Chapter 16
Circannual Rhythms in Insects

Yosuke Miyazaki, Tomoyosi Nisimura, and Hideharu Numata

Abstract Although many insects adapt to seasonal changes by photoperiodism, a small proportion of insect species use a circannual rhythm for seasonal adaptations. The circannual pupation rhythm of the varied carpet beetle *Anthrenus verbasci* shows a periodicity of approximately 40 weeks under constant conditions, and the change in photoperiod acts as a zeitgeber. The circannual rhythm of *A. verbasci*, of which the larval duration varies from one to several years, probably plays an important role for synchronizing the pupation and breeding times with spring each year. There are only slight differences in the critical daylength for circannual entrainment among geographically distinct populations in Japan, and they pupate in the same period under natural conditions in Osaka. Therefore, *A. verbasci* can adapt to seasonal changes in different regions without changing the parameters of the circannual rhythm. Long-term endogenous rhythms have also been reported in oviposition and pupation of some ant species. These insects are considered to refer not only to external cues but also to the phase of an endogenous clock for maintaining appropriate seasonality.

Keywords *Anthrenus verbasci* • Geographic variation • Phase-response curve • Photoperiodism • Seasonal adaptation

16.1 Introduction

Many species adapt to seasonal environmental changes by a direct response to photoperiod, that is, photoperiodism, but some species are known to use a circannual rhythm for seasonal adaptations (Gwinner 1986; Goldman et al. 2004; Paul et al. 2008).

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Circannual rhythms regulating various physiological and behavioral functions have been reported in a variety of organisms. However, our understanding of circannual rhythms is still poor compared to that of photoperiodism and circadian rhythms.

Insect species that have a life cycle shorter than a year seem to have no reason to use a circannual rhythm. Some of these insects enter diapause in a specific developmental stage of one generation to avoid the production of an additional generation in the season unfavorable for growth and reproduction, such as winter. For diapause induction and termination, many temperate insects use photoperiod and temperature as reliable seasonal cues from the environment (Danilevskii 1965; Danks 1987; Saunders 2010).

Some insect species of which the life cycles take two or more years may adopt a circannual rhythm for seasonal adaptations (Saunders 2010). The circannual rhythm in insects was first reported in the varied carpet beetle *Anthrenus verbasci* by Blake (1958, 1959, 1960, 1963). *A. verbasci* is widely distributed in temperate regions and known as a pest that feeds on woolen goods, dried animal products, and zoological specimens (Griswold 1941; Hinton 1945). The primary natural habitat of this beetle is the nests of birds (Yokoyama 1929; Hinton 1943, 1945; Woodroffe 1953). Adults emerge, mate, and lay eggs from spring to early summer. Larvae grow by feeding mainly on dried animal matter during warm seasons. Pupation occurs in spring. The duration of the life cycle varies from one to several years depending on the local climate and nutritional conditions (Yokoyama 1929; Griswold 1941; Blake 1958; Kiritani 1958; Miyazaki et al. 2009a). Blake (1958, 1959) showed that pupation of this species occurs with a periodicity of approximately 40 weeks under constant conditions. Similar long-term rhythms have been reported in pupation of another carpet beetle, *Anthrenus sarnicus* (Coombs and Woodroffe 1983; Armes 1990), and in queen oviposition and larval pupation of some ant species (Kipyatkov 1993, 1995). Chronobiological analyses, however, have not been conducted in detail in these reports. Since 1996, we have been running further tests and new experiments based on the present chronobiological views in the circannual rhythm of *A. verbasci*. In this chapter, with a central focus on the results obtained in *A. verbasci*, we outline the characteristics of the circannual rhythm in insects and discuss its adaptive significance.

16.2 Periodicity Under Constant Conditions

Constant 24-h photoperiods have been routinely used as seasonally constant conditions under which the circannual rhythm should be demonstrated to persist for at least two cycles with periods different from 12 months (Gwinner 1981a, 1986). Nisimura and Numata (2001) observed the pupation rhythm of *A. verbasci* with a period of approximately 37 weeks, when larvae within 1 week after hatching were continuously reared on bonito powder under a constant photoperiod of 12-h light and 12-h darkness (LD 12:12) at 20 °C and 66 % relative humidity (Fig. 16.1a). In circadian rhythms, the daily periodicity found in the once-in-a-lifetime event such as hatching,
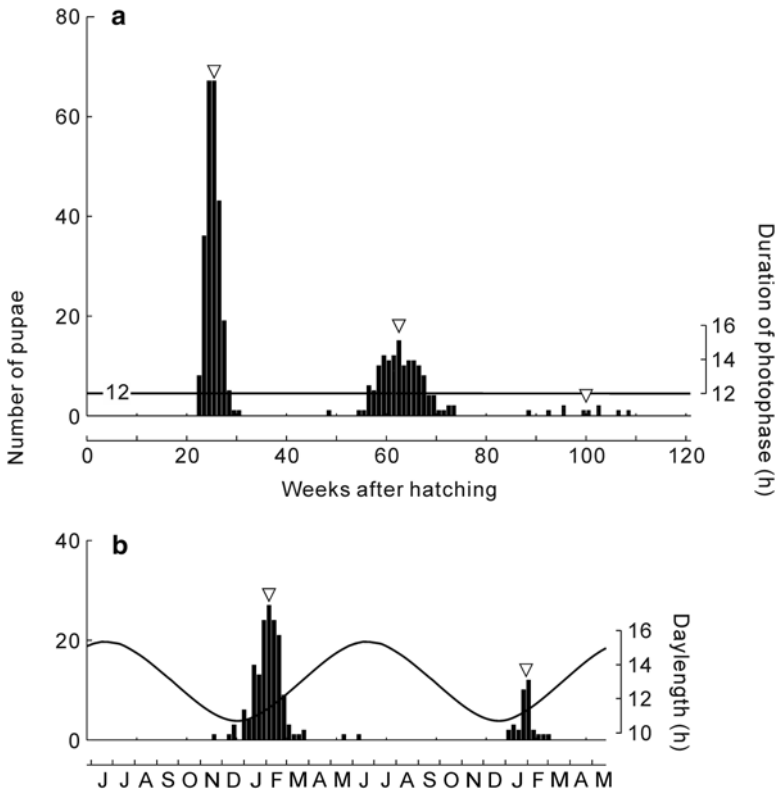
Fig. 16.1 Self-sustainability (**a**) and entrainability (**b**) in the circannual pupation rhythm of *Anthrenus verbasci* at 20 °C. Larvae were kept under light–dark (LD) 12:12 (**a**) or under naturally changing daylength (**b**). *Triangles* indicate median of each pupation group. *Solid line* in each panel indicates the duration of photophase or the natural daylength, including 1 h of twilight in Osaka, Japan (35°N). ((**a**) Modified from Nisimura and Numata 2001; (**b**) modified from Miyazaki et al. 2006)

