Title

Genetic variation and phenotypic plasticity in circadian rhythms of an armed beetle, *Gnatocerus cornutus* (Tenebrionidae)

Authors

Kentarou Matsumura[1], Masato S Abe[2], Manmohan D Sharma[3], David J. Hosken[3], Takashi Yoshii[4], Takahisa Miyatake[1]*

Institutions

1. Graduate School of Environmental and Life Science, Okayama University, Okayama, Japan.

2. Center for Advanced Intelligence Project, RIKEN, Tokyo, Japan.

3. Centre for Ecology & Conservation, School of Biosciences, University of Exeter, Tremough, Penryn, UK.

4. Graduate School of Natural Science and Technology, Okayama University, Okayama, Japan.

Short running title

Circadian rhythm variation in armed beetle

*** Corresponding Author**

E-mail: miyatake@okayama-u.ac.jp

Abstract

Circadian rhythms, their free-running periods and strength of the rhythm are often used

as indicators of biological clocks, and there is evidence that the free-running periods of circadian rhythm are not affected by environmental factors like temperature. However, there are few studies of environmental effects on the power of rhythms and it is not clear if temperature compensation is universal. Additionally, genetic variation and phenotypic plasticity in biological clocks are important for understanding the evolution of biological rhythm, but genetic and plastic effects are rarely investigated. Here, we used 18 isofemale lines (genotypes) of *Gnatocerus cornutus* to assess rhythms of locomotor activity, while also testing for temperature effects. We found that total activity and power of circadian rhythm were affected by interactions between sex and genotype or sex, genotype and temperature, so that while males tended to be more active and showed greater increases in activity, this effect varied across both genotypes and temperatures. The period of activity only varied by genotype and was thus independent of temperature. The complicated genotype-sex-environment interactions we recorded stress the importance of investigating circadian activity in more integrated ways.

Keywords

circadian rhythm, power of circadian rhythm, isofemale line, *Gnatocerus cornutus*

Introduction

Many species show a rhythmicity of activity, from the timing of flowering in plants to foraging behaviour in animals (Saunders 2002). Examining these rhythms in sexually dimorphic animals is interesting because males and females could have different life-history strategies and thus different daily patterns of activity (e.g., Anderson 1994; Emlen 2015). A rhythmicity approaching 24h is called the circadian rhythm, and many studies have identified the genes underpinning circadian rhythms (Dunlap 1996). Additionally, the time it takes for an organism to repeat endogenous rhythms in the absence of environmental cues is known as the free-running period and, amongst other things, this provides information on the accuracy of internal clocks (Klarsfeld et al. 2003). The power of circadian rhythm (=strength of rhythm; hereafter "power") is another measure of activity cycles, and indicates the inherent strength of the rhythm (Klarsfeld et al. 2003). However, while many previous studies have explored free-running periods, few have investigated the power of circadian rhythm (but see Cavey et al. 2016; Fujioka et al. 2017; King et al. 2017).

Free-running periods of circadian rhythms can vary with light intensity (Aschoff 1960, 1965), but rhythms can remain robust over a wide range of temperatures, a situation known as temperature compensation (e.g., Zimmerman et al. 1968; Dunlap et al. 2004; Pittendrigh 1954). Be that as it may, there are a few published reports of temperature compensation in the power of circadian rhythms (e.g., Saunders 1976, Sorek and Levy 2012).

In addition to environmental affects, circadian rhythms can also differ between the sexes. For example, in the fly *Drosophila melanogaster*, the free-running period of males is shorter than that of females (Helfrich-Förster 2000). This suggests that males have

different periods of rhythm, possibly to facilitate searching for females (Helfrich-Förster 2000). It is also possible that the power is different between males and females. To date however, no investigation of the effect of sex on power has been reported, although in species with strong sexual selection (e.g., those with pronounced dimorphism in sexually selected morphology) there may well be dimorphism in the strength and period of bio-rhythms as a result of sex-specific selection.

