Involvement of the circadian system in photoperiodic control of pubertal development in female deer mice, *Peromyscus maniculatus*[1]

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Summary. Pubertal development in female deer mice, *Peromyscus maniculatus*, was stimulated by exposure to light cycles of LD 16:8, LD 6:30 or LD 6:54, but not by exposure to cycles of LD 6:18 or LD 6:42. These results support the hypothesis that female deer mice use a circadian rhythm of responsiveness to light to measure photoperiodic time.

The reproductive system of individuals in many species of vertebrates is responsive to the external photoperiod[2]. Two hypotheses have been proposed to account for the ability of an organism to measure day length. According to the 'hourglass' hypothesis, the organism measures the length of either the light period or the dark period. The 2nd hypothesis, attributable to Bünning[3], assumes a circadian rhythm in sensitivity to light, such that a pulse of light triggers the photoperiodic response only when presented at certain times in the circadian cycle. In photoperiodic mammals that have been examined, photoperiodic time is measured in accordance with the Bünning hypothesis[3]; all of the supporting data, however, were derived from studies of males.

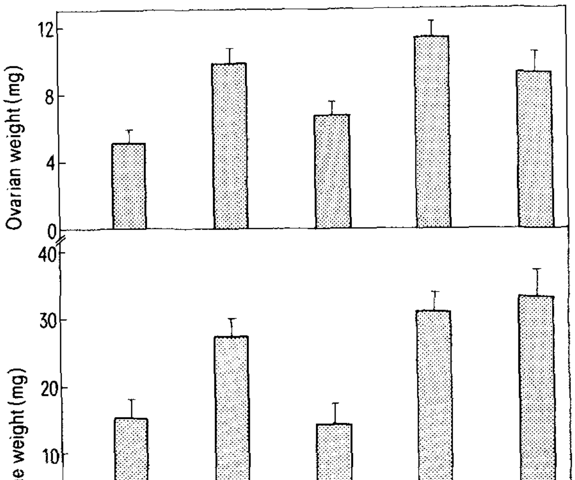
Here we report an investigation of the mechanisms of photoperiodic time measurement in a female mammal. The test was based on the use of a standard 'resonance' protocol, the rationale of which has been discussed in detail previously[5]. Briefly, however, this protocol involves coupling a short photoperiod (e.g., 6 hrs) to different durations of darkness to generate light-dark (LD) cycles with periods (T) of multiples or half-multiples of 24 h (e.g., $T = L + D = 24, 36, 48, 60$). The circadian system of animals exposed to any of these LD cycles is assumed to be entrained with a period of 24 h. The 6-h photoperiod in the 24- and 48-h cycles occurs at the same time every day or every other day and is sufficient to entrain the rhythm to a 24-h period. In the 36- and 60-h cycles there is an alteration of light in the morning with light in the evening the following day (LD 6:30) or 2 days later (LD 6:54). If the morning and evening pulses occurred on the same day they would form a 'skeleton' of an 18-h photoperiod. In actuality, the day ($T = 36$) or 2 ($T = 60$) of darkness between light pulses is assumed to have little effect on the pattern of entrainment. These assumptions relative to the effects of resonance cycles on the circadian system have been validated in the most thoroughly studied case, the golden hamster[4]. Assuming that a photosensitive phase for photoperiodic induction exists in an animal's subjective night (Bünning's hypothesis), those cycles which illuminate, on alternate cycles, the subjective night (e.g., LD 6:30 and LD 6:54) will be stimulatory to the reproductive system whereas those cycles which illuminate only the subjective day (LD 6:18 and LD 6:42) will not.

