the number of male gametes which produced offspring. Since the spermatozoa produced 12 days after irradiation of 1-day-old adult males yielded less crossovers[9], the broods produced by irradiated adults were limited to 12 days.

A dose of 14 Gy was found to be lethal to larvae and the amount of radiation was lowered to 8 Gy which enabled some of the larvae to survive and become adults. The adult males which emerged from irradiated larvae were mated as soon as possible to *ru h th st cu sr* e^s *ca* homozygous females for three broods 0–3, 3–6, and 6–9 days. Crossovers were scored when at least two mutant or wild type alleles were involved in the induced recombination, for the presence or absence of a single mutant could represent a deletion or mutation[10]. If the phenotype of the induced recombinant flies involved two breaks, these were scored twice.

Results and discussion. As previously reported[9], the most frequently induced crossovers involved the breaks in the region surrounding the centromere between *st* and *cu* with appearance of *ru h th st* and *cu sr* e^s *ca* offspring. The hyperthermia treatment alone did not induce crossing-over in the adult males of the control *yw*, however, there was a significant increase in cross-overs induced in the repair deficient *y mei-9*^{a} males (table 1). The hyperthermia treatment also increased the radiation-induced cross-overs in the three broods of repair proficient *yw* but only in one 8–10-day brood of *y mei-9*^{a} (table 1). These data support the hypothesis that one of the methods that hyperthermia increases radiation induced damage is that heat may affect a repair process, hence more damage, but if the repair process is already defective the hyperthermia cannot increase the radiosensitivity. The gamma-ray irradiation alone induced more crossing-over in the *y mei-9*^{a} strain compared to *yw* and this may be due to the mutagen sensitivity[11] of *mei-9*^{a}.

The 1-h hyperthermia treatment alone had no effect upon induced crossing-over in the 6-day-old larvae (table 2) in both *yw* and *y mei-9*^{a} experiment. However, the heat treatment significantly increased the radiation induction of crossovers in all broods from the *yw* male larvae but none in the broods produced by the *y mei-9*^{a} males (table 2). Again more support for the hypothesis that one of the actions of hyperthermia in inducing radiosensitivity may be due to the interference of excision repair process, hence more radiation induced damage.

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Thermoperiodically induced diapause in a mite in constant darkness is vitamin A dependent

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Summary. By rearing the predacious mite *Amblyseius potentillae* in a daily temperature cycle in constant darkness it could be shown that diapause may be thermoperiodically induced. When the same experiments were performed using diets without vitamin A it appeared that vitamin A is necessary to achieve a state of reproductive diapause in this mite.
Key words. Thermoperiodism; photoperiodism; diapause; carotenoids; vitamin A; predacious mites; *Amblyseius potentillae*.

The perception of environmental cues such as photoperiod and the transduction of photoperiodic 'information' into a neuroendocrine signal in insects and mites is still poorly understood at a physiological level; the photoperiodic mechanism has so far been described only in terms of formal models, dealing mostly with the possible kinetics of the photoperiodic clock[1-3]. From genetic studies with spider mites[4,5] and from dietary studies with predacious mites[6-8], two moth species[9,10] and a parasitoid wasp[11] it appeared that carotenoids are somehow involved in the photoperiodic response. In the case of the predacious mite[8] and the wasp[11], vitamin A could be substituted for β-carotene, from which it was concluded that a rhodopsin might function as the photoreceptor for the photoperiodic clock. However, direct evidence for a photoreceptor function is still lacking. From action spectrum studies in an aphid[12] and a whitefly[13] it was concluded that possibly a carotenoprotein, but not rhodopsin, might function as the photoperiodic photoreceptor.

Apart from photoperiod in some insects daily fluctuating temperatures (thermoperiods) were found to be capable of inducing diapause in the complete absence of light[14-21]. Temperature may have various direct as well as indirect effects on the photoperiodic mechanism, but apparently it may also act as a 'zeitgeber' in its own right[1,2,22]. Although very little concrete information is available on thermoperiodic induction of diapause it has been argued that the same clock mechanism may be involved in both photoperiodism and thermoperiodism in insects[16,19,22]. Here we report for the first time a thermoperiodic response in a mite in constant darkness; moreover, this response proved to be dependent on the presence of vitamin A in the diet of these mites, just as shown previously for the photoperiodic response in the same species.

Materials and methods. The strain of the phytoseid mite *Amblyseius potentillae* used for the experiments originated from an apple orchard in the Netherlands[23]. *A. potentillae* exhibits a facultative reproductive diapause which is expressed only in the females and which is induced normally by short-day photoperiods experienced during the immature stages[6-8]. Mites were kept on rectangles of black plastic (8 × 15 cm), surrounded by a barrier of moist filter paper. Cultures were supplied with pollen of the broad bean, *Vicia faba*, as the only source of food, which constitutes a carotenoid-depleted diet for these mites[7,8]. β-Carotene and vitamin A acetate (retinol acetate) were added in crystalline form (5% w/w) and mixed with the pollen. The maternal generation of mites

