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ORIGINAL PAPER

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Endogenous timing mechanism controlling the circannual pupation rhythm of the varied carpet beetle *Anthrenus verbasci*

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Abstract This paper describes the detailed characteristics of the circannual pupation rhythm in *Anthrenus verbasci* determined by laboratory experiments under various photoperiods and temperatures. The frequency distribution of larval duration showed a periodic pattern over 2–3 years and the period was 37–40 weeks under a constant short-day photoperiod (light:dark 12:12) at 20°C. This rhythm showed temperature compensation to some extent under a short-day photoperiod between 17.5°C and 27.5°C. Under alternations of a long-day (light:dark 16:8) and a short-day photoperiod, pupation occurred 21–24.5 weeks after transfer from a long-day to a short-day photoperiod. Therefore, we concluded that the timing of pupation in *A. verbasci* is controlled by a circannual rhythm and its zeitgeber is a change in photoperiod. Furthermore, when larvae were transferred from a long-day to a short-day photoperiod at various ages, the larval duration after the photoperiodic transfer depended on the time of the transfer. This difference can be explained by phase-dependent phase shifts in the circannual rhythm.

Keywords Circannual rhythm · *Anthrenus verbasci* · Endogenous rhythm · Temperature · Photoperiod

Abbreviations *DD* constant darkness · *LD* light/dark · *LL* constant light

Introduction

Pengelley and Fisher (1957) first showed circannual onset and cessation of hibernation under constant conditions in the golden-mantled ground squirrel, *Spermophilus lateralis*. Since then circannual rhythms have been shown to control the seasonal development of some birds and mammals (see Gwinner 1986 for review). In insects, Blake (1958, 1959) reported evidence of a circannual rhythm in a British population of the varied carpet beetle *Anthrenus verbasci* L. (Insecta, Coleoptera, Dermestidae). Larvae of this species grow in the summer and enter diapause in the winter, taking one or more years to pupate in the early spring. When larvae were reared under conditions of constant temperature and humidity under constant darkness (DD), rhythmic pupation was observed (Blake 1958, 1959). This periodicity can be explained by the concept of 'gates', which was originally introduced for the allowed zone for eclosion in the circadian clock (Pittendrigh 1966). However, these results have not been verified for more than 40 years, even though this periodicity has been regarded as a typical example of circannual rhythm in insects (e.g., Saunders 1982; Gwinner 1986).

On the other hand, Kuwana (1951) observed no rhythmic pupation under DD at a constant temperature in a Japanese population of *A. verbasci*. This raises the question of whether Japanese populations lack circannual rhythms in the control of pupation. In the present study, we first reared larvae originating from a Japanese population of *A. verbasci* under constant light-dark cycles, because this method has been used routinely to detect circannual rhythms in vertebrates (see Gwinner 1986). These insects showed periodical pupation under constant light-dark cycles.

Although temperature compensation is one of the prerequisites of biological rhythms, it has been examined in circannual rhythms of vertebrates only in *S. lateralis* (Pengelley and Asmundson 1969; Mrosovsky 1980). Although Blake (1958, 1959) demonstrated temperature compensation in the circannual rhythms of *A. verbasci*, we examined the temperature dependence of the rhythmicity in this species, because poikilotherms are better subjects in which to examine this characteristic.

Blake (1960, 1963) stated that a decrease in day length inhibits the metamorphosis of the second pupation

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group and an increase in day length shortens the larval duration of the first group in *A. verbasci*, based on the results under natural changing day length, DD, and their combinations. In the present study, we transferred larvae from a constant long-day to a short-day photoperiod or vice versa at various ages, to determine whether the change in photoperiod is the actual zeitgeber for the periodicity.

Materials and methods

Adults of *A. verbasci* were collected from flowers of the marguerite *Chrysanthemum frutescens* in Osaka City (34.7° N, 135.5° E), Japan, between May and June 1996–1998. Thirty to fifty adults were kept on diluted honey as food and a piece of wool as a substrate for oviposition in 200-ml plastic cups under a long-day photoperiod of 16 h light and 8 h darkness (LD 16:8) at 25 ± 1°C. The pieces of cloth were replaced every other day. The pieces of cloth with eggs were transferred to plastic boxes (61 mm×43 mm×17 mm) and kept under the same conditions. Hatching of larvae was examined every other day.

Within a week after hatching, plastic boxes accommodating larvae were transferred to various photoperiods and temperatures for the experiments. The photoperiod was produced by white fluorescent lamps (NEC Lighting, Tokyo) and timers (Omron, Kyoto), and the light intensity during the photophase was about 0.9 W m^{-2}. To produce constant dim light of about 0.006 W m^{-2}, the fluorescent lamp was covered with sheets of black polyethylene. The temperature was kept constant within a range of ±1.0°C. Dried yeast (Asahi Beer Pharmaceutical, Tokyo) and powder of dried bonito were provided as larval food. The boxes were put in airtight containers (155 mm in diameter; 85 mm in depth) with a saturated solution of $NaNO_2$ to keep the relative humidity at about 66%. Thereafter dried bonito powder was provided ad libitum.

