**Symposium:**
**Physiological mechanisms underlying environmental adaptation in insects**
シンポジウム「昆虫の環境適応の生理学」

アメリカ・オハイオ州立大学の David L. Denlinger 博士をお迎えし，昆虫の環境適応にかかわる生理学的なしくみ，特にカの休眠を制御する分子生物学的機構についてご講演いただきます．また，その後，学生・ポスドク有志による発表も行います．ふるってご参加くださいますよう，よろしくお願いします．

主催：大阪市立大学大学院理学研究科情報生物学研究室
共催：日本応用動物昆虫学会

2013 年 3 月 30 日（土）
大阪市立大学　学術情報総合センター 1F　文化交流室
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14:45-14:50　Shin G. Goto (Osaka City University, Japan)
**Opening remarks**

14:50-15:35　David L. Denlinger (Ohio State University, USA)
**The long sleep of winter: a role for insulin signaling in mosquito diapause**

Break (15:35-15:45)

15:45-16:00　Masatoshi Nagata (Osaka City University, JAPAN)
**The effect of RNAi of *Clock* on circadian rhythm and photoperiodism in the band-legged ground cricket *Dianemobius nigrofasciatus***

16:00-16:15　Qiushi Wang (Kobe University, JAPAN)
**Serotonin receptor B may lock the gate of PTTH release in the Chinese silk moth, *Antheraea pernyi***

16:15-16:30　Ahmed A. M. Mohamed (Kobe University, JAPAN)
***N*-acetyltransferase (*nat*) is a critical conjunct of photoperiodism in *Antheraea pernyi* with circadian system and endocrine axis**

Break (16:30-16:40)

16:40-16:55 Yukirou Murai (Kyoto University, JAPAN)

**Quantitative analysis of singing time in two Japanese cicadas, *Graptopsaltria nigrofuscata* and *Cryptotympana facialis***

16:55-17:15 Satoshi Yamamoto (Kyoto University, JAPAN)

**Insipient speciation of winter moth: a consequence of life cycle adaptation to seasonal environmental rhythm**

17:45-19:45 Party at Sugimoto or Abiko (To be announced)

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## The long sleep of winter:
## a role for insulin signaling in mosquito diapause

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The shortening days and lower temperatures of late summer and early autumn are widely used as environmental cues to program an overwintering developmental arrest (diapause) in insects. This presentation will present a quick overview of insect diapause and then focus on the overwintering reproductive diapause of the mosquito *Culex pipiens.* Mosquitoes programmed for diapause are not attracted to vertebrate hosts and instead feed extensively on nectar sources that fuel an impressive accumulation of lipid stores. Insulin signaling appears to be a key regulatory pathway leading to the diapause phenotype. Current experiments are focused on segments of the pathway leading from the use of clock genes to monitor daylength through to the signaling pathways responsible for the enhanced stress resistance, increased longevity, accumulation of fat reserves, and other features that are linked to the diapause phenotype. RNA interference (RNAi) works well in this system and has been the primary tool we have used for identifying the regulatory components involved in the diapause response.

## The effect of RNAi of *Clock* on circadian rhythm and photoperiodism in the band-legged ground cricket *Dianemobius nigrofasciatus*

Masatoshi Nagata and Shin G. Goto
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A photoperiodic response is crucial for determining various developmental programs, such as diapause, growth and seasonal morphs. Although involvement of a circadian clock in the photoperiodic response has long been suggested, it has yet been verified at the molecular level. Here, we approach this issue by using RNA interference (RNAi) in the band-legged ground cricket *Dianemobius nigrofasciatus* which shows a clear photoperiodic response and a distinct circadian activity rhythm. At first, we focused on the circadian clock controlling the activity rhythm. The activity rhythm of intact crickets free-ran under constant darkness (DD) and entrained to light-dark cycles of LD 12:12. When crickets were injected with double-stranded RNA (dsRNA) of *Clock* (*Clk*), one of the circadian clock genes, and transferred to DD, most crickets showed clear rhythmicity in their activity for 5-8 days, but later the rhythmicity disappeared. When the dsRNA-treated crickets were transferred from DD to LD 12:12 subsequently, however, clear activity rhythm appeared. Next, we investigated the effect of RNAi of *Clk* on the photoperiodic response. When crickets were reared under short-day conditions (LD 12:12) and then transfered to long-day conditions (LD 16:8) upon adult emergence, the adults laid diapause eggs for 12-15 days, but later they laid nondiapause eggs. On the other hand, crickets maintained under short-day conditions laid diapause eggs continuously. When crickets reared under short-day conditions were injected with *Clk* dsRNA on the day of adult emergence and maintained under the same photoperiodic conditions, they showed unique response, i.e., they laid nondiapause eggs first, later they turned to lay diapause eggs, and finally they laid nondiapause eggs again. We will discuss the roles of *Clk* gene in the photoperiodic response.