Here we investigated the effects of temperature on the free-running period, power and circadian rhythm of locomotor activity in the broad-horned flour beetle *Gnatocerus cornutus* [Fabricius], while also testing for sex-specific effects. In *G. cornutus*, only males developed mandibles and they often fight with rival males to obtain matings using these condition-dependent weapons (Okada et al. 2006; House et al. 2016). Furthermore, there is significant genetic variation in mandible size and this affects fighting and mating outcomes (Harano et al. 2010). Thus this species is ideal to assess whether strong sexual selection has resulted in sex differences in activity rhythms, power and periods, because beetles generally show evidence of circadian activity (Harano and Miyatake 2011). Our investigation used 18 iso-female lines (genotypes), making it possible to also test for genetic variation in circadian rhythms of locomotor activity, their free-running period and power. We also investigated the plasticity of the activity rhythm by measuring it at different temperatures.

Materials & Methods

Stock population and isolines

The *G. cornutus* beetle culture originated from adults collected in Miyazaki City (31° 54′N, 131° 25′ E), Japan, in 1957 (see Okada et al. 2006), and has been maintained in the

91 laboratory of the National Food Research Institute and Okayama University for ~50 years
92 on whole meal enriched with yeast as food. Beetles were reared on whole meal enriched
93 with brewer's yeast and kept at 25–27ºC and 60% relative humidity under a photoperiod
94 of 14:10 h light:dark (House et al. 2016). We established 18 iso-female lines (isolines =
95 standardized genotypes) from the stock population. Initially, 18 males and 18 females
96 were selected at random and paired. Subsequent full-sib-matings within each family were
97 used to propagate each line for 37 generations until the present study was conducted.
98 Differences between lines are then largely genetic and any within line variance is largely
99 environmental (David et al. 2005). Lines can effectively be thought of as different
100 genotypes (David et al. 2005). To obtain adults for the present experiments, one final
101 instar larva was placed in a separate well of a 24-well tissue culture plate because pupation
102 in *G. cornutus* is inhibited under high larval density (Okada et al. 2006).
103
104 *Locomotor activity*
105 To assess circadian rhythm, we maintained beetles at 14L10D conditions for more than
106 20 days in an incubator kept at 25ºC before the measurement of locomotor activity, and
107 we then measured the locomotor activity of *G. cornutus* for 10 days in darkness. A beetle
108 from each isoline with enough food described above was put in a clear plastic Petri dish
109 (30 × 10 mm) in an incubator maintained at 25ºC or 29ºC. The beetles develop faster at
110 29ºC (unpublished observation, TM), and almost investigation of the beetle's behaviour
111 has been conducted under 25ºC (Okada et al. 2006; Okada and Miyatake 2009, 2010),
112 and thus we choose the two temperatures, 25ºC and 29ºC in this experiment. The
113 locomotor activity of each individual was monitored using an infrared actograph. An
114 infrared light beam was passed through a clear Petri dish, and the beam was projected

115 onto a photomicrosensor (E3S-AT11; Omron, Kyoto, Japan) that detected all
116 interruptions of the light beam. Signals of interruption of the infrared light beam were
117 recorded every 6 min. Sample size of each isoline is shown in S1 Table.
118
119 *Statistical analysis*
120 To determine the circadian rhythm, the locomotor activity data for 10 days (= total
121 activity) in constant dark condition was analyzed. The free-running period of circadian
122 rhythm was established using a χ^2 periodogram test (Sokolove and Bushell 1978) for data
123 on locomotor activity between 20–28 h (Halberg 1969). Circadian rhythmicity was
124 determined using χ^2 periodgram analysis, and used "power" as an index for the strength
125 of rhythms. Power of circadian rhythm was defined as the maximum difference between
126 the χ^2 value and the significance threshold line $P = 0.05$, that is the size of the peak above
127 5% threshold (see Fig. 1 in Klarsfeld et al. 2003). Power is high when rhythm is clear and
128 strong, and power of less than 0 indicates a statistically arrhythmic state.
129 To analyze the effects of temperature, isoline and sex on the period, power of the
130 circadian rhythm and total activity, we used MANOVA and post-hoc analysis of variance
131 (ANOVA). All statics were based on R version 3.4.3 (R Development Core Team, 2017).
132
133 **Results**
134 MANOVA revealed significant main effects for all predictors and interactions on the
135 multivariate combination of power, period and total activity. All variables were significant
136 (see S2 Table). Post-hoc tests showed that power and total activity were the main drivers
137 of these effects, with all predictors and interactions being significant for power, while sex,
138 temperature and isoline plus the sex-by-isoline interaction being significant for total

activity (Table 1).

