Photoperiodic induction of diapause in an insect is vitamin A dependent

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Summary. A photoperiodic response was found to be absent in larvae of the parasitoid wasp *Apanteles glomeratus* when its host (caterpillars of *Pieris brassicae*) was reared on a low-carotenoid artificial diet. Addition of vitamin A to the host's diet restored the response to short-day photoperiods in the wasp larvae, thus showing that vitamin A is essential for photoperiodic induction of diapause. Possibly vitamin A or a derivative of vitamin A functions as the photoreceptor pigment for the photoperiodic reaction in this species of insect.
Key words. Photoperiodism; diapause; photoreception; carotenoids; vitamin A; *Apanteles glomeratus; Pieris brassicae.*

In several studies, aimed at the localization of the photoreceptors involved in photoperiodic time measurement in insects, it was found that the photoreceptors are brain-centered; it appeared that photoperiodic photoreception takes place directly in the brain itself[1-5]. In two instances, however, the compound eyes were shown to be involved in the reception of photoperiod regulating seasonal development[6,7]. Apparently, both retinal and extraretinal photoreceptors may be utilized, depending on the species[8]. Elements with the morphological appearance of photoreceptors have been found in the brain and optic lobes, respectively, of two insect species; although the function of these structures is unknown, it has been suggested that they might be engaged in the reception of photoperiodic signals controlling either photoperiodic responses or circadian rhythms[9,10].

The photoreceptor pigments involved in the reception of photoperiod in insects have not yet been identified. The first indications that carotenoids or carotenoid derivatives might function in photoperiod reception came from genetic experiments with a spider mite[11,12] and from dietary studies with a moth[13]. These results were corroborated in dietary studies with a predacious mite[14] and another moth species[15]. Using a carotenoid-deficient diet supplemented with various pure carotenoids or carotenoid derivatives, it was shown that vitamin A or carotenoids with provitamin A function are essential for photoperiodic induction in the predacious mite[16]. These results suggest that vitamin A, or possibly a rhodopsin, functions as the photoperiodic receptor in these mites; however, evidence for a direct link between vitamin A and the photoreceptor is still wanting.

The physiological basis of photoperiodism in insects and mites is still largely unknown, and several theoretical mechanisms have been proposed to explain photoperiodic induction[17-19]. Evidently, it is of great interest if common elements are found in the induction mechanism of different arthropods. We therefore investigated whether or not vitamin A might also be involved in the photoperiodic reaction of an insect. As a test animal we chose the parasitoid wasp *Apanteles glomeratus,* which shows a clear photoperiodic response and whose host, the larvae of *Pieris brassicae,* can be reared on an artificial diet lacking all but traces of carotenoids[20-22].

Materials and methods. A. glomeratus is a gregarious endoparasitoid of the larvae of the large white butterfly, *P. brassicae.* It overwinters in the prepupal stage in a sulphur-yellow cocoon, spun outside its host. Winter diapause is induced by short-day photoperiods at moderate temperatures, and is independent of the induction of diapause in the host[23,24]. The critical daylength for the Dutch strain used for the experiments is 15 h at 19 °C; diapause is completely absent in wasp larvae reared in continuous darkness.

Host larvae were parasitized in the second instar and reared either on cabbage *(Brassica oleracea)* or on an artificial diet without cabbage, containing sinigrin as a phagostimulant[20]. Although the diet is not completely carotenoid-free, carotenoids are drastically reduced[25]; the carotenoid content proved to be sufficiently low for the purpose of the present experiments. Parasitized host larvae were reared in a climatic room at 19 °C in short-day (light/dark, 10:14 h) (LD 10:14) and long-day (LD 18:6) conditions respectively. Apart from the controls reared on cabbage, parasitized host larvae were reared on the artificial diet, either with or without a supplementation of vitamin A palmitate (water-dispersable, ICN Nutritional Biochemicals, Cleveland, Ohio, 250,000 IU/g), in a concentration of 0.1 mg/g diet. Newly-spun cocoons were collected and kept for three weeks under the same experimental regime as the larvae had been exposed to during their development. After this time all non-diapausing wasps had emerged as adults. Diapause incidence was then determined from the number of living larvae within their cocoons, expressed as a percentage of the total number of cocoons. Mortality of the stages inside the cocoons was negligible.

Results and discussion. When larvae of *P. brassicae,* parasitized by *A. glomeratus,* were reared on cabbage, 100% diapause incidence was found in the parasitoid larvae under short-day conditions, whereas diapause induction was virtually absent under long-day conditions (table). However, rearing the parasitized caterpillars on a low-carotenoid artificial diet resulted in a much reduced diapause incidence under short-day conditions; the response under long-day conditions was comparable to that of the controls reared on cabbage. Although the diapause frequency in the short-day experiments with the low-carotenoid diet was rather variable, ranging from 7.6 to 35.3% in the four replicate experiments, the diet significantly reduced diapause induction. Supplementing the diet with vitamin A restored the response to short-day photoperiods completely and resulted in 100% diapause; the response to long-day photoperiods did not differ from that in the other experiments (table). Apparently, vitamin A is able to relieve the deficiency of the artificial diet with regard to photoperiodic induction. These results show that vitamin A is essential for photoperiodic induction of diapause in *A. glomeratus.* Vitamin A deficiency does not seem to have any influence on the normal development of the wasps under long-day conditions.

