# Photoperiodic and Food Signals Control Expression Pattern of the Clock Gene, Period, in the Linden Bug, Pyrrhocoris apterus

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# Photoperiodic and Food Signals Control Expression Pattern of the Clock Gene, *Period*, in the Linden Bug, *Pyrrhocoris apterus*

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*Abstract* The temporal expression pattern of the circadian clock gene *period* was compared between heads of the linden bug, *Pyrrhocoris apterus*, kept under diapause-promoting short days (SD) and diapause-preventing long days (LD) using a real-time PCR quantification. Diapause or reproduction was programmed by photoperiod during the larval stage, but the first difference in *per* mRNA abundance between SD and LD insects was observed only after adult ecdysis. The expression level of *per* mRNA was markedly higher, up to more than 10-fold, in the destined-to diapause animals compared with those scheduled for reproduction. Up-regulation of *per* transcript was restricted to an early diapause peak, with the maximum expression on days 3 to 5 after adult ecdysis. Starvation reduced the peak level of *per* mRNA to about 50% of the value found in feeding females in the SD conditions, but *per* mRNA abundance was similarly low in fasting and feeding females in LD. Photoperiodic refractoriness in either wild-type postdiapause adults or in a selected nondiapause variant of *P. apterus* was associated with reproduction and low, LD-like levels of *per* mRNA under both SD and LD. Overall, the data suggest that the photoperiodic programming itself has no direct effect on *per* mRNA abundance, but it does determine the response of *per* transcript to food signals during subsequent expression of diapause/reproduction physiology.

*Key words* photoperiodic programming, photoperiodic refractoriness, feeding, starvation, diapause, reproduction, *period* gene expression

Photoperiodic regulation of development requires a timing mechanism to distinguish long days from short days (a photoperiodic clock), a mechanism to count the number of long or short days (a counter), and endocrine outputs governing the final expression of the developmental mode (diapause vs. continuous development or reproduction) (Denlinger, 2002; Saunders, 2002). A substantial body of evidence suggests that night-length measurement is a function of the circadian system (Saunders et al., 2004; Saunders, 2005). A potential role of circadian clock genes in photoperiodism is the subject of extensive discussions (Tauber and Kyriacou, 2001; Saunders et al., 2004; Mathias et al., 2005).

Remarkable progress has been made in the genetic dissection of the circadian clock in *Drosophila melanogaster* (Dunlap, 1999; Panda et al., 2002). One aspect of these wide-ranging studies was the

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demonstration that levels of *period* (*per*) mRNA in heads of *D. melanogaster* are regulated by both photoperiod and temperature (Majercak et al., 1999; Collins et al., 2004). However, the class *period* mutant called $per^{01}$ showed arrhythmic patterns of adult eclosion (Konopka and Benzer, 1971); nevertheless, $per^{01}$ females were able to discriminate photoperiods for the induction of diapause (Saunders et al., 1989). Unfortunately, *D. melanogaster* shows only a shallow ovarian diapause at a low temperature of 12 °C (Saunders and Gilbert, 1990) and may not be the best model for the molecular description of photoperiodism (Tauber and Kyriacou, 2001; Denlinger, 2002). An involvement of circadian clock genes in photoperiodism is indicated in few other species by different levels of gene transcripts in heads of wild-type (WT) versus nondiapause (ND) strains. The genes that have been assayed accordingly are *per* and *timeless* (*tim*) in the drosophilid fly *Chymomyza costata* (Kostal and Shimada, 2001; Pavelka et al., 2003) and the flesh fly *Sarcophaga bullata* (Goto et al., 2006), as well as *per* and *Clock* (*Clk*) in the linden bug *Pyrrhocoris apterus* (Hodkova et al., 2003; Syrová et al., 2003). The expression of *tim* in whole bodies of the pitcher-plant mosquito *Wyeomyia smithii* varies consistently according to latitude of origin, suggesting that *tim* has the potential to affect photoperiodic response (Mathias et al., 2005).

