers, and only those with >10 in a peak were used to compare the extent of spread of eclosion days. In total, 30 cases were compared for the first peak, and 25 cases for the second peak. For both peaks of eclosion, the $M_{0–3}$ treatment had significantly smaller variation than the M_c in their eclosion days (Mann–Whitney *U* Test, $p = 0.04$ and 0.03, respectively; Fig. 4). Paired comparisons were used to compare peaks 1 and 2 in individual beakers, and the variation was significantly smaller in the former than in the latter (Night light: $p < 0.01$, control: $p = 0.02$, Wilcoxon signed rank test; Fig. 4). In other words, the second peak of eclosion is less concentrated than the first one.

Temperature compensation

The first eclosion began on Days 25, 26 and 27 of the 3 treatments at 30, 27 and 24°C, respectively. The mean eclosion days for the first peaks, 28.0, 29.2 and 28.9 (with n = 1011, 365 and 113), respectively, varied significantly among treatments ($p < 0.01$, ANOVA), although by only 1.2 d. The last days of eclosion were 31, 32 and 32 in the 3 treatments, respectively. The treatment with the highest temperature (30°C) began earlier and ended earlier, and had an earlier mean eclosion day than the other 2 treatments.

All second eclosion peaks began on Day 37, although the last days of eclosion were 44, 45 and 47 (n = 381, 244, 410) upon the 3 treatments at 30, 27 and 24°C, respectively. The mean eclosion days, 40.3, 40.9 and 40.8 under the 3 treatments, varied significantly

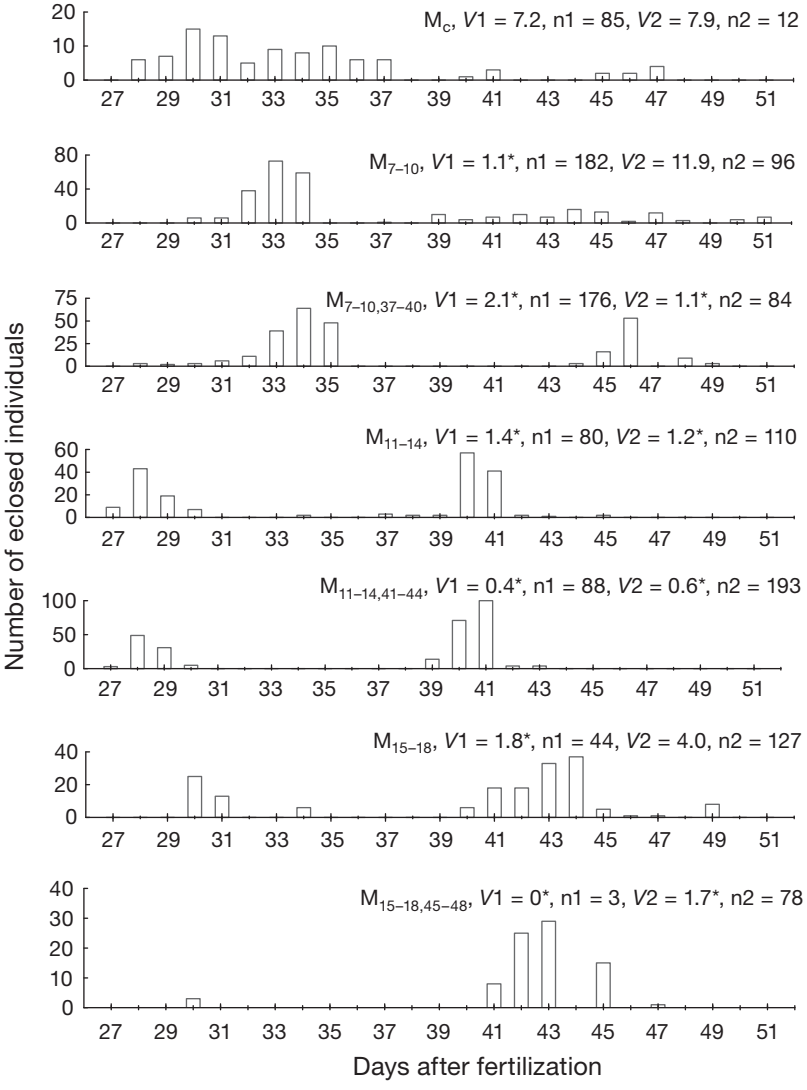

Fig. 3. *Pontomyia oceana*. Frequency distribution of eclosion days upon exposure to 1 and 2 bouts of night light in E2. Subscript of M refers to ranges of nights with artificial moonlight. *V*1 and *V*2: variance of eclosion days of peaks 1 and 2, respectively; *p < 0.01, significantly smaller than that of control group, M_c

(p < 0.01, ANOVA), although the difference was <1 d.