Strictly speaking it is the highest order interactions that need to be interpreted, which for power was the three-way interaction between sex, temperature and isoline, indicating that there is a genotype-by-environment affect that varies by sex affecting rhythm power (Figure 1 A ; S1 Figure A; Table 1). Males showed higher power than females at 25ºC, while the difference looks disappear at 29ºC (Figure 1 A).

The period of activity was only significantly affected by isoline; large variances with a weak trend for a temperature-isoline effect and all other effects being non-significant (Figure 1 B; S1 Figure B; Table 1). Examples of activity rhythm figures of male and female under two temperatures were shown in S3 Figure.

While for total activity there is a sex-by-isoline interaction suggesting genetic variation for activity that varies across the sexes (Figures 1 C, S1 Figure C; Table 1). Males showed higher activity than females at both temperatures (Figures 1 C, S2 Figures; Table 1).

Discussion

In *G. cornutus*, only males fight with rival males to obtain matings using the developed mandibles (Okada et al. 2006), mandibles are condition dependent (House et al. 2016), and the significant genetic variation in mandible size affects fighting and mating outcomes (Harano et al. 2010). This could generate sexual difference in activity and circadian related traits. Furthermore, sex difference in circadian characters may be especially prevalent across different environments because male sexual traits tend to be condition dependent and thus males may be susceptible to stressors like elevated temperatures (Rashed & Polak 2010). To test these ideas, we measured the locomotor activity of a number of *G. cornutus* genotypes (isolines) at 25ºC and 29ºC, assessing the

free-running period and power of the circadian rhythm, as well as total activity, across genotype, sex and temperature environments. Findings suggest that males tended to be more active and showed greater increases in activity than females in this species. However, we found complicated interactions as well as main effects (temperature, sex, genotype) for the multivariate combinations of these activity measures, as well as very strong univariate effects that also included interactions between some of our predictors. The exception was the period of the activity rhythm, which was only affected by genotype (isoline).

Many previous studies have suggested that the free-running period of activity rhythm should show temperature compensation and remain robust over a wide range of temperatures (e.g., Zimmerman et al. 1968; Dunlap et al. 2004; Pittendrigh 1954), and this is what we also find. Temperature had no impact on the activity rhythm period (rhythm was temperature compensated), which was only impacted by genotype, suggesting period can evolve in spite of temperature compensation, and that the environmental impacts on it are buffered across the range of temperatures we tested. Additionally, there were no sex effects or interactions affecting period, so the genetic effects were the same across environments and were the same for males and females, but genotypes differ in their period of circadian rhythm. Given the strong sexual selection in this beetle (Okada and Miyatake 2009, 2010; Harano et al. 2010; Okada et al, 2014), it was surprising that the sexes did not differ in their activity period, especially since studies of other taxa have found sex difference in this measure, even if it only relates to development and emergence times (e.g., Simmons et al. 1994).

While many previous studies have investigated temperature effects on free-running periods, few have focused on the power of circadian rhythms. We found all main effects

and their interactions affected power, generating a genotype-by-temperature–by-sex interaction for power - which measures how much activity increases during active periods. So for example, the power of male circadian rhythms was much higher at 25ºC than at 29ºC, while females tended to be about the same at either temperature, but this effect differed across genotypes, so that for some genotypes females were more active at higher temperature for example. The sex element of this interaction affecting the power of circadian rhythm response probably relates to reproductive behaviours, with males searching for mates and rivals, generating a much greater up-lift in male-activity during the circadian cycle (e.g., Parker 1978; Muniz et al. 2018; Matsumura et al. 2019). This affect seems to be very pronounced in some genotypes, which may relate to genetic variation in male aggression (Harano et al. 2010; Okada and Miyatake 2010).

It seems plausible that all this explains why circadian activity has greater power in males, but not why male power falls to female levels at 29ºC. Perhaps this decrease is stress-related, as stress can shift resource from activity to maintenance, and effects can be genotype specific (Mitton 1997). Stress could be important here because beetles are normally reared at 25-27ºC, and because males have large condition-dependent mandibles (House et al. 2016), they may be especially sensitive to stress (David et al. 2000). We need to further investigate the effect of temperature on male-male competition and sexual selection more generally, but in any case, this seems to be the first report of a sex difference in response of the power of circadian rhythm across temperature environments.

The genotype effect indicates genetic variance for power of circadian rhythm. Similar findings have been reported in other studies, including recent work investigating output pathways of the circadian clock in *Drosophila* (Cavey et al. 2016; King et al. 2017). That work revealed molecular genetic components that affect the power of circadian rhythm

and perhaps our findings similarly involve output pathways. Furthermore, if the output pathways were effected in our beetles, it seems that the power, but not the period, of circadian rhythm was impacted. More work on across environment effects needs to be undertaken to test for genotype-environment-interactions, matching calls made in other fields of study (Hunt and Hosken 2014). Additionally, although the power of circadian rhythm has been investigated in several previous studies (Fujioka et al. 2017; Malpel et al. 2004; Meshi and Bloch 2007), there are few studies that have investigated the fitness impacts of variation in power. It would seem prudent to conduct such investigations across environments.

We found no significant effect of temperature on the free-running period. This is consistent with previous studies, which report temperature compensation in the free-running period of circadian rhythm in other species (e.g., Pittendrigh 1954; Zimmerman et al. 1968; Dunlap et al. 2004). We also found no sex-differences. This contrasts with *D. melanogaster* where males had a shorter period of circadian rhythm than females (Helfrich-Förster 2000). This probably relates to male *Drosophila* searching for females (Helfrich-Förster 2000), while in *G. cornutus*, the period of mate searching may be less beneficial because of male-male fighting and strong territoriality (Okada et al. 2014; Yamane et al. 2010). Additionally, males that lose fights do not engage in fighting behaviour for four days (Okada and Miyatake 2010), and losers transferred more sperm to females during copulation (Yamane et al. 2010). Therefore, males may invest more in fighting than searching for females in *G. cornutus*. However, males do seem to generally increase power more than females (see above), so rather than increase the duration of activity, they instead increase how active they are during periods of activity. This suggests finding females at the right times, when they are receptive, is important, noting that for

many insects males cannot force copulations after adulthood cuticles have hardened (Markow 2000; Eberhard 2002). In any case, males seem to increase the amount of activity within a period, rather than increase the duration of activity, and there was significant genetic variation in the free-running period of circadian rhythm - this was the only significant main effect. This again implies period can evolve which is arguably as expected for a behavioural phenotype (Hosken et al. 2019) and all the more so given that there is variation in animal activity periods across taxa (e.g., diurnal vs. nocturnal).

The total locomotor activity over 10 days was affected by the interaction between genotype (isoline) and sex, with males tending to be more active than females over both temperatures, although this affect varied with genotype. Again this interaction could be understood in terms of stress and the condition dependence of male sexual-traits and how both vary across genotypes. There was also an independent (of the interaction) effect of temperature such that activity was higher at 25^{o}C than 29^{o}C, which again supports the notion of the higher temperature being more stressful. And again, the genotype (isoline) effect on activity shows there is genetic variation for this trait. We need to undertake additional work to uncover the details of all these effects to more fully understand some of our findings, especially as they relate to mechanism and fitness.

To conclude, we found strong effects of sex, genotype and environment (temperature) on a range of circadian activity measures. These effects interacted and did not act consistently on all the activity elements we measured. Thus it appears that activity is like many other behaviours, being affected by genes and environment and their interaction and elements of activity seem to be somewhat independent of each other. However, additional work is needed to both clarify the detail of our findings and to test their generality across taxa.

259

Acknowledgements

This work was supported by Grant-in-Aid for Scientific Research, KAKENHI 16K14810 and 18H02510, MEXT, JSPS to T.M.

Compliance with ethical standards

Competing interests

The authors declare that they have no conflict of interest.

Ethical approval

All procedures performed in studies involving animals were in accordance with the ethical standards of Okayama University and University of Exeter which the studies were conducted.

References

Anderson M. 1994. *Sexual Selection*. Princeton University Press, Princeton.

Aschoff J. 1960. Exogenous and endogenous components in circadian rhythms. Cold Spring Harbor Symposia on Quantitative Biology. 25:11-28.

Aschoff J. 1965. Circadian rhythms in man. Science. 148:1427-1432.

Cavey M, Collins B, Bertet C, Blau J. 2016. Circadian rhythms in neuronal activity propagate through output circuits. Nat. Neurosci. 19:587-95.

David JR, Gibert P, Legout H, Pétavy G, Capy P, Moreteau B. 2005. Isofemale lines in *Drosophila*: an empirical approach to quantitative trait analysis in natural populations. Heredity. 94:3-12.

David P, Bjorksten T, Folwer K, Pomiankowski A. 2000. Condition-dependent signaling of genetic variation in stalk-eyed flies. Nature. 406:186-188.

Dunlap JC. 1996. Genetic and molecular analysis of circadian rhythms. Annu. Rev. Genet. 30:579-601.

Dunlap JC, Loros JJ, DeCoursey PJ. 2004. *Chronobiology: Biological Timekeeping*. Sinauer Associates, Inc.

Eberhard WG. 2002. The function of female resistance behaviour: intromission by male coercion vs. female cooperation in sepsid flies (Diptera: Sepsidae). Rev. Biol. Trop. 52:485-505.

Emlen DJ. 2015. *Animal Weapons: the Evolution of Battle*. Picador USA.

Fujioka H, Abe MS, Fuchikawa T, Tsuji K, Shimada M, Okada Y. 2017. Ant circadian activity associated with brood care type. Biol. Lett. 13: https://doi.org/10.1098/rsbl.2016.0743

Halberg F. 1969. Chronobiology. Annl. Rev. Physiol. 31:675-726.

297 Harano T, Okada K, Nakayama S, Miyatake T, Hosken DJ. 2010. Intralocus sexual
298 conflict unresolved by sex-limited trait expression. Curr. Biol. 20:2036-2039.
299 Harano T, Miyatake T. 2011. Independence of genetic variation between circadian rhythm
300 and development time in the seed beetle, *Callosobruchus chinensi*s. J. Insect. Physiol.
301 57:415-420.
302 Helfrich-Förster C. 2000. Differential control of morning and evening components in the
303 activity rhythm of *Drosophila melanogaster*-sex-specific differences suggest a different
304 quality of activity. J. Biol. Rhythms. 15:135-154.
305 Hosken DJ, Hunt J, Wedell N. 2019. *Genes and Behaviour: Beyond Nature-Nurture*.Wiley.
306 Hunt J, Hosken DJ (2014) *Genotype-by-Environment Interactions in Sexual Selection*.
307 Wiley Blackwell.
308 House CM, Jensen K, Rapkin J, Lane S, Okada K., Hosken DJ, Hunt J. 2016.
309 Macronutrient balance mediates the growth of sexually selected weapons but not
310 genitalia in male broad-horned beetles. Funct. Ecol. 30:769-779.
311 King AN, Barber AF, Smith AE, Dreyer AP, Sitaraman D, Nitabach MN, Cavanaugh DJ,
312 Sehgal A. 2017. A peptidergic circuit links the circadian clock to locomotor activity. Curr.
313 Biol. 27:1915-1927.
314 Klarsfeld A, Leloup JC, Rouyer F. 2003. Circadian rhythms of locomotor activity in
315 *Drosophila*. Behav. Processes. 64:161-175
316 Malpel S, Klarsfel A, Rouyer F. 2004. Circadian synchronization and rhythmicity in larval
317 photoperception-defective mutants of *Drosophila*. J. Biol. Rhythms. 19:10-21.
318 Markow TA. 2000. Forced matings in natural populations of *Drosophila*. Am. Nat.
319 156:100-103.
320 Matsumura K, Archer CR, Hosken DJ, Miyataka T. 2019. Artificial selection on walking

distance suggests a mobility-sperm competitiveness trade-off. Behav. Ecol. (in press)

Meshi A, Bloch G. 2007. Monitoring circadian rhythms of individual honey bees in a social environment reveals social influences on postembryonic ontogeny of activity rhythms. J. Biol. Rhythms. 22:343-355.

Mitton JB. 1997. Selection in Natural Populations. Oxford University Press.

Muniz DG, Baena ML, Macías-Ordóñez R, Machado G. 2018. Males, but not females, perform strategic mate searching movements between host plants in a leaf beetle with scramble competition polygyny. Ecol. Evol. 8:5828-5836.

Okada K, Miyanoshita A, Miyatake T. 2006. Intra-sexual dimorphism in male mandibles and male aggressive behavior in the broad-horned flour beetle *Gnatocerus cornutus* (Coleoptera: Tenebrionidae). J. Insect. Behav. 19:457-467.

Okada K, Miyatake T. 2009. Gernetic correlations between weapons, body shape and fighting behaviour in the horned beetle *Gnatocerus cornutus*. Anim. Behav. 77:1057-1065.

Okada K, Miyatake T. 2010. Effect of losing on male fights of broad horned flour beetle, *Gnatocerus cornutus*. Behav. Ecol. Sociobiol. 64:361-359.

Okada K, Katsuki M, Sharma MD, House CM, Hosken DJ. 2014. Sexual conflict over mating in *Gnatocerus cornutus*? Females prefer lovers not fighters. Proc. R. Soc. B. 281: https://doi.org/10.1098/rspb.2014.0281

Parker GA. 1978. The evolution of competitive mate searching. Annu. Rev. Entomol. 23:173-196.

Pittendrigh CS. 1954. On temperature independence in the clock system controlling emergence time in *Drosophila*. Proc. Natl. Acad. Sci. USA. 40:1018-1029.

R Development Core Team. 2017. R: A language and environment for statistical

computing. R Foundation for Statistical Computing, Vienna, Austria. R version 3.4.3.

Rashed A Polak M. 2010. Differential condition dependence among morphological traits inferred from responses to heat stress is predicted by sexual selection theory. Biol. J. Linn. Soc. 101:566-574.

Saunders DS. 2002. Insect Clocks, 3rd edn. Elsevier, Amsterdam.

Simmons LW, Llorens T, Schinzig M, Hosken D, Craig M. 1994. Sperm competition selects for male mate choice and protandry in the bushcricket, *Requena verticalis* (Orthoptera: Tettigoniidae). Anim. Behav. 47:117-122.

Sokolove PG, Bushell WN. 1978. The chi square periodogram: its utility for analysis of circadian rhythms. J. Theor. Biol. 72:131-160.

Sorek M, Levy O. 2012. The effect of temperature compensation on the circadian rhythmicity of photosynthesis in Symbiodinium, coral-symbiotic alga. Sci. Rep. https://doi.org/10.1038/srep00536

Yamane T, Okada K, Nakayama S, Miyatake T. 2010. Dispersal and ejaculatory strategies associated with exaggeration of weapon in an armed beetle. Proc. R. Soc. B. 277:1705-1710.

Zimmerman WF, Pittendrigh CS, Pavilidis T. 1968. Temperature compensation of the circadian oscillation in *Drosophila pseudoobscura* and its entrainment by temperature cycles. J. Insect. Physiol. 14:669-684.

Ethical notes

We followed all of the COPE guidelines.

Conflict of Interest

Kentarou Matsumura, Masato S Abe, Manmohan D Sharma, David J. Hosken, Takashi Yoshii, and Takahisa Miyatake declare that they have no conflict of interