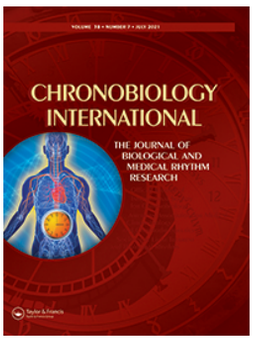

Taylor & Francis
Taylor & Francis Group

Chronobiology International

The Journal of Biological and Medical Rhythm Research

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/icbi20

Off with their heads: analysis of the circadian clock genes expression in the body of *Aedes aegypti*

Rayane Teles-de-Freitas, Liliane Barboza & Rafaela V. Bruno

To cite this article: Rayane Teles-de-Freitas, Liliane Barboza & Rafaela V. Bruno (2021) Off with their heads: analysis of the circadian clock genes expression in the body of *Aedes aegypti*, Chronobiology International, 38:7, 994-1001, DOI: 10.1080/07420528.2021.1903030

To link to this article: https://doi.org/10.1080/07420528.2021.1903030

CHRONOBIOLOGY INTERNATIONAL
2021, VOL. 38, NO. 7, 994–1001
https://doi.org/10.1080/07420528.2021.1903030

Off with their heads: analysis of the circadian clock genes expression in the body of *Aedes aegypti*

Rayane Teles-de-Freitas[a], Liliane Barboza[a], and Rafaela V. Bruno[a,b]

[a]Laboratório de Biologia Molecular de Insetos, Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro, Brazil; [b]Instituto Nacional de Ciência e Tecnologia em Entomologia Molecular (INCT-EM)/CNPq, Rio de Janeiro, Brazil

ABSTRACT

The circadian clock of mosquitoes can influence physiological and behavioral processes linked to disease transmission. Currently, we know how clock genes are expressed in the head of the *Aedes aegypti* in different light and temperature regimens, but we still do not know anything about the expression of these genes in the body. The present work aims to contribute to this understanding. We observed that the expression of clock genes in the body of *Aedes* can be different from that in the head. Additionally, we found that temperature cycles have greater influence on the clock genes of the body of *Aedes* than light/dark cycles.

ARTICLE HISTORY
Received 29 November 2020
Revised 8 March 2021
Accepted 9 March 2021

KEYWORDS
Aedes aegypti; circadian clock; peripheral tissues; temperature cycles; light/dark cycles; clock genes expression

Introduction

Since the first organisms appeared on the planet, they have been subjected to daily cycles of light and temperature. These selective pressures have led beings to evolve strategies to adapt to environmental fluctuations. They developed a daily pacemaker for controlling their physiological and behavioral rhythms (Moore-Ede et al. 1982). In the insect model, *Drosophila melanogaster*, this clock is composed of a set of genes that regulate themselves, as well as other genes related to various phenotypes (reviewed by Patke et al. 2020). Microarray studies estimate that 10% of the *Drosophila* genome is cyclically expressed, which underscores the importance of the clock in controlling a broad spectrum of cellular pathways. However, when the rhythmic expression was compared in different tissues, less than 10% of the genes that cycle in the head were found to do so in the body (Ceriani et al. 2002; Claridge-Chang et al. 2001; Keegan et al. 2007; McDonald and Rosbash 2001).

As in *Drosophila*, hematophagous mosquitoes exhibit activity, reproduction, and feeding rhythms under the control of an endogenous circadian clock (Saunders 2002). Mosquitoes are vectors of important tropical diseases. Their activity and blood-feeding rhythms are crucial for the transmission of various pathogens (Clements 1999; Saunders 2002). Our,, as well as other groups, have been able to elucidate the expression of the core clock genes in the central pacemaker (head) of *Aedes aegypti*, the main vector of Dengue, Chikungunya, and Zika arboviruses (Gentile et al. 2009; Leming et al. 2014; Rivas et al. 2018; Teles-de-freitas et al. 2020), but we still do not know how these genes are expressed in the body. Head and body comparative studies have shown differences in clock gene expression in vector insects, such as *Lutzomyia longipalpis* and *Anopheles gambiae* (Meireles-Filho et al. 2006a, 2006b; Rund et al. 2011, 2013). Since synchronization of temperature cycles begins in the peripheral tissues mainly located in the body (based on the *Drosophila* model, Sehadova et al. 2009) and *Aedes aegypti* appears to be more sensitive to temperature cycles than other mosquitoes (Rivas et al. 2018), we were motivated to understand how clock genes are expressed in the body of this vector species. Thus, we now present the expression in the body of the core genes (*period, timeless, cryptochrome 2* and *cycle*) of the circadian clock of *Aedes*. Our objective is not to accomplish an exhaustive understanding on *Aedes* clock, but to provide an initial model about the way it functions in peripheral tissues, comparing it to what is already known about the expression of these genes in the head.

Materials and methods

Mosquitoes

The IBEx (Instituto de Biologia do Exército, Rio de Janeiro, Brazil) donated the eggs of *Ae. aegypti*

CONTACT Rafaela V. Bruno rafaelav@ioc.fiocruz.br; rafaelabruno@gmail.com Laboratório de Biologia Molecular de Insetos, Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro, Brazil.