pupation, and eclosion in insects can be explained by the concept of "gate," which was first introduced for the allowed time for eclosion in the fruit fly *Drosophila pseudoobscura* (Pittendrigh 1966). According to this concept, the gate is opened periodically by a circadian rhythm and only sufficiently developed individuals can hatch, pupate, or eclose during the period of gate opening (Saunders 2002). As described in more detail later, the pupation rhythm in *A. verbasci* has some similarities to the circadian eclosion rhythm of *D. pseudoobscura*, although the time scale is not days but years. Therefore, *A. verbasci* has an endogenous circannual rhythm and the gate for pupation is controlled by this rhythm (Blake 1958, 1959; Nisimura and Numata 2001). Larval growth inhibition by a low-nutrient diet has little or no influence on the timing of pupation in each circannual cycle but markedly changes the percentage of pupae in each cycle (Miyazaki et al. 2009a).

Most physiological processes depend strongly on temperature. However, temperature compensation of period length is needed to accurately measure time

and is known as a key characteristic of biological rhythms (Saunders 2002; Johnson et al. 2004). This property was first clearly shown in eclosion of *D. pseudoobscura* in circadian rhythms (Pittendrigh 1954) and in pupation of *A. verbasci* in circannual rhythms (Blake 1958, 1959). Temperature compensation is relatively easy to examine in insects, as compared to mammals or birds, because they are poikilotherms. Nisimura and Numata (2001) kept larvae of *A. verbasci* under LD 12:12 at various constant temperatures. Larvae began to pupate 21–27 weeks after hatching as the first pupation group at temperatures between 17.5 and 27.5 °C, and the second group pupated about 40 weeks after the first. High temperatures did not result in earlier pupation. Other developmental processes of *A. verbasci*, for example, the rate of larval development, the proportion of pupation, and the lengths of egg and pupal stages, depend on temperature as usual physiological processes do (Griswold 1941; Blake 1958; Kiritani 1958). Therefore, the timing of pupation of *A. verbasci* is clearly and specifically temperature compensated. Moreover, in *A. sarnicus*, three pupation peaks with intervals of 32–42 weeks were observed at both 15 and 25 °C, although the first and third peaks at 25 °C consisted of only two to four pupae (Armes 1990). This observation suggests that temperature compensation of the period also exists in the pupation rhythm of *A. sarnicus*.

There is a pronounced influence of photoperiod on the degree of persistence and the period length of the circannual rhythm of *A. verbasci*, as in other organisms with circannual rhythms (Gwinner 1986). The pupation rhythm was clear under LD 12:12 but was obscure under LD 15:9 and LD 16:8 and arrhythmic under LD 14:10. Although a clear rhythm was observed under LD 13:11, the circannual period was 5 weeks longer than under LD 12:12 (Fig. 16.2) (Nisimura and Numata 2003). There is still no convincing interpretation of these photoperiodic effects on the degree of persistence and the period length (see also Gwinner 1981a, 1986).

Many circadian rhythms in insects do not persist under constant light (Saunders 2002). Miyazaki and Numata (2010) examined whether the circannual pupation rhythm of *A. verbasci* persists under constant light. Rhythmic pupation did not occur under constant light when hatching larvae were continuously reared under these conditions. However, the pupation rhythm oscillated with a circannual pattern under constant light when hatching larvae had been exposed to LD 12:12 for 8 weeks before transfer to constant light. Therefore, preexposure to LD 12:12 was required for this circannual rhythm to be exhibited under constant light. As described next, the photoperiod is a zeitgeber for this circannual rhythm. It is possible that the initial circannual phase of newly hatched larvae varies among individuals, but exposure to LD 12:12 synchronizes the circannual phase of the larvae, and the circannual rhythm of pupation is exhibited under constant light.

