Pea Aphids (Hemiptera: Aphididae) Have Diurnal Rhythms When Raised Independently of a Host Plant

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Abstract

Seasonal timing is assumed to involve the circadian clock, an endogenous mechanism to track time and measure day length. Some debate persists, however, and aphids were among the first organisms for which circadian clock involvement was questioned. Inferences about links to phenology are problematic, as the clock itself is little investigated in aphids. For instance, it is unknown whether aphids possess diurnal rhythms at all. Possibly, the close interaction with host plants prevents independent measurements of rhythmicity. We reared the pea aphid Acerthosiphon pisum (Harris) on an artificial diet, and recorded survival, moulting, and honeydew excretion. Despite their plant-dependent life style, aphids were independently rhythmic under light–dark conditions. This first demonstration of diurnal aphid rhythms shows that aphids do not simply track the host plant’s rhythmicity.

Key words: circadian clock, hourglass clock, Acerthosiphon pisum, artificial diet, photoperiodism

Throughout latitudes and altitudes, day- and night-time temperatures and their respective durations vary, and species are adapted to make best use of the temporal niches via diurnal or nocturnal activity (Bennie et al. 2014). Diurnal rhythms can dictate whether species meet, and can contribute to presence or absence of biotic interactions (Stich and Lampert 1981, Fleury et al. 2000). Due to the relevance of a correct timing, nearly all organisms examined so far possess an endogenous mechanism called circadian clock (Moore-Ede et al. 1982, but see Lu et al. 2010), and the circadian clock affects all major physiological processes (Moore-Ede et al. 1982).

One remaining question is whether the clock is involved in seasonal timing (phenology) via day length (photoperiod) measurements (Bunning 1936). Supporters of clock involvement have proposed two models, which describe how phase relations of clock and environment (external coincidence, Pittendrigh and Minis 1964), or of multiple clocks (internal coincidence, Pittendrigh and Minis 1972) could govern photoperiodism. Numerous studies indeed correlated clock gene expression with photoperiodism (Schulz and Kay 2003), including in hemipterans (Ikeno et al. 2010), but this correlation can be at least partially attributed to research bias in favor of clock genes (Bradshaw and Holzapfel 2010). Hence, despite accumulating correlative evidence, the debate is still not fully settled (Danks 2005).

An alternative to clock involvement in photoperiodism is the hour glass model (Garner and Allard 1920). In this model, steady, clock-independent accumulation of a molecule triggers a response upon reaching a threshold. Aphids played a prominent role in the discussion of clock involvement in photoperiodism, as they were seen as first evidence for such an hour glass model (Lees 1973). Careful re-evaluation contradicted this view, and suggested that aphid photoperiodism depends on the circadian clock (Hardie and Vaz Nunes 2001). The clock was proposed to damp quickly, i.e., disappear within few cycles. However, very little empirical data are available about damping or other properties of the aphid clock itself, let alone studies on how the clock affects aphid behavior. Before settling the argument on circadian clock involvement in photoperiodism, the first logical question is whether aphids have a diurnal rhythm driven by an endogenous clock.

The lack of research on diurnal rhythms of aphids may in part be explained by the high degree of food specialization, which complicates studies of an independent rhythm. Aphids are known for their remarkable phenotypic plasticity, and asexual forms (morphs) can bear sexual offspring if induced by a short photoperiod (Lees 1973). Because the sexual offspring is less dependent on host plants, experiments with various aphid species have been conducted on such sexual morphs (Eisenbach and Mittler 1980, Thieme and Dixon 1996). However, if the ultimate aim is the link of circadian clock and photoperiodism, tests on long-day (asexual) aphids are needed. While some experiments have been also conducted on asexual morphs, the aphids were always held on living plants (Gomez et al. 2005).
of the nymphs were light-entrained under a photoperiod of 16:8 (L:D) h (i.e., the circadian clock was synchronized with the light–dark cycle), but reared on plants. We started the first measurement period at 4 d age. Every 1.5 d (during lights-on in both chambers), we placed surviving nymphs into new Petri dishes with new food and counted the accumulated honeydew and exuviae. We counted the number of honeydew drops but did not estimate the volumes due to the low visibility and the small sizes; we noticed, however, no trend in drop diameters, and variability in drop volumes can be considered low (Auclair 1958). The two climate chambers (Sanyo MLR-H series) provided 18.1 ± 0.9°C and 81.3 ± 2.8 humidity and a photoperiod of 16:8 (L:D) h at 19.7 ± 0.7 klux.