(Rockefeller strain). We raised mosquitoes from the egg stage in LD 12:12 under constant 25°C. Females were separated from males while newly emerged and still virgins. In all experiments, we used 1- to 3-day-old virgin females.

Simulation of light/dark and temperature cycles

For light/dark cycles we used the HLT Powerbus USB station (Hoenig Lichttechnik Ltd.), with which we were able to simulate LD12:12 conditions with gradual increases and decreases in light intensity during dawn and dusk. Light intensity increased from 0 – to 1000 lux, from ZT0 to ZT1.5, and remained stable in 1000 lux from ZT1.5 to ZT10.5. Establishment of semi-natural temperature cycles was possible due to the data generated by the Instituto Nacional de Meteorologia (INMET) at the equinoxes in Rio de Janeiro, Brazil; we used the thermal ramp system of the Solab incubator (SL225/334) – of which minimum and maximum temperature were 20°C and 30°C, respectively. Both the simulation of light/dark cycles and temperature cycles were based on the protocol established in our previous studies (Teles-de-freitas et al. 2020).

Expression of circadian clock genes

Female mosquitoes were kept for two days under set conditions (Table 1). On the third day, we collected ten individuals every 4 h, over 24 h. For almost all regimens the set conditions were the same as those of the day of collection. The only exception was mosquitoes in free running. They were subjected to two days under gradual LD cycles at 25°C and then collected on the first day under DD at 25°C. For each experiment, there were six time points and this procedure was repeated three or four times. Total RNA of the bodies was extracted by the TRIzol method (Invitrogen, Carlsbad, CA), and cDNA was synthesized with TaqMan Reverse Transcription Reagents (Applied Biosystems, Foster City, CA) following the methods described by Teles-de-freitas et al. (2020). The final cDNA concentration was 1ng/μL. Then, we made a relative quantification via real-time PCRs (qPCRs), using Power SYBR Green PCR Master Mix (Thermo Fisher, Waltham, MA) in a StepOnePlus Real-Time PCR System (Thermo Fisher, Waltham, MA). We amplified *per, tim, cry2, cyc*, and *rp49* genes using oligonucleotides designed by Gentile et al. (2009). We used the *rp49* gene as a constitutive control and calculated the relative mRNA abundance with the comparative C_T method (Pfaffl 2001). The values obtained for the relative abundance of mRNA were plotted in Excel graphs. We also calculated if the expression of the relative abundance of the genes varied significantly throughout the 24 h period of each regimen. We considered a gene presented rhythmic expression if the mRNA abundance differed significantly among the six time-point samples using a One-Way ANOVA (p = .05). All statistical analyses were conducted with GraphPad Prism 5 (Prism, La Jolla, CA).

Table 1. Conditions under which mosquitoes were entrained and collected.

	Synchronization days	Collect days
DD at 25°C	1st and 2nd days under gradual LD 25°C	3rd day under DD 25°C
gradual LD at 25°C	1st and 2nd days under gradual LD 25°C	3rd day under gradual LD 25°C
DD with seminatural TC	1st and 2nd days under DD with seminatural TC	3rd day under DD with seminatural TC
gradual LD with seminatural TC	1st and 2nd days under gradual LD with seminatural TC	3rd day under gradual LD with seminatural TC

Results

The purpose of our research is to understand how clock genes are expressed in the body of *Aedes aegypti*. To this end, we used the same strain (Rockefeller), as well as light/dark and temperature cycles previously used by our group in the study of clock gene expression in the head of *Aedes aegypti* (Teles-de-freitas et al. 2020). We intended to compare the data obtained from the body with the expression described in the head of *A. aegypti*.

Initially, we subjected mosquitoes to a regimen under which their main environmental cues were absent, that is, constant dark and temperature (DD 25°C), in order to observe the expression of *period* (*per*), *timeless* (*tim*), *cryptochrome 2* (*cry2*), and *cycle* (*cyc*) in the body. We noted only *per* presented cyclic expression, with an expression peak at CT13 and trough at CT5. The other clock genes were arrhythmic under this condition (Figure 1, Table 2). When we maintained the temperature constant (25°C), but added the 12 h light/12 h dark cycle (gradual LD 25°C), we observed a pattern very similar to that produced under DD 25°C, that is, the expressions of *tim, cry2*, and *cyc* were arrhythmic in the body, but the expression of *per* cycled with a peak in ZT13 and trough at ZT5 (Figure 2, Table 2).

Then we exposed the mosquitoes to constant darkness, but this time we added the temperature cycles to the regimen, which we usually call DD with