The linden bug *P. apterus* exhibits adult (reproductive) diapause with a robust photoperiodic response even at a high temperature of 25 °C—that is, diapause is not caused by unfavorable environmental conditions (Hodek, 1968). In heads of 1-week-old WT females of *P. apterus*, the level of *per* mRNA is about 10-fold higher under diapause-inducing short-day photoperiod compared with diapause-preventing long-day photoperiod. In ND females that do not undergo diapause, even under short days, levels of the transcript are low under both photoperiods (Hodkova et al., 2003; Syrová et al., 2003). Although the adults of *P. apterus* remain sensitive to photoperiod, the onset of photoperiodic perception occurs much earlier, during the late larval stage (Hodek, 1971). The first week of adult life represents an important physiological transition period when hormonal changes resulting in the actual expression of diapause or reproduction occur.

If different levels of *per* mRNA under short versus long days, noted in 1-week-old adult females (Hodkova et al., 2003; Syrová et al., 2003), were a direct consequence of the photoperiodic perception in the larval stage, the up-regulation of *per* transcript under short days would become apparent already at the onset of the adult stage. To define the exact timing of changes in *per* gene expression, we monitored temporal patterns of gene expression from the onset of the adult stage in females held under 2 contrasting photoperiods. The physiological transition, including changes in the activity of corpus allatum (Hodkova, 1982, 1992) and in the metabolic rate (Sláma, 1964), depends on feeding and does not occur when adults are starved. Although freshly ecdysed adults are already programmed for diapause or reproduction, they continue to perceive and count short versus long days independently of food intake (Hodek and Hodkova, 1986; Hodkova and Hodek, 1987). To discriminate between changes in *per* gene expression related to the food-dependent physiological transition and those related to the food-independent changes within the photoperiodic clock and counter, we compared *per* expression patterns in feeding and fasting females of *P. apterus*.

## MATERIAL AND METHODS

### Insects

Colonies of *P. apterus* (L.) (Heteroptera) were reared at 25 ± 2 °C and supplied ad libitum with linden seeds and water. The WT strain was maintained under a diapause-preventing long-day photoperiod of 18 h light/6 h darkness (LD). The ND strain, lacking diapause response at 25 °C, was selected under a diapause-promoting short-day photoperiod of 12 h light/12 h darkness (SD) from a few reproducing individuals (Socha and Hodkova, 1994; Hodkova and Socha, 1995). Both strains originated from Ceske Budejovice (Czech Republic). Experimental insects were reared from eggs to adulthood under LD 25 ± 2 °C, SD 25 ± 2 °C, or SD 20 ± 2 °C. Females were used in all experiments. Photoperiodic, thermal, and food conditions for adults are defined in individual experiments. Conditions hereafter referred to as "low temperature" are as follows: insects were exposed to gradually decreasing temperatures: 20 °C (day)/10 °C (night) during the first week, followed by 15 °C/5 °C and 10 °C/0 °C during the second and third weeks, respectively (all 3 weeks in SD), followed by 7 weeks at constant 0 °C and continuous darkness. Such an acclimation protocol simulates the natural drop of temperatures during autumn.

### Molecular Techniques

Heads of insects (without antennae or rostrum) were cut off, immediately placed on dry ice, and kept

at –85 °C until analysis. All samples were collected 6 to 9 h after lights-on. Previous studies noted only a very weak diurnal rhythm in *per* mRNA abundance under LD and no rhythm under SD (Hodkova et al., 2003; Syrová et al., 2003). RNA was isolated with Trizol (Sigma, St. Louis, MO) or RNA Blue (Top Bio, Prague, Czech Republic) from 5 or 10 heads and diluted in 100 µL $H_2O$ (pigments are copurified but do not affect subsequent enzymatic procedures; RNA concentration was determined after gel electrophoresis). Then, 0.1 to 1 µg of total RNA was used for cDNA synthesis using SuperscriptII (Invitrogen, Carlsbad, CA) reverse transcriptase according to the manufacturer's instruction (42 °C incubation step was 60 min) with oligo $dT_{24}$ primer.