The pupation of larvae was recorded each week, although pupae were removed every day or every other day to prevent cannibalism. The pupae were kept under LD 16:8 at 20°C. Newly emerging adults were kept under the same conditions as those under which field-collected adults were kept. Their eggs were also collected for the experiments. Remaining larvae were counted after about 1 year and 2 years, and at the end of the experiments.

Results

Constant temperature and photoperiod

We transferred larvae to LD 12:12 at 20°C within a week after hatching. The frequency distribution of larval duration showed a periodic pattern. Larvae pupated 23–31 weeks after hatching as the first pupation group, and the median larval duration was 26 weeks. Larvae of the second group pupated 49–74 weeks after hatching, and the median larval duration was 63 weeks. A third pupation group was also observed 89–109 weeks after hatching, and the median larval duration was 100.5 weeks. Thus, the intervals between the medians were 37 weeks and 37.5 weeks (Fig. 1A). Even under constant dim light, two pupation groups were distinguished, although the periodic pattern was less clear than that under LD 12:12. To calculate the median for each group, we accepted a border between the two pupation groups 64–65 weeks after hatching, during which no larvae pupated. The median larval durations for the first and second groups were 37 and 80 weeks, respectively; the interval between them being 43 weeks (Fig. 1B).

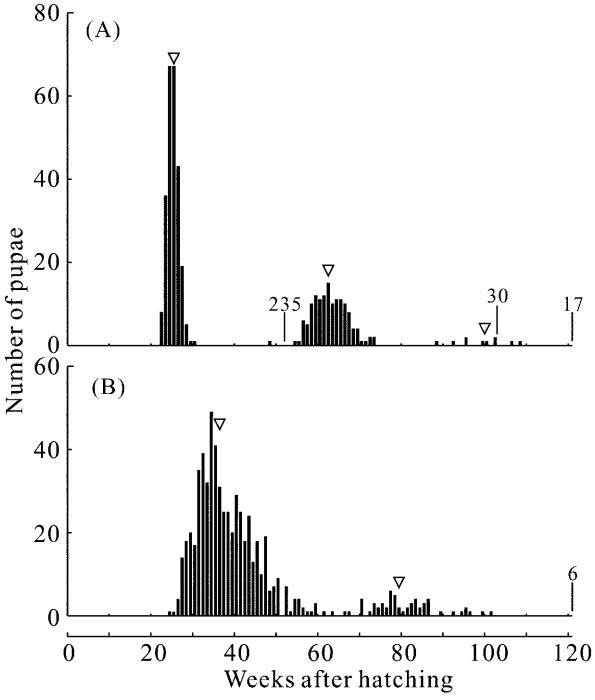

Fig. 1 Frequency distribution of larval duration under light/dark (LD) 12:12 (**A**) and constant dim light (**B**) at 20°C in *Anthrenus verbasci*. Eggs laid in September 1997 by adults emerging in the laboratory were kept under LD 16:8 at 25°C and transferred to the experimental conditions within a week after hatching. *Numerals with vertical lines* show the numbers of insects remaining as larvae. Each *triangle* indicates the median for each pupation group

We examined the possibility that the existence of two or three genetic strains within the same species with different developmental periods might be responsible for the apparent periodic pattern. Several adults of the second pupation group were allowed to breed and their progeny were reared under LD 12:12 at 20°C. The frequency distribution of larval duration separated into two pupation groups in a similar pattern to Fig. 1A (data not shown). Therefore, the above hypothesis is unlikely.

To examine temperature dependency of the timing of pupation, we reared larvae under LD 12:12 at various constant temperatures. At all temperatures except 15°C, pupation began 21–27 weeks after hatching and continued for about 10 weeks (Fig. 2). Larvae of the second group pupated about 40 weeks after the first. High temperatures did not produce earlier pupation as might be expected in normal developmental processes. More precisely, the first pupation peaks were delayed slightly at higher temperature. Consequently, we concluded that the timing of pupation showed temperature compensation to some extent. Furthermore, pupation of the first group was less synchronous at 25°C and 27.5°C than at 20°C and 22.5°C. At both 25°C and 27.5°C, only one larva pupated after pupation of the first group, even