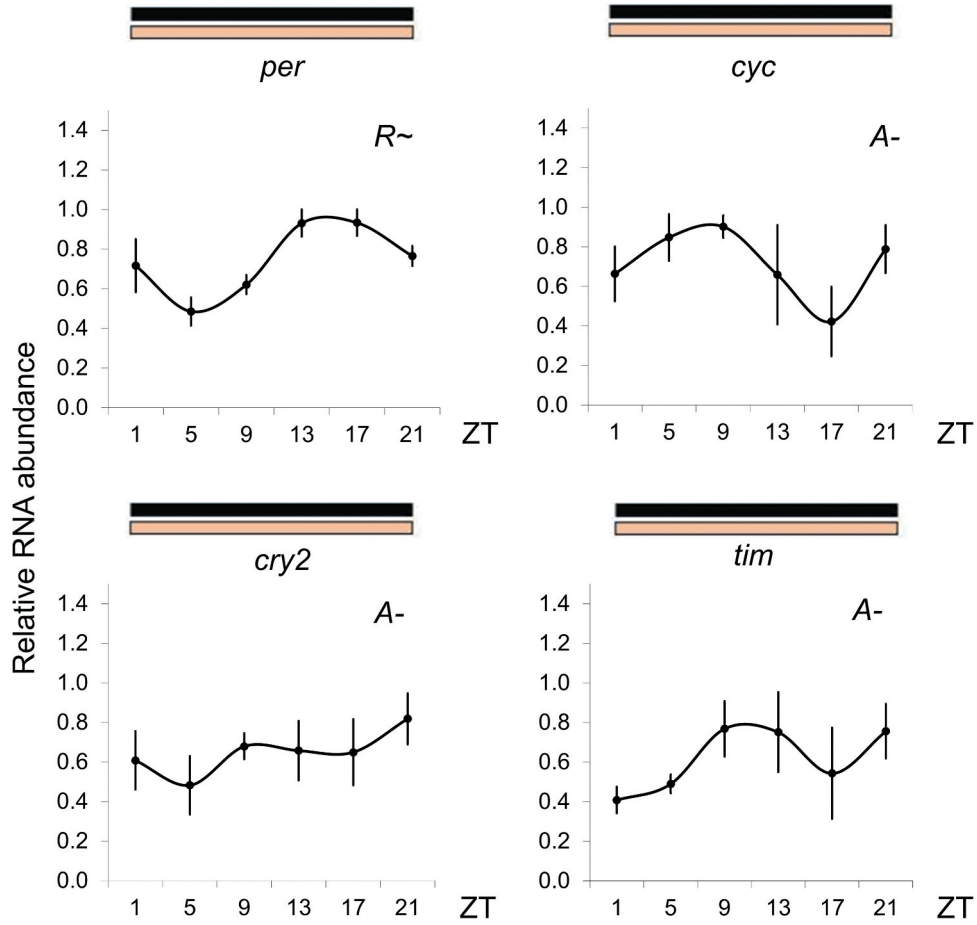

Figure 1. Expression of circadian clock genes in the body of *Ae. aegypti* in DD 25°C. The graphs show the expression of *per* (*period), cyc* (*cycle); cry2* (*cryptochrome2*) and *tim* (*timeless*). We used three replicates, with a pool of 10 individuals/group. The vertical bars represent the standard deviation. The black horizontal bars indicate the darkness constants and the orange bars the constant temperature (25°C). Symbols R~ and A- represent rhythmic genes and arrhythmic genes, respectively, in accordance with the One-Way ANOVA statistical test.

Table 2. Statistical analysis of the circadian expression of clock genes in the body of *Ae. aegypti.*

		Gene			
		per	*cyc*	*cry2*	*tim*
DD 25°C	$F_{5,18}$	5.02	1.24	0,62	1.09
	P	**<0.005**	0.35 (ns)	0.68 (ns)	0.42 (ns)
gradual LD 25°C	$F_{5,18}$	11.45	0.16	0.91	0.84
	P	**<0.001**	0.97 (ns)	0.50 (ns)	0.54 (ns)
DD with seminatural Tc	$F_{5,18}$	11.34	1.20	2.02	58.88
	P	**<0.001**	0.37 (ns)	0.15 (ns)	**<0.001**
gradual LD with seminatural Tc	$F_{5,24}$	31.06	24.38	14.60	18.24
	P	**<0.001**	**<0.001**	**<0.001**	**<0.001**

One-way analysis of variance. ns = non-significant rhythmicity of mRNA transcription. We highlighted in bold the rhythmic gene expressions.

seminatural TC. Under these conditions, we observed some differences in the expression of the analyzed genes when compared to the DD 25°C and gradual LD 25°C regimens. Under DD with seminatural TC, although *cry2* and *cyc* maintained the arrhythmic pattern, *tim* presented cyclic expression in the body with a peak between ZT9 and ZT13, and a trough between ZT1 and ZT5. We also noted that *period* continued to present cyclic expression in DD with seminatural TC. However, interestingly, this gene had a peak of expression at ZT17 and a trough at ZT 1 under these conditions (Figure 3, Table 2).

Our next step was to unite the two environmental indicators. We subjected mosquitoes to 12 h light/12 h dark cycles and temperature cycles simultaneously (gradual LD with seminatural TC). Under these conditions,