*period* PCR primers were designed to anneal to exon sequences separated by large (~1-kb) intron, and conditions were optimized so that only cDNA product (~200 bp) was amplified with forward (5'ACAGCTAGTGGTGGTGAAGAGG) and reverse (5'AAAAGTTGTTCAGTAAGAGCAGTAG) primers. As a reference transcript, we amplified ribosomal protein 49 (RP49) with a forward primer designed to anneal specifically only to cDNA (intron position is marked with ^): 5'CCGATATGTAAAACTGAGG^AGAAAC and reverse primer: 5'GGAGCATGTGCCTGGTCTTTT.

To reduce pipetting errors, 5 µL of diluted cDNA was added to a tube containing 15 µL of PCR mastermix (final reaction concentrations: Ex Taq HS polymerase [Takara, Madison, WI] 1.6 U/100 µL, Ex Taq buffer 1×, dNTPs 200 µM each, Syber green 1:25,000, primers 400 nM each). PCRs for *per* and RP49 were done in separate tubes (20 µL per tube) in triplicate for each primer combination and each cDNA sample. Real-time PCR (Rotor-Gene 3000, Corbett Research, Sydney, Australia) started with initial 5-min cDNA denaturation and enzyme activation (95 °C), followed by 40 to 50 cycles, each consisting of denaturation (94 °C, 15 sec), primer annealing (59 °C, 30 sec), extension, and acquiring on the Syber green channel (74 °C, 40 sec), followed by acquiring on the Syber green channel at 80 °C (15 sec). Melting analysis was performed when all cycles were completed; PCR product sizes were verified by 2% agarose gel electrophoresis. We always run 3 to 4 reactions without cDNA (substituted with 5 µL $H_2O$) for each primer combination as a negative control. Data were analyzed and quantified with the Rotor-Gene analysis software. Relative values were standardized to RP49 and normalized to the sample with the highest expression. Values represent the mean of independent experiments ± standard deviation.

### Statistical Analysis

GraphPad PRISM (Version 4) software was used for the *t* test, 1-way analysis of variance (ANOVA), and 2-way ANOVA analysis.

## RESULTS

### Diapause Programming and Intensity

The intensity of diapause was measured as preoviposition period (PRE-OP) after the transfer of adult females from SD 25 °C to LD 25 °C. PRE-OP was compared among 4 groups of females: those continuously reared at LD (LD females) and 3 groups transferred from SD to LD (SD/LD females) (Fig. 1). SD/LD females were transferred to LD (1) on the day of adult ecdysis, (2) after 10-day starvation at SD, and (3) after 10-day feeding at SD. Under LD conditions, all groups of females were fed. One-way ANOVA and analysis by Tukey's posttest revealed significant difference between the mean PRE-OP of LD females and all groups of SD/LD females ($p < 0.001$) (Fig. 1). Females continuously kept at SD did not oviposit at all. The mean PRE-OP of SD/LD females transferred to LD on the day of adult ecdysis was almost 3 times longer compared with the PRE-OP of LD females (17.5 ± 0.5 d vs. 6.7 ± 0.2 d), thus indicating that females were programmed to diapause during the larval stage.

The mean PRE-OP of SD/LD females transferred to LD on the day of adult ecdysis (17.5 ± 0.5 d) was significantly shorter ($p < 0.001$) than the PRE-OP of SD/LD females transferred to LD 10 days after adult ecdysis, irrespective of whether females were fed (22.3 ± 0.7 d) or starved (24.8 ± 1.5 d) before the transfer. Food conditions at SD had no significant effect on the subsequent PRE-OP at LD (Fig. 1). This suggests that (1) the intensity of diapause increased during 10 days of adult life at SD, and (2) the increase of diapause intensity is not dependent on food intake.