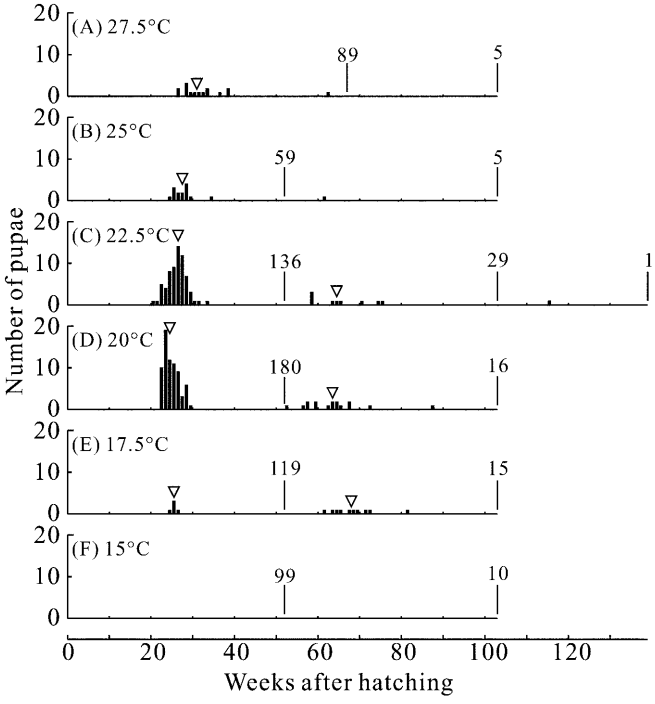

Fig. 2 Frequency distribution of larval duration under LD 12:12 at various constant temperatures in *A. verbasci*. Eggs laid in January 1997 by adults emerging in the laboratory (**A**) and laid in June 1996 by field-collected adults (**B–F**) were kept under LD 16:8 at 25°C and transferred to the experimental conditions within a week after hatching. *Numerals with vertical lines* show the numbers of insects remaining as larvae. Each *triangle* indicates the median for each pupation group

though many larvae survived. Therefore, high temperature inhibited pupation.

Next, we reared larvae under LD 16:8 at 20°C or 25°C. The medians of larval duration in the first pupation group at 20°C and 25°C were 48 weeks and 46.5 weeks, respectively, which were longer by about 20 weeks than those under LD 12:12 (Fig. 3, cf. Fig. 2B, D). The second group pupated about 80 weeks after hatching at 20°C. The period between the medians of the two groups was 32 weeks. At 25°C, only one larva pupated 80 weeks after hatching even though many larvae survived after pupation of the first group. Furthermore, the pupation was less synchronous under LD 16:8 than under LD 12:12. Therefore, long-day conditions also inhibited pupation. Despite this inhibition of pupation, we observed a periodic pattern and temperature compensation in the timing of pupation under long-day conditions.

Alternating photoperiods

To determine the zeitgeber for this periodicity, we reared larvae under alternation of a long-day (LD 16:8) and a short-day (LD 12:12) photoperiods, i.e., square-type photoperiodic cycles, at 20°C. Periods of photoperiodic cycles used were 36 weeks, 52 weeks, and 72 weeks,

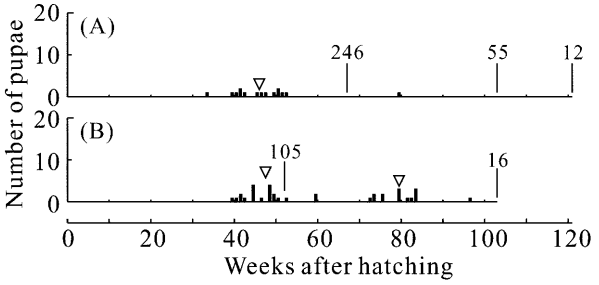

Fig. 3 Frequency distribution of larval duration under LD 16:8 at 25°C (**A**) and 20°C (**B**) in *A. verbasci*. Eggs laid in January 1997 by adults emerging in the laboratory (**A**) and laid in June 1996 by field-collected adults (**B**) were kept under LD 16:8 at 25°C and transferred to the experimental conditions within a week after hatching. *Numerals with vertical lines* show the numbers of insects remaining as larvae. Each *triangle* indicates the median for each pupation group

which were shorter than a year, close to a year, and longer than a year, respectively.

When larvae were exposed to LD 16:8 first, the pupation of the first group was delayed compared to that under constant LD 12:12 (Figs. 2D, 4A–C). The medians in the larval duration of the first pupation group were 33 weeks, 36 weeks, and 41 weeks, when the periods of photoperiodic cycles were 36 weeks, 52 weeks, and 72 weeks, respectively. The phase at which pupation occurred differed among these three conditions. However, the median period from the first transfer from LD16:8 to LD 12:12 to pupation of the first group was 23 weeks or 24 weeks under all conditions (Fig. 4A–C). Furthermore, the median period from the second transfer from LD 16:8 to LD12:12 to pupation of the second group was 24.5 weeks under a photoperiodic cycle of 36 weeks in which a clear second pupation group was observed (Fig. 4A). Under the 52-week photoperiodic cycle the second pupation group was not clear, and under the of 72-week cycle no larvae pupated in the second group (Fig. 4B, C).