Q_{10} estimation is based on the regression formula for the above experiment (Fig. 5). Temperature ranges between 24 and 30°C were used. The Q_{10} of the first peak of eclosion was 1.09, the second peak 1.02, and that of the period of semilunar rhythm, based on difference between the first and the second peak, was estimated at 0.86.

Lunar or semilunar rhythm

All treatments in experiment R1 (LD of 10:14, 12:12 and 14:10 h) had 2 discreet peaks of eclosion (Soong & Leu 2005). However, the 2 peaks may differ greatly in the number of emerging individuals. For example, in the treatment with the 12:12 h LD period, the first and second peaks had 336 and 65 emerging individuals, respectively. Most midges eclosed at the first peak, ~1 mo after fertilization. The Cosinor analyses revealed significant rhythms in 2 out of the 3 treatments. The optimum periods in the 10:14 and 14:10 h LD treatments were 13 and 14 d, respectively (Fig. 6, Table 1). The difference between the 2 peaks in numbers of eclosions was the greatest in the 12:12 h LD treatment, and no significant rhythm was found (p = 0.17, Cosinor analysis). However, when the numbers of eclosed midges were normalized for each peak, a significant rhythm was identified by the Cosinor method (unpubl. data). The fertilized eggs for this experiment were collected around full moon.

In R2, which involved LL and DD light conditions without lunar cues, the optimum periods were 13 and 15 d (p = 0.02, < 0.01, Cosinor analyses; Fig. 6), respectively. Fewer eclosions occurred in LL (n = 102) than in DD (n = 412). The experiment used fertilized eggs that were collected around a full moon.

For R3, many midges (n = 3493) successfully completed eclosion (Fig. 7). The numbers of eclosions ranged from 367 to 817 in the peaks of individual treatments. All 3 treatments yielded obvious eclosion peaks and the optimal periods were all 12 d (p < 0.01, all cases, Cosinor analysis; Fig. 6). The treatment with night light entrainment (LDN) was associated with a significantly narrower distribution of eclosion days in both peaks than were the other 2 treatments (LD and DD) without lunar cues (p < 0.01, in all 4 comparisons, *F*-tests; Table 1). The experiment used fertilized eggs that were collected around a new moon.

DISCUSSION

The present study shows that the semilunar cycles of the eclosion of the short-lived *Pontomyia oceana* is controlled endogenously. Furthermore, this endogenous control has properties, i.e. entrainment, temperature compensation and free-running, similar to those of circadian rhythms (Pittendrigh 1965).

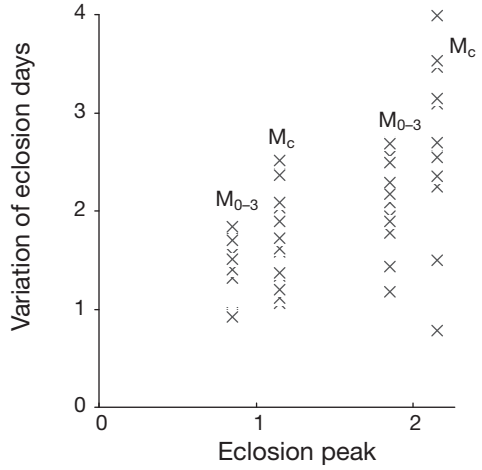

Fig. 4. Spread of eclosion days between M_{0-3} and the control without night light (M_c) in E3. Data points: beakers with >10 ind. $M_{0-3} < M_c$ in variation in both peaks; 1st peaks < 2nd peaks variation in both groups