Although the circannual rhythm in pupation of *A. verbasci* is detected only in populations, an individual insect can also display a circannual rhythm. Blake (1958) reported not only the pupation rhythm but also the alternation of the active (molting) and resting periods in the larvae of this species. Figure 16.3 shows molting, pupation, and body weight in seven larvae of *A. verbasci* that were individually reared under LD 12:12 at 22.5 °C (Nisimura and Numata, unpublished data). Although results in more individuals are necessary for conclusions, it seems that the alternation
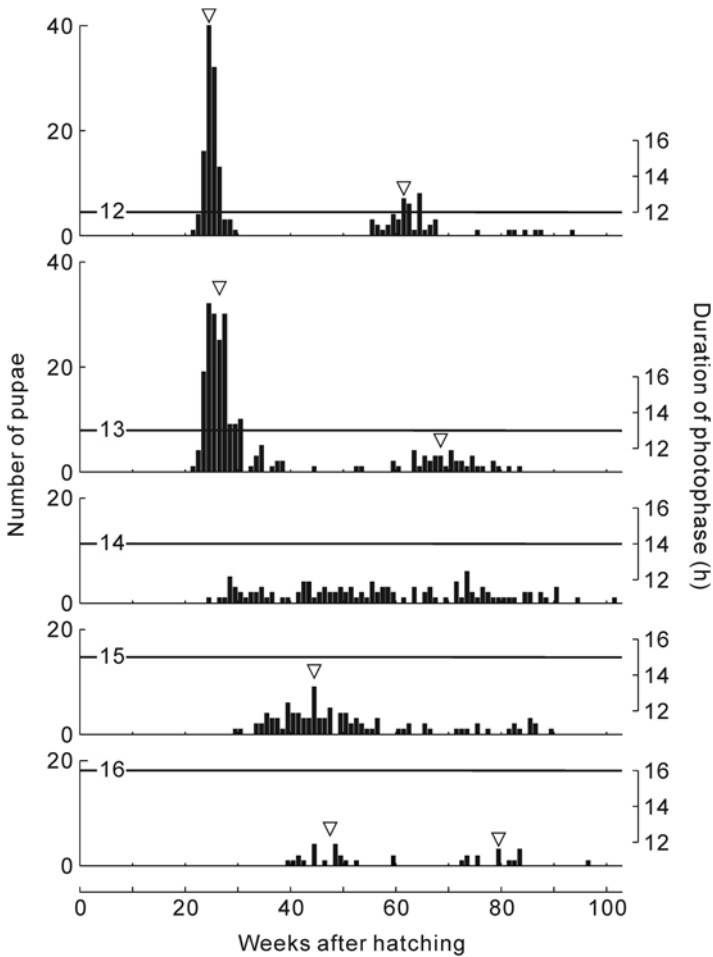
Fig. 16.2 Pupation of *Anthrenus verbasci* under various constant photoperiods at 20 °C. *Triangles* indicate median of each pupation group. *Solid line* in each panel indicates the duration of photophase. (Modified from Nisimura and Numata 2003)

of the active and resting periods was observed in terms of molting and the increase of the body weight. Thus, observation and analysis of the circannual rhythm in an individual would be feasible in the frequency of molting and the increase of body weight in *A. verbasci*.

In some species of ants, a queen stops oviposition and resumes it after an interval of several weeks or months, and such cycles are spontaneously repeated under constant photoperiod and temperature. Many of these rhythms show great variation in the endogenous period over several years (Kipyatkov 1993, 1995). In the red wood ants *Formica aquilonia* and *Formica polyctena*, for example, an endogenous period of the oviposition rhythm varied between 90 and 525 days (mean, 212 days)

Fig. 16.3 Change in body weight (**a**) and the timing of molting and pupation (**b**) of *Anthrenus verbasci* larvae individually reared under LD 12:12 at 22.5 °C. Each *solid line* shows the growth of an individual
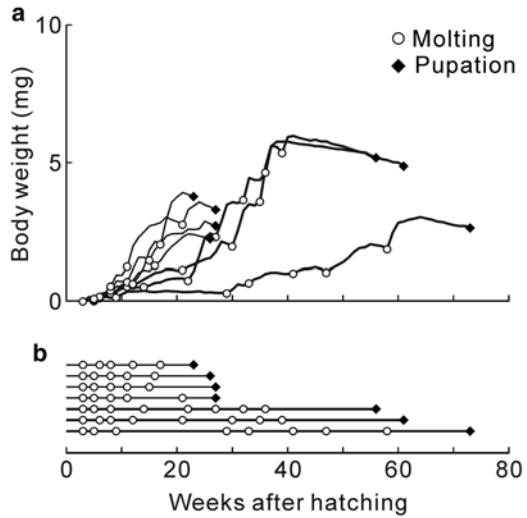
and between 60 and 345 days (mean, 179 days), respectively, even under optimal conditions (Kipyatkov and Shenderova 1990). Considerable intraindividual variability in the period is also reported in circannual rhythms of other animals (Gwinner 1986). In *F. aquilonia* and *F. polyctena*, the mean periods of oviposition rhythms did not depend on temperature between 20 and 25 °C and did so only slightly between 17 and 30 °C. Moreover, the mean periods were not different between short-day and long-day conditions (Kipyatkov and Shenderova 1990). It is considered of value to examine whether the stability of the period of the endogenous rhythm to temperature and photoperiod also exists in queens of other ant species.

16.3   Response to Seasonal Environmental Cues

Timing of pupation in *A. verbasci* and *A. sarnicus* is modulated by naturally changing daylength (Blake 1960, 1963; Armes 1990; Nisimura and Numata 2003; Miyazaki et al. 2006). The circannual period of *A. verbasci* is considerably shorter than 1 year, but continuous exposure to naturally changing daylength entrains the pupation rhythm to 1 year (Fig. 16.1a, b). Such entrainment is attributed to phase resetting of the circannual rhythm in response to the zeitgeber of photoperiodic changes but not to the direct regulation of development and diapause by classical photoperiodism (Nisimura and Numata 2001, 2003); this is similar to the entrainment of circadian rhythms to a daily cycle achieved by resetting of a circadian clock in response to light.