When larvae were exposed to LD 12:12 first, the median pupation time in the second group was 23 weeks after the first change from LD 16:8 to LD 12:12 (Fig. 4D, E). Under the 36-week photoperiodic cycle, the third group of pupation was also observed, and the median period from the second transfer from LD 16:8 to LD 12:12 to pupation was 24 weeks (Fig. 4D). Therefore, pupation occurred 23–24 weeks after the change from a long-day to a short-day photoperiod. Only four larvae pupated in the first group under the 36-week photoperiodic cycle, in which they were transferred to LD 16:8 9 weeks after hatching. Thus, exposure to a long-day photoperiod in the early larval period suppressed or delayed pupation (Fig. 4D). This was in accordance with the results in larvae kept under LD 16:8 continuously, in which the shortest larval period was 40 weeks (Fig. 3B). When the periods of photoperiodic cycles were 52 weeks and 72 weeks, the medians of the larval period in the first pupation group were 21 weeks and 24 weeks, respectively. In both of these two exper-

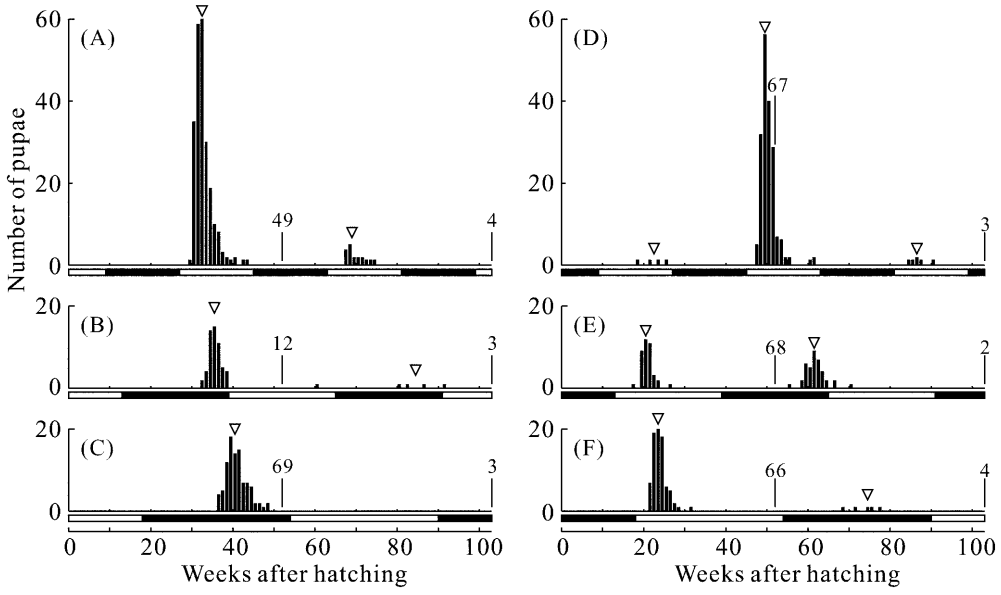

Fig. 4A–F Frequency distribution of larval duration at 20°C under alternating photoperiods of LD 16:8 (*empty bars*) and LD 12:12 (*filled bars*) with various constant periods in *A. verbasci*. The period of photoperiodic alternation was 36 weeks (**A**, **D**), 52 weeks (**B**, **E**), and 72 weeks (**C**, **F**). Eggs in June 1996 laid by field-collected adults were kept under LD 16:8 at 25°C and transferred to the experimental conditions within a week after hatching. *Numerals with vertical lines* show the numbers of insects remaining as larvae. Each *triangle* indicates the median for each pupation group

imental series, the larval period in the first pupation was significantly shorter than that under constant LD 12:12 (Mann-Whitney *U*-test, $P < 0.05$; Figs. 2D, 4E, F). Therefore, the change from a short-day to a long-day photoperiod advanced the timing of pupation.

Change from a long-day to a short-day photoperiod

The results under alternating photoperiods suggested that a change from a long-day to a short-day photoperiod sets the pupation rhythm to a certain phase. To examine this hypothesis, photoperiodic transfer from LD 16:8 to LD 12:12 was performed, 9 weeks, 24 weeks, 30 weeks, or 36 weeks after hatching. When we changed photoperiod 9 weeks after hatching, pupation in the first and second groups was delayed as compared to that under constant LD 12:12 (Fig. 5A, B). The medians of the larval duration in the first and second pupation groups were 34 and 76 weeks, respectively; the period between them was 42 weeks (Fig. 5B). Thus, the rhythm of pupation persisted even when the pupation time was shifted by exposure to a long-day photoperiod. As the photoperiodic transfer was delayed, pupation of the first group was delayed, although the second group was unclear because one or a few larvae pupated after pupation of the first group (Fig. 5C–E).