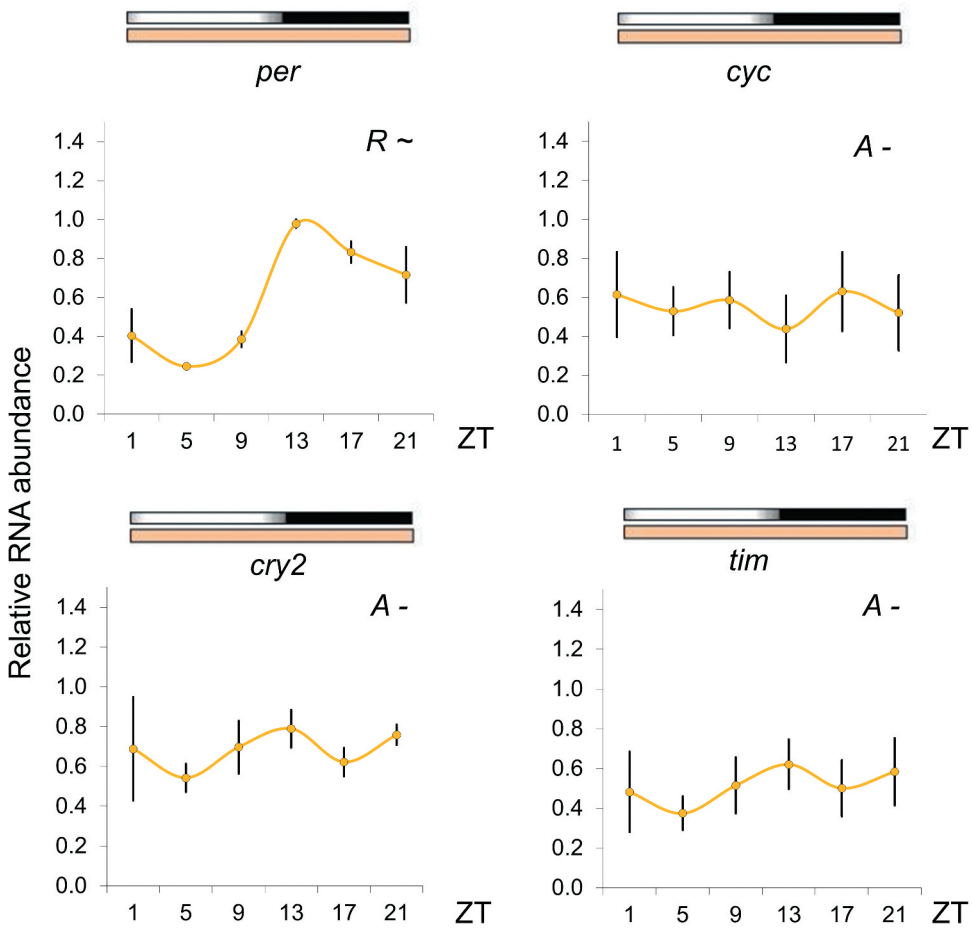

Figure 2. Expression of circadian clock genes in the body of *Ae. aegypti* in gradual LD 25°C. The graphs show the expression of *per* (*period), cyc* (*cycle); cry2* (*cryptochrome2*) and *tim* (*timeless*). We used three replicates, with a pool of 10 individuals/group. The vertical bars represent the standard deviation. In the horizontal bars, white, black, and orange colors indicate the light phase, dark phase, and constant temperature (25°C), respectively. R~ represents rhythmic genes and A- represents in accordance with the One-Way ANOVA statistical test.

all of the genes analyzed curiously exhibited cyclic expression in the body of *Aedes aegypti; per* had a peak of expression at ZT17 and a trough at ZT5, while *tim* presented a peak of expression at ZT13 and a trough at ZT5. The expression of *cry2* produced a trough at ZT9 and a unique peak at ZT1, and *cyc* exhibited a peak of expression between ZT1 and ZT5 and a trough at ZT13 (Figure 4, Table 2).

Discussion

Circadian clocks impose daily periodicity on many physiological and behavioral processes in a wide variety of organisms. In animals, several tissues are known to contain circadian pacemakers. In mammals, coordination is performed in the head by the central clock of the suprachiasmatic nucleus (SCN) by means of pathways that include endocrine, neural, and even thermal signals (Albrecht 2012; Buhr et al. 2019; Schibler et al. 2015; Yoo et al. 2004). In insects, the knowledge about central and peripheral clocks is almost exclusively derived from studies done on *Drosophila*. Unlike mammals, most peripheral pacemakers in *Drosophila* are autonomous, and their synchronization is accomplished through exposure to signals such as light – which can penetrate the translucent exoskeleton – or temperature (Ito and Tomioka 2016). However, before this study, nothing was known about the expression of clock genes in peripheral tissues of *Aedes aegypti*, an insect so important for the transmission of various diseases.

We selected four of the most important clock genes in *Aedes aegypti* and observed their expression in the mosquito body. We observe that the expression of the analyzed genes was very similar in DD 25°C and gradual LD 25°C. Under these conditions, only *period* presented cyclic expression, showing a peak and a trough at the same time in both regimens – ZT13 and ZT5, respectively, while the other genes, *tim, cyc*, and *cry2*, were