Materials and methods. The subjects of the experiment were female prairie deer mice, *Peromyscus maniculatus bairdii*. Husbandry and breeding were as described earlier[5]. Female deer mice, previously housed on a photoregimen of LD 6:18, were weaned at 20–23 days of age and, 6 days later, were randomly assigned to 1 of 9 light-tight wooden chambers[5]. Each chamber, which held 9 cages of individually housed females, received one of the following treatments: LD 6:18, LD 6:30, LD 6:42, LD 6:54, or LD 16:8. There was 1 chamber containing long-day control females (LD 16:8) and 2 replicate chambers for the other treatments. At 48–51 days of age, females were killed and frozen at −20°C for later measurement of ovarian and uterine weights. Data were analyzed by F tests calculated by the general linear models procedure of the Statistical Analysis System[6]. Organ weights were adjusted for body weight by analysis of covariance and are presented as least squares means[6] in the figure. Overall F's were statistically significant [ovaries: $F(4,72) = 5.62$, $p < 0.0005$; uterus; $F(4,72) = 6.75$, $p < 0.0001$], and all statements below are based on significant differences between means in pairwise comparisons.

Results and discussion. Females reared from weaning on a lighting regimen of LD 6:18 had smaller reproductive organs than did females reared on LD 16:8 (fig.), confirming the inhibition of pubertal development in female deer mice by a short photoperiod[7]. Relative to the control shortday cycle (LD 6:18), the LD 6:42 cycle was not stimulatory, whereas cycles of LD 6:30 and LD 6:54 clearly stimulated growth of ovaries and uterus.

If the female deer mouse were to measure time in accordance with the hourglass hypothesis, no treatment involving 6-h photoperiods should have stimulated reproductive organ growth. If, however, the female deer mouse measures time according to the Bünning hypothesis, cycles of LD 6:18 and LD 6:42 should be non-stimulatory, because light pulses always fall outside the sensitive region of the circadian rhythm of photoperiodic photosensitivity, but cycles of LD 6:30 and LD 6:54 should be stimulatory because every other pulse of light falls within the photo-sensitive region of the circadian rhythm[5].

Our results clearly conform to the predictions of the Bünning hypothesis. The major conclusion is identical to that of an earlier study of male deer mice[5]. In addition, the present investigation provides stronger evidence than the previous one that a photoregimen of LD 6:54 is fully stimulatory; these females, which received in 3 weeks only 4 pulses of light within the photo-sensitive portion of their circadian cycle, had reproductive organs as large as those of females on cycles of LD 6:30 and LD 16:8. Male and female deer mice, despite their many functional differences, use a common mechanism for measurement of photoperiodic time. Perhaps this is also true of other mammalian species.



Influence of light:dark cycles on reproductive organs in female deer mice at 7 weeks of age. Data presented as least squares means (adjusted for body weight) +SE.

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Oviposition behavior in the Australian stick insect *Extatosoma tiaratum*

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Summary. The Australian stick insect *Extatosoma tiaratum* (MacLeay) (Insecta: Phasmida) oviposits by throwing the eggs to a distance of circa 0.80–2.00 m by a strong flick with the abdomen. The initial speed is circa 2.7 m/sec. The rather immobile female just drops her feces, and as these may act as olfactory attractants to predators, the eggs that are flicked away will be safe, lying in a ring around the central deposit of feces.

Different types of oviposition are known to occur in the stick- and leaf-insects (Phasmida)[1], but little is known about the mechanism and biological significance of these. Eggs may be passively dropped, actively flicked away, glued singly or together, stuck into parts of plants, placed singly or together on a substrate. Only a few species are known to flick away their eggs[2–6]. The distance which the egg is thrown varies from 6–8 cm in *Bacillus rossius* (Rossi) to 5–6 m in *Phasma gigas* (Linné)[3,5,6]. In this paper the oviposition in the Australian stick-insect *Extatosoma tiaratum* (MacLeay) is considered.