### Temporal Pattern of *per* mRNA Abundance in WT Females

#### *Feeding Females*

In the adults aged 1 to 4 hours or 1 day, the *per* mRNA levels were similar at LD 25 °C and SD 25 °C (Fig. 2A). Under LD 25 °C, there was a small peak of *per* mRNA abundance 1 to 3 days after adult ecdysis (Fig. 2A). Under SD 25 °C, *per* mRNA level sharply

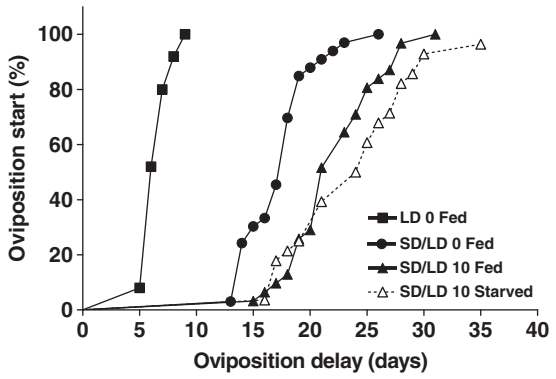


**Figure 1. Oviposition delay in females of *Pyrrhocoris apterus* transferred from short days (SD) to long days (LD). Oviposition delay represents the number of days to the first oviposition after the adult ecdysis in females continuously kept under long days (LD 0 Fed, $n = 33$) or after the transfer of females from short to long days. Females were transferred on the day of adult ecdysis (SD/LD 0 Fed, $n = 24$), after 10-day feeding (SD/LD 10 Fed, $n = 30$), or after 10-day starvation (SD/LD 10 Starved, $n = 27$). Under long days, all groups of females were fed. Difference between mean preoviposition period was analyzed by 1-way analysis of variance and Tukey's multiple-comparison test. LD 0 Fed versus SD/LD 0 Fed ($p < 0.001$), LD 0 Fed versus SD/LD 10 Fed ($p < 0.001$), LD 0 Fed versus SD/LD 10 Starved ($p < 0.001$), SD/LD 0 Fed versus SD/LD 10 Fed ($p < 0.001$), SD/LD 0 Fed versus SD/LD 10 Starved ($p < 0.001$), and SD/LD 10 Fed versus SD/LD 10 Starved (*ns*). For other explanations, see the text.**

increased, peaked 3 to 5 days after adult ecdysis (more than 10-fold compared with LD), then decreased and, 2 weeks after adult ecdysis, dropped to the level measured during LD (Fig. 2A). A similar sharp increase of *per* mRNA level was recorded under SD 20 °C (Fig. 2D), but the decline was less rapid compared with SD 25 °C.

Once *per* mRNA abundance dropped, the *per* expression level remained consistently low for the remainder of the experiment (Fig. 2D) and did not change in females transferred from the diapause-maintaining conditions of SD 20 °C to the diapause-terminating conditions of LD 25 °C (photoperiodic activation) (Table 1). Reduced *per* mRNA abundance was also noted throughout 70-day (group I: days 14-84, group II: days 60-120, and group III: days 120-190) exposures of diapause adults to low temperature (Fig. 2D). Diapause was completed during the low-temperature exposure, but females remained in a postdiapause quiescence (photoperiodic responsiveness was lost, but reproduction was prevented, and diapause syndrome was maintained by low temperature). After transfer of the postdiapause females from low temperature to 25 °C, vitellogenesis was resumed within 1 week, and low *per* mRNA abundance persisted under both LD and SD (Table 1).

These data highlight 3 important points: (1) increase in *per* mRNA abundance is not a direct consequence of diapause programming during the larval stage. (2) This molecular up-regulation of *per* mRNA is restricted to a short period in prediapause/early diapause. (3) Levels of *per* mRNA are consistently low throughout late diapause, postdiapause quiescence, and reproduction. Previously, an intensive feeding activity was noted in reproducing and early diapause females of *P. apterus*, while feeding was greatly reduced in diapause females older than 10 days (Šula et al., 1998). Thus, up-regulation of *per* mRNA coincides with intensive feeding in early diapause, while low levels of *per* transcript are associated with either reduced feeding in diapause females or intensive feeding in reproducing females.