## Serotonin receptor B may lock the gate of PTTH release in the Chinese silk moth, *Antheraea pernyi*

Qiushi Wang, Ahmed A. M. Mohamed, Makio Takeda
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The release of prothoracicotropic hormone, PTTH, is the major endocrine switch for photoperiodism regulating pupal diapause of the Chinese silk moth, *Antheraea pernyi*. Serotonin receptor A (5-HTR$_A$)-, serotonin receptor B (5-HTR$_B$) and PTTH like immunohistochemical reactivities (-ir) coexisted in two pairs of neurosecretory cells at the dorsolateral region of the protocerebrum (DL). 5-HTR$_B$- and eclosion hormone (EH)-ir co-exist in the same cells at deutocerebrum (DC) in *A. pernyi*, suggesting that 5-HTRB is not only involved in the release PTTH but also involved the EH release. Under LD 16:8, mRNA level of serotonin receptor (5-HTR) decreased, the level after 10 days was lower than 0 and 5 cycles. When pupae were injected with both luzindole an antagonist of melatonin receptor, and 5-HT, the termination of diapause occurred later than normal pupae. dsRNA$^{5\text{-HTRB}}$ were injected into pupa under LD 16:8, diapause. However, when dsRNA$^{5\text{-HTRA}}$ were injected into pupae, diapause termination occurred earlier than the untreated as normal pupae. Under LD 12:12, after injection of 5, 7-DHT, a serotonin antagonist, pupae failed to stay in diapause. The results strongly suggest that 5-HTR$_B$ functions to keep diapause from termination.

## N-acetyltransferase (nat) is a critical conjunct of photoperiodism in *Antheraea pernyi* with circadian system and endocrine axis

Ahmed A. M. Mohamed[a,*], Wang Qiushi[a,*], Jadwiga Bembenek[b,*], Takeshi Suzuki[b], Taketo Tsugehara[b], Susumu Hiragaki[b], Naoyuki Ichihara[b,✝] and Makio Takeda[a,b]

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The localization of immunohistochemical reactivity to antisera against clock gene products was described in the brain of *Antheraea pernyi*. PER-, DBT-, Clk- and Cyc-like antigens were expressed in the same cells at dorsolateral protocerebrum in A. pernyi, suggesting that these neurons are the central circadian neurons of this species. Melatonin, NAT- and HIOMT- like antigens coexisted in these cells, suggesting that the indolamine pathway is a cogwheel of circadian output pathway. Also, melatonin receptor-like reactivity was demonstrated in PTTH producing neurons, suggesting that melatonin is involved in the gating of PTTH release. Melatonin content showed a day/night fluctuation with a peak at night under both LD16:8 and LD12:12 but the baseline level was higher under LD 16:8 than under LD 12:12, a photoperiodic influence. Along diapause termination by either the exposure to LD16:8, 25oC or to 4oC, NAT activity increased: ten cycles of long-day exposure or 6 month chilling turns on the ecdysone release. The promoter region of *nat* gene contained E-boxes to that Cyc/Clk heterodimer binds since NAT transcription was turned off when Clk or Cyc dsRNA was injected. RNA-i against *nat* under LD 16:8 dysfunctioned photoperiodic activation of diapause. Transcriptional profiles of *nat* and *clk* had the same phase. The results strongly suggest that *nat* serves as a critical link between circadian clock and endocrine switch. In conclusion, photoperiodism involves transcriptional regulation of *nat* based on the circadian clock that supports endocrine switch to release or not to release PTTH. This system contains all the basic functional units, i.e., clock, photoperiodic counter, and endocrine switch in photoperiodism regulating pupal diapause in *A. pernyi*.

## Quantitative analysis of singing time in two Japanese cicadas, *Graptopsaltria nigrofuscata* and *Cryptotympana facialis*

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It is widely accepted that different cicada species sing at different times of the day. To quantitatively analyze their singing time, we obtained continuous recordings in a forest in Oji-town, Nara-prefecture, Japan, which captured multispecific cicada songs. We then input the recordings to a computer program to extract singlespecific volume sequences for *Graptopsaltria nigrofuscata* and *Cryptotympana facialis*.

The results showed that *C. facialis* sang from 06:00 to 11:00, a timeframe from which *G. nigrofuscata* seemed to schedule its singing time away, resulting in a virtual lack of overlapping singing times between the two species. We also found that the beginning and the end of *G. nigrofuscata* singing time transitioned according to the daily changes in sunrise and sunset time, respectively. In *C. facialis*, the sound volume showed a sharp increase at the beginning of the singing time, but a fluctuating and gradual decrease at the end of the singing time.

The results agreed with a hypothesis that the significant loudness of the songs of *C. facialis* may cause *G. nigrofuscata* to avoid singing simultaneously with *C. facialis*, which, if proven, may partly explain a marked decrease of *G. nigrofuscata* populations and increase of *C. facialis* recently taking place in southeast Japan.

## Insipient speciation of winter moth: a consequence of life cycle adaptation to seasonal environmental rhythm

Satoshi Yamamoto
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Insects have the adaptive life-cycle schedule to the seasonal environmental rhythm of their habitats: each insect has specific timings of hatching, growth, and reproduction of year. The adaptive evolution of insect's life cycle provides with the chance of not only physiological studies but also ecological and evolutionary studies.

Adults of winter moths emerge and reproduce during winter (cool season). The environmental conditions of winter (e.g. length of cool season, lowest temperature) vary among habitats. Therefore, each population of winter moth adapts to winter conditions of their habitats. For example, the winter moth species emerge in mid winter (during coldest period) in the habitats with mild winter condition (short cool season and mild temperature) while they emerge either in early or late of winter in the habitat with harsh winter condition. Emergence timings in harsh habitat are usually fixed by species. However, a Japanese winter moth, *Inurois punctigera*, emerges and reproduces in both early and late winter in harsh habitat although they emerge and reproduce in mid winter in mild habitat. This situation would suggest that winter harshness disrupts the reproductive period of *I. punctigera*.

I examined the genetic isolation between the early and late populations of *I. punctigera*, and geographic pattern of their evolution by molecular population genetics and phylogeographic analyses. As a result, we revealed the reproductive isolation even between sympatric seasonal populations and the differentiations of life cycle have taken place in distant regions in parallel, suggesting that winter harshness plays import role in the differentiation and speciation between early- and late-winter *I. punctigera*.