Therefore, we concluded that larvae responded to a change from a long-day to a short-day photoperiod at any age examined. However, the period from photoperiodic transfer to pupation of the first group was not constant as expected from the results under alternating photoperiods. Figure 6 shows the median interval from the change from LD 16:8 to LD 12:12 to pupation in the first group as a function of the time under LD 16:8 before transfer, based on the results shown in Figs. 4

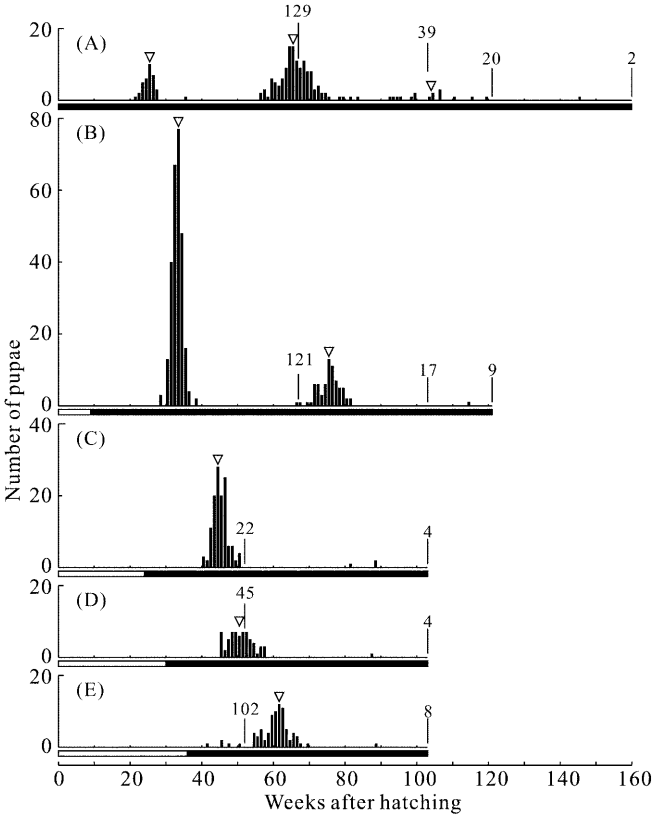

Fig. 5 Frequency distribution of larval duration when insects were transferred from LD 16:8 (*empty bars*) to LD 12:12 (*filled bars*) 0 weeks (**A**), 9 weeks (**B**), 24 weeks (**C**), 30 weeks (**D**), or 36 weeks (**E**) after hatching at 20°C in *A. verbasci*. Eggs laid in January 1997 by adults emerging in the laboratory (**A**, **B**) and laid in June 1998 by field-collected adults (**C–E**) were kept under LD 16:8 at 25°C and transferred to the experimental conditions within a week after hatching. *Numerals with vertical lines* show the numbers of insects remaining as larvae. Each *triangle* indicates the median for each pupation group

and 5. The period to pupation gradually decreased from 26.5 weeks to 21 weeks as the photoperiodic transfer was delayed until 30 weeks after hatching. When photoperiodic transfer was performed 36 weeks after hatching, however, the period to pupation increased to 26 weeks, which was similar to the value under constant LD 12:12. From these results, we concluded that the physiological state in the pupation rhythm periodically changed even under LD 16:8, and after 36 weeks returned to the initial state.

Environmental factors except for temperature and photoperiod

Although we first regarded the periodicity under constant photoperiod and temperature as evidence of an endogenous rhythm, it is still possible that certain exogenous factors other than temperature and photoperiod are responsible for the periodicity. If a certain geophysical factor related to the astronomical year determines the periodicity, the results must depend on the time at which the experiment started. Table 1 summarizes the results of the experiments under LD 12:12 at 20°C beginning at various times of the year. Although the third pupation group was observed in only two of five experimental series, the duration of larval period in each group was similar. The coincidence of the periods in the first and second cycles indicated a constant periodicity of the ongoing rhythm. Therefore, we concluded that *A. verbasci* possesses an endogenous and persistent rhythm controlling pupation.

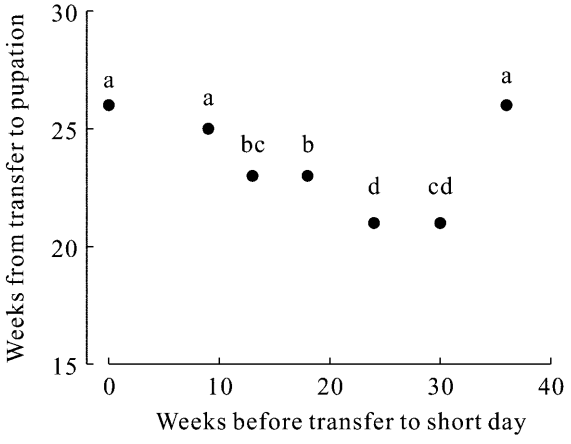

Fig. 6 Effects of the period before transfer from LD 16:8 to LD12:12 on the larval duration in *A. verbasci* at 20°C. *Closed circles* show the median (weeks) from photoperiodic transfer to pupation in the first group. Values with the same letter are not significantly different ($P > 0.05$, by nonparametric multiple comparison (see Zar 1999, pp 223–226))

Environmental conditions within a week after hatching

In all the above experiments, parent adults were reared under LD 16:8 at 25°C, and their progeny larvae were transferred to various experimental regimes within a week after hatching. Therefore, all insects experienced LD 16:8 at 25°C at the beginning of their life, and the change in photoperiod and/or temperature possibly affected the rhythm of pupation. To exclude this possibility, some field-collected adults were maintained under LD 12:12 at 20°C in June. Their progeny were reared from eggs under the same conditions. Simultaneously, we started a control series in which progeny of adults kept under LD 16:8 at 25°C were transferred to LD 12:12 at 20°C within a week after hatching. The frequency distribution of larval duration was similar between these two series (Fig. 7A, B). Therefore, exposure to long-day conditions and high temperature at an early stage had no effect on the pupation rhythm.