### *Starving Females*

Females were deprived of food on the day of adult ecdysis. Starvation under SD 25 °C resulted in a significant depression of *per* mRNA levels. In fasting females, the peak level of *per* mRNA was reduced to about 50% relative to that found in feeding females (Fig. 2B). Thus, the up-regulation of *per* transcript in early diapause is food dependent and is probably not related to the intensification of diapause that is food independent (Fig. 1).

In contrast to SD, starvation under LD 25 °C had no effect on the *per* mRNA levels (Fig. 2C). Consequently, the difference between SD and LD females in the temporal pattern of *per* mRNA abundance was much less pronounced under fasting compared with feeding conditions (Fig. 2B,C). Starvation under LD impairs ovarian maturation (Hodkova, 1982). The stimulating effect of feeding on *per* expression noted in the absence of reproduction under SD (Fig. 2B), together with similarly low levels of *per* mRNA in feeding, reproducing females and in fasting, nonreproducing females (Fig. 2C) under LD, indicates an antagonism between feeding and reproduction in relation to *per* mRNA abundance.

## *per* mRNA Abundance in WT versus ND Females 1 Week after Adult Ecdysis

In WT females, *per* mRNA level was about 10 times higher under SD than under LD. In contrast,

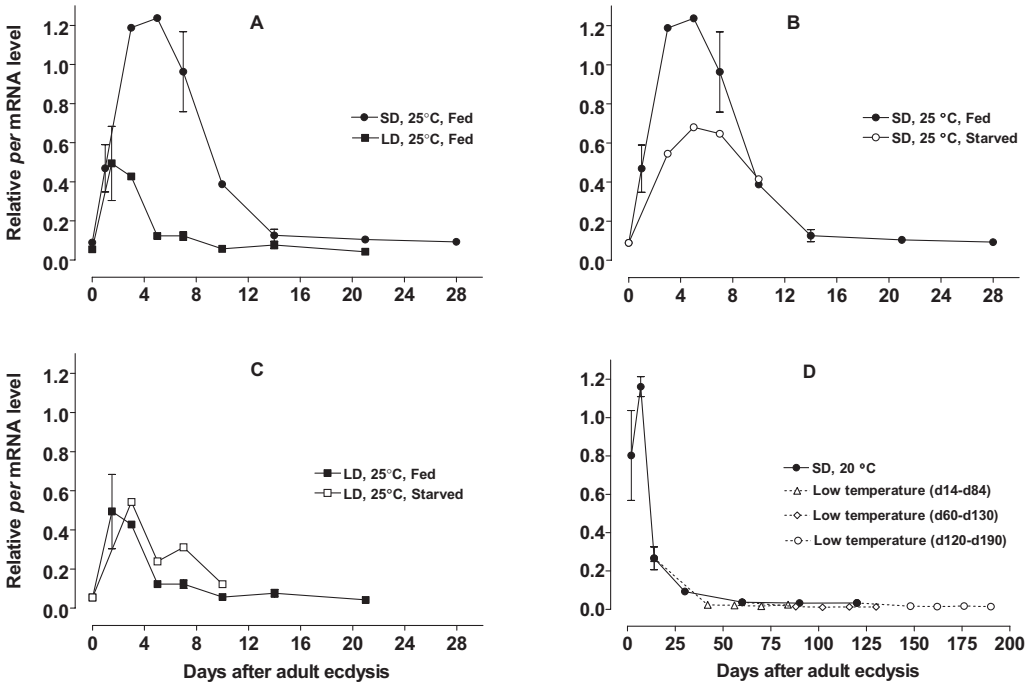


**Figure 2. Temporal pattern of *per* mRNA abundance in feeding and starving females of *Pyrrhocoris apterus* kept under 2 contrasting photoperiods. (A) Short days (SD) versus long days (LD), feeding, 25 °C; (B) feeding versus starving, SD, 25 °C; and (C) feeding versus starving, LD, 25 °C. Values for feeding females aged 1, 7, and 14 days represent a mean of 2 to 3 analyses (1 analysis = 10 heads) ± standard deviation. Other points represent 1 analysis (10 heads). (A) Two-way analysis of variance revealed a significant effect of both photoperiod ($F = 58.92$, DFn = 1, DFd = 10, $p < 0.0001$) and time ($F = 