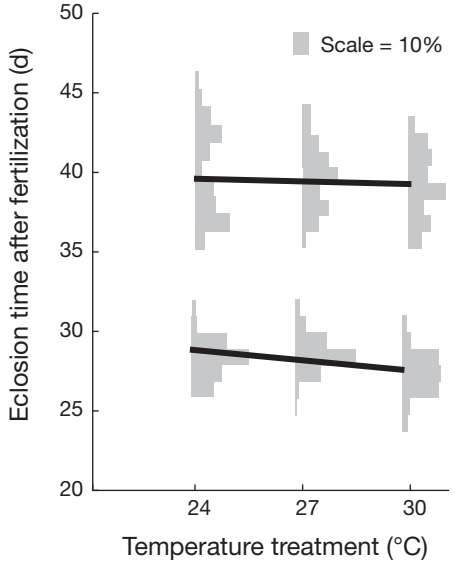

Fig. 5. *Pontomyia oceana*. Regression analyses of eclosion days under treatments at different temperatures. Entraining night-light applied on Days 11–14. First eclosion peak: $y = -0.25x + 35.5$, $p < 0.01$, $R^2 = 0.12$, N = 1489. Second eclosion peak: $y = -0.07x + 42.6$, $p < 0.01$, $R^2 = 0.007$, N = 1035

The entrainment of the semilunar eclosion rhythm in *Pontomyia oceana* was demonstrated with 4 evenings of night light. The midges that were subjected to night light on Days 11–14 eclosed in a narrow range of days ~16 d later (Fig. 2). Moreover, the synchronization persisted to the second peak of eclosion. This result is evidence of free-run, an important property of endogenous rhythms. The ability to entrain >1 peak of eclosion is important, because night light occurs around full moons only, but the *P. oceana* populations have 2

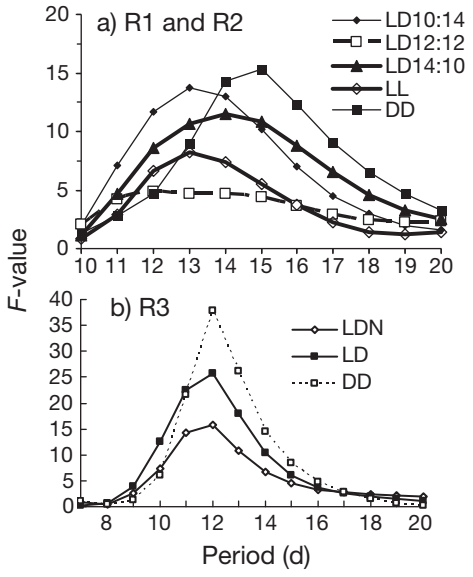

Fig. 6. *Pontomyia oceana*. Cosinor periodograms of eclosion days under different light regimes (LD: light dark cycles, LL: continuous light, DD: continuous darkness, LDN: light dark cycles with night light between Days 11–14) for experiments (a) R1, R2, and (b) R3. $p < 0.01$ for all peak *F*-values, after multiple-test corrections. Threshold in single tests is $F = 5.6$ at significance level of 0.01

peaks of eclosion in a month (Soong et al. 1999). If the endogenous rhythms were damped too quickly, the eclosion around full moons would be less concentrated, from a lack of entraining night light half a month before, and all night light they could have detected was available a full month (2 cycles of semilunar rhythm) before. On the other hand, in E3, where night light was applied at the beginning of the midges' lives, the degree of concentration was significantly lower about 3 semilunar cycles later. Since only 2 peaks of eclosion exist in a cohort of this midge, the lack of selection to further inhibit damping may be the case in this short-lived midge.

Entrained cultures of the marine midge *Clunio marinus* of northern Spain eclosed synchronously for a third and even a fourth peak during free-running (Neumann 1976b, Neumann & Heimbach 1985). In this species with a longer generation time, fast damping of the endogenous circasemilunar rhythm must be selected against.

In inferring from the results of E1, a 2 d difference in birth date results in only a 1 d difference in eclosion, on average. (1) This phenomenon suggests that midges of the same cohort did not have random phases in circasemilunar rhythms, even when they saw no night light in the laboratory. Had the midges raised without lunar cues been random in phases of their circasemilunar rhythms, the intervals between the entraining night light and the eclosion would have been a constant in

Table 1. *Pontomyia oceana*. Rhythm experiments. LD: light–dark cycle, LL: continuous light, DD: total darkness, LDN: light–dark cycles with 4 evenings of night light. *p < 0.01 for greater variance than that of the control (LDN) (*F*-test). Optimum period estimated using the Cosinor method

