

[REVIEW]

Chronobiology of Crickets: A Review

Kenji Tomioka*

Graduate School of Natural Science and Technology, Okayama University, Okayama 700-8530, Japan

Crickets provide a good model for the study of mechanisms underlying circadian rhythms and photoperiodic responses. They show clear circadian rhythms in their overt behavior and the sensitivity of the visual system. Classical neurobiological studies revealed that a pair of optic lobes is the locus of the circadian clock controlling these rhythms and that the compound eye is the major photoreceptor necessary for synchronization to environmental light cycles. The two optic lobe clocks are mutually coupled through a neural pathway and the coupling regulates an output circadian waveform and a free-running period. Recent molecular studies revealed that the cricket's clock consists of cyclic expression of so-called clock genes and that the clock mechanism is featured by both *Drosophila*-like and mammalian-like traits. Molecular oscillation is also observed in some extra-optic lobe tissues and depends on the optic lobe clock in a tissue dependent manner. Interestingly, the clock is also involved in adaptation to seasonally changing environment. It fits its waveform to a given photoperiod and may be an indispensable part of a photoperiodic time-measurement mechanism. With adoption of modern molecular technologies, the cricket becomes a much more important and promising model animal for the study of circadian and photoperiodic biology.

Key words: circadian rhythm, photoperiodism, clock gene, molecular oscillation, cricket

INTRODUCTION

Adaptation to periodically changing environment is prerequisite for insects' life. They show daily rhythms in their physiology including behavioral activity. Some insects show seasonal changes in circadian rhythms as well as in their reproductive activity (Tauber et al., 1986; Saunders, 2002). The mechanisms underlying these physiological changes have been studied at behavioral, cellular, and biochemical levels. Recent development of technology in molecular biology promotes studies on these issues at the molecular level with analysis of functional roles of genes involved (Reppert et al., 2010; Tomioka and Matsumoto, 2010; Mito et al., 2011).

Crickets provide an especially good model for these studies for several reasons. They show clear behavioral rhythms with seasonal modulations and clear photoperiodic responses (Tomioka and Abdelsalam, 2004). Some of these are suitable for surgical manipulation due to their large size, and have been used for search for the circadian clock that drives the physiological rhythms and for the photoreceptors that are necessary for the clock to synchronize with environmental light cycles. The RNAi is particularly effective in crickets (Mito et al., 2011) so that functional roles of clock genes have been so far investigated (Tomioka et al., 2009).

In the cricket, the clock tissue has been identified and the photoreceptor for entrainment has also been clarified with neurobiological techniques (Tomioka and Abdelsalam, 2004). Recent molecular studies have revealed that the cricket clock oscillates with cyclical expression of clock genes, as has been suggested for other insect species, but has some unique features as well (Moriyama et al., 2008; Danbara et al., 2010; Moriyama et al., 2012; Uryu et al., 2013). The cricket possesses multiple clocks that distribute both in the central nervous system and peripheral tissues and produce an appropriate temporal structure of an insect at large through mutual interactions (Uryu and Tomioka, 2010, 2014). The present review provides an overview of the cricket circadian and photoperiodic systems, focusing on physiological and recent molecular approaches to the clock system. Issues of potential interest for future studies will be also discussed.

Overt circadian rhythms

Crickets show robust circadian rhythms in various behavioral and physiological functions (Fig. 1). Locomotor activity rhythms have been extensively studied in several species such as *Gryllus bimaculatus*, *Teleogryllus commodus*, *Modicogryllus siamensis*, *Grylloides sigillatus*, and *Dianemobius nigrofasciatus* (Sokolove, 1975; Tomioka and Chiba, 1982b; Abe et al., 1997; Shiga et al., 1999; Sakamoto et al., 2009). Crickets show clear locomotor rhythms as adult in both males and females. Stridulatory activity is also under circadian control in many cricket species (Loher, 1972; Nakatani et al., 1994). Such rhythms may

* Corresponding author. Tel. : +81-86-251-8498;
Fax : +81-86-251-8498;
E-mail: tomioka@cc.okayama-u.ac.jp
doi:10.2108/zs140024

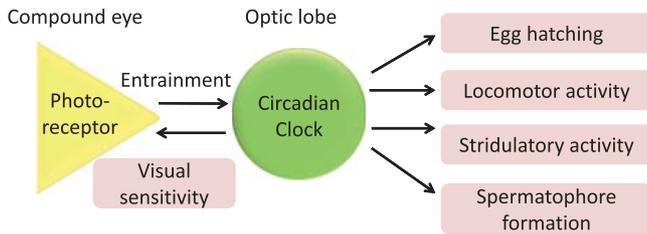


Fig. 1. The circadian system in the cricket. The circadian clock in the optic lobe is entrained by light information perceived by photoreceptors in the compound eye, and regulates daily rhythm in various physiological functions such as egg hatching, locomotor and stridulatory activity, spermatophore formation, and visual sensitivity.

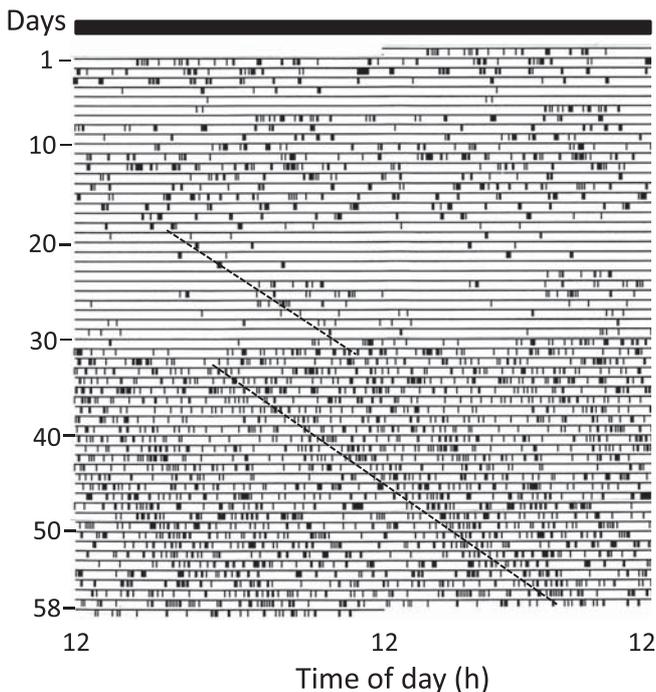


Fig. 2. Double-plotted actogram of locomotor activity of a cricket (*Gryllus bimaculatus*) in which bilateral optic lobe-compound eye complexes were surgically replaced with adult ones at the 7th instar nymphal stage. The locomotor activity was recorded in constant darkness at a constant temperature of $25 \pm 0.5^\circ\text{C}$. Recording was started on the day of the operation. The locomotor activity was arrhythmic for about 20 days, then a weak rhythm appeared, and another clear and anti-phased rhythm subsequently started around day 32 with an increase of activity level. Oblique lines indicate onsets of rhythmic activity.

contribute to increase the chance to mate. In fact, in *T. commodus*, female locomotor activity and male calling occur at approximately the same time (Loher, 1979). In tropical areas, many species, also including bush crickets, are colocalized in a narrow area. Some species are known to sing in a manner of temporal compartmentalization (Diwakar and Balakrishnan, 2007). In other cases, inter-specific interaction apparently determines the daily temporal singing pattern. The male crickets *Hemisaga denticulate*, for example, are known to stop singing when the other dominant species *Mygalopsis marki* starts to sing in the evening, while they

continue to sing during the entire evening when *M. marki* is absent (Römer et al., 1989).