16.92$, DFn = 7, DFd = 10, $p < 0.0001$). Bonferroni posttests showed significant differences between LD and SD at d3 ($p < 0.01$), d5 ($p < 0.001$), and d7 ($p < 0.001$). (A-C) Difference between means was analyzed by *t* test with data from d3, d5, and d7 combined. SD Fed versus LD Fed ($t = 8$, $df = 8$, $p < 0.0001$), SD Fed versus SD Starved ($t = 3$, $df = 6$, $p < 0.02$), LD Fed versus LD Starved ($t = 1.71$, $df = 6$, *ns*), and SD Starved versus LD Starved ($t = 2.59$, $df = 4$, *ns*). (D) SD 20 °C or constant darkness, low temperature. All points represent means of 3 analyses (1 analysis = 5 heads) ± SEM. Relative *per* mRNA levels refer to the *per*/*RP49*.**

Table 1. Abundance of *per* mRNA in Females of *Pyrrhocoris apterus* during the Photoperiodic Activation or the Postdiapause Resumption of Reproduction under 2 Contrasting Photoperiods

| *Physiological State* | *Conditions before Transfer* | *Conditions after Transfer* | *Days after Adult Edysis* | *Days after Transfer* | *Relative* per *mRNA Level* (per/RP49)[a] |
|---|---|---|---|---|---|
| Diapause | Short days, 20 °C | Long days, 25 °C[b] | 60 | 0 | 0.037 ± 0.009 |
| | (days 0-60) | | 63 | 3 | 0.073 ± 0.003 |
| | | | 67 | 7 | 0.059 ± 0.002 |
| | | | 74 | 14[c] | 0.065 ± 0.031 |
| Postdiapause | Short days, 20 °C | Long days, 25 °C[d] | 130 | 0 | 0.013 ± 0.004 |
| quiescence | (days 0-60) | | 133 | 3 | 0.053 ± 0.025 |
| | followed by | | 137 | 7[c] | 0.091 ± 0.038 |
| | Low temperature | Short days, 25 °C[d] | 130 | 0 | 0.013 ± 0.004 |
| | (days 60-130) | | 133 | 3 | 0.051 ± 0.017 |
| | | | 137 | 7[c] | 0.063 ± 0.038 |

a. Mean of 3 analyses (1 analysis = 5 heads) ± standard deviation.
b. One-way analysis of variance (ANOVA) and Tukey's multiple-comparison test revealed no significant differences.
c. Presence of vitellogenic oocytes.
d. Two-way ANOVA revealed significant effect of time ($F = 6.52$, DFn = 2, DFd = 12, $p < 0.02$) and nonsignificant effect of photoperiod ($F = 0.50$, DFn = 1, *ns*).