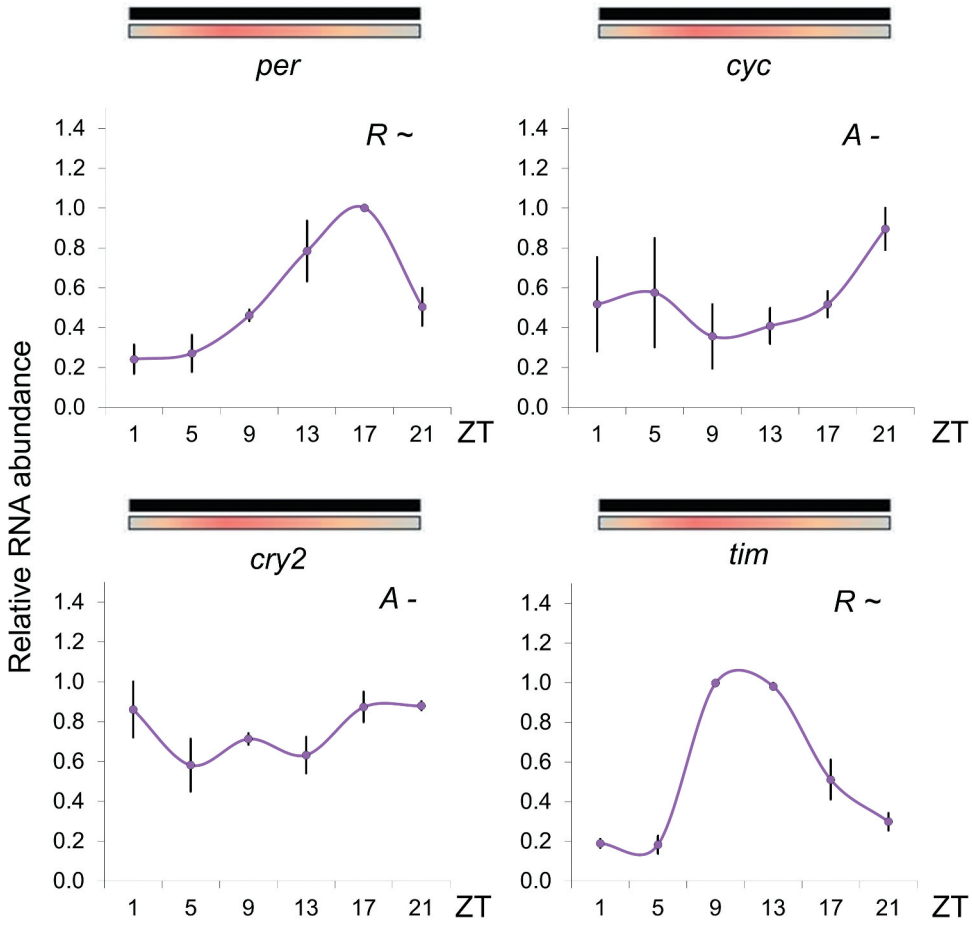

Figure 3. Expression of circadian clock genes in the body of *Ae. aegypti* in DD with seminatural TC. The graphs show the expression of *per* (*period), cyc* (*cycle); cry2* (*cryptochrome2*) and *tim* (*timeless*). We used three replicates, with a pool of 10 individuals/group. The vertical bars represent the standard deviation. The black horizontal bar indicates the constant dark. The bar in gradient colors shows the temperature cycles (red = 30°C, orange = 25°C, blue = 20°C). Symbols R~ and A- represent rhythmic genes and arrhythmic genes, respectively, in accordance with the One-Way ANOVA statistical test.

arrhythmic (Figures 1 and 2, Table 2). These results suggest that the expression of *per* in the body is regulated endogenously by the circadian clock even in the absence of environmental factors and that the addition of gradual LD cycles, alone, was not able to alter the expression of any of the analyzed genes.

The expression of the genes in the body was quite similar in LD and DD 25°C. However, we observed many differences when we compared these findings with those of the head under both conditions. Most of the analyzed genes presented cyclical expression in the head under LD and DD at 25°C (except for *tim* in LD, which was of borderline statistical difference) (Gentile et al. 2009), but we found only *period* presented cyclical expression in the body under these conditions (Figures 1 and 2, Table 2). Furthermore, even *period* presented a different expression phase in the body, compared to the head. This gene showed a peak of expression at ZT17 in LD and DD in the head (Gentile et al. 2009).

The fact that we observed almost no changes in the body of *Aedes* when comparing the gene expression under LD and DD at 25°C suggests that LD cycles are a weak *Zeitgeber* in the body, at least for the clock genes analyzed. On the other hand, in the body of *Anopheles gambiae, per, tim, cyc*, and *cry2* presents cyclical expression under LD with constant temperature and seem to suffer more from the effects of this environmental indicator (Rund et al. 2011, 2013). Hence, it is possible that the lack of sensitivity to synchronize its peripheral clock with the LD cycles is specific to *Aedes aegypti* and should not be extrapolated to other mosquitoes.

Alternatively, we observed that temperature cycles seem to have a greater influence than light/dark cycles on clock genes in the body of *Aedes*. This is