Mating activity is generally higher during the night. During the copulation, a male cricket transfers a spermatophore to his mate. If unable to find a mate, the male replaces the spermatophore once a day with a newly formed one several hours before the onset of stridulation. This spermatophore formation is under circadian control and persists with an approximately 24 h interval in constant conditions (Loher, 1974).

The visual system of crickets also shows rhythmic changes in sensitivity. The retinal photoreceptive systems show daily rhythms in their responsiveness to light (Fig. 1): Electroretinogram (ERG) analysis of the compound eyes shows a daily change in amplitude with a peak during the night (Tomioka and Chiba, 1982a; Tomioka, 1985a). This change is probably based on daily morphological changes in the retina (Sakura et al., 2003). The visual interneurons in the optic lobe also show a circadian rhythm in their responsiveness to light stimuli again with a peak during the night (Tomioka and Chiba, 1986).

Loci of the clock

The circadian clock that regulates various physiological rhythms is known to reside in the optic lobe (Fig. 1). When the optic lobe is surgically removed bilaterally, the rhythms of all overt activity are abolished. The locomotor and stridulatory rhythms, for example, disappeared after removal of the optical lobe and were never restored in the cricket, *T. commodus* and *G. bimaculatus* (Sokolove and Loher, 1975; Tomioka and Chiba, 1984). The locomotor rhythm was restored by transplantation of the optic lobe into a cricket with its optic lobes surgically removed (K. Tomioka, unpublished observation). Interestingly when adult compound eye-optic lobe complex was bilaterally transplanted to nymphal crickets whose complexes had been removed bilaterally, the transplanted crickets restored a weak rhythm after about three weeks, and the rhythm replaced with a stronger and antiphase one (Fig. 2). The change of the rhythm seemed to occur after adult emergence, such as rhythm reversal associated with sexual maturation in intact crickets. The restoration of the rhythm may be attributable to neural regeneration between the transplanted optic lobe and the brain, as in cockroaches (Page, 1982). Although the fact strengthened the view that the optic lobe is the locus of the clock, the final proof for the optic lobe clock hypothesis came from in vitro recording of neural activity from an isolated and cultured optic lobe. The efferent neuronal activity of the optic lobe toward the central brain exhibited a clear circadian rhythm with a peak during the night (Tomioka and Chiba, 1992). The importance of the optic lobe in circadian rhythm generation has been confirmed in many other crickets, including *Hemideina thoracica*, *G. sigillatus*, and *D. nigrofasciatus* (Waddell et al., 1990; Abe et al., 1997; Shiga et al., 1999).

The circadian clock in the optic lobe regulates the sensory system as well. After severance of the optic stalk, the circadian ERG rhythm of the two compound eyes could be entrained separately by light-dark cycles (Tomioka, 1985a). Visual interneurons showed circadian rhythms in their photoresponsiveness in an optic lobe isolated and cultured in vitro (Tomioka and Chiba, 1986; 1992; Tomioka, 2000).

A single optic lobe fully controls the circadian locomotor

rhythm. A cricket with a single optic lobe showed a robust locomotor rhythm that synchronized with the light-dark cycle (Yukizane and Tomioka, 1995; Shiga et al., 1999; Koga et al., 2005). The fact suggests that a single optic lobe includes all constituents required for locomotor rhythm generation and may contribute toward survival in the daily changing biotic and abiotic environment, even if the clock in one side is damaged.

However, involvement of extra-optic lobe oscillators has been postulated in locomotor rhythms in *G. bimaculatus*. After the optic lobes were bilaterally removed, a weak rhythm often persisted for a while in locomotor rhythms (Tomioka, 1985b). The rhythm soon disappeared in constant darkness, but could be established again by photic entrainment when a neural connection was regenerated between the optic stalk and the compound eye (Tomioka and Chiba, 1989c). This rhythm can be explained as regulated by extra-optic lobe weak oscillators that are normally coupled by the optic lobe but run with slightly different periods when not controlled from the optic lobe. Similar but more clear optic lobe-independent rhythms were reported for stridulatory activity in *T. commodus* (Rence and Loher, 1975); crickets with optic lobes bilaterally removed exhibited a stridulatory rhythm with characteristic circadian properties under temperature cycles.

The circadian photoreceptors

The compound eye is the major photoreceptor necessary for photic entrainment of the locomotor rhythm (Fig. 1). This is supported by the fact that bilateral severance of the optic nerves resulted in a free-run of the rhythm even in LD cycles (Tomioka and Chiba, 1984; Yukizane and Tomioka, 1995). Dissociation of two rhythms after unilateral severance of the optic nerve also provides evidence for the compound eye as the circadian photoreceptor (Tomioka, 1993).

The photoreceptors for the photic entrainment appear to be distributed over whole compound eye. Even after the dorsal, mid-, or ventral one-third of the compound eye was removed, the locomotor rhythm can synchronize with the light dark cycle (Tomioka et al., 1990). However, synchronizability to the light cycle depends on the number of ommatidia: the more ommatidia that survive the partial removal, the more quickly the rhythm synchronized to the light cycle (Tomioka et al., 1990). This suggests that the photic signals from those ommatidia are additively provided to the circadian clock to achieve effective synchronization.

Crickets have three ocelli but these play no apparent role in the photic entrainment of the rhythm in *G. bimaculatus*. Severance of three ocellar nerves had no clear effects on the photic entrainment (Yukizane and Tomioka, 1995). However, in *T. commodus* and *Acheta domestica*, the period of free-running stridulatory rhythms was slightly modulated under constant dim light by the severance of ocellar nerves. Ocelli were shown to modulate the light sensitivity of compound eyes that may result in the modulation of free-running period (Rence et al., 1988). Extra-retinal photoreceptors may be involved in *D. nigrofasciatus*. The rhythm remained to be entrained even after removal of the compound eyes and all ocelli (Shiga et al., 1999). Thus, the contribution of retinal and extra-retinal photoreceptors may differ among cricket species. In some species, all of the photoreceptors,

i.e. the compound eyes, ocelli and extra-retinal photoreceptors may contribute to photic entrainment, as has been shown for *Drosophila* (Helfrich-Förster et al., 2001).