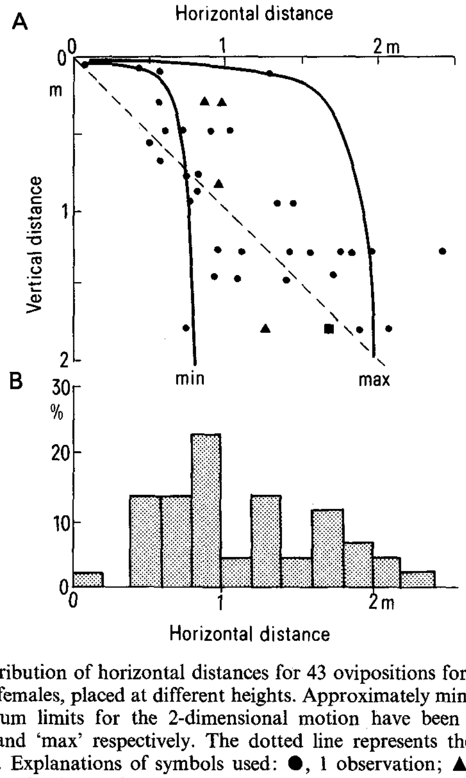
Material and methods. The adult female *E. tiaratum* is a large insect, length and mass of body, 105–130 mm and 10–20 g[7]. The egg is more or less round; height, 3.50–4.50 mm; width, 2.50–3.50 mm; mass, 37–40 mg[8,9]. Females were placed upside-down in their normal resting and ovipositing position on twigs, supplied with leaves of *Quercus robur* Linné. The twigs were arranged at various heights. 5-cm-high obstacles with a 10-cm-distance between them were placed on the ground, giving a ±5 cm range of measurements. The ordinary Newtonian formula for 2-dimensional motion with constant acceleration was used, assuming an inclination angle of 0°.

Results and discussion. When comparing the distance that the eggs were flicked by females placed at different heights (fig. A), a dispersal could be observed, but minimum and maximum limits could be estimated at 0.80 and 2.00 m, respectively. The large length values from low heights show that the females are capable of throwing the eggs long distances from low initial heights. This suggests an inclination angle somewhat larger than 0°, giving a small incline in the initial phase of oviposition. The initial speed was around 2.7 m/s (range: 0.49–7.17 m/s, $\bar{X} \pm$ SD:2.68 ± 1.11 m/s, CV:42%, n: 43). Since the eggs are thrown away by a strong flick of the abdomen, some of the variations observed (fig. A) could be due to different body-lengths. When comparing the ratio d/bl (d = distance which the egg is thrown away and bl = bodylength) the following results were obtained: 8–15 for *E. tiaratum* (bl = 105–130 mm), 24–36 for *Phyllium bioculatum* (bl = circa 84 mm) and 26–32 for *P. gigas* (bl = circa 190 mm)[3,7]. However, authors reporting distances without describing the experimental conditions must be taken with a pinch of salt, since the egg may well continue to roll along on the ground after landing, and therefore no intepretation can be made of the results.

The surface egg density (SED), previously discussed[1] as the biological reason for flicking away the eggs, states that the immobile female[10] is in the center of a circular area (*A*), where the radius (*r*) is the horizontal distance which the eggs are flicked away, and is defined as:

$$SED = n/A = n/\pi r^2 \quad (1)$$

where n = number of eggs laid; $\pi \approx 3.14$. The present results (fig.) shows that SED is low for $0.00 \leq r \leq 0.80$ and for



A Distribution of horizontal distances for 43 ovipositions for 12 *E. tiaratum* females, placed at different heights. Approximately minimum and maximum limits for the 2-dimensional motion have been indicated, 'min' and 'max' respectively. The dotted line represents the slope of − 1.00. Explanations of symbols used: ●, 1 observation; ▲, 2 observations; ■, observations.

B Percentage distribution of horizontal distances. When summing all primary data from *A* the lower values become overrepresented ($\bar{X} \pm$ SD: 1.13 ± 0.51 m, CV: 45%, n: 43). When excluding some of these lower values, a new distribution was found ($\bar{X} \pm$ SD: 1.35 ± 0.49 m, CV: 33%, n: 28). Furthermore, it was not stastistically significantly different from the original population (t = 1.866, df: 69, $0.050 < p < 0.025$).