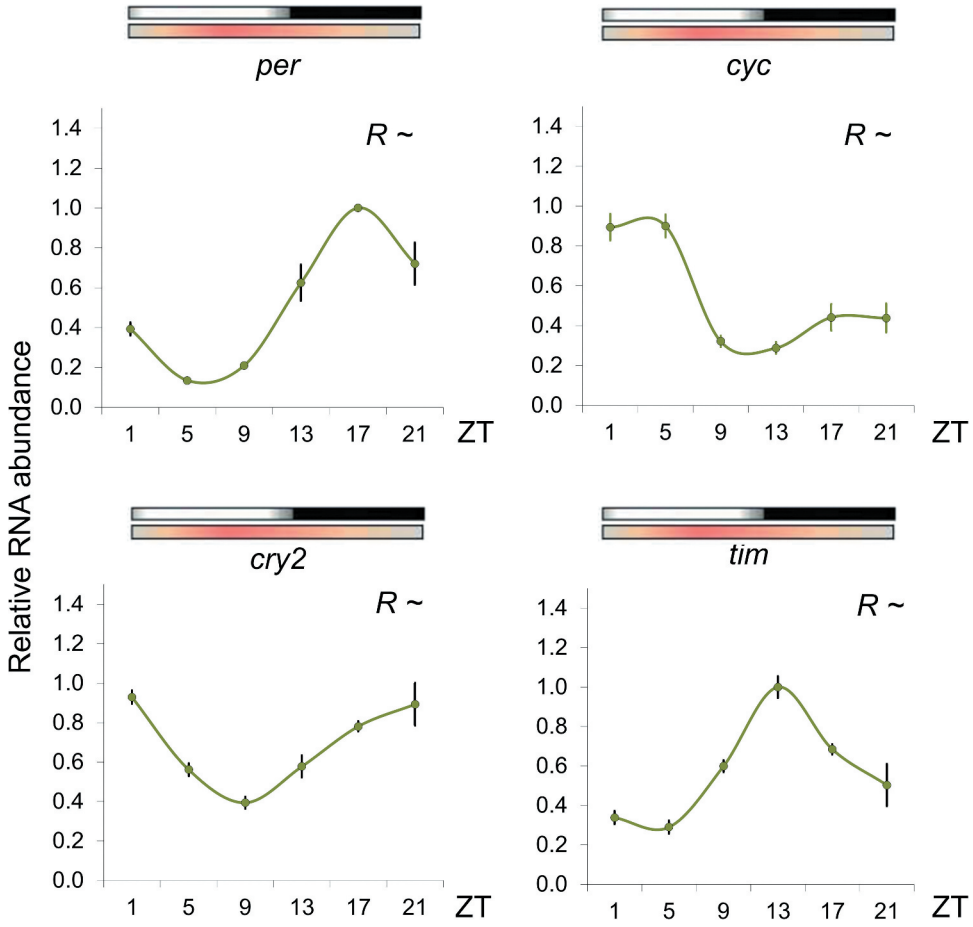

Figure 4. Expression of circadian clock genes in the body of *Ae. aegypti* in gradual LD with seminatural TC. The graphs show the expression of *per* (*period), cyc* (*cycle); cry2* (*cryptochrome2*) and *tim* (*timeless*). We used four replicates, with a pool of 10 individuals/group. The vertical bars represent the standard deviation. The white horizontal bar indicates the light phase, while the black bar shows the dark phase. The bar in gradient colors shows the cycles of temperature (red = 30°C, orange = 25°C, blue = 20°C). Symbols R~ and A- represent rhythmic genes and arrhythmic genes, respectively, in accordance with the One-Way ANOVA statistical test.

because the DD with seminatural TC regimen was able to both promote the cyclic expression of *timeless* and shift the expression peak of *period* (Figure 3, Table 2). Under this condition, the expression peaks of *per* and *tim* in the body occurred, respectively, at ZT17 and ZT13-17. Both genes showed the same peak of expression under DD with seminatural TC when we analyzed the head of *Aedes* (Teles-de-freitas et al. 2020).

Moreover, although temperature cycles appear to have greater influence than light/dark cycles on the regulation of the clock in the body of *Aedes*, it is likely that they do not act alone. The fact that all analyzed genes recovered their cyclical expression under gradual LD with seminatural TC (Figure 4, Table 2) suggests that for the full functioning of the clock in the body there must be synergy between temperature cycles and light/dark cycles. To reinforce this hypothesis, when mosquitoes are under LD with seminatural TC, rather than under the other conditions observed previously, the expression of clock genes in the head and the body is more similar. We highlight only one exception: *cry2*. This gene showed a clear difference when we compared its expression in the head with that in the body of mosquitoes under LD with seminatural TC (Figure 4, Table 2) (Teles-de-freitas et al. 2020). In the head, *cry2* is known to present an expression peak at ZT1 and another one at ZT17 under LD with seminatural TC (Teles-de-freitas et al. 2020). However, we observed only one peak of expression at ZT1 in the body under the same condition (Figure 4, Table 2).

Gentile et al. (2009) had suggested that *cry2* expression in the head of *Ae. aegypti* is controlled by two different circadian response elements, one responsible for the morning peak and the other for the evening peak. They proposed that *cry2* had distinct promoter regions, where different transcriptional factors would bind depending on the time of day. The morning peak of

cry2 is viewed as being controlled by the same transcriptional factors of *cyc*, while the afternoon peak is viewed as being controlled by factors responsible for the transcription of *per, tim*, and other genes. This would justify why, in the head, the morning peak of *cry2* occurred at the same time as the expression peak of *cyc*, while the evening peak of *cry2* was similar to the expression peak of *per*. However, in the body, *cry2* showed only one peak at ZT1, which is similar to the expression of *cyc* in LD with seminatural TC. The fact that *cry2* did not present an afternoon peak, while *period* and *timeless* kept their canonical expressions in LD with seminatural TC, suggests that at least in the body the regulation mechanism of *cry2* is different from the previously proposed model of the clock regulation in the head (Figure 4, Table 2).