The phase of circadian rhythms advances or delays depending on the phase at which a zeitgeber stimulus is applied. A phase-response curve (PRC) is a plot of these phase shifts as a function of the phase of the stimulus. PRCs provide useful information

regarding the entrainment of the rhythm and the underlying mechanism. In most cases, a circadian PRC to light is constructed by administering a light pulse to a circadian rhythm under constant darkness (Saunders 2002; Johnson et al. 2004). From systematic experiments in the eclosion rhythm of *D. pseudoobscura*, Winfree (1970) realized that the resetting pattern of the circadian clock can be divided into two major types. He called these types type 1 and type 0, when pulse lengths are relatively short and long, respectively. Type 1 PRCs have low amplitude and a continuous transition between delays and advances (Fig. 16.4a). Type 0 PRCs have high amplitude and a breakpoint at the transition between delays and advances (Fig. 16.4b).
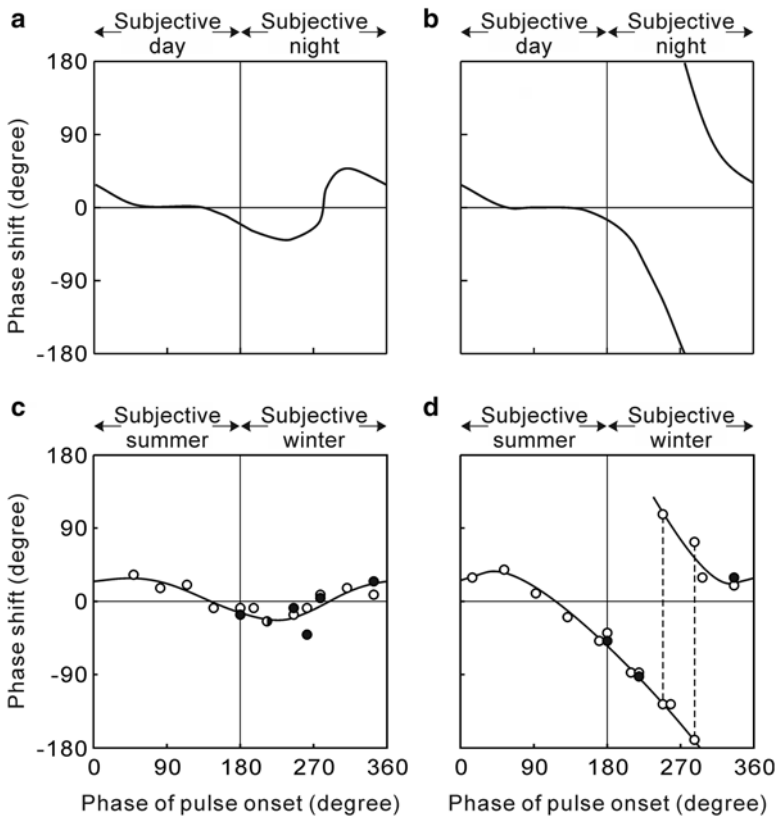

Fig. 16.4 Comparison of phase-response curves for circadian and circannual rhythms. (**a**, **b**) Phase-response curves in circadian rhythms, type 1 (**a**) and type 0 (**b**). (**c**, **d**) Phase-response curves in the circannual pupation rhythm of *Anthrenus verbasci*, one curve for 2-week long-day pulses (**c**) and one curve for 4-week long-day pulses (**d**). Larvae were kept under LD 12:12 at 20 °C and exposed to LD 16:8 for 2 weeks (**c**) or 4 weeks (**d**) at various phases in the circannual rhythm. The circannual period under continuous LD 12:12 is shown in terms of angle degrees (0–360°), and the initial phase under LD 12:12, i.e., the beginning of this experiment, is represented as 180°. *Open* and *closed circles* represent the phase shifts in the first and second pupation group after pulse perturbation, respectively. *Broken lines* in (**d**) show the split into advanced and delayed groups. (**c**) From Miyazaki et al. 2007; (**d**) from Miyazaki et al. 2005)

In addition, Winfree (1970) found that when a light pulse of intermediate length was applied at the transition between delays and advances in the circadian PRC, the eclosion of *D. pseudoobscura* became arrhythmic. Phase resetting dependent on pulse lengths and arrhythmicity evoked by a singular stimulus have also been confirmed in circadian rhythms of various species (Saunders 2002; Johnson et al. 2004).

To construct PRCs for a circannual rhythm, Miyazaki et al. (2005, 2007) kept *A. verbasci* larvae under LD 12:12 and exposed the larvae to LD 16:8 for 2 or 4 weeks (long-day pulse) during various phases of the rhythm. A long-day pulse clearly caused a phase advance or delay of the circannual rhythm in a phase-dependent manner. Circannual PRCs to 2-week and 4-week long-day pulses resembled type 1 and type 0 PRCs of circadian rhythms, respectively (Fig. 16.4c, d). Moreover, Miyazaki et al. (2007) found that a 4-week long-day pulse administered at the transition between delays and advances in the circannual PRC can evoke arrhythmicity in pupation of *A. verbasci*. These results demonstrated that the circannual rhythm of *A. verbasci* is entrained to an environmental cycle by the same mode of phase resetting as circadian rhythms and indicated that the circannual rhythm of *A. verbasci* is derived from a biological clock obtained for adaptation to annual environmental changes, that is, a circannual clock (see Miyazaki et al. 2012 for details).

Phase responses of circannual rhythm in *A. verbasci* are observed not only under constant conditions but also under naturally changing daylength. Miyazaki et al. (2006) applied 4-week long-day pulses between early August and late November under natural daylength at a constant temperature of 20 °C and observed phase shifts in the first pupation group. A long-day pulse applied on 4 August, 1 September, and 29 September caused 3-, 5-, and 5.5-week delays, respectively. A pulse applied on 27 October and 24 November caused 2- and 3-week advances, respectively. A pulse applied on 29 September also induced much less synchronous pupation than other conditions. Thus, the circannual phase of *A. verbasci* changes at least from early August to late November under natural daylength, as under LD 12:12.

Temperature and more infrequently photoperiod serve as environmental factors to accelerate or cease egg-laying and pupation in ant species (Kipyatkov 1993). The regulation of the life cycle by external factors is very important for ants with endogenous periods that deviate considerably from 1 year with great variability. However, it has still not been clarified whether temperature and photoperiod directly regulate the initiation and cease of oviposition and pupation or act to reset the phase of the endogenous rhythm as zeitgebers.