Thermoperiodic induction of diapause in *Amblyseius potentillae* reared on diets with and without β-carotene or vitamin A

Diet supplement	Temperature regime 15 °C constant		27 °C constant		16 h (15 °C):8 h (27 °C)		16 h (27 °C):8 h (15 °C)	
	%	(n)	%	(n)	%	(n)	%	(n)
None	0	(70)	0	(67)	5	(226)	0	(109)
β-Carotene	16	(113)	0	(88)	100	(139)	0	(70)
Vitamin A acetate	20	(105)	0	(124)	99	(130)	14	(117)
Test generation none, maternal generation vitamin A acetate	1	(98)	1	(115)	99	(87)	4	(106)

Duration of warm and cool phases in hours (h); temperature in degrees centigrade; %, percentage diapause incidence; n, number of females per test.

was reared in a regime of 16h light:8h dark (LD 16:8) at 25 °C; in all experiments except those in the bottom row of the table mites of the parental generation were fed the same diets as those of the test generation. Experiments were started with eggs, ranging from 0- to 24-h-old, which had been laid in the LD 16:8 regime at 25 °C. All experiments were performed in constant darkness, which was maintained until day 4 after the final molt. No visual inspections were made before that time; the date of the last molt was estimated from controls. At day 4 of adult life the mites were transferred to long-day conditions (LD 16:8) at 22 °C; mites which had not started egg-laying after 10 days under this regime were considered to be in diapause. Controls reared throughout in this regime laid their first egg on day 2 after the final molt. The experiments were done in temperature controlled incubators (Heraeus, West-Germany), placed in a dark room. Temperatures were maintained constant within ± 0.5 °C. Temperature transitions in the thermoperiodic experiments took about 1 h.

Results and discussion. In the experiments presented in the table the response to thermoperiods was investigated in *A. potentillae,* using the above pollen diet with and without a supplement of β-carotene or vitamin A. A clear thermoperiodic reaction (99–100% diapause) in constant darkness was found in mites, reared on either the β-carotene or vitamin A supplemented diet, in response to a 'short-day' thermoperiod, i.e. a thermoperiod with a short (8 h) warm phase and a long (16h) cool phase; no diapause or only a very low incidence of diapause (0–14%) was found in mites reared in a 'long-day' thermoperiod, consisting of a 16-h thermophase and an 8-h cryophase. Diapause was not observed when mites were kept in continuous darkness at a constant temperature of 27 °C, the upper temperature of the thermoperiod employed, whereas only a low incidence of diapause (16–20%) was found in mites which experienced the lower temperature of 15 °C throughout development. A thermoperiodic response to the 'short-day' thermoperiod mentioned above was found to be absent in mites reared in continuous light instead of continuous darkness (not shown in the table). These results demonstrate, for the first time in a mite, the presence of a thermoperiodic response similar to that found in some species of insects: substitution of a 'short-day' photoperiod by a 'short-day' thermoperiod in constant darkness results in the same high incidence of diapause. Comparison with the controls shows that it is the periodic aspect of the temperature signal which is of importance, since no diapause or only a low incidence of diapause was obtained when the upper and lower temperatures of the thermoperiod were employed as a constant temperature throughout. Comparison between the constant temperature controls on the one hand and the results of the 'long-day' and 'short-day' thermoperiods on the other shows that it is not just the fluctuation of temperature causing diapause induction, but the duration of the warm and cool phases that appears to be of prime importance. As in the photoperiodic response, a clock measuring the duration of either the cryo- or thermophase (or both) is apparently also involved in the thermoperiodic response of these mites.

When, however, the same experiments were performed with mites reared on broad bean pollen without any addition of β-carotene or vitamin A, the thermoperiodic response appeared to be virtually absent, a diapause incidence of only 5% being found under the 'short-day' thermoperiodic regime (mean of three repetitions of the experiment, yielding 0, 5 and 11% diapause, respectively, with between 70 and 80 females per test). The slightly inducing effect of constant low temperatures had even completely disappeared on the carotenoid-depleted diet. These results show that vitamin A is somehow involved in the perception or transduction of temperature cues in these mites, as it has previously been shown to be involved in the mediation of photoperiodic information in the same species of mites.

In the last experiment, presented in the bottom row of the table, the same temperature regimes were tested with mites whose parents had received ample supplies of vitamin A acetate in their diet, but which were themselves reared on broad bean pollen alone. The results show the same high thermoperiodic response (99% diapause) as in mites which received vitamin A both from their mother via the egg and as a supplement to their own diet. The response to constant low temperature, however, was as low as that of mites which had not received any pigment supplement for two generations (table), which again shows that, although constant low temperature may induce some diapause, it is nevertheless a much weaker signal than fluctuating temperatures. The fact that a full thermoperiodic response was present in mites whose only source of vitamin A was the amount deposited by the mother in the eggs shows that only minimal amounts of vitamin A are required to achieve a state of reproductive diapause. A comparable maternal effect has been demonstrated previously for the photoperiodic response in these mites[6] and in two species of moths[9,10]. A maternal effect on the photoperiodic reaction was found to be absent in a parasitoid wasp; this might be explained, however, by the virtual absence of yolk in the wasp's eggs[11].