Experiment	Moon phase when eggs collected	Light treatment	Optimum period (d)	Variance of eclosion days; 1st, 2nd peak	N; 1st, 2nd peak	Data source
R1	Full	LD, 10:14	13	2.1, 3.0	202, 110	Soong & Leu (2005)
	Full	LD, 12:12	Ns	1.7, 4.0	336, 65	
	Full	LD, 14:10	14	1.6, 3.7	271, 132	
R2	Full	LL	13	1.3, 11.5	26, 76	This study
	Full	DD	15	4.3, 10.7	265, 147	
R3	New	LD, 12:12	12	8.4*, 4.1*	516, 480	This study
	New	DD	12	2.6*, 2.6*	817, 635	
	New	LDN	12	1.4, 1.0	367, 678	

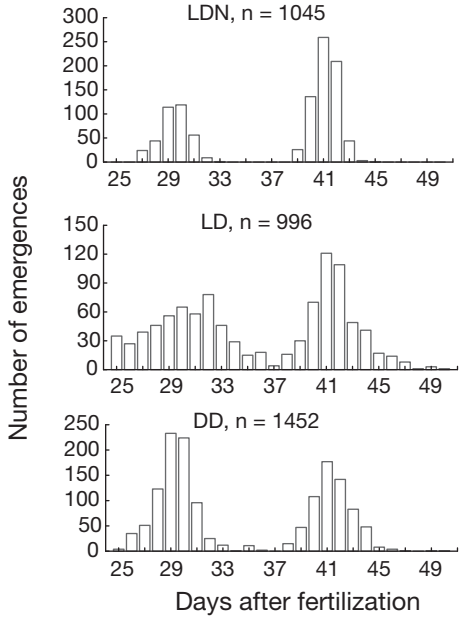

Fig. 7. *Pontomyia oceana*. Frequency distribution of eclosion days of midges under LDN (light–dark cycles with 4 evenings of night light), LD (light–dark cycles), and DD (continuous darkness)

different treatments. (2) The less than full response in eclosion days to a shift in the entraining evenings is similar to that in circadian rhythms in which several cycles of new light regimes are necessary to allow the organisms to adjust fully to the new conditions (e.g. Alleva & Alleva 2002). The mechanism that underlies this phenomenon is unclear, although it may serve the function of synchronizing midges that are born on different evenings that bracket the same full or new moons. For example, midges that are born earlier may see the entraining moon light when they are 14 d old and take another 14.4 d to eclose, but those born 3 d later see the same night light at 11 d old, and eclose 15.8 d later. There is only 1.4 d difference in average eclosion days. The net effect is those born late are prompted to emerge sooner; thus, more overlap in eclosion days with those born earlier are expected.

The intervals between the nights of artificial entraining and the eclosion days were sometimes much longer than expected, such as in treatments in which night light was applied much earlier than usual: on Day 7 to 10 in E2 (Fig. 3). In this treatment, synchronization was effective, but eclosion dates were later than expected. The midges in this treatment were probably not ready in the first window and could eclose only when the second window became available. Mean eclosion is supposed to occur 16 d after the first night light, i.e. on Day 21 of treatment M_{7-10}, but the earliest eclosion of all experiments was Day 24. The next available lunar window allowing emergence depends on the period of the circasemilunar rhythm, i.e. ~12 to 15 d later. The actual eclosion days of the M_{7-10} treatment fits this prediction (Fig. 3). There should be no surprise that the second eclosion peak was not synchronized. As articulated previously, the entraining night light occurred 3 cycles before, and the endogenous rhythms damped.

Several marine species exhibited temperature compensation in their endogenous lunar/semilunar rhythms (Franke 1985, Neumann 1988). The Q_{10} values in these species were very close to 1.0. A Q_{10} value of <1.2 means that they require temperature compensation mechanisms (see Hastings & Sweeney 1957). Although some effects of temperature on the length of the period of the circasemilunar rhythms were found in *Pontomyia oceana*, the deviation of Q_{10} from 1.0 was very small. It is equivalent to a ~1 d shift in eclosion days with a change of 6°C in water temperature. Given the number of evenings that are typically associated with an eclosion peak (Fig. 7), most individuals still eclose on the same evenings, even when the tidal pools

they inhabit have very different temperatures. The same argument applies to eclosion evenings in different seasons: the midges can eclose at very similar phases of spring/neap tide cycles (Soong et al. 1999) using this endogenous mechanism.