Molecular oscillatory mechanism of the circadian clock

One of the most important and challenging questions is the identity of the molecular oscillatory mechanism of the clock. This has been extensively studied in *Drosophila* and is postulated to consist of rhythmic expression of so-called clock genes (Stanewsky, 2002; Hardin, 2009; Tomioka and Matsumoto, 2010). One major part of the clock is rhythmic expression of *period* (*per*) and *timeless* (*tim*) genes which is regulated by negative feedback of their product proteins PERIOD (PER) and TIMELESS (TIM). Their transcription is activated by transcription factors called CLOCK (CLK) and CYCLE (CYC). The CLK/CYC heterodimer binds to a promoter region, called E-box, located upstream region of *per* and *tim* and activates their transcription during late day to early night. The translation occurs during the night and the product proteins PER and TIM accumulate in the cytoplasm during the night. The PER and TIM form heterodimers and enter nucleus to suppress the transcriptional activity of CLK/CYC, reducing the *per* and *tim* transcripts, which results in a reduction of PER and TIM. The CLK/CYC thus restores its transcriptional activity, and the oscillatory loop moves to the next round.

Cricket's oscillatory mechanism: Central pacemaker

In the cricket *G. bimaculatus*, the clock machinery has been so far substantially clarified (Moriyama et al., 2008; Danbara et al., 2010; Moriyama et al., 2012; Uryu et al., 2013). It includes at least four clock genes, *per*, *tim*, *Clk*, and *cyc* (Fig. 3). These were rhythmically expressed in the optic lobe, except *Clk*. The mRNAs of *per* and *tim* peaked at early night and *cyc* transcripts did late at night. Basically similar expression patterns were reproduced in the constant dark conditions. RNAi based functional analyses revealed that they are involved in the circadian clock oscillatory mechanism (Fig. 3). The RNAi of *per* and *Clk* resulted in a loss of locomotor rhythm in constant darkness (Moriyama et al., 2008; Moriyama et al., 2012). In both cases, the level of respective mRNA was severely knocked down. In *Clk* RNAi crickets, the *per* and *tim* transcripts were downregulated (Moriyama et al., 2012). This is consistent with the *Drosophila* clock model, in which *Clk* is a transcriptional activator for *per* and *tim*.

The RNAi of *tim* or *cyc* genes never abolished the locomotor rhythm but changed the free-running period; *tim* RNAi shortened and *cyc* RNAi lengthened it in DD (Danbara et al., 2010; Uryu et al., 2013). Quantitative PCR revealed that in *tim* RNAi crickets, mRNA levels of both *tim* and *per* were knocked down and no significant rhythm was observed in LD. The mRNAs of *Clk* and *cyc* also stayed at lower levels and no significant rhythm was observed. Thus we assume an oscillatory loop that consists of a component other than those clock genes and regulates the locomotor rhythm (Fig. 3). In *cyc* RNAi crickets, the mRNA levels of both *per* and *tim* were downregulated (Uryu et al., 2013), being consistent with the *Drosophila* clock model hypothesizing that *cyc* is the transcriptional activator for these two genes (Fig. 3). Interestingly, *Clk*, *per* and *tim* were rhythmically expressed

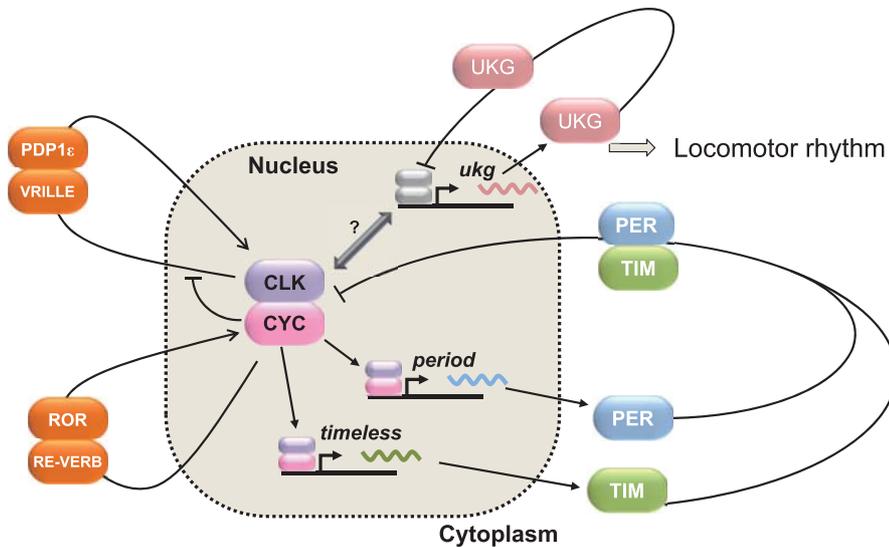


Fig. 3. A model of molecular oscillatory mechanism of the circadian clock in the cricket *Gryllus bimaculatus*. The clock genes *period* (*per*) and *timeless* (*tim*) form an oscillatory loop, in which their product proteins PER and TIM suppress the transcription factors CLOCK (CLK) and CYCLE (CYC). Negative feedback produces the rhythmic expression of *per* and *tim*. CYC is rhythmically expressed, and CLK is so in certain conditions, but the rhythmic expression of CLK may be normally concealed by abundant expression of CYC. We assume that their rhythmic expression is achieved by loops including ROR and RE-VERB orthologs, and PDP1 and VRILLE orthologs, respectively, in analogy to mammalian and *Drosophila* clock models. We also assume an existence of another oscillatory loop that consists of yet unknown gene (*ukg*) and regulates the locomotor rhythm since the rhythm persists even in the crickets with *tim* or *cyc* RNAi. The relationship between this loop and the PER/TIM loop remains to be elucidated. For further explanations see text.

in DD (Uryu et al., 2013). Thus, at least in *cyc* RNAi crickets, it seems plausible that *Clk* is rhythmically expressed by some yet unknown mechanism and regulates the rhythmic expression of *per* and *tim* (Fig. 3). In intact animals, the *Clk* oscillation may be concealed by abundant expression of *cyc*.

The translational control of this system has not been analyzed in the cricket but translation is apparently involved in the circadian rhythm. When chronically treated with translational inhibitor, cycloheximide (CHX), the circadian electrical activity rhythm of the optic lobe showed changes in a dose-dependent manner (Tomioka, 2000). Treatment with lower doses lengthened the free-running period in DD, while higher doses abolished the rhythm. The effect was observed not only for the rhythm of spontaneous electrical activity, but also for that of light-induced responses. Short-period treatment with CHX induced phase shifts of the circadian clock in the optic lobe in a phase-dependent manner: it induced phase advances during the late night to early morning but phase delays in the rest of the day (Tomioka, 2000). This suggests that there may be circadian regulation of the translation of different sets of proteins.