Discussion

Circannual rhythms governing seasonal cycles of migration, molting and breeding in vertebrates have been postulated for several decades (see Gwinner 1981, 1986 for review). Early evidence of circannual rhythms among invertebrates came from results in *A. verbasci*. The gate to pupation opens periodically under constant temperature and humidity, and under DD (Blake 1958, 1959). The period of the rhythm was between 10 months and 11 months and considerably shorter than a year, as reported in most species shown to have circannual rhythms (Gwinner 1986). The present results confirmed the circannual rhythm of pupation in a Japanese population of *A. verbasci*, although the following new findings should be noted. We used 24-h LD cycle and constant dim light that contained no information about

Table 1 Summary of the experiments beginning at various times of the year in the larval duration of *Anthrenus verbasci* under LD 12:12 at 20°C

Beginning of experiments	Figure no[a]	Larval duration (median, weeks)			Period (weeks)	
		1st group	2nd group	3rd group	1st cycle	2nd cycle
June, 1996	2D	25.0	64.0	–	39.0	–
January, 1997	5A	26.0	66.0	104.5	40.0	38.5
June, 1997	–	26.5	64.0	–	37.5	–
September, 1997	1A	26.0	63.0	100.5	37.0	37.5
June, 1998	7B	25.0	62.0	–	37.0	–

[a]Figure numbers showing the frequency distribution of larval duration

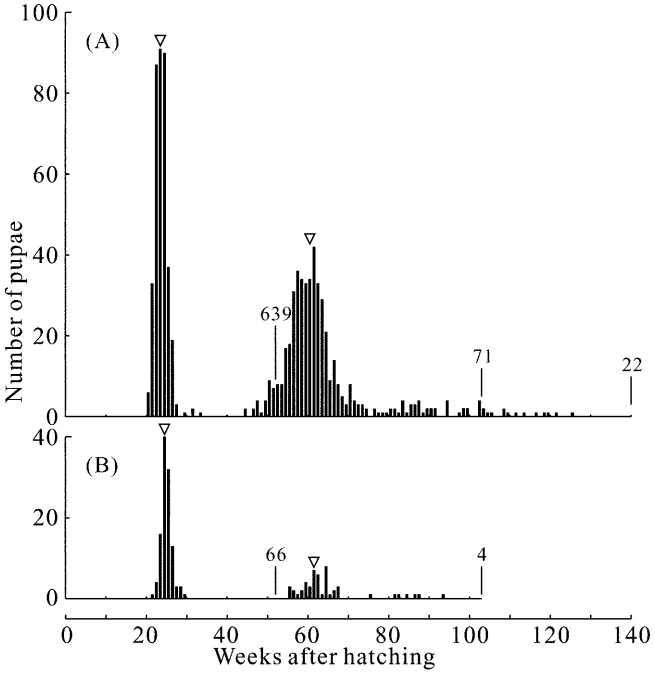

Fig. 7A,B Effects of environmental conditions in the early larval period on larval duration under LD 12:12 at 20°C in *A. verbasci*. Eggs laid in June 1998 by field-collected adults were kept under LD 12:12 at 20°C (**A**) or kept under LD 16:8 at 25°C and transferred to LD 12:12 at 20°C within a week after hatching (**B**). *Numerals with vertical lines* show the numbers of insects remaining as larvae. Each *triangle* indicates the median for each pupation group

the duration of a year, whereas Blake (1958, 1959) kept larvae in darkness as a constant condition but occasionally checked their pupation with light. Some larvae pupated in the third gate as in the present study, although no larvae pupated in the third gate and only one larva pupated in the fourth gate in the study reported by Blake (1958, 1959). Our results satisfied the criterion of circannual rhythms proposed by Gwinner (1981) that the characteristics should be demonstrated over at least two cycles. As Blake (1958, 1959) stated, it is unlikely that the existence of two or three genetic strains within the same species with different developmental periods causes the apparent periodic pattern.

Temperature compensation has been demonstrated in the period of circannual rhythm of a hibernating homeotherm, *S. lateralis*, which spends many months each year in deep torpor with body temperature close to environmental temperature. The first period of circannual cycles in body weight and hibernation increased slightly with decreasing temperature, although the differences between temperature conditions disappeared in the second and third cycles (Pengelley and Asmundson 1969). Much larger differences would be expected among environmental temperatures if the processes underlying circannual rhythms showed a temperature dependence known for many other physiological processes. Pengelley and Asmundson (1969) concluded that the circannual rhythm in *S. lateralis* is temperature compensated to some extent. However, Gwinner (1986) stated that a more conspicuous example of temperature-compensated circannual rhythms should be found in poikilotherms, as already shown in *A. verbasci* by Blake (1958, 1959). Our results under LD 12:12 agreed with those reported by Blake (1958, 1959) under DD.