Statistics were performed with R 3.1.1 (R Core Team 2014). We applied a mixed-effects model including chamber, time, and their interaction as factors, and Petri dish as random term. As we expected alternating slopes (Fig. 1), a significant interaction term with reversing slopes would evidence rhythmicity. We corrected honeydew excretion for the number of surviving aphids. We additionally corrected honeydew excretion for moultin aphids, which are not expected to produce honeydew. This yielded the combined activity estimate

\[ \text{Survivors - Exuviae} \]

Results

Honeydew excretion was overall low, with one to three drops per aphid per 1.5 d, but the nutrient uptake was sufficient for experimental animals to develop into adults and to survive for 2 wk. Survival declined over time (65–89% survival rates between measurements), leaving on average 38% (11.4 ± 0.4 aphids) at the fourth measurement (see Supp. Fig. 1 [online only]). Under a photoperiod of 16:8 (L:D) h, survival, moultin, and honeydew excretion individually did not significantly alternate with changing treatments, but the slopes of the combined activity estimate crossed significantly (i.e., significant statistical interaction, Tables 1 and 2; Fig. 2). This interaction conforms to our prediction of diurnal rhythmicity. In the L treatments (with 28 h light in 36 h), the median of observed activity (drops per nonmouling survivor) was 1.80 drops, 31% higher than in the D treatments (with 20 h light in 36 h, 1.37 drops). The ratio of L/D = 1.31 allows estimates on how active aphids were during lights-on and during lights-off (x and y in eq. 1). For instance, if aphids were purely day-active (x = 1, y = 0), one would expect activity for 28 h in the L treatment and for 20 h in the D treatment, so that the ratio L/D between the treatments would be 28/20 = 1.4, or 40% higher in the L treatment. Solving eq. (1) with L/D = 1.31 yields 1 y = 7.2 x. We conclude that aphids are seven times more active during day than during night.

Discussion

The experiment is to our knowledge the first to measure aphid diurnal rhythms independent of host plants by feeding artificial diets. Although independent diurnal rhythms have been observed in sexual morphs, after photoperiodic induction (Eisenbach and Mittler 1980, Thieme and Dixon 1996), in all studies on asexual morphs aphids were reared on living plants (Gomez et al. 2006, Cortes et al. 2010, Taylor et al. 2012). Aphids can be described as plant parasites (The International Aphid Genomics Consortium 2010), so aphids might well hitch-hike the plant rhythm instead of using the light–dark cycle (LD). The present study indicates that this is not the case (evidenced by the statistical significant interaction term), and that aphids have diurnal rhythms even on constant food sources.
Common to all studies in various species is an activity peak during daytime, and our results show that this is generally also true for the pea aphid. However, further experiments are needed to determine how the activity distributes over the course of the day. Knowledge of the activity pattern of aphids has implications for pest control, because it assists more specific treatment with insecticide in a circadian manner (Hooven et al. 2009). Furthermore, it is interesting to know whether the plant modulates the aphid rhythmicity, because exploitation of diurnal changes in host receptivity and quality can lead to coevolution among the circadian clocks (Goodspeed et al. 2012, Martinez-Bakker and Helm 2015). Changes in diurnal timing are also accompanied by changes in the abiotic conditions, which aphid experience. For example, day-activity might be an explanation of fitness constraints under short days (Joschinski et al. 2015). Overall, our study lays the foundation for future studies on aphid diurnal rhythms and the interaction with their host plants.