Peripheral oscillator

In crickets, many tissues outside the optic lobe show rhythmic expression of the clock genes like in *Drosophila* (Plautz et al., 1997; Uryu and Tomioka, 2010). This indicates that the tissues include the circadian clock, which may regulate their physiological function in a daily manner. How-

ever, their oscillations were found to depend on the central clock in the optic lobe to various degrees (Uryu and Tomioka, 2010). The oscillation in the brain and the terminal abdominal ganglion (TAG) persisted even after the optic lobes were removed. However, the phase of the oscillation shifted significantly. In the TAG, *per* lost its oscillatory expression but *tim* maintained it. In the mid-gut and anterior stomach, the rhythmic expression disappeared after the optic lobe removal. These findings suggest that the circadian oscillation of extra-optic lobe tissues depends on the central clock to various degrees in a tissue dependent manner. They also suggest that the molecular oscillatory mechanism in the extra-optic lobe tissues differs from that of the optic lobe. In *Drosophila*, some peripheral oscillators include *cryptochrome* (*cry*) as a core clock component which, however, serves only as a photoreceptor in the central clock (Emery et al., 1998; Stanewsky et al., 1998; Collins et al., 2006). The molecular oscillatory mechanism of the cricket's extra-optic lobe tissues and its regulation by the central clock should be addressed in future studies.

Circadian organization

Crickets have two circadian clocks, one in each optic lobe. The two optic lobe clocks are coupled to each other to produce a stable circadian locomotor rhythm. The existence of coupling was confirmed by an experiment with crickets in which a single optic nerve was severed. When placed in constant light conditions, two circadian components appeared to run with different free-running periods (Fig. 4A) (Tomioka et al., 1991). Similar results have been reported for another cricket species *T. commodus* (Wiedenmann, 1983). The two components are apparently controlled by the two circadian clocks, because either of the component disappeared when an optic lobe was surgically removed (Fig. 4A) (Tomioka et al., 1991). The mechanism of the interaction has been investigated by synchronizing the clock in the intact side with a light cycle longer or shorter than 24 hrs (Tomioka, 1993). Figure 4B exemplifies a result in LD12.5:12.5. The clock on the blind side showed a free-running rhythm of which period systematically changed depending on the phase of activity onset driven by the intact circadian clock. When the entrained component occurred in the subjective night the period of the blinded component was lengthened, while it was shortened when the other component occurred during the subjective day. In intact animals this should occur mutually, and this should be the mechanism for mutual synchronization. Another important point is that the two clocks inhibit the locomotor activity during their subjective day (Fig. 4B) so that the stable nocturnal rhythm

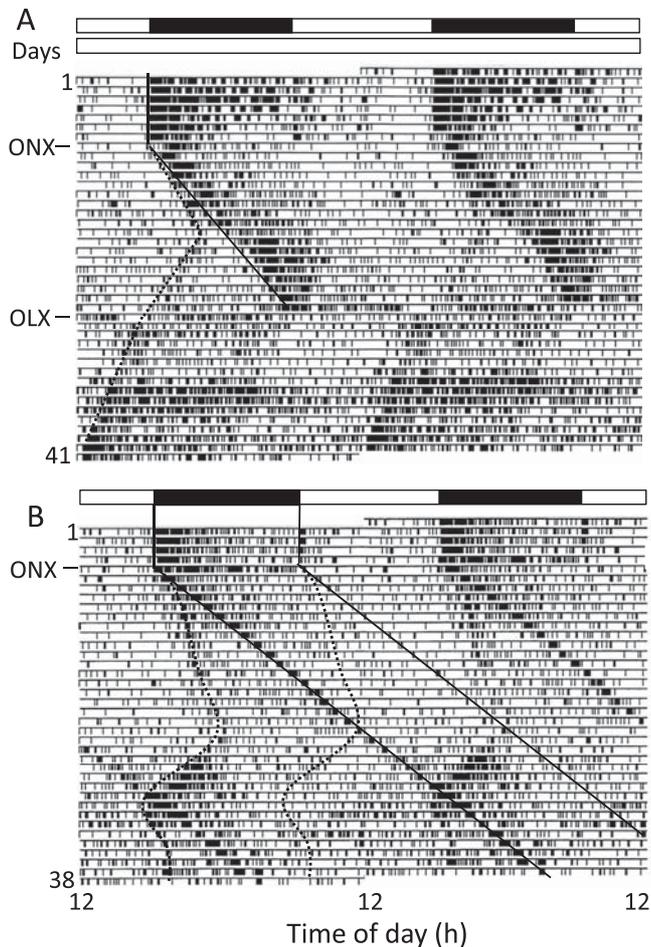


Fig. 4. Two examples of activity rhythms from crickets (*Gryllus bimaculatus*) that received unilateral optic nerve severance on the day (ONX) of transfer from LD12:12 to LL (**A**) or to LD12.5:12.5 (**B**). Both animals showed activity rhythm splitting into two rhythmic components in LL or LD12.5:12.5. In **A**, immediately after optic lobe removal on the intact side (OLX), a component running with a period longer than 24 h (solid line) disappeared and only a short period component (broken line) continued to run. In **B**, a component driven by intact side steadily entrained to the LD12.5:12.5 and another component driven by the blinded side ran free, but the free-running period changed as a function of the phase relationship between the two rhythmic components. This lengthened when the onset of the entrained component was in its subjective night, while shortened when it was in the subjective day. Activity levels of both components were reduced when they occurred in the subjective day of the other component and increased when they occurred in the subjective night. Solid lines indicate the dark phase and broken lines indicates the subjective night of free-running component. Panel **A** reproduced from Tomioka et al. (1991).

would be maintained (Tomioka et al., 1991).

This coupling is most likely mediated by a group of about 25 neurons, called medulla bilateral neurons (MBN), which are visually responding neurons and directly connect the bilateral medulla area of the optic lobe (Fig. 5B) (Yukizane and Tomioka, 1995). Their cell bodies are located in the ventral region of the optic lobe close to the accessory medulla and their dendritic fields cover various area of the medulla forming specific receptive fields in the compound

eye. They are roughly divided into four groups according to their receptive fields, i.e., anterosuperior, anteroinferior, posterosuperior, and posteroinferior area (Yukizane et al., 2002). Their responsiveness to light is regulated in a highly circadian manner, increasing during the night (Saifullah and Tomioka, 2002; Uemura and Tomioka, 2006). The circadian modulation of their responsiveness is regulated by two neurotransmitters, serotonin (5-HT) and pigment-dispersing factor (PDF). Serotonin suppresses the response during the day and PDF enhances it during the night. These effects have been confirmed by pharmacological experiments using serotonin, 5-HT antagonists and agonists, or PDF and PDF antibodies (Saifullah and Tomioka, 2002; Saifullah and Tomioka, 2003a; Saifullah and Tomioka, 2003b).

The role of MBNs in the coupling of the two clocks has been examined in a surgical lesion experiment. When the tract including the MBNs was abolished, the period modulation by coupling was totally disrupted and at the same time, the rhythmic component driven by the clock on the severed side disappeared (Yukizane and Tomioka, 1995). This suggests that the MBNs mediate the clock signal driving the locomotor rhythm, and indeed they have collaterals in the central brain region that may be the output area for this purpose (Yukizane et al., 2002). The MBNs that have a receptive field in the posterosuperior region are the most likely candidate for the period modulator, as destruction of this region of the compound eye abolished the period modulation (Tomioka and Yukizane, 1997). Again serotonin is the most likely neurotransmitter used in this coupling mechanism (Fig. 5A). The content of serotonin in the optic lobe showed a rhythmic profile peaking during the night (Tomioka et al., 1993). Serotonin treatment shifted the optic lobe circadian clock in vitro in a phase-dependent manner, inducing phase advance during the subjective day and phase delay during the subjective night (Tomioka, 1999). The phase response curve thus obtained is basically similar to that caused by the mutual entrainment. The pharmacological experiment also revealed that the different 5-HT receptors mediate the responses for the subjective night and day. 5-HT_{1A} like receptor mediates the response during the subjective night but the other type receptor for the subjective day (Tomioka, 1999).

Adaptation to seasonal changes

Photoperiodic modulation of locomotor rhythms

Crickets living in temperate zones are exposed to seasonal environmental changes and must adapt their daily and seasonal life to this seasonally changing environment. They show seasonal changes not only in their locomotor rhythms but also in efferent electrical activity rhythms in the optic lobe (Fig. 6). The nocturnally active cricket, *G. bimaculatus*, showed a modulation of activity rhythm of which duration of active phase changed dependent on the night length: This was longer in long night conditions (Tomioka and Chiba, 1989b, a; Koga et al., 2005). The modulated rhythm persisted in the ensuing DD for long period and the strength of modulation depended on the number of cycles exposed to a particular photoperiodic condition. Even a single exposure had a significant effect, but maximal effect occurred on exposure to 10 cycles, suggesting that there is a system accumulating the effect of each exposure (Koga et al.,

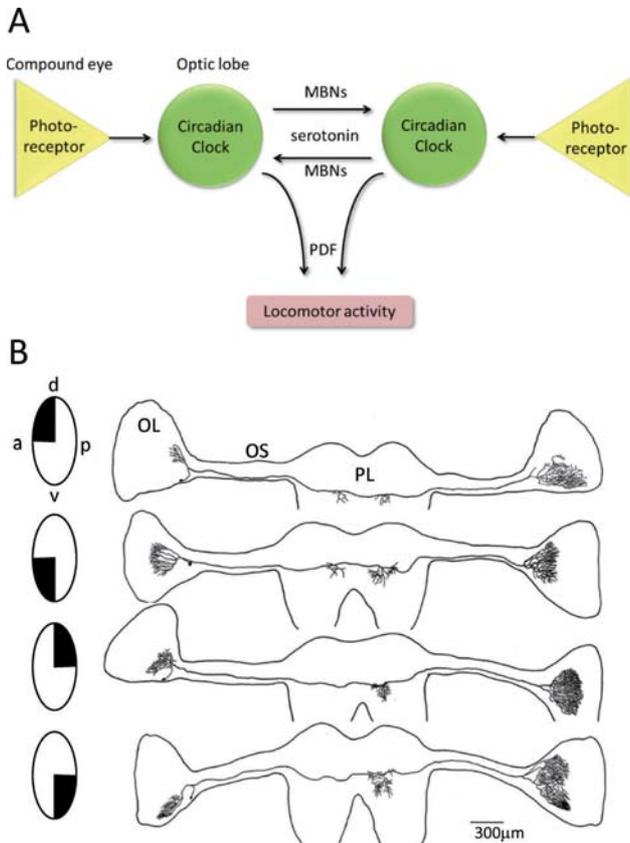


Fig. 5. The multi-clock system in the cricket, *Gryllus bimaculatus* (**A**) and the putative coupling mediator, the medulla bilateral neurons (**B**). (**A**) The two optic lobe circadian clocks are coupled to each other to regulate the overt activity rhythm. The coupling is most likely mediated by the medulla bilateral neurons (MBNs) and serotonin is used as the neurotransmitter. PDF is a candidate of output signal molecule regulating the locomotor activity. For further explanations see text. (**B**) The MBNs can be classified into four types according to their receptive field and morphology. Their dendritic fields roughly correspond to the receptive fields and their axon projects to the contralateral optic lobe. Ellipses on the left indicate the receptive field (black area). a, anterior; p, posterior; d, dorsal; v, ventral; OL, optic lobe; OS, optic stalk; PL, protocerebral lobe. Panel **B** reproduced from Yukizane et al. (2002).

2005). The effect can be detected in the efferent neuronal activity of the optic lobe. The duration of subjective night apparently correlated with the given night length of the 24 h photoperiod (Tomioka and Chiba, 1989a; Koga et al., 2005).

The circadian waveform is determined by mutual interaction between the two optic lobe clocks. After removal of a single optic lobe, the duration of subjective night became significantly longer than that of intact crickets (Koga et al., 2005). This suggests that the interaction is required to optimize the waveform modulation and/or to maintain the modulated waveform. When the optic nerve was unilaterally severed and the intact eye was exposed to a short night photoperiod, the optic lobes of both blind and intact side showed an intermediate length of subjective night between crickets kept in short night and DD conditions (Koga et al., 2005). Thus, the mutual interaction of the two optic lobe clocks shown in Fig. 4B is involved in determination of the circadian waveform.

Photoperiodic time-measurement

The circadian clock is also involved in the photoperiodic time measurement in the cricket (Fig. 6). The cricket, *Modicogryllus siamensis*, shows clear photoperiodic responses in its nymphal development (Taniguchi and Tomioka, 2003). In long-day conditions, the crickets become adult about 50 days after hatching undergoing seven moultings. In short-day conditions, however, they take longer periods to become adult with an increased number of moultings. The sensitive period for the photoperiodic response is limited to the first two instar periods, i.e. about 10 days after hatching.

The photoreceptors involved in the response include the compound eye (Fig. 6). When the compound eye was surgically removed, the long-day response was disrupted to a certain degree (Sakamoto and Tomioka, 2007). The importance of the compound eye has been confirmed by the results of molecular experiments (Tamaki et al., 2013). There are three opsins, opsin-UV, opsin-Blue, and opsin-Long Wave, expressed in the compound eye. RNAi of these opsin genes prohibited the long-day response at least partially.