Just as in the predacious mite mentioned earlier[14,16], it seems that wasps deficient in vitamin A have become 'blind' to the photoperiod and behave as if reared in continuous darkness. It is not unlikely, therefore, that vitamin A (or possibly a rhodopsin) is involved in photoperiodic photoreception. However, proof of this hypothesis will need further experimentation and is not to be expected from dietary studies alone. The fact that vitamin A has

Diapause incidence (%) in *Apanteles glomeratus* larvae reared in hosts (caterpillars of *Pieris brassicae)* which were fed either cabbage or an artificial diet with and without vitamin A

Diet of host	LD 10:14		LD 18:6	
	%	(n)	%	(n)
Cabbage	100	(1299)	0.2	(1845)
Diet without vitamin A	18.4	(1117)	2.4	(1063)
Diet with vitamin A	100	(866)	0.3	(888)

LD: h of light and darkness respectively in a light/dark cycle. Percentages of diapause are the means of four replicates. n: total number of animals tested.

now been shown to be functionally involved in the photoperiodic reaction of both an insect and a mite might indicate that the photoperiodic mechanism in different classes of arthropods may comprise certain common functional elements.

It is remarkable that the effect of carotenoid deficiency on the photoperiodic response is fully expressed already in the first generation reared on the diet; in all cases investigated so far the effect was delayed for one or more generations, because of the transmission of small but sufficient amounts of carotenoids from the mother via the eggs[11–15]. However, the eggs of *Apanteles*, like those of other parasitoid wasps, contain very little yolk[26], which may explain the absence of a maternal effect in our work with carotenoid-free diets. The rather variable incidence of diapause in wasps reared under short-day conditions on the diet without vitamin A (table) may be explained by slight variations in the carotenoids present in the natural products (wheat germ, casein, agar) used in the diet. In some studies, carotenoid deprivation did not show an effect on the photoperiodic response or on the entrainment of circadian rhythms; these negative results, however, may well have been due to the presence of maternally derived carotenoids. Rearing the insects for one or more further generations on the carotenoid-free diet might have shown a different effect, but this was not always possible[21,27,28].

When its host is reared on cabbage, the *Apanteles* larvae spin yellow cocoons. However, *A. glomeratus* larvae emerging from caterpillars reared on the artificial diet, both with and without vitamin A, spin white cocoons. Preliminary analyses of the yellow cocoons indicate that lutein is the main carotenoid pigment present. All carotenoids present in *A. glomeratus* are necessarily sequestered from its host; a rather close conformity has been found between the carotenoids present in *A. glomeratus* and in *P. brassicae*[29].

The larvae of *Apanteles* have no external ocelli. It is likely, therefore, that photoperiodic photoreception occurs extraretinally, possibly directly in the brain. The photoreceptors must be very sensitive, considering the fact that light has to pass through the rather heavily pigmented cuticle of the host before it reaches the parasitoid larvae inside. Nevertheless, photoperiodic time measurement is executed by the wasp larvae with remarkable accuracy, quite independent of the host's own response to the photoperiod[23,24]. One of the main goals of further research will be the localization of the photoperiodic photoreceptor in *Apanteles*.

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Prokaryote-eukaryote interactions in trace element metabolism. *Desulfovibrio* sp. in *Helix aspersa*

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Summary. Snails *(Helix aspersa)* contain sulphate-reducing bacteria in their crops. Feeding such animals on food containing sulphate and molybdate ions does not induce a copper deficiency and in fact the bacteria appear to facilitate metal absorption. This is in contrast to the effects of these bacteria in ruminant cattle.
Key words. Copper metabolism; sulphate-reducing bacteria; molybdate; snails.

There are at least 16 metals that are essential for all prokaryotic and eukaryotic forms of life and about 12 of these are only required in trace amounts. Deficiencies in these trace elements are, however, among the most common of naturally occurring diseases probably because most minerals cannot be absorbed in their natural state by cells. Trace elements are to a large extent absorbed in a complexed form and what attaches to the metal ion frequently determines its degree of absorption by the organism[1].

Microorganisms have evolved a wide range of systems for accumulating the trace elements that they require and low mol.wt molecules in the form of water soluble chelates (siderochromes) are secreted by many microorganisms in order to assimilate metals from the environment[2]. The importance of these microbial systems in facilitating metal ion uptake by other organisms is well known and is frequently a crucial influence in the rhizosphere region around plant roots. In fact the microbe-plant interaction is so important in its effect on the growth, devel-