16.4 Photoperiodic Time Measurement for Entrainment

As described here, the photoperiod considerably affects the periodicity and phase setting of circannual rhythm in *A. verbasci*. Therefore, the photoperiodic time measurement system is involved in the regulation of the circannual rhythm. This system seems identical to that used for usual photoperiodism in insects without circannual rhythms, because it is probable that a circannual clock was originated later than the basic form of the photoperiodic time measurement system in the course of evolution.

A critical daylength is one of the important characteristics in insect photoperiodism. Behavioral and physiological responses are shown depending on whether the daylength is above or below this value (Danilevskii 1965; Danks 1987; Saunders 2002, 2010). In the Osaka population of *A. verbasci*, when larvae were kept under constant photoperiods, the critical daylength for synchronous pupation was between 13 and 14 h (Fig. 16.2) (Nisimura and Numata 2003). Similarly, a clear phase delay, which is pivotal for the appropriate entrainment of a circannual rhythm with a period considerably shorter than 1 year, was induced when the photoperiodic change exceeded a critical value in the photophase between 13 and 14 h (Miyazaki and Numata 2009). It is, therefore, considered that the photoperiodic time measurement system of *A. verbasci* in Osaka adopts this critical value as a criterion for determining whether the daylength is long or short.

The photoperiodic time measurement system generally involves a circadian clock. The Nanda–Hamner protocol, in which organisms are subjected to light–dark cycles with a fixed short photophase followed by a variable scotophase to give cycle lengths (T) of up to 72 h or more, has often revealed the involvement of a circadian system in photoperiodic responses. If short-day responses are induced when T is a multiple of 24 h, but not induced when T is not a multiple of 24 h, it is considered that a circadian system is involved in photoperiodic time measurement (Saunders 2002; Goldman et al. 2004). By this protocol, Miyazaki et al. (2009b) examined whether a circadian clock is involved in photoperiodic entrainment of the circannual rhythm of *A. verbasci*. Exposure to LD 12:36 or LD 12:60, for which T is a multiple of 24 h, had effects similar to exposure to LD 12:12. In contrast, exposure to LD 12:24 or LD 12:48, for which T is not a multiple of 24 h, had effects clearly different from exposure to LD 12:12. These results show the involvement of a circadian system in photoperiodic entrainment of the circannual rhythm of *A. verbasci*.

16.5   Adaptive Significance

Circannual rhythms with a period deviating significantly from 12 months and with relatively weak self-sustainability appropriately function only in the presence of seasonal zeitgebers entraining the rhythms to the natural annual cycle (Gwinner 1986). Gwinner (1981b, 1986) stated that as yet there appears to be no convincing answer to the question whether external seasonal factors could not do the same job alone. Naturally and more seriously, such a question also arises in insects that are more susceptible to environmental factors than are homeotherms. In insects inhabiting temperate regions, other systems that depend exclusively on external proximate factors such as temperature and photoperiod are predominantly more common than circannual rhythms (Danilevskii 1965; Danks 1987; Saunders 2010). Unfortunately, we cannot convincingly explain why a small proportion of insect species have evolved a circannual rhythm for the control of their life cycle. However, at least, we can discuss some significance of the circannual rhythm in the control of the life cycle.

The circannual clock probably allows the organism to better predict and adapt to seasonal changes and increase the chances of survival and reproductive success
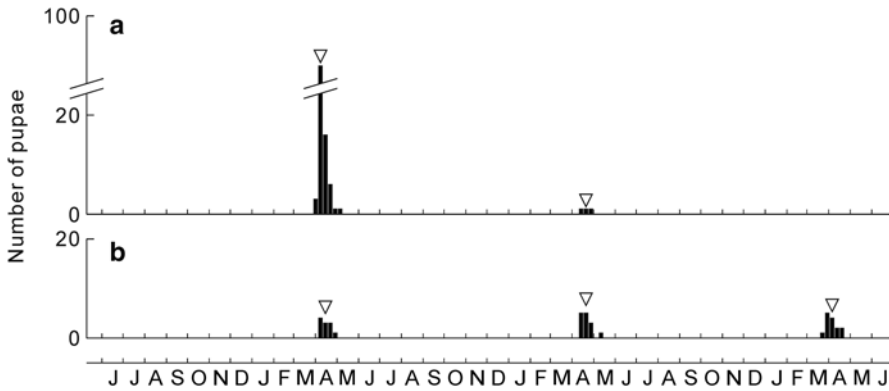
Fig. 16.5 Effects of dietary nutrition on the pupation of *Anthrenus verbasci* under natural daylength and temperature in Osaka, Japan. Dried bonito powder (**a**) or feathers of the domestic pigeon (**b**) were provided as larval food. *Triangles* indicate median of each pupation group. (Modified from Miyazaki et al. 2009a)

(Gwinner 1981a, 1986). *A. verbasci* can refer not only to ambient photoperiod and temperature but also to the phase of a circannual clock and thereby may show more accurate seasonal responses in the field than other insects that do not have this clock (Miyazaki et al. 2012). Although the pupation peak of *A. verbasci* exists in early February under natural photoperiod at 20 °C (see Fig. 16.1b), pupation occurs in April under natural photoperiod and temperature in Osaka because of the suppression of development by low temperature during winter (Fig. 16.5) (Nisimura and Numata 2003). Nisimura and Numata (2003) placed larvae of *A. verbasci*, within a week after hatching, at various times of the year under natural temperature and photoperiod in Osaka. The results showed that larvae that had hatched between October and mid-April (i.e., broadly speaking in "winter") could not grow to pupae, whereas larvae that had hatched between late April and September (i.e., in summer) could pupate in March or April, of the next year or the year after. In addition, pupal weight was relatively high (approximately 4 mg) in individuals that had hatched between late April and July, but only approximately 2 and 1 mg in individuals reared from August and September, respectively. Therefore, it was suggested that in *A. verbasci*, synchronous pupation and adult emergence in spring achieve the sufficient growth of offspring larvae during warm seasons so that the larvae overwinter and the relatively large adults with high fecundity emerge. Armes (1990) also reported that all the first-instar larvae of *A. sarnicus* introduced into the pigeon loft in southern England between November and February died within a few weeks, but larvae introduced between March and September could successfully develop to pupation between May and July, of the next year or the year after. Thus, *A. verbasci* and *A. sarnicus* should synchronize the breeding time with the beginning of the warm season to enhance the survival and reproductive rates, and therefore the circannual clock regulating pupation timing is likely a beneficial adaptive mechanism.