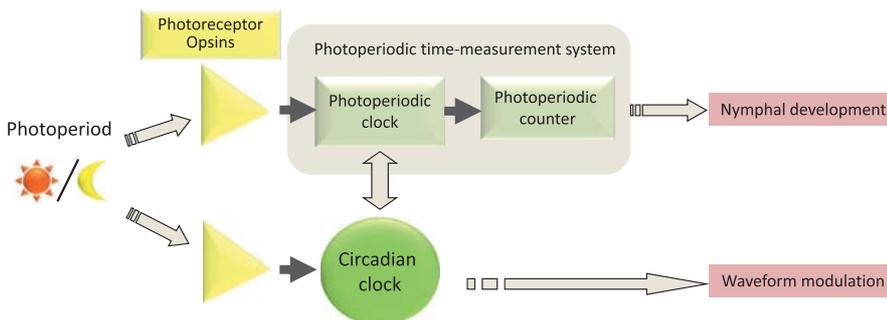


Fig. 6. A hypothesis for seasonal adaptation of the cricket timing system. In the cricket *Modicogryllus siamensis*, the time course of nymphal development is highly dependent on the photoperiod detected by the opsins expressed in the compound eye. The photoperiodic clock that measures day length includes the circadian clock, since disruption of its oscillation by clock gene's RNAi abolished the photoperiodic responses. The circadian clock in *Gryllus bimaculatus*, on the other hand, changes its output waveform, expressed in locomotor rhythms and electrical activity rhythms in the optic lobe, in response to a given photoperiod. The magnitude of the change depends on the number of cycles exposed to the photoperiod. For further explanations see text.

Most important and challenging issue is the mechanism underlying the photoperiodic time measurement. There are several hypothesis for the mechanism incorporating the circadian clock (Goto, 2013). One notable hypothesis is the external coincidence model that assumes a photoinducible phase in the circadian clock. When the photoinducible phase is exposed to light, the animal produces a long-day response, while, if it is in the dark, the animal shows a short-day response. By disrupting the circadian clock with *per* RNAi, we have tested the role of the circadian clock in the cricket's photoperiodism. We first examined the effect of *per* RNAi on the locomotor rhythm in *M. siamensis* and confirmed that the RNAi resulted

in a reduction of *per* mRNA levels and disrupted the circadian locomotor rhythm (Sakamoto et al., 2009). For nymphal treatment with RNAi, the parental RNAi was employed: *per* dsRNA was injected into the adult female and the nymphal development of her progeny was examined. The treated nymphs showed a pattern of adult emergence under long-day or short-day conditions that resembled the pattern neither of long-day nor short-day conditions, but had close resemblance to that in constant darkness (Sakamoto et al., 2009), which clearly suggests that the circadian clock is a prerequisite for photoperiodic time measurement in this species. Importance of the circadian clock has been stressed by subsequent studies used different insect species (Ikeno et al., 2010; Ikeno et al., 2011). However, the cricket would be an excellent model for the molecular study of photoperiodic time measurement because, as mentioned above, it shows clear photoperiodic responses and clear behavioral rhythms as a gauge of the circadian clock, and its clock machinery as well as photoperiodic receptors has been studied.

Future perspectives

Recent progress of physiological and molecular studies substantially promoted our understanding of the cellular and molecular mechanisms underlying the insect circadian systems. In the cricket, we have shown that basically similar molecular constituents are involved in the clock machinery and some of them play a role similar to those in *Drosophila* clock but some may play different roles. Of particular interest is the mechanism through which the clock maintains its oscillation even after treated with *tim* or *cyc* dsRNA (Danbara et al., 2010; Uryu et al., 2013). Interestingly, *Clk* can oscillate in certain conditions (Uryu et al., 2013), thus crickets have both *Drosophila*-type and mammalian-type oscillatory components. Investigation of the two components may lead to understanding the diversification of insect clocks.

An important question remains to be answered is how the molecular oscillation is reflected in behavioral rhythms. The output pathway controlling overt rhythms also remains to be explicated. Cerebral PDF neurons are apparently involved in the clock's output pathway, because RNAi of *pdf* gene reduced the nocturnal activity with reduced amount of PDF (Hassaneen et al., 2011). PDF is more strongly involved in control of overt activity rhythms in *Drosophila* and cockroaches. The fruit fly mutant lacking PDF and the cockroaches treated with *pdf* RNAi both lost their locomotor rhythms in constant darkness (Renn et al., 1999; Lee et al., 2009). However, *pdf* RNAi never cause arrhythmicity in the cricket, suggesting existence of another pathway that controls behavioral rhythms. This view is also consistent with our earlier finding that partial removal of the optic lobe resulted in a loss of locomotor rhythms without ablation of PDF neurons (Okamoto et al., 2001). Humoral factors secreted by cerebral neurosecretory cells may be involved, as destruction of the pars intercerebralis containing those cells lead to a loss of locomotor rhythm and the brain transplantation restored the rhythm in *Acheta domestica* (Cymborowski, 1981).

The resetting mechanism of the clock is another important issue in insects. The CRY pathway has been extensively studied in *Drosophila*, while other pathways including the external photoreceptors are not well understood. The

cricket is a good model for addressing this issue as its circadian photoreceptor is found within the compound eyes. Recently, photoreceptor molecules in the compound eye have been identified in *G. bimaculatus* (Henze et al., 2012). Thus the role of each molecule may be investigated in the near future.

The most challenging issue is the role of circadian clock in seasonal adaptation. The clock plays a central role in photoperiodic control of development and physiology (Koga et al., 2005; Sakamoto et al., 2009). Since considerable progress has been made in our knowledge on the molecular oscillatory mechanism in the clock, the mechanism of the clock's response to seasonally changing photoperiod may be studied at a molecular level. The study will promote our understanding on the insect photoperiodism, which remains largely mysterious.

Genomic sequencing and transcriptome using next generation DNA sequencers are now in progress in some cricket species (Danley et al., 2007) and will provide useful information on genes related to the circadian and photoperiodic clocks. In addition to the accumulating genomic information, analysis with modern technology including gene silencing, knockout, and knock-in will promote studies on above mentioned issues at a molecular level. Thus the cricket is an important and promising model animal for the study on the internal timing mechanism and its role in adaptation to daily and seasonally changing environment.

ACKNOWLEDGEMENTS

The author thanks Dr. Akira Matsumoto of Juntendo University for critically reading an earlier version of the manuscript. He also thanks two anonymous reviewers for their comments on the manuscript. This work was supported in part by grants from JSPS.

REFERENCES

- Abe Y, Ushirogawa H, Tomioka K (1997) Circadian locomotor rhythm of the cricket *Grylloides sigillatus*. I. Localization of the pacemaker and the photoreceptor. *Zool Sci* 14: 719–727
- Collins B, Massoni EO, Stanewsky R, Blau J (2006) *Drosophila* CRYPTOCHROME is a circadian transcriptional repressor. *Curr Biol* 16: 441–449
- Cymborowski B (1981) Transplantation of circadian pacemaker in the house cricket, *Acheta domestica* L. *J Interdiscipl Cycle Res* 12: 133–140
- Danbara Y, Sakamoto T, Uryu O, Tomioka K (2010) RNA interference of *timeless* gene does not disrupt circadian locomotor rhythms in the cricket *Gryllus bimaculatus*. *J Insect Physiol* 56: 1738–1745
- Danley PD, Mullen SP, Liu F, Nene V, Quackenbush J, Shaw KL (2007) A cricket gene index: a genomic resource for studying neurobiology, speciation, and molecular evolution. *BMC Genomics* 8: 109
- Diwakar S, Balakrishnan R (2007) The assemblage of acoustically communicating crickets of a tropical evergreen forest in southern India: call diversity and diel calling patterns. *Bioacoustics* 16: 113–135
- Emery P, So WV, Kaneko M, Hall JC, Rosbash M (1998) CRY, a *Drosophila* clock and light-regulated cryptochrome, is a major contributor to circadian rhythm resetting and photosensitivity. *Cell* 95: 669–679
- Goto SG (2013) Roles of circadian clock genes in insect photoperiodism. *Entomol Sci* 16: 1–16
- Hardin P (2009) Molecular mechanisms of circadian timekeeping in *Drosophila*. *Sleep Biol Rhythms* 7: 235–242