In view of the present state of our knowledge of the physiological mechanism of diapause induction in insects and mites any statement on the possible function of vitamin A can only be speculative. The fact that we have now found that vitamin A is not only functionally involved in the photoperiodic reaction but is also essential for a thermoperiodic response raises doubt about earlier suppositions that vitamin A, or possibly a rhodopsin, might function in the photoperiodic photoreceptor. Unless the vitamin functions in both the photoreceptor and thermoreceptor (or in a photoreceptor which functions equally well as a thermoreceptor), it seems more likely that vitamin A, or a derivative of vitamin A, is active in a more central part of the mechanism. The results might be explained just as well by assuming that traces of vitamin A are required to establish or maintain a state of reproductive diapause in these mites. Although vitamin A is clearly not necessary for normal development and reproduction, it might be involved in the prevention of ovarial development. Experiments will be devised to discriminate between the possibilities that vitamin A either works on the induction level or is necessary to establish or maintain the state of diapause.

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Insect photoperiodism: the linden bug, *Pyrrhocoris apterus*, a species that measures daylength rather than nightlength

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Summary. Independent variation of the dark and light components of the daily photocycle has shown that the linden bug, *Pyrrhocoris apterus*, unlike other species, 'measures' daylength rather than nightlength. Greatly extended dark periods coupled with a short photophase (a Nanda-Hamner protocol) shows peaks and troughs of diapause at about 16-h intervals, an extremely short period for a circadian clock. If circadian oscillations are involved in photoperiodic time measurement in this species, a photoinducible phase might lie in the early rather than the late subjective night.
Key words. Diapause; photoperiodism; daylength measurement; circadian system.

Photoperiodic regulation of diapause or seasonal morphs is very widespread in the insects, particularly among those in the temperate zones[1]. In those species that have been adequately investigated, it is concluded that the duration of the scotophase or dark (D) component of the daily photocycle is more important than the duration of the photophase or light (L)[2–5]. In the European corn borer, *Ostrinia nubilalis,* for example, Beck[6] showed that larval diapause induction was maximal with D between 10 and 14 h, but when D was held constant at these values, the duration of L could be varied from 5 to 18 h. In the green vetch aphid, *Megoura viciae,* the range of L giving a high incidence of the autumnal egg-laying morph (the ovipara), when coupled to 12 h of D, was 12 to 36 h, whereas, with a constant 8 h of light, the duration of darkness reached a sharp critical nightlength at 9.5 h[7]. These observations suggest very strongly that the principal element of the photoperiodic clock begins at the light-off signal and the clock measures nightlength rather than daylength.
These conclusions are further supported by the results of 'resonance' or Nanda-Hamner experiments in which L is kept short but the length of the dark component is greatly extended to give LD cycles up to 3 or 4 days in duration. Experiments of this type may reveal two apparently different forms of nightlength measurement: hourglass or oscillatory. In *M. viciae,* for example, a dark period hourglass is indicated because the incidence of ovipara production rises sharply after 9.5 h of darkness and then remains consistently high[7]. In other species, such as the flesh-fly *Sarcophaga argyrostoma*[8] and the red spider mite, *Tetranychus urticae*[9] diapause incidence rises and falls with a circadian periodicity as D is extended, and the circadian oscillator(s) involved are clearly phase-set from the beginning of darkness.
The experiments reported here were carried out with the linden bug, *Pyrrhocoris apterus,* which overwinters in the adult instar with an ovarian diapause if the last nymphal instar and the young adults are exposed to short days[10,11]. In the central Bohemian strain (Prague, 50° N) used in this investigation, the 'critical' daylength for diapause induction occurs in a LD cycle of about 15.75 h of light and 8.25 h of darkness[11]. Groups of about 40–50 5th instar nymphs were maintained in glass Kilner jars with dry lime seed *(Tilia cordata)* and water, and exposed to a variety of experimental light regimes at 25°C. Three weeks later, when the bugs had developed to the adult instar, the females were dissected in saline to ascertain the status of their ovaries, according to the criteria described by Hodek[12]. By this time, non-diapausing bugs had either laid eggs (Hodek's stage 6), contained chorionated eggs (stage 5), or showed various stages of vitellogenesis (stages 3 or 4). Diapausing bugs, on the other hand, possessed ovaries with undifferentiated oocytes (stage 1) or small oocytes lacking yolk (stage 2). The proportion of diapause in the group was expressed as a percentage of bugs in stages 1 and 2.
Bugs were exposed to light cycles with the photophase (L) held constant (L = 12, 15, 16 or 17 h) and the scotophase (D) varied, or with D held constant (D = 7, 8, 9 or 12 h) and L varied (fig. 1). In each experiment the incidence of diapause was compared with the critical day or nightlength obtained from 24-h cycles[11].
The results show that when D was held constant (right hand panels) diapause was consistently high when L was less than about 15 h, but consistently low when L was more than 16 h. This compares well with the known critical photophase of 15.75 h/24. On the other hand, when L was held constant at