Circannual rhythms are expressed within a narrow range of environmental conditions in some animals (Gwinner 1986). The annual testicular cycle of the red-billed dioch *Quelea quelea* persisted for 2 years under LD 12:12, but disappeared under LD 8:16 (Lofts 1962, 1964). Sika deer *Crevus nippon* replaced their antlers with a circannual rhythmicity if exposed to constant light, constant LD 18:6 or LD 6:18, although this rhythm disappeared under LD 12:12 (Goss 1969b). In the Japanese population of *A. verbasci*, the circannual rhythm in pupation was clear under constant LD 12:12, but was less evident under constant dim light, although a clear rhythm was shown under DD in the British population (Blake 1958, 1959). Moreover, the Japanese population of *A. verbasci* showed rhythmic pupation even under LD 16:8, although the interval between the first and the second pupation groups was shorter under LD 16:8 than under LD 12:12.

The annual cycle of photoperiod seems the most powerful zeitgeber for annual rhythms, at least in most vertebrate species examined (Gwinner 1986). Not only sinusoidal changes, but also square-type and saw-tooth cycles can be effective photoperiodic zeitgebers for various circannual functions in different species (e.g., Goss 1969a, 1976; Lincoln 1979; Legan and Karsch 1983). In the present study, larvae at all stages of development responded to an abrupt decrease in the photophase and pupated with some delay. However, a non-rhythmic timer reset by a decrease in the photophase does not explain this rhythm, because a decrease in photophase delayed the pupation not only in the first group but also in the second group. Furthermore, an abrupt decrease in the photophase did not always set the phase of this rhythm to the same point. A decrease in the photophase induces a phase shift in an endogenous timing mechanism that underlies the circannual rhythm in this species. The time of pupation was advanced or delayed depending on the time at which the photophase was decreased (see Fig. 6). Blake (1960) explained the delay of pupation under naturally decreasing day length in *A. verbasci* by direct inhibition of metamorphosis by decreasing day length apart from the timing mechanism. However, we can explain the results reported by Blake (1960) under naturally decreasing day lengths as a shift in the phase of the circannual rhythm when natural daylength decreases below a critical value.

Moreover, naturally increasing day lengths within 13 weeks after hatching shortened the larval period in the first pupation group (Blake 1963). An abrupt increase in the photophase before pupation in the first group also shortened the larval period in the present study. However, it is still unclear whether an increase in the photophase advances the phase of the circannual rhythm.

Circadian and circannual rhythms share many common features with regard to their behavior in the synchronized state (Gwinner 1986). Here, we showed that the pupation rhythm in *A. verbasci* shows a self-sustaining feature, temperature compensation and entrainment to a zeitgeber, and its period is a little shorter than a year. It is likely, therefore, that this rhythm is based on an annual biological oscillator. A similar gating phenomenon in population rhythms was shown in the circadian eclosion rhythm in *Drosophila pseudoobscura*. This rhythm is damped out under constant light (LL), and is reset to a unique phase, i.e., circadian time Ct 12, at or soon after a subsequent return to DD (Pittendrigh 1966). If the LL and DD in the eclosion rhythm of *D. pseudoobscura* are equivalent to long-day and short-day photoperiods in the pupation rhythm of *A. verbasci*, respectively, the circannual rhythm of *A. verbasci* would stop under a long-day photoperiod and restart at a constant phase when transferred to a short-day photoperiod. However, this is not the case because (1) larvae showed a circannual periodicity in pupation even under a long-day photoperiod, and (2) the period from photoperiodic transfer to pupation of the first group differed depending on the duration under a long-day photoperiod. The circannual rhythm of *A. verbasci* does not stop under a long-day photoperiod. In some animals, circannual rhythms persist even under LL (Goss 1969b; Pengelly et al. 1976; see Gwinner 1986). In the Japanese population of *A. verbasci*, however, the unclear pupation rhythm under constant dim light may result from damping out of the circannual rhythm as suggested in the circadian eclosion rhythms under LL in *D. pseudoobscura* and the flesh fly *Sarcophaga argyrostoma* (Pittendrigh 1966; Saunders 1976).

