Circadian rhythmicity is involved in photoperiodic time measurement in the aphid *Megoura viciae**

M. Vaz Nunes and J. Hardie

Department of Biology, Imperial College at Silwood Park, Ascot, Berkshire SL5 7PY (Great Britain)

Received 2 March 1993; accepted 20 April 1993

Abstract. The photoperiodic clock in the vetch aphid *Megoura viciae* is generally accepted to be based on a non-circadian mechanism or "hourglass", as no evidence has been found for the involvement of the circadian system in the photoperiodic response. By using a recently-devised protocol which discriminates between single and repeated night length measurement, we demonstrate here that long-night measurement in *Megoura* is executed in a repetitive way, and thus that its photoperiodic clock is based on a circadian oscillator after all. However, it is also apparent that the determination of short nights is not repetitive.

Key words. Photoperiodism; photoperiodic clock; circadian rhythmicity; aphid, *Megoura viciae.*

It is well known that the circadian system somehow plays a role in the process of photoperiodic induction in many organisms[1-3]. A notable exception in this respect is the vetch aphid *Megoura viciae*: despite extensive investigations into the photoperiodic response of this species, no evidence has been found for the involvement of the circadian system[4-8]. The photoperiodic clock in *M. viciae* is, therefore, generally accepted to be a non-circadian clock or "hourglass"[3,6,7,9-11] (although some authors prefer the equivalent term "rapidly damped oscillator"[9,10]). In *M. viciae*, parthenogenetic females (virginoparae) occur in long days (short nights), while the short days (long nights) of autumn induce the sexual females (oviparae). The mated oviparae lay diapausing eggs, which are the overwintering stage in the life cycle.

Photoperiodic induction comprises at least two mechanisms, a clock which measures photoperiodic time, most commonly night length, and a counter which accumulates photoperiodic information contained in subsequent light-dark cycles[12]. Recently it was found that for ovipara-induction in *M. viciae* a minimal number of long-night cycles is required[13]. In the absence of long-night cycles all aphids developed into virginopara-producers (0% ovipara-producers), whereas a minimum of about 8 long nights (12 h light: 12 h dark, abbreviated as 12L:12D) was required for 100% ovipara-producers (0% virginopara-producers). The counter mechanism in *M. viciae* has recently been analysed more thoroughly[14]. In continuous dark (DD) an average of 30% virginopara-producers (range: 0–76%) was observed[6]. If DD is equivalent to one single long night (as would be the case if the clock were an hourglass), then 80–90% virginopara-producers would have been expected. In fact a range of 0–76% implies that DD is equivalent to between 2 long nights (76%) and 8 or more long nights (0%). A 30% response is expected when DD is equivalent to 5–6 long nights. This suggests that *Megoura's* clock measures night length in a repetitive way, and therefore could be a circadian oscillator rather than an hourglass.

To investigate the possibility of a circadian-based clock, an experimental protocol[15] was used which discriminates between single and repeated night length measurement by comparing the inductive effects of scotophases of 12, 36, 60 and 84 h duration (i.e. $12 + n \times 24$ h; $n = 0, 1, 2$, or 3). In the case of an hourglass clock, scotophases of $12 + n \times 24$ h are all measured only once and are therefore regarded by the counter as a single long night. On the other hand, in the case of a circadian oscillator clock a 12-h night is measured once, a 36-h night twice, a 60-h night three times and an 84-h night four times. This leads to the following prediction – if the clock is an hourglass *one* cycle of, for example, 12L:60D is expected to result in the same percentage of virginopara-producers as *one* cycle of 12L:12D, but if the clock is a circadian oscillator *one* cycle of 12L:60D is expected to result in the same percentage of virginopara-producers as *three* cycles of 12L:12D.

Materials and methods

A stock culture of an English clone of *M. viciae*[16] was reared in Fisons' environmental cabinets in long days (16L:8D) at 15 °C. For the experiments, long-day fourth-instar apterous larvae (generation P) were isolated on tick-bean seedlings (*Vicia faba*) and then transferred to short days (12L:12D). Once these aphids had undergone the final moult and had started to larviposit, they were placed on fresh seedlings at the beginning of the photophase and allowed to larviposit. Larvae born (generation G1) during the photophase were transferred to continuous light and then, for the remainder of the 10-day postnatal sensitive period, to various numbers of long-night cycles of the general form 12L:$(12 + n \times 24)$D ($n = 0, 1, 2$ or 3). The first ten progeny born (generation G2) were distinguished as virginoparae or oviparae. If there were five or more virginoparae in the test progeny the mother was classified as virginopara-producer[13]. Normally, 18 mothers were used per experiment. For the exact details of experiments, see table. For further information, see reference 13.