- Hassaneen E, Sallam A, Abo-Ghalia A, Moriyama Y, Karpova S, Abdelsalam S, Matsushima A, Shimohigashi Y, Tomioka K (2011) Pigment-dispersing factor affects nocturnal activity rhythms, photic entrainment and the free-running period of the circadian clock in the cricket *Gryllus bimaculatus*. *J Biol Rhythms* 26: 3–13
- Helfrich-Förster C, Winter C, Hofbauer A, Hall JC, Stanewsky R (2001) The circadian clock of fruit flies is blind after elimination of all known photoreceptors. *Neuron* 30: 149–261
- Henze MJ, Dannenhauer K, Kohler M, Labhart T, Gesemann M (2012) Opsin evolution and expression in arthropod compound eyes and ocelli: insights from the cricket *Gryllus bimaculatus*. *BMC Evol Biol* 12: 163
- Ikeno T, Tanaka SI, Numata H, Goto SG (2010) Photoperiodic diapause under the control of circadian clock genes in an insect. *BMC Biol* 8: 116
- Ikeno T, Numata H, Goto SG (2011) Circadian clock genes period and cycle regulate photoperiodic diapause in the bean bug *Riptortus pedestris* males. *J Insect Physiol* 57: 935–938
- Koga M, Ushirogawa H, Tomioka K (2005) Photoperiodic modulation of circadian rhythms in the cricket *Gryllus bimaculatus*. *J Insect Physiol* 51: 681–690
- Lee C-M, Su M-T, Lee H-J (2009) Pigment dispersing factor: an output regulator of the circadian clock in the german cockroach. *J Biol Rhythms* 24: 35–43
- Loher W (1972) Circadian control of stridulation in the cricket *Teleogryllus commodus* Walker. *J Comp Physiol* 79: 173–190
- Loher W (1974) Circadian control of spermatophore formation in the cricket *Teleogryllus commodus* Walker. *J Insect Physiol* 20: 1155–1172
- Loher W (1979) Circadian rhythmicity of locomotor behavior and oviposition in female *Teleogryllus commodus*. *Behav Ecol Sociobiol* 5: 253–262
- Mito T, Nakamura T, Bando T, Ohuchi H, Noji S (2011) The advent of RNA interference in entomology. *Entomol Sci* 14: 1–8
- Moriyama Y, Sakamoto T, Karpova SG, Matsumoto A, Noji S, Tomioka K (2008) RNA interference of the clock gene *period* disrupts circadian rhythms in the cricket *Gryllus bimaculatus*. *J Biol Rhythms* 23: 308–318
- Moriyama Y, Kamae Y, Uryu O, Tomioka K (2012) *Gb'Clock* is expressed in the optic lobe and required for the circadian clock in the cricket *Gryllus bimaculatus*. *J Biol Rhythms* 27: 467–477
- Nakatani I, Adachi T, Murayama O (1994) Selection of light or darkness, locomotor, and stridulatory activities in the cricket, *Gryllus bimaculatus* Degeer (Orthoptera: Gryllidae). *J Insect Physiol* 40: 1007–1015
- Okamoto A, Mori H, Tomioka K (2001) The role of optic lobe in generation of circadian rhythms with special reference to the PDH immunoreactive neurons. *J Insect Physiol* 47: 889–895
- Page TL (1982) Transplantation of the cockroach circadian pacemaker. *Science* 216: 73–75
- Plautz JD, Kaneko M, Hall JC, Kay SA (1997) Independent photoreceptive circadian clocks throughout *Drosophila*. *Science* 278: 1632–1635
- Rence BG, Loher W (1975) Arrhythmically singing crickets: thermoperiodic reentrainment after bilobectomy. *Science* 190: 385–387
- Rence BG, Lisy MT, Garves BR, Quilan BJ (1988) The role of ocelli in circadian singing rhythms of crickets. *Physiol Entomol* 13: 201–212
- Renn SCP, Park JH, Rosbash M, Hall JC, Taghert PH (1999) A *pdf* neuropeptide gene mutation and ablation of PDF neurons each cause severe abnormalities of behavioral circadian rhythms in *Drosophila*. *Cell* 99: 791–802
- Reppert SM, Gegear RJ, Merlin C (2010) Navigational mechanisms of migrating monarch butterflies. *Trends Neurosci* 33: 399–408
- Römer H, Bailey W, Dadour I (1989) Insect hearing in the field. III. Masking by noise. *J Comp Physiol A* 164: 609–620
- Saifullah ASM, Tomioka K (2002) Serotonin sets the day state in the neurons that control coupling between the optic lobe circadian pacemakers in the cricket, *Gryllus bimaculatus*. *J Exp Biol* 205: 1305–1314
- Saifullah ASM, Tomioka K (2003a) 5-HT₇ like receptors mediate serotonergic modulation of photo-responsiveness of the medulla bilateral neurons in the cricket, *Gryllus bimaculatus*. *Zool Sci* 20: 303–309
- Saifullah ASM, Tomioka K (2003b) Pigment-dispersing factor sets the night state of the medulla bilateral neurons in the optic lobe of the cricket, *Gryllus bimaculatus*. *J Insect Physiol* 49: 231–239
- Sakamoto T, Tomioka K (2007) Effects of unilateral compound eye removal on the photoperiodic responses of nymphal development in the cricket *Modicogryllus siamensis*. *Zool Sci* 24: 604–610
- Sakamoto T, Uryu O, Tomioka K (2009) The clock gene *period* plays an essential role in photoperiodic control of nymphal development in the cricket *Modicogryllus siamensis*. *J Biol Rhythms* 24: 379–390
- Sakura M, Takasuga K, Watanabe M, Eguchi E (2003) Diurnal and circadian rhythm in compound eye of cricket (*Gryllus bimaculatus*): changes in structure and photon capture efficiency. *Zool Sci* 20: 833–840
- Saunders DS (2002) *Insect Clocks* 3rd Edn. Elsevier, Amsterdam
- Shiga S, Numata H, Yoshioka E (1999) Localization of the photoreceptor and pacemaker for the circadian activity rhythm in the band-legged ground cricket, *Dianemobius nigrofasciatus*. *Zool Sci* 16: 193–201
- Sokolove PG (1975) Locomotory and stridulatory circadian rhythms in the cricket, *Teleogryllus commodus*. *J Insect Physiol* 21: 537–558
- Sokolove PG, Loher W (1975) Role of the eyes, optic lobes and pars intercerebralis in locomotory and stridulatory circadian rhythms of *Teleogryllus commodus*. *J Insect Physiol* 21: 785–799
- Stanewsky R (2002) Clock mechanisms in *Drosophila*. *Cell Tissue Res* 309: 11–26
- Stanewsky R, Kaneko M, Emery P, Beretta B, Wager-Smith K, Kay SA, et al. (1998) The *cry^b* mutation identifies cryptochrome as a circadian photoreceptor in *Drosophila*. *Cell* 95: 681–692
- Tamaki S, Takemoto S, Uryu O, Kamae Y, Tomioka K (2013) Opsins are involved in nymphal photoperiodic responses in the cricket *Modicogryllus siamensis*. *Physiol Entomol* 38: 163–172
- Taniguchi N, Tomioka K (2003) Duration of development and number of nymphal instars are differentially regulated by photoperiod in the cricket *Modicogryllus siamensis* (Orthoptera: Gryllidae). *Eur J Entomol* 100: 275–281
- Tauber TJ, Tauber CA, Masaki S (1986) *Seasonal Adaptations of Insects*. Oxford University Press, New York
- Tomioka K (1985a) Optic lobe-compound eye system in cricket: a complete circadian system. *J Interdiscipl Cycle Res* 16: 73–76
- Tomioka K (1985b) Residual circadian rhythmicity after bilateral lamina-medulla removal or optic stalk transection in the cricket, *Gryllus bimaculatus*. *J Insect Physiol* 31: 653–657
- Tomioka K (1993) Analysis of coupling between optic lobe circadian pacemakers in the cricket *Gryllus bimaculatus*. *J Comp Physiol A* 172: 401–408
- Tomioka K (1999) Light and serotonin phase-shift the circadian clock in the cricket optic lobe in vitro. *J Comp Physiol A* 185: 437–444
- Tomioka K (2000) Protein synthesis is a required process for the optic lobe circadian clock in the cricket *Gryllus bimaculatus*. *J Insect Physiol* 46: 281–287
- Tomioka K, Abdelsalam SA (2004) Circadian organization in hemimetabolous insects. *Zool Sci* 21: 1153–1162