We are well aware that the rhythm in diurnal behavior is no evidence for circadian clock involvement yet, as it needs also continuation ("free-runs") under constant conditions (Moore-Ede et al. 1982). Future experiments need to test aphid rhythmicity under constant darkness, and in particular need to quantify how the

Table 1. ANOVA results

| Response | Factor       | df | F     | P       | Significance level |
|----------|--------------|----|-------|---------|--------------------|
| Survival | Chamber      | 1,26 | 1.40 | 0.25    |                    |
|          | Time         | 3,77 | 85.70| <0.0001 | ***                |
|          | Chamber:time | 3,77 | 1.59 | 0.20    |                    |
| Moulting | Chamber      | 1,26 | 1.19 | 0.29    |                    |
|          | Time         | 3,77 | 3.24 | 0.03    | *                  |
|          | Chamber:time | 3,77 | 0.67 | 0.16    |                    |
| Honeydew | Chamber      | 1,26 | 0.08 | 0.78    |                    |
|          | Time         | 3,69 | 5.37 | <0.01   | **                 |
|          | Chamber:time | 3,69 | 2.43 | 0.07    | *                  |
| Activity | Chamber      | 1,26 | 0.99 | 0.33    |                    |
|          | Time         | 1,69 | 1.27 | 0.29    |                    |
|          | Chamber:time | 1,69 | 3.02 | 0.04    | *                  |

The three responses aphid survival, moulting, and honeydew excretion were combined to one estimate that expresses activity as honeydew excretion per nonmoulting survivor. We expected a significant interaction of chamber and time (see main text).

a Significance levels: ***p < 0.001; **p < 0.01; *p < 0.05; p < 0.1.
oscillation of the clock damps out. On the one hand, studies suggest an hour glass mechanism, i.e., no clock involvement, in aphids (Lees 1973); on the other hand, correlative evidence from other species suggests that this clock mechanism is not the norm (e.g., Ikeno et al. 2010). These apparent differences could be unified by a quickly damping clock (Hardie and Vaz Nunes 2001), and a damped clock might be exemplified by damping activity under constant darkness. The molecular mechanism of the aphid clock has been investigated, and some parts of the core clockwork (CRY and the PER/TIM feedback loop) are indeed undergoing accelerated changes (Cortes et al. 2010). Our current protocol, which requires feeding every 36 h to maintain high honeydew excretion rates, does not yet allow working under constant darkness, because feeding in darkness proved impossible. Hopefully further advances in rearing methods will allow studying aphid clock properties in depth. Yet, the demonstration of independent diurnal behavior is a crucial first step in understanding aphid clocks.

In conclusion, we showed that pea aphids produce honeydew and moult during daytime, and maintain the rhythm independently of host plants. We think that pea aphids are worth investigating for the involvement of clock and photoperiodism in aphid physiology, and our study with artificial diets are a first step in understanding its mechanisms.

Supplementary Data
Supplementary data are available at Journal of Insect Science online.