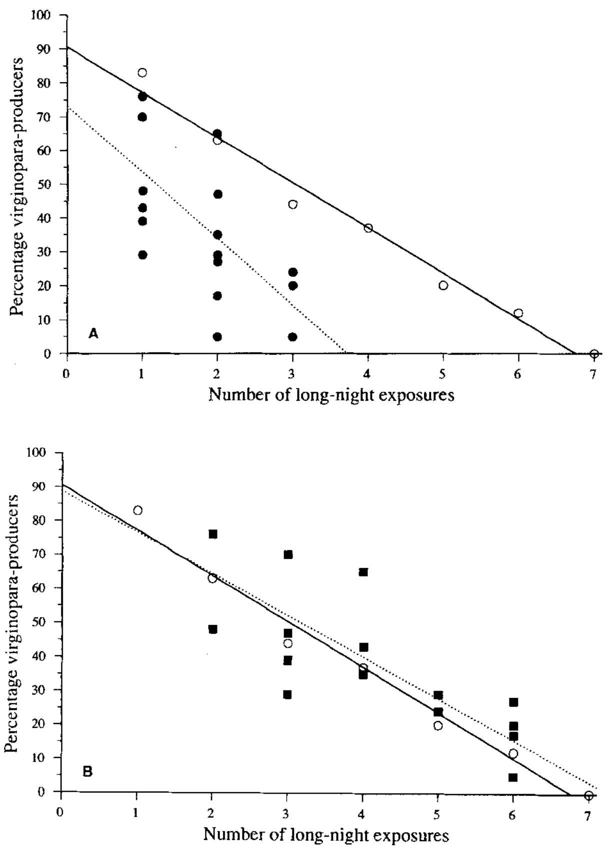
Results and discussion

The results are shown in the table. The data of the 12L:12D controls are from Hardie[13]. It is clear that the observed incidences of virginopara-producers are not predicted by an hourglass clock. For example, two cycles of 12L:60D (17 and 27% virginopara-producers) agree better with six cycles of 12L:12D (12%) than with two (63%). In the figure the experimental data are plotted against the number of long-night exposures as predicted by an hourglass clock (A) or by a circadian oscillator clock (B) (i.e. the "hourglass" and "circadian oscillator" columns in the table). If the clock were an hourglass, then the observed incidences of virginopara-producers would be expected to overlap the 12L:12D control. This is not the case: in figure A the determined regression line lies well below that of the control and the slope is much steeper. In figure B, on the other hand, the data all fall close to and around the 12L:12D control, and the regression line overlaps that of the control. The results, therefore, show clearly that in *Megoura* long nights are measured repetitively, and that the clock cannot be an hourglass. So, although previous experiments failed to demonstrate any involvement of the circadian system in the photoperiodic induction in this species[6-8], the present results indicate very clearly that the photoperiodic clock is based on a circadian oscillator rather than an hourglass.

Experiments similar to the above have been performed with the mite *Tetranychus urticae*[15] and three species of insect, *Pieris brassicae*[17], *Sarcophaga argyrostoma* and *Calliphora vicinia*[18]. In all these species, circadian rhythmicity has been found to play some role in the process of photoperiodic induction, as responses to Nanda-Hamner protocols (i.e. a photophase of constant length is combined with scotophases of up to 72 h) revealed "peaks" and "troughs" at circadian intervals[17,19-21]. The Veerman-Vaz Nunes experiment, however, showed that in two of the species (*T. urticae* and *P. brassicae*) the clock was an hourglass[15,17], whereas in the two flies (*S. argyrostoma* and *C. vicina*) the clock was shown to be a (damped) circadian oscillator[18]. These results could be explained by assuming that circadian rhythmicity plays an *indirect* role in the photoperiodic response of the species with an hourglass clock (i.e. has an effect downstream of the clock, e.g. ref. 22), but plays a *direct* role (i.e. within the clock mechanism) in the two species of fly[18,19].