- Tomioka K, Chiba Y (1982a) Persistence of circadian ERG rhythms in the cricket with optic tract severed. *Naturwissenschaften* 69: 355–356
- Tomioka K, Chiba Y (1982b) Post-embryonic development of circadian rhythm in the cricket, *Gryllus bimaculatus*. *J Comp Physiol A* 147: 299–304
- Tomioka K, Chiba Y (1984) Effects of nymphal stage optic nerve severance or optic lobe removal on the circadian locomotor rhythm of the cricket, *Gryllus bimaculatus*. *Zool Sci* 1: 385–394
- Tomioka K, Chiba Y (1986) Circadian rhythms in the neurally isolated lamina-medulla complex of the cricket, *Gryllus bimaculatus*. *J Insect Physiol* 32: 747–755
- Tomioka K, Chiba Y (1989a) Light cycle during post-embryonic development affects adult circadian parameters of the cricket (*Gryllus bimaculatus*) optic lobe pacemaker. *J Insect Physiol* 35: 273–276
- Tomioka K, Chiba Y (1989b) Photoperiod during post-embryonic development affects some parameters of adult circadian rhythm in the cricket, *Gryllus bimaculatus*. *Zool Sci* 6: 565–571
- Tomioka K, Chiba Y (1989c) Photoperiodic entrainment of locomotor activity in crickets (*Gryllus bimaculatus*) lacking the optic lobe pacemaker. *J Insect Physiol* 35: 827–835
- Tomioka K, Chiba Y (1992) Characterization of optic lobe circadian pacemaker by in situ and in vitro recording of neuronal activity in the cricket *Gryllus bimaculatus*. *J Comp Physiol A* 171: 1–7
- Tomioka K, Matsumoto A (2010) A comparative view of insect circadian clocks. *Cell Mol Life Sci* 67: 1397–1406
- Tomioka K, Yukizane M (1997) A specific area of the compound eye in the cricket *Gryllus bimaculatus* sends photic information to the circadian pacemaker in the contralateral optic lobe. *J Comp Physiol A* 180: 63–70
- Tomioka K, Okada Y, Chiba Y (1990) Distribution of circadian photoreceptors in the compound eye of the cricket *Gryllus bimaculatus*. *J Biol Rhythms* 5: 131–139
- Tomioka K, Yamada K, Yokoyama S, Chiba Y (1991) Mutual interactions between optic lobe circadian pacemakers in the cricket *Gryllus bimaculatus*. *J Comp Physiol A* 169: 291–298
- Tomioka K, Ikeda M, Nagao T, Tamotsu S (1993) Involvement of serotonin in the circadian rhythm of an insect visual system. *Naturwissenschaften* 80: 137–139
- Tomioka K, Sakamoto T, Moriyama Y (2009) RNA interference is a powerful tool for chronobiological study in the cricket. *Sleep Biol Rhythms* 7: 144–151
- Uemura H, Tomioka K (2006) Postembryonic changes in circadian photo-responsiveness rhythms of optic lobe interneurons in the cricket *Gryllus bimaculatus*. *J Biol Rhythms* 21: 1–11
- Uryu O, Tomioka K (2010) Circadian oscillations outside the optic lobe in the cricket *Gryllus bimaculatus*. *J Insect Physiol* 56: 1284–1290
- Uryu O, Tomioka K (2014) Post-embryonic development of the circadian oscillations within and outside the optic lobe in the cricket, *Gryllus bimaculatus*. *Zool Sci* 31: 237–243
- Uryu O, Karpova SG, Tomioka K (2013) The clock gene *cycle* plays an important role in the circadian clock of the cricket *Gryllus bimaculatus*. *J Insect Physiol* 59: 697–704
- Waddell B, Lewis RD, Engelmann W (1990) Localization of the circadian pacemakers of *Hemideina thoracica* (Orthoptera; Stenopelmatidae). *J Biol Rhythms* 5: 131–139
- Wiedenmann G (1983) Splitting in a circadian activity rhythm: the expression of bilaterally paired oscillators. *J Comp Physiol* 150: 51–60
- Yukizane M, Tomioka K (1995) Neural pathways involved in mutual interactions between optic lobe circadian pacemakers in the cricket *Gryllus bimaculatus*. *J Comp Physiol A* 176: 601–610
- Yukizane M, Kaneko A, Tomioka K (2002) Electrophysiological and morphological characterization of the medulla bilateral neurons that connect bilateral optic lobes in the cricket, *Gryllus bimaculatus*. *J Insect Physiol* 48: 631–641

(Received January 30, 2014 / Accepted February 28, 2014)