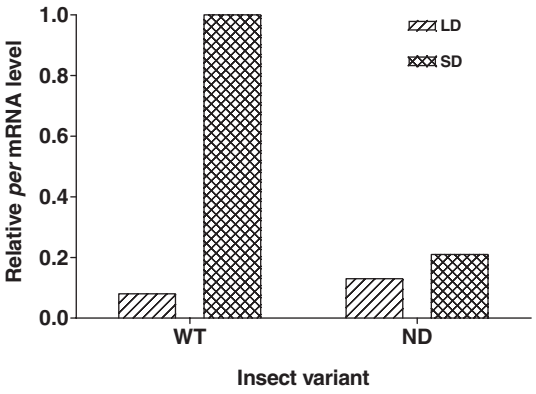


**Figure 3. Comparison of *per* mRNA abundance in wild-type (WT) females and nondiapause variant (ND) of *Pyrrhocoris apterus* kept under 2 contrasting photoperiods. Each bar represents 1 analysis (10 heads) of 1-week-old females continuously kept under long days (LD) or short days (SD). Relative *per* mRNA levels refer to the *per*/*RP49*.**

photoperiod had no effect on *per* gene expression in ND females, which do not undergo diapause even under SD; the *per* mRNA level in both LD and SD was low, similar to that found under LD in WT females (Fig. 3). The question of whether the expression of *per* would decline further if ND females were starved was not addressed, but this possibility seems unlikely, given that no decline in *per* mRNA levels was noted under LD in fasting WT females (Fig. 2C). The results confirm previously reported data (Hodkova et al., 2003; Syrová et al., 2003) suggesting that the up-regulation of *per* mRNA in early diapause reflects SD-like physiology rather than the ambient photoperiod.

## DISCUSSION

A dramatic increase in *per* mRNA transcript associated with diapause in *P. apterus*, demonstrated in previous studies by the RNase protection assay (Hodkova et al., 2003; Syrová et al., 2003), was confirmed by real-time PCR quantification (Fig. 3). The current study builds on previous work by characterizing the exact timing of *per* gene expression changes in the head of adult females and revealed an interaction between photoperiodic and food signals in the regulation of *per* transcript abundance.

### Responses of Clock Genes to Photoperiodic and Food Signals

Although freshly ecdysed adults were already programmed for diapause (Fig. 1), the up-regulation of the *per* gene started only after adult ecdysis (Fig. 3A). The data indicate that, in *P. apterus*, the up-regulation of the *per* gene under SD is not a direct consequence of diapause programming (a covert process within photoperiodic clock and/or counter) during the larval stage.

The up-regulation of the *per* transcript is restricted to a short period in early diapause. Changes in *per* mRNA abundance in feeding SD females (Fig. 2A) exactly mirror the temporal pattern of their feeding activity (Šula et al., 1998). Expression of this gene was high during intensive feeding at the beginning of adult life, and it declined with gradual cessation of feeding and accumulation of nutritive reserves in diapause females. This early diapause peak of *per* mRNA abundance was considerably reduced in fasting females (Fig. 2B). In contrast, feeding had no effect on *per* expression in LD, and *per* mRNA levels were low in both feeding and starving females (Fig. 2C). Consequently, remarkable differences in *per* mRNA levels between SD and LD noted in feeding females did not occur in starving females. Given that adult females of *P. apterus* continue to perceive and count short versus long days independently of food intake (Hodek and Hodkova, 1986; Hodkova and Hodek, 1987), the magnitude of *per* expression in the head seems to be involved in the food-dependent output pathways to diapause or reproduction physiology. A recent study on the sand fly, *Lutzomyia longipalpis*, shows a significant down-regulation of *per* and *tim* in heads and bodies after a blood meal (Meireles-Filho et al., 2006). Interestingly, food intake affects both the peak time (Stokkan et al., 2001) and the expression level (Kobayashi et al., 2004) of circadian clock genes (*Per1, Per2*) in mammalian peripheral tissues.