In summary, the results obtained from the expression of clock genes in the body of *Aedes aegypti* differ considerably from those in its head. First, because, unlike what occurs in the head, clock genes in the body seem to be more affected by temperature cycles than by light/dark cycles (Rivas et al. 2018; Teles-de-freitas et al. 2020). Secondly, in DD 25°C, all clock genes analyzed cycled in the heads (Gentile et al. 2009). On the other hand, in the body, we observed rhythmicity for all genes only in LD with seminatural TC. Thus, it is possible that, contrasting to the central pacemaker in the brain, peripheral clocks are much more dependent on environmental factors, especially temperature cycles. Due to the epidemiological importance of the vector *Aedes aegypti*, increasingly diverse research groups around the world are studying its peripheral tissues, such as fat bodies, ovaries, testis, etc. But most of these studies are not concerned with the circadian clock of peripheral tissues in *Aedes*. This is at least curious, since for many insects the circadian clock controls genes involved in growth, development, immunity, response to insecticides, among other phenotypes (Leming et al. 2014; Matthews et al. 2016; Price et al. 2011; Ptitsyn et al. 2011; Roberts et al. 1974; Zhang et al. 2017).

Here we show for the first time how clock genes are expressed in the body of *Aedes aegypti*. Our experiments were not designed toprovide an exhaustive understanding of the expression the clock genes of *Aedes*; rather, their findings were intended to serve as an initial model of the functioning of the clock genes in the body, in relation to what is already known about the expression of the clock genes in the head. We think that upcoming studies focusing on clock genes in specific tissues in the body will also be interesting.

The clock gene expression in the body not only differs from what is known about the heads of *Aedes* but it also seems to be more sensitive to temperature cycles than to light/dark cycles. It leads us to question why most studies that investigate peripheral tissues in *Aedes* insist on exposing these mosquitoes to constant temperatures (Matthews et al. 2016; Price et al. 2011; Zhang et al. 2017). We believe investigations using gradual temperature cycles – which are closer to what is found in nature than the constant temperature conditions traditionally used in experiments – will allow us to better perceive the effects on the physiology and development of mosquitoes.

Acknowledgements

We would like to thank Robson Costa da Silva for technical support. This study was partially financed by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001. We also thank the CNPq and the FAPERJ for the financial support to RTF and RVB.

Funding

This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ).

ORCID

Rayane Teles-de-Freitas http://orcid.org/0000-0003-2613-0631
Liliane Barboza http://orcid.org/0000-0001-5899-005X
Rafaela V. Bruno http://orcid.org/0000-0002-7082-9768

Authors contributions

Conceptualization and design of study, RTF, LBS, RVB; Performance and formal analysis, RTF, LBS, RVB; Writing - original draft, RTF; Writing - revision & editing, RTF, LBS, RVB; Supervision, RVB; Funding acquisition, RVB.

Declaration of interest statement

The authors report no conflict of interest.

References

Albrecht U. 2012. Timing to perfection: the biology of central and peripheral circadian clocks. Neuron. 74:246–260. doi:10.1016/j.neuron.2012.04.006.

Buhr ED, Vemaraju S, Diaz N, Lang RA, Van Gelder RN. 2019. Neuropsin (OPN5) mediates local light-dependent induction of circadian clock genes and circadian photoentrainment in exposed murine skin. Curr Biol. 29:3478–3487.e4. doi:10.1016/j.cub.2019.08.063.

Ceriani MF, Hogenesch JB, Yanovsky M, Panda S, Straume M, Kay SA. 2002. Genome-wide expression analysis in *Drosophila* reveals genes controlling circadian behavior. J Neurosci. 22:9305–9319. doi:10.1523/JNEUROSCI.22-21-09305.2002.

Claridge-Chang A, Wijnen H, Naef F, Boothroyd C, Rajewsky N, Young MW. 2001. Circadian regulation of gene expression systems in the *Drosophila* head. Neuron. 32:657–671. doi:10.1016/s0896-6273(01)00515-3.

Clements AN. 1999. The biology of mosquitoes Vol. 2: sensory reception and behaviour. New York (NY): CABI Publishing. https://www.cabdirect.org/cabdirect/abstract/19990505183 .

Gentile C, Rivas GB, Meireles-Filho AC, Lima JB, Peixoto AA. 2009. Circadian expression of clock genes in two mosquito disease vectors: *cry2* is different. J Biol Rhythms. 24:444–451. doi:10.1177/0748730409349169.

Ito C, Tomioka K. 2016. Heterogeneity of the peripheral circadian systems in Drosophila melanogaster: a review. Front Physiol. 7:8. doi:10.3389/fphys.2016.00008.

Keegan KP, Pradhan S, Wang J-P, Allada R. 2007. Meta-analysis of *Drosophila* circadian microarray studies identifies a novel set of rhythmically expressed genes. PLoS Comput Biol. 3:e208. doi:10.1371/journal.pcbi.0030208.

Leming MT, Rund SSC, Behura SK, Duffield GE, O'Tousa JE. 2014. A database of circadian and diel rhythmic gene expression in the yellow fever mosquito *Aedes aegypti*. BMC Genomics. 15:1128. doi:10.1186/1471-2164-15-1128.