The nature of the oscillator of circannual rhythms remains unknown (Gwinner 1986). The phase-response curve provides one experimental assay of the oscillation's phase, and is the best available characterization of its time course (Saunders 1982; Gwinner 1986). In circadian rhythms, one full circadian cycle of the organism free-running under DD is systematically perturbed by single light pulses to construct a phase response curve. Depending on the phase of the circadian rhythm at which it is presented, a single pulse can advance or delay the phase, or have no effect. The relationship between the phase of light exposure and the size and direction of the ensuing phase shift is represented graphically in the form of a phase response curve (Pittendrigh 1981; Saunders 1982). In circannual rhythms, however, there have been only a few reports of phase-dependent phase shifts in response to a stimulus. Randall et al. (1998) reported that when the rainbow trout *Oncorhynchus mykiss* under natural day length were exposed to 2 months of LL, spawning was advanced or delayed depending on the time of year at which exposure occurred. They described these effects in the form of a circannual phase response curve. Similarly, testicular development of the European starling *Sturnus vulgaris* was enhanced, inhibited or unaffected by exposure to 1 month of LL or DD, the response again depending on the time of year of the transfer from natural day length (Gwinner 1973). However, these results are not sufficient to construct a circannual phase response curve, because the circannual rhythms before exposure to the stimuli are entrained to seasonally changing day length.

In the circadian rhythm of the Syrian hamster *Mesocrcetus auratus*, phase-dependent phase shifts also occur in response to transfer from LL to DD and vice versa and phase response curves were described (Albers 1986). In circannual rhythms, there has been one attempt to derive a circannual phase response curve for step transitions from the results of experiments in animals kept under constant conditions. Duston and Bromage (1988) kept female rainbow trout for various periods under a constant long-day photoperiod, and then changed the photoperiod abruptly to a constant short day. They described the effects of exposure to these conditions on the timing of sexual maturation. In the present study, we observed that the larvae under a constant long-day photoperiod showed phase-dependent phase shifts in response to step transitions to a short-day photoperiod. When the larvae were transferred to a short-day photoperiod 24 weeks or less after hatching, the timing of pupation was advanced as compared to that under a constant long-day photoperiod. When the photoperiodic transfer was performed 30 or more weeks after hatching, however, the timing of pupation was delayed. By calculating advances and delays relative to the median duration under a constant long-day photoperiod, we described here a circannual phase response curve that has not been reported in invertebrates (Fig. 8). This curve resembles the curves obtained in rainbow trout (Duston and Bromage 1988; Randall et al. 1998).

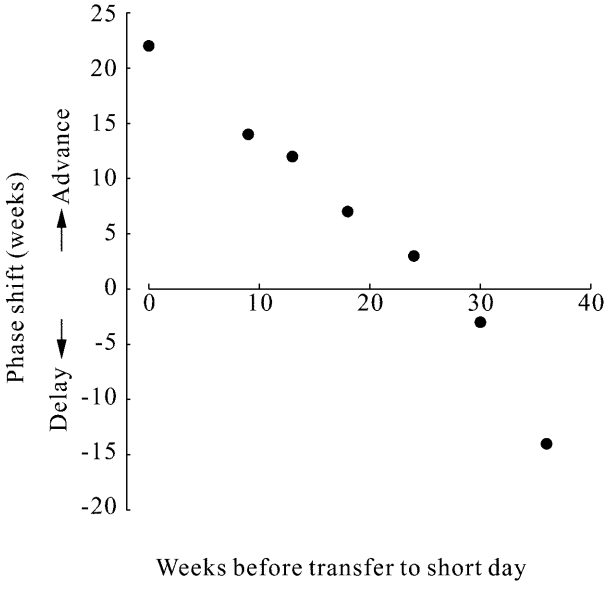

Fig. 8 Phase-response curve for the circannual pupation rhythm in *A. verbasci*. Closed circles show phase shifts induced by photoperiodic transfer from LD 16:8 to LD 12:12 at 20°C

These circannual phase response curves differed from circadian phase response curves in shape. In the circadian response curve, a light pulse in early subjective night generally acts as a 'new dusk' and causes a phase delay, whereas a light pulse in late subjective night acts as a 'new dawn' and causes a phase advance. Pulses applied in subjective day have little or no effect on the phase (Pittendrigh 1981; Saunders 1982). In the present study, photoperiodic transfer at any time of year caused a shift to the phase 21–26.5 weeks before pupation. As pupation occurs in early spring under natural conditions in *A. verbasci*, the season 21–26.5 weeks previously corresponds to autumn. Therefore, we propose that a decrease in the photophase acts as an 'autumn signal'. A decrease in the photophase causes a phase advance in subjective summer, a phase delay in subjective winter, and has no effect in subjective autumn. Therefore, the phase-response curve constantly decreases along the abscissa, even though there were significant differences in the time from photoperiodic transfer.

In conclusion, the circannual rhythm in *A. verbasci* can be well understood on the basis of the oscillator model of circadian rhythm. In circadian rhythms, there are six possible ways to obtain data for phase response curves, and the measured response curve depends on the type of signal and on the type of experiment (Aschoff 1965). To clarify the mechanisms of entrainment of the circannual rhythm, it is necessary to describe phase response curves by other methods. For example, it is feasible to construct a phase-response curve by an increase in the photophase, because the present study showed that an abrupt increase in the photophase shortened the larval period in the first pupation group.

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