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References Cited
Auclair, J. L. 1958. Honeydew excretion in the pea aphid, Acyrthosiphon pisum (Harr.) (Homoptera: Aphididae). J. Insect Physiol. 2: 330–337.
Bennie, J. J., J. P. Duffy, R. Inger, and K. J. Gaston. 2014. Biogeography of time partitioning in mammals. Proc. Natl. Acad. Sci. USA 111: 13727–13732.
Bradshaw, W. E., and C. M. Holzapfel. 2010. What season is it anyway? Circadian tracking vs. photoperiodic anticipation in insects. J. Biol. Rhythms 25: 155–165.
Bunning, E. 1936. Die endogene Tagesrhythmik als Grundlage der photoperiodischen Reaktion. Berichte der Deutschen Botanischen Gesellschaft 54: 590–607.
Cortes, T., B. Ortiz-Rivas, and D. Martinez-Torres. 2010. Identification and characterization of circadian clock genes in the pea aphid Acyrthosiphon pisum. Insect Mol. Biol. 19: 123–139.
Danks, H. V. 2005. How similar are daily and seasonal biological clocks? J. Insect Physiol. 51: 609–619.
Eisenbach, J., and T. E. Mittler. 1980. Photoperiodic time measurement in the aphid Aphidoletes aphidimyza. Proc. R. Soc. Lond., Ser. B: Biol. Sci. 267: 511–515.
Fevvay, G., B. Delobel, and Y. Rahbé. 1988. Influence of the amino acid balance on the improvement of an artificial diet for a biotype of Acyrthosiphon pisum (Homoptera: Aphididae). Can. J. Zool. 66: 2449–2453.
Fleury, F., R. Allemand, F. Vavre, P. Foullet, and M. Bouletreau. 2000. Adaptive significance of a circadian clock: Temporal segregation of activities reduces intrinsic competitive inferiority in Drosophila parasitoids. Proc. R. Soc. Lond., Ser. B: Biol. Sci. 267: 1005–1010.
Garner, W. W., and H. A. Allard. 1920. Effect of the relative length of day and night and other factors of the environment on growth and reproduction in plants. J. Agric. Res. 18: 553–606.
Gomez, S. K., D. M. Oosterhuis, D. L. Hendrix, D. R. Johnson, and D. C. Steinkraus. 2006. Diurnal pattern of aphid feeding and its effect on cotton leaf physiology. Environ. Exp. Bot. 55: 77–86.
Godspred, D., E. W. Chehab, A. Min-Venditti, J. Braam, and M. F. Covington. 2012. Arabidopsis synchronizes jasmonate-mediated defense with insect circadian behavior. Proc. Natl. Acad. Sci. 109: 4674–4677.
Hardie, J., and M. Vaz Nunes. 2001. Aphid photoperiodic clocks. J. Insect Physiol. 47: 821–832.
Hodgson, C. J., and I. R. Lane. 1981. Some effects of photoperiod on larviposition and fresh weight gain in Myzus persicae. Physiol. Entomol. 6: 21–25.
Hooven, L. A., K. A. Sherman, S. Butcher, and J. M. Giebultowicz. 2009. Does the clock make the poison? Circadian variation in response to pesticides. PLoS ONE 4: e6469.
Ikono, T., S. Tanaka, H. Numata, and S. Goto. 2010. Photoperiodic diapause under the control of circadian clock genes in insects. BMC Biol. 8: 116.
Joscinski, J., T. Hovestadt, and J. Krauss. 2015. Coping with shorter days: Do phenology shifts constrain aphid fitness? PeerJ 3: e1103.
Lees, A. D. 1973. Photoperiodic time measurement in the aphid Megoura viciae. J. Insect Physiol. 19: 2279–2316.
Lu, W. Q., Q. J. Meng, N. J. C. Tyler, K. A. Stokkan, and A. S. I. Loudon. 2010. A circadian clock is not required in an arctic mammal. Curr. Biol. 20: 533–537.
Martinez-Bakker, M., and B. Helm. 2015. The influence of biological rhythms on host–parasite interactions. Trends Ecol. Evol. 30: 314–326.
Mittler, T. E., and R. H. Dadd. 1963. Studies on the artificial feeding of the aphid Myzus persicae (Sulzer)—I. Relative uptake of water and sucrose solutions. J. Insect Physiol. 9: 623–645.
Moore-Ede, M. C., F. M. Sultzman, and C. A. Fuller. 1982. The clocks that time us. Harvard University Press, Cambridge, MA.

Pittendrigh, C. S., and D. H. Minis. 1964. The entrainment of circadian oscillations by light and their role as photoperiodic clocks. Am. Nat. 98: 261–294.

Pittendrigh, C. S., and D. H. Minis. 1972. Circadian systems: longevity as a function of circadian resonance in Drosophila melanogaster. Proc. Natl. Acad. Sci. USA 69: 1537–1539.

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Schultz, T. F., and S. A. Kay. 2003. Circadian clocks in daily and seasonal control of development. Science 301: 326–328.

Stich, H.-B., and W. Lampert. 1981. Predator evasion as an explanation of diurnal vertical migration by zooplankton. Nature 293: 396–398.

Taylor, S. H., W. E. Parker, and A. E. Douglas. 2012. Patterns in aphid honeydew production parallel diurnal shifts in phloem sap composition. Entomol. Exp. Appl. 142: 121–129.

The International Aphid Genomics Consortium. 2010. Genome sequence of the pea aphid Acyrthosiphon pisum. PLoS Biol. 8: e1000313.

Thieme, T., and A. F. G. Dixon. 1996. Mate recognition in the Aphis fabae complex: Daily rhythm of release and specificity of sex pheromones. Entomol. Exp. Appl. 79: 85–89.

Van Emden, H. F., and R. Harrington. 2007. Aphids as Crop Pests, Cabi, Wallingford, United Kingdom.