The periods of the endogenous rhythm, as estimated by the Cosinor methods, differed among the experiments and ranged between 12 and 15 d (Table 1). These variable estimations may be contributed by several factors. (1) The assessment is based on population data, and so any mortality due to environmental variations is likely to affect the estimation. (2) Individual midges can obviously eclose in a window of 4 to 5 evenings, as clearly evidenced by synchronized natural populations (Soong et al. 1999) and laboratory cultures with entraining night lights (Fig. 7). Both factors contribute to the variability of estimated periods. The deviation of the estimated periods from the intervals of actual semilunar cycles parallels that of circadian rhythms in which the endogenous periods are not exactly 24 h (see Saunders 2002).

Some treatments without entraining night lights still eclosed with certain extent of concentration and rhythms, although the extent of concentration was significantly lower than when night light cues were present in our investigation (Figs. 6 & 7). Since rhythmic eclosion is a population-level phenomenon, some synchronizing mechanisms must be present. Experiments on *Clunio marinus* in northern Europe, on the other hand, revealed no eclosion rhythm for cultures without entraining night light under unchanging LD (Neumann 1976b). However, those cultures were of mixed cohorts and generations, and so the lifetimes of individual midges were unclear. In the present study, all individuals were fertilized on the same evening eliminating such variation. Therefore, the concentration and rhythm of eclosion in the cue-less lab environment suggest that the synchronization mechanism may be related to their common developmental stages. For example, if the endogenous rhythms of various individuals all began at the same phase at certain stage of development, then a cohort of midges would have similar eclosion dates, even without synchronizing cues or entraining factors. This mechanism, if confirmed, would still need external cues, otherwise subsequent generations would be even more scattered than their parents. Moreover, this mechanism would only work for individuals born on the same evenings and would produce more scattered eclosion days in nature where a peak of parent midges covers 4 to 5 consecutive evenings of eclosion. Without some external factors to synchronize the midges, the eclosion peak would be 8 to 10 d wide in just one generation.

Eclosion may be concentrated because nocturnal light of the moon entrained midges during the egg string stage. This is supported by E3. Thus, midges collected around a new moon, when no night light is available, would be expected to eclose on a wider range of days than those collected around a full moon. This is corroborated under LD culturing conditions, although no such pattern was observed under DD culturing conditions (Table 1). The maternal effect is another possibility. A semilunar eclosion window is 4 to 5 d wide; thus, early and late offspring are 4 to 5 d apart in development. The early offspring either in egg strings or hatched can perceive the moon light, but the late ones are still inside their mothers when the moon light is present. These late offspring, however, can only be synchronized by the same entraining night light. Hence, either the eggs directly sense the moon light through their mothers' body wall, or a maternal effect needs to be invoked. More experiments are needed to distinguish the hypotheses.

An additional possibility is that environmental factors other than night light are involved in entraining the circasemilunar rhythms of eclosion. This phenomenon occurs in European marine midges at higher latitudes (Neumann 1976a,b, 1978, 1985). In temperate latitudes >54° N, the dark nights become shorter during summer and dim light remains continuously above the Northern horizon, making moon light an unreliable monthly zeitgeber cue. At these latitudes, a tidal disturbance pattern in combination with the 24 h day–night-period (resulting in identical phase relations every 14.7 d) entrain the circasemilunar rhythms of eclosion of *Clunio marinus* (Neumann 1976b). Temperature cycles that are caused by tides along with the 24 h light–dark cycle also act as cues that control the semilunar eclosion of a Norwegian population of *C. marinus* (Neumann & Heimbach 1984). The involvement of multiple and a combination of factors must be an adaptation in which single factors do not always reliably synchronize eclosion. At lower latitudes, such as in Japan, *C. tsushimensis* responded to night light, but was insensitive to tidal disturbance (Neumann 1985). So far, no midge population at low latitudes is known to use cues other than night light. Use of additional environmental factors may be very helpful to those species with short generation times, since they cannot afford to wait. Our preliminary tests involving both temperature and LD cycles, however, could not concentrate the semilunar eclosion into 4 to 5 evenings (unpubl. data).

The night light-entrained mechanisms identified herein probably represent only some of the mechanisms that control the semilunar eclosion cycles of this short-lived marine midge *Pontomyia oceana*. The short generation time of the Taiwanese *P. oceana* must have given it great potential to propagate (Fisher 1930). The tidal fluctuation in intertidal and shallow water habi-

tats requires much adaptation for organisms living here. Endogenous semilunar rhythm, demonstrated here for the marine midge *P. oceana*, is an important trait that not only helps the adults find exposed substrate at low tide for the next generation, but also helps the short-lived adults find mates by synchronizing their emergence.

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