The photoperiodic response of *P. apterus* presents a few unusual features with regard to the photoperiodic time measurement. This species appears to "measure" day length rather than length of night, and Nanda-Hamner results indicate an extremely short free-running period (16 h) of the constituent oscillators. Furthermore, no clear daily changes in *per* mRNA abundance were noted in the head of adult females in either LD or SD (Hodkova et al., 2003; Syrová et al., 2003), although they remain sensitive to photoperiod (Hodek and Hodkova, 1986; Hodkova and Hodek, 1987), suggesting, perhaps, that the *per* gene plays no central role as a photoperiodic clock component. On the other hand, a possibility that daily rhythms in a small proportion of *per* mRNA in the head are masked by a large proportion of *per* activity with a nonclock (physiological) function is still open to question. Distinct daily patterns of *per*

and/or *tim* expression under different photoperiodic conditions were recorded in 2 dipteran species, *D. melanogaster* (Majercak et al., 1999, 2004; Collins et al., 2004; Shafer et al., 2004), and the flesh fly, *Sarcophaga crassipalpis* (Goto and Denlinger, 2002). However, it is not known whether these patterns may be used to determine diapause responses to photoperiod.

### Functional Relationship between Clock Genes and Photoperiodism

Although the results reported here demonstrate that the magnitude of *per* gene expression in *P. apterus* is correlated with diapause or reproduction physiology, no evidence of a functional relationship has yet been provided. Genetic linkage analysis revealed that a defect responsible for the block to diapause photoresponsiveness in ND females of *P. apterus* is in the single locus but not in the *per* gene (Doležel et al., 2005). Up-regulation of the *per* gene in young WT females under SD may still provide an important link in the physiological transition to diapause, but this possibility cannot be directly tested because mutants in the *per* gene are not available in *P. apterus*. It cannot be excluded that *per* expression in ND females (Fig. 3) could be lowered by an affected ND locus independently of imposed physiology. However, a similarly low *per* expression in postdiapause WT females, which also lack diapause photoresponsiveness (Table 1), cannot be explained by a defective genotype.

It is likely that distinct patterns of *per* gene expression under different photoperiodic conditions are correlated with changes in other circadian clock genes that deserve further attention. A previous study noted the up-regulation of the *Clock* gene in SD (Syrová et al., 2003), and our preliminary results indicate the up-regulation of *cryptochrome* and the down-regulation of *Par Domain Protein 1* in 1-week-old diapause females of *P. apterus* (Doležel, unpublished).

Abnormal expression levels of *per* and *tim* were noted in nondiapause variants of the drosophilid fly, *C. costata*, and the flesh fly, *S. bullata*. In *C. costata*, the loss of diapause response to photoperiod was associated with constantly low abundance of *per* mRNA (Kostal and Shimada, 2001), and no *tim* mRNA was detected (Pavelka et al., 2003). Genetic analysis demonstrated that the *tim* (Pavelka et al., 2003), but not the *per* gene (Kostal and Shimada, 2001), was causally linked with the photoperiodic response. In a nondiapause variant of *S. bullata*, the expression level of both *per* and *tim* was elevated (Goto et al., 2006). The loss of diapause response was associated with arrhythmicity of adult eclosion in both *C. costata* (Lankinen and Riihimaa, 1992) and *S. bullata* (Goto et al., 2006), suggesting that the same clock component(s) is involved in circadian rhythms and photoperiodism. In contrast to *C. costata* and *S. bullata*, circadian rhythms of locomotor activity and their coupling to the entraining effect of the light/dark cycle are maintained in the ND variant of *P. apterus* (Hodkova et al., 2003). Although the possibility that the photoperiodic time measurement system shares some molecular components with the circadian clock mechanism cannot be dismissed, the pathways involved in the photoperiodic regulation of *per* mRNA abundance and the entrainment of circadian rhythms are apparently different in *P. apterus*.

### Conclusion

Although the exact site of *per* action remains to be identified, the current study clearly demonstrates that distinct patterns of *per* gene expression in the head of WT females of *P. apterus* under different day lengths are food dependent and reflect physiological conditions of adults (with respect to diapause or reproduction). Photoperiodic programming of diapause and reproduction during the photosensitive larval stage has no direct effect on the expression level of the *per* gene, but the program in question determines the subsequent response of *per* transcripts to food intake in young adults (increase under SD, no change under LD). Further studies on individual tissues should unravel the exact site of *per* action and its regulation in female physiological response to photoperiod and food intake.

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