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Short communication

‘Bistability’ experiments and the photoperiodic clock in the spider mite *Tetranychus urticae*

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Introduction

In insects and mites, photoperiodic induction of diapause comprises at least two processes: night-length measurement by means of a photoperiodic ‘clock’ and the subsequent accumulation of photoperiodic information contained in a sequence of light-dark cycles (photoperiodic ‘counter’). It has been shown that in many species the circadian system is involved in photoperiodic induction, but whether it plays a role in night-length measurement itself (i.e. as the clock) or is more indirectly involved in the induction process, is still largely a matter of dispute.

Many experimental protocols have been designed to try to solve this problem, and one of those is the so-called ‘bistability’ protocol. This protocol consists of so-called ‘symmetrical skeleton’ photoperiods (i.e. consisting of two short light pulses per 24 h (Pittendrigh, 1966)), with both light pulses close to 12 h apart, for example L1:D10:L1:D12. Work on circadian rhythms (e.g., the eclosion rhythm in *Drosophila pseudoobscura* (Pittendrigh, 1966)) demonstrated that symmetrical skeleton photoperiods simulated ‘complete’ photoperiods, such that the shorter of the two dark phases was always ‘interpreted’ as light. For example, L1:D8:L1:D14 and L1:D14:L1:D8 both always simulated L10:D14. However, when the light pulses were placed close to 12 h apart, both the shorter and the longer dark phase could be interpreted as light. This region with two possible ‘interpretations’ was called the ‘zone of bistability’ (Pittendrigh, 1966). Which interpretation was adopted, depended on (1) the circa-

dian time at which the first light pulse started and (2) the length of the first dark phase.

The bistability protocol was applied to the spider mite, *Tetranychus urticae*. Another experimental protocol had already shown, that the mite’s clock is based on a non-circadian, or ‘hourglass’, mechanism (Veerman & Vaz Nunes, 1987). It was expected, therefore, that bistability would not occur in photoperiodic induction in this species.

Materials and methods

Experiments were performed with the Dutch strain of *T. urticae* called ‘Sambucus’. The mites were reared on bean plants (*Phaseolus vulgaris*) under long-day (L17:D7) illumination and 26 °C. For the experiments mites were kept on detached leaf cultures (Veerman, 1977).

The experiments were done in light-proof wooden cabinets placed in constant temperature rooms of either 18.5 ± 0.5 °C or 22.5 ± 0.5 °C. Air of constant temperature was forced through the cabinets by means of small electric fans. Each cabinet was equipped with two day-light fluorescent tubes of 8 W, wired to externally mounted time clocks. Light intensities at the level of the mites ranged from 500–700 lux. Approximately 200 mites were used in each photoperiodic treatment.

Experimental mites were placed in either L1:D10:L1:D12 or L1:D12:L1:D10 after an initial dark phase of between 0 and 20 h, so that (1) the first light pulse started at different circadian times and

Figure 1. Incidences of diapause observed with *Tetranychus urticae* in symmetrical skeleton photoperiods in the expected 'zone of bistability' at 18.5 °C (circles, uninterrupted line) and 22.5 °C (triangles, dashed line), with the first light pulse starting after a variable initial dark phase. For each point approximately 200 mites were used.

(2) the first dark phase seen was either 10 or 12 h. To ensure that the initial dark phase began at the same circadian time in all of the experiments, newly deposited eggs, differing not more than 6 h in age, were maintained in continuous light and 25 °C during the 4 days of embryonic development, before transfer to the experimental conditions.

Results and discussion

The incidence of diapause in the photoperiods L12:D12 and L14:D10 was determined at both temperatures and the results are shown in Table 1. The results of the bistability experiments at 18.5 and 22.5 °C are presented in Figure 1. Both L1:D10:L1:D12 and L1:D12:L1:D10 were expected to simulate either L12:D12 or L14:D10, depending on the length of the initial dark phase experienced before the first 10- or 12-h dark interval, if the mite's clock were based on a circadian oscillator.

At 18.5 °C diapause incidences vary between 0 and 34% (Figure 1) instead of being either 50 or

Table 1. Incidence of diapause in *Tetranychus urticae* in control photoperiods at 18.5 °C and 22.5 °C

Photoperiod	Percent diapause	
	18.5 °C	22.5 °C
L12:D12	100	49
L14:D10	50	1

100% depending on the length of the initial dark phase and, although the incidences are slightly higher in L1:D10:L1:D12 than in L1:D12:L1:D10, no bistability is observed.

At 22.5 °C no diapausing mites have been observed, in either of the photoperiodic cycles (Figure 1), in spite of the observation that the complete photoperiodic cycle L12:D12 resulted in 49% diapause (Table 1). These results show that bistability does not occur in photoperiodic induction in *T. urticae*.

Bistability experiments have been applied to several insects and a plant. A clear bistability effect was demonstrated in the plant *Lemna perpusilla* (Hillman, 1964), in the flies *Sarcophaga argyrostoma* (Saunders, 1973) and *Calliphora vicina* (Vaz Nunes et al., 1990), and in the cabbage moth, *Mamestra brassicae* (Kimura & Masaki, 1993). Bistability was not found, however, in the pink bollworm *Pectinophora gossypiella* (Pittendrigh & Minis, 1971), the vetch aphid *Megoura viciae* (Hillman, 1973), the black bean aphid *Aphis fabae* (Vaz Nunes & Hardie, 1989), and in *Drosophila triauraria* (Yoshida & Kimura, 1993). These experiments have been used to determine whether the photoperiodic clock is based on a circadian oscillator. A positive bistability effect would be a strong indication that this is the case. A negative effect, however, as found for *T. urticae*, does not necessarily preclude the possibility for a circadian-based clock, as was shown by Vaz Nunes et al. (1991): in their pacemaker-slave model for the photoperiodic clock, a strong bistability effect was found only when the 'slave' was coupled strongly to the circadian pacemaker, but not when the coupling was weak (in which case the clock would be a rapidly damping oscillator).

Night-length measurement in *T. urticae* has already been shown to be based on a non-repetitive (i.e. non-circadian or 'hourglass') mechanism (Veerman & Vaz Nunes, 1987), which, in terms of the pacemaker-slave model (Vaz Nunes et al., 1991), would mean that coupling between the pacemaker and the slave is so weak, that the resulting clock is indistinguishable from an

'hourglass'. The negative results of the present bistability experiments agree with this conclusion and give, therefore, further support in favour of the non-circadian nature of the photoperiodic clock in *T. urticae*.

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