Table. Comparison of the effect on virginopara-production in short-day born *Megoura viciae* of various sequences of long-night cycles with a 12-h photophase ($T = n \times 24$ h), applied toward the end of the ten-day sensitive period, following continuous light.

Photoperiodic regimes following continuous light	% Virginopara-producers observed	No. of long-night exposures if clock is:	
		Hourglass	Circadian Oscillator
1 × 12L:12D (control)	***83***	***1***	***1***
1 × 12L:36D	76	1	2
1 × 12L:36D	76	1	2
1 × 12L:36D	48	1	2
1 × 12L:60D	39	1	3
1 × 12L:60D	70	1	3
1 × 12L:60D	29	1	3
1 × 12L:84D	43	1	4
2 × 12L:12D (control)	***63***	***2***	***2***
1 × 12L:36D + 1 × 12L:12D	47	2	3
2 × 12L:36D	35	2	4
2 × 12L:36D	65	2	4
1 × 12L:60D + 1 × 12L:36D	29	2	5
2 × 12L:60D	17	2	6
2 × 12L:60D	27	2	6
1 × 12L:84D + 1 × 12L:36D	5	2	6
3 × 12L:12D (control)	***44***	***3***	***3***
2 × 12L:36D + 1 × 12L:12D	24	3	5
3 × 12L:36D	20	3	6
3 × 12L:36D	5	3	6
4 × 12L:12D (control)	**37**	**4**	**4**
5 × 12L:12D (control)	**20**	**5**	**5**
6 × 12L:12D (control)	**12**	**6**	**6**
7 × 12L:12D (control)	**0**	**7**	**7**



Percentages of virginopara-producers in *Megoura viciae* plotted against the number of long-night exposures experienced during a series of 12L:12D photoperiods (open circles; regression line is Y = 90.6 − 13.4 X ($r^2 = 0.980$)), *a* compared to the number of long-night exposures predicted by an hourglass clock in regimes 12L:variable D (solid circles). Regression line is Y = 73.2 − 19.6 X ($r^2 = 0.422$) and *b* compared to the number of long-night exposures predicted by a circadian oscillator based clock in regimes 12L:variable D (solid squares). The regression line is Y = 88.8 − 12.2 X ($r^2 = 0.680$).

The situation in *M. viciae*, however, is very different. No involvement of circadian rhythmicity could be demonstrated in the photoperiodic response, and yet the present experiments show very clearly that the clock measures long night lengths in a repetitive manner and could, therefore, be regarded as a circadian oscillator. But if the clock is a circadian oscillator, then why is there no indication of circadian rhythmicity in Nanda-Hamner experiments[7,8]? A possible solution to this paradox might be the following. If the circadian photoperiodic clock were, for instance, based on the external coincidence model[9] then the night length would be determined by whether a photo-inducible phase (ϕ_i) fell in light or in darkness. Consequently, ϕ_i is positioned at the end of the critical night length (CNL): a "short" night (i.e. shorter than the CNL) causes ϕ_i to fall in the light, a "long" night (i.e. longer than the CNL) causes ϕ_i to fall in the dark. As ϕ_i occurs in every circadian cycle, CNL occurs, therefore, also at circadian intervals, thus resulting in "long" and "short" nights being determined at circadian intervals, causing the peaks and troughs in Nanda-Hamner experiments for a number of species. The absence of such peaks and troughs in *M. viciae* suggests that, irrespective of ϕ_i falling in light or dark, a night is only regarded as "short" if its *actual* length is shorter than CNL, and "long" if it is longer. A very long scotophase may, in addition, be regarded as equivalent to a *number* of long-night (12L:12D) cycles, viz when ϕ_i falls in the dark a number of times. A consequence of this assumption is, however, that for the determination of night lengths shorter than the CNL, some other (hourglass?) mechanism may be involved. It seems that, although it is now possible to examine the underlying basis of the photoperiodic clock more directly, there is still no unified mechanism which can account for all the phenomena observed.

* Dedicated to our dear friend, the late Professor A. D. (Tony) Lees who, sadly, died before we had a chance to discuss the data reported here.

Acknowledgments. We thank AFRC for financial support.

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