Matthews BJ, McBride CS, DeGennaro M, Despo O, Vosshall LB. 2016. The neurotranscriptome of the *Aedes aegypti* mosquito. BMC Genomics. 6:17–32. doi:10.1186/s12864-015-2239-0.

McDonald M, Rosbash M. 2001. Microarray analysis and organization of circadian gene expression in *Drosophila*. Cell. 107:567–578. doi:10.1016/S0092-8674(01)00545-1.

Meireles-Filho ACA, Amoretty PR, Souza NA, Kyriacou CP, Peixoto AA. 2006a. Rhythmic expression of the cycle gene in a hematophagous insect vector. BMC Mol Biol. 7:38. doi:10.1186/1471-2199-7-38.

Meireles-Filho ACA, Rivas GBDS, Gesto JSM, Machado RC, Britto C, De Souza NA, Peixoto AA. 2006b. The biological clock of an hematophagous insect: locomotor activity rhythms, circadian expression and downregulation after a blood meal. FEBS Lett. 580:2–8. doi:10.1016/j.febslet.2005.11.031.

Moore-Ede MC, Sulzman FM, Fuller CA. 1982. The clocks that time us: physiology of the circadian timing system. Harvard University Press. doi:10.4271/820832.

Patke A, Young MW, Axelrod S. 2020. Molecular mechanisms and physiological importance of circadian rhythms. Nat Rev Mol Cell Biol. 21:67–84. doi:10.1038/s41580-019-0179-2.

Pfaffl MW. 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 29:e45. doi:10.1093/nar/29.9.e45.

Price DP, Nagarajan V, Churbanov A, Houde P, Milligan B, Drake LL, Gustafson JE, Hansen IA. 2011. The fat body transcriptomes of the yellow fever mosquito *Aedes aegypti*, pre- and post- blood meal. PLoS One. 6:e22573. doi:10.1371/journal.pone.0022573.

Ptitsyn AA, Reyes-Solis G, Saavedra-Rodriguez K, Betz J, Suchman EL, Carlson JO, Black WC. 2011. Rhythms and synchronization patterns in gene expression in the *Aedes aegypti* mosquito. BMC Genomics. 12:153. doi:10.1186/1471-2164-12-153.

Rivas GBS, Teles-de-freitas R, Pavan MG, Lima JBP, Peixoto AA, Bruno RV. 2018. Effects of light and temperature on daily activity and clock gene expression in two mosquito disease vectors. J Biol Rhythms. 33:272–288. doi:10.1177/0748730418772175.

Roberts D, Smolensky MH, Hsi B, Scanlon JE. 1974. Circadian pattern in susceptibility of *Aedes aegypti* (L.) larvae to Dursban. In: Scheving LE, Halberg F, Pauly JE, editors. Chronobiology. Tokyo (Japan): Igaku Shoin. p. 612 6.

Rund SSC, Gentile JE, Duffield GE. 2013. Extensive circadian and light regulation of the transcriptome in the malaria mosquito *Anopheles gambiae*. BMC Genomics. 108:E421–30. doi:10.1073/pnas.1100584108.

Rund SSC, Hou TY, Ward SM, Collins FH, Duffield GE. 2011. Genome-wide profiling of diel and circadian gene expression in the malaria vector *Anopheles gambiae*. Proc Natl Acad Sci USA. 108:E421–30. doi:10.1073/pnas.1100584108.

Saunders DS. 2002. Insect clocks. 3rd ed. Elsevier. https://www.elsevier.com/books/insect-clocks-third-edition/saunders/978-0-444-50407-4 .

Schibler U, Gotic I, Saini C, Gos P, Curie T, Emmenegger Y, Sinturel F, Gosselin P, Gerber A, Fleury-Olela F, et al. 2015. Clock-talk: interactions between central and peripheral circadian oscillators in mammals. Cold Spring Harb Symp Quant Biol. 80:223–232. doi:10.1101/sqb.2015.80.027490.

Sehadova H, Glaser FT, Gentile C, Simoni A, Giesecke A, Albert JT, Stanewsky R. 2009. Temperature entrainment of *Drosophila*'s circadian clock involves the gene *nocte* and signaling from peripheral sensory tissues to the brain. Neuron. 64:251–266. doi:10.1016/j.neuron.2009.08.026.

Teles-de-freitas R, Rivas GBS, Peixoto AA, Bruno RV. 2020. The summer is coming: *nocte* and *timeless* genes are influenced by temperature cycles and may affect *Aedes aegypti* locomotor activity. Front Physiol. 11:614722. doi:10.3389/fphys.2020.614722.

Yoo SH, Yamazaki S, Lowrey PL, Shimomura K, Ko CH, Buhr ED, Siepka SM, Hong HK, Oh WJ, Yoo OJ, et al. 2004. PERIOD2::LUCIFERASEreal-time reporting of circadian dynamics reveals persistent circadian oscillations in mouse peripheral tissues. Proc Natl Acad Sci U S A. 101:5339–5346. doi:10.1073/pnas.0308709101.

Zhang X, Aksoy E, Girke T, Raikhel AS, Karginov FV. 2017. Transcriptome-wide microRNA and target dynamics in the fat body during the gonadotrophic cycle of *Aedes aegypti*. Proc Natl Acad Sci U S A. 114:E1895–E1903. doi:10.1073/pnas.1701474114.