A circannual clock is the repetitive system oscillating for a number of years. This clock may facilitate production of consistent seasonal responses every year. If the rate of larval development of *A. verbasci* differs considerably among individuals in natural environments, it is considered that the gate controlled by the circannual clock is of advantage in restricting pupation to the appropriate season each year. In rearing experiments under outdoor conditions in Osaka, however, most individuals pupated in the next spring (Fig. 16.5a) (Nisimura and Numata 2003; Miyazaki et al. 2009a). In these experiments, bonito powder was provided as a sufficient larval diet, as in our other experiments. Dried bonito is favorable for growth of *A. verbasci* larvae (Kiritani 1958). Under natural conditions, however, such nutrient-rich food is unlikely to be available frequently. In bird nests, *A. verbasci* larvae feed on feathers and other dry animal materials (Hinton 1943; Woodroffe 1953). The nutrient quality of these foods is probably poorer, except for the carcasses of dead birds. When larvae of *A. verbasci* were supplied with pigeon feathers as food under outdoor conditions in Osaka, larval development was slower than on bonito powder, and the pupation times varied over 3 years or more. Although most larvae did not develop to pupae and died, similar numbers of the surviving larvae pupated around April each year for 3 years (Fig. 16.5b). There was also a larva that pupated in spring of the fourth year (Miyazaki et al. 2009a). Therefore, it is probable that the larval development of *A. verbasci* in the natural habitat often varies and takes 1, 2, or more years to complete. In nutrient-poor and unstable environments where the duration of the life cycle differs considerably among individuals, the gate for pupation controlled by a circannual clock would have greater significance as a seasonal adaptation.

With higher latitudes, insects are subjected to longer, colder winters and shorter, cooler summers. Because the developmental rate of insects is correlated with environmental temperature, insect species that are univoltine at lower latitudes may extend the duration of the life cycle over a number of years at higher latitudes (Saunders 2009). In fact, the life cycle of *A. verbasci* takes 2 years to complete in southern England even though favorable food for growth is supplied (Blake 1958, 1960), whereas it takes 1 year in central Japan (Yokoyama 1929; Nisimura and Numata 2003). The significance of internal time-of-year information provided by the circannual rhythm seems higher with higher latitudes because of shorter warm seasons and the longer life cycle.

In some insects living in further high latitudes, however, expression of seasonal traits regulated by long-term endogenous rhythms may decline under constant laboratory conditions. Many ant species distributed in the temperate climatic zone spontaneously resume oviposition and development under constant conditions. In the ant species penetrating to extremely high latitudes, such as the northernmost areas of the Palaearctic region, spontaneous reactivation under constant conditions is possible but could not be sufficient to maintain the colony. These species require a long exposure to low temperature for complete restoration of oviposition and development as a result of adaptation to the climate with a long and cold winter. Under such environments, endogenous rhythms in ants effectively control the physiological process and change the internal tendency for seasonal traits (Kipyatkov 1993, 1995), suggesting

that persistence of endogenous seasonal rhythms becomes weak and dependency on external seasonal factors becomes strong in insects living in extremely high latitudes, although it has been shown only in some ants.

16.6 Geographic Variation

Insect species distributed in different areas often show geographic variation in the seasonal timekeeping mechanism (Danilevskii 1965; Saunders 2002, 2009). In many insects, for example, the critical daylength in photoperiodism for induction of winter diapause increases by approximately 1.0–1.5 h for every 5° of latitude, because diapause must be induced earlier in cooler climate zones (Danilevskii 1965; Danks 1987). Adjustment of the pupation timing in *A. verbasci* by photoperiod is attributed to phase resetting of the circannual rhythm rather than induction and termination of larval diapause (Nisimura and Numata 2001, 2003). To examine whether geographic variation exists in the seasonal timekeeping mechanism of *A. verbasci*, Matsuno et al. (2013) examined, under various environmental conditions, the pupation time in four geographically distinct populations in Japan: Takanabe (32.1°N), Osaka (34.7°N), Sendai (38.3°N), and Sapporo (43.1°N) populations.

The initial phase and endogenous period of the circannual pupation rhythm under continuous LD 12:12 differed only slightly among the four populations. When larvae were exposed for 12 weeks to various longer photophases between 13 and 16 h and then transferred to LD 12:12, in all procedures the first pupation peak significantly was delayed, compared to that under continuous LD 12:12. Figure 16.6 shows results in the Osaka population. The magnitude of the delay depended on the photophase duration of 12-week exposure. Exposure to LD 14:10, LD 15:9, or LD 16:8 caused 9-week delays. Exposure to LD 13:11 and LD 13.5:10.5 caused 3- and 6-week delays, respectively. Therefore, LD 13:11 and LD 13.5:10.5 induced intermediate delay responses.

The degree of the delay evoked by the same stimulus depended on the geographic population. Exposure to LD 16:8 induced the maximum delays of 9–10 weeks in all four geographic populations. This maximum delay was regarded as 100 %, and the critical daylength was calculated as the photophase duration under which the magnitude of the delay was 50 % (Fig. 16.7a). The critical daylength was 12.8 h in the Takanabe population, 13.2 h in the Osaka population, and 13.6 h in the Sendai and Sapporo populations. Thus, there was a correlation between the critical daylength for entrainment of the circannual rhythm in *A. verbasci* and habitat latitude, but the correlation was much weaker than that obtained in photoperiodism for diapause induction in other insects, for example, larval diapause of the rice stem borer *Chilo suppressalis* (Kishino 1970) (Fig. 16.7b).

The gate for pupation of *A. verbasci* is set approximately a half year after transfer from long days to short days by a phase shift in the circannual rhythm (Nisimura and Numata 2001; see also Fig. 16.6), and the development and pupation are suppressed by low temperature during winter (Nisimura and Numata 2003). These findings
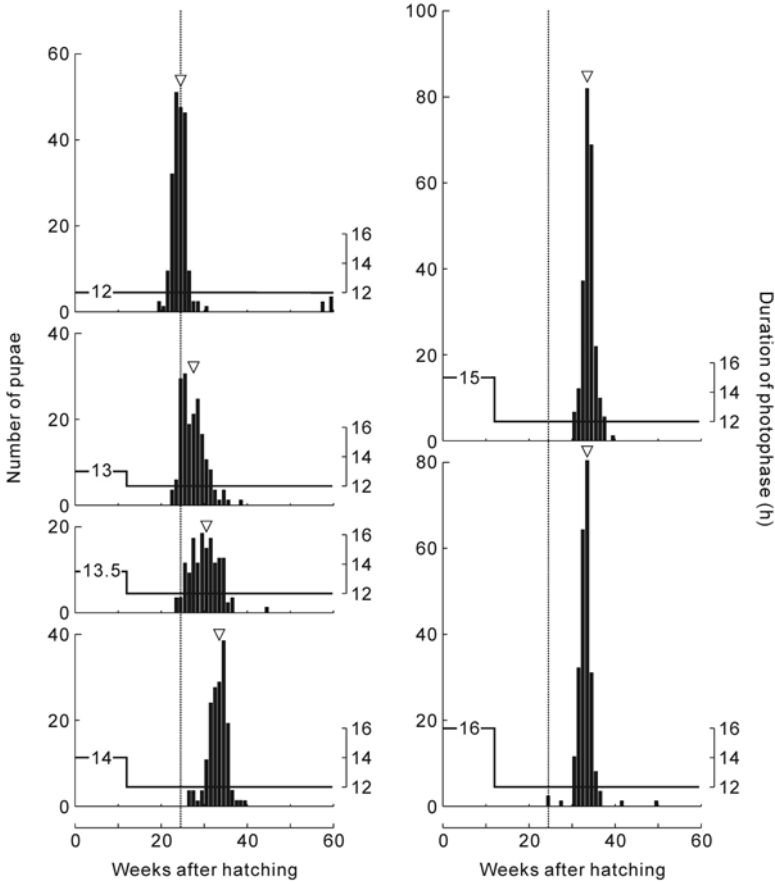
Fig. 16.6 Effects of photoperiodic changes on the timing of pupation in the Osaka population of *Anthrenus verbasci* at 20 °C. *Triangle* indicates median of each pupation group. *Vertical dotted lines* show median of the first pupation group under continuous LD 12:12. *Solid line* in each panel indicates duration of photophase. (Modified from Matsuno et al. 2013)

suggest that even though the geographic cline in the critical daylength is slight, *A. verbasci* can avoid pupation and adult eclosion during autumn and winter. Therefore, selective pressures on the critical daylength for phase delay of the circannual rhythm of *A. verbasci* might be weak compared to those on the critical daylength for diapause induction in many other insects. In fact, when newly hatched larvae were reared from spring under outdoor conditions in Osaka, in all four geographic populations many larvae survived and showed a pupation peak in April of the following year (Fig. 16.8) (Matsuno et al. 2013). Thus, *A. verbasci* appears capable of adapting to seasonal changes in different geographic regions without changing the parameters of the circannual rhythm. Such a mechanism may be one factor contributing to the geographically widespread distribution of *A. verbasci* (Griswold 1941; Hinton 1945).
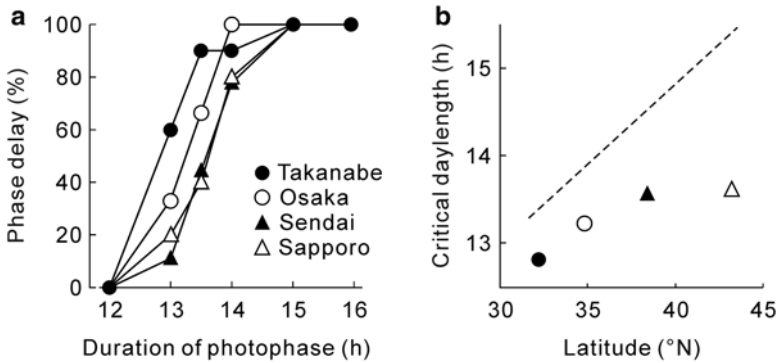
Fig. 16.7 Responsiveness to photoperiod for the timing of pupation in Takanabe, Osaka, Sendai, and Sapporo populations of *Anthrenus verbasci* at 20 °C. (**a**) Relationship between the photophase before transfer to LD 12:12 and the degree of phase delays. (**b**) Relationship between the habitat latitude and the critical daylength for phase delay. Phase delays were calculated from the first pupation peak when larvae were exposed for 12 weeks to various longer photophases and transferred to LD 12:12, compared to the first pupation peak under continuous LD 12:12 (see Fig. 16.6). The magnitude of phase delay induced by LD 16:8 exposure was regarded as 100 %, and the critical daylength was regarded as a photophase duration under which the magnitude of the delay was 50 %. The *broken line* designates the critical daylength for induction of larval diapause in *Chilo suppressalis* at 25 °C (Kishino 1970). (Modified from Matsuno et al. 2013)

16.7 Conclusions and Perspectives

The circannual pupation rhythm of *A. verbasci* shows self-sustainability, temperature compensation of the period, and entrainability to a zeitgeber, and the shapes of the circannual PRCs in this rhythm resemble those of circadian PRCs. These results indicate that a circannual clock of *A. verbasci* is the endogenous oscillator that has some parallels with a circadian clock (Miyazaki et al. 2012). It is likely that this circannual clock arose in the evolutionary process during which the life-cycle duration has been extended to a few or several years, and involved, as part of the input pathway for entrainment, a photoperiodic time measurement system almost identical to that used for usual photoperiodism exhibited by many temperate insects (Miyazaki et al. 2009a, b, 2012). Because this circannual clock sets the gate for pupation approximately a half year after the decrease of photoperiod in late summer and development is suppressed by low temperature during winter, even though the geographic cline in the critical daylength is slight, pupation adequately occurs in spring or early summer in many local areas (Matsuno et al. 2013). In this way, *A. verbasci*, and probably *A. sarnicus*, accomplish synchrony with the seasons by the circannual rhythm. The circannual clock of *A. verbasci* is also likely to regulate other developmental and physiological parameters, including larval molting. Therefore, the primary role of the circannual clock(s) may be to maintain the appropriate relationship between a temporal sequence of physiological stages and natural local seasons.
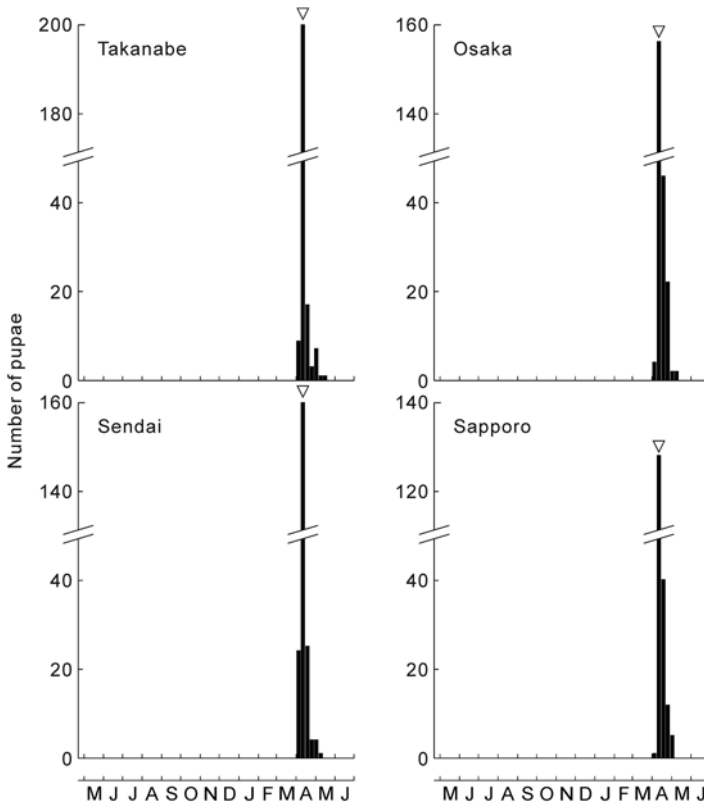
Fig. 16.8 Pupation of Takanabe, Osaka, Sendai, and Sapporo populations of *Anthrenus verbasci* under natural daylength and temperature in Osaka, Japan. Newly hatched larvae were reared from spring, and many larvae showed a pupation peak in April of the following year regardless of the geographic population. *Triangles* indicate median of each pupation group. (Modified from Matsuno et al. 2013)

It is a future subject to uncover in more details the physiological mechanisms and functions of the circannual rhythm of *A. verbasci*. One possible way would be to identify molecular candidates involved in this circannual rhythm by utilizing omics approaches such as RNA-seq and then reveal the precise role of the candidates by RNA interference, which inhibits gene expression in various physiological responses, or by genome editing technology such as zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) to disrupt the candidate genes.

The circannual clock is an internal provider of the approximate time of year, and its oscillation is affected by external time cues. Therefore, annual rhythms displayed by many organisms can be regarded as a consequence of the interaction between internal and external time-of-year information. The understanding of the adaptive value of circannual rhythms depends on the understanding of this interrelationship (Gwinner 1986). Concerning seasonality in ants, Kipyatkov (1993, 1995) described

it as follows. (1) Many species of tropical and subtropical ants do not clearly show annual rhythms in ontogenetic events, but in some other species endogenous rhythms may have an important role in the regulation of seasonal development under conditions of tropical and subtropical climates. (2) In species distributed in lower latitudes of the temperate zone, reactivation induced by the endogenous rhythms occurs in autumn but approaching cool weather makes the resumption impossible until warm seasons. (3) In species distributed in much higher latitudes, true reactivation caused by cold weather is developed and reactivation induced by the endogenous rhythms is incomplete. This broad overview on the relationship among habitat latitudes in ant species, influences of external seasonal factors, and the role of endogenous rhythms in the control of the life cycle may be helpful to investigate the ecological and evolutionary aspects of circannual rhythms in insects, although more investigations are required.

Carpet beetles and ants are holometabolous insects, but circannual rhythms may exist in hemimetabolous insects, although the evidence is still insufficient (Mao and Henderson 2007; Saunders 2010). Investigations of insects of which the life cycles take several or more years require a long time and are scare compared to investigations of insects that have a life cycle shorter than a year. Therefore, how far seasonal strategy with circannual rhythms is distributed over insect species is still veiled. To clarify the physiological and adaptive significance of circannual rhythms in insects, further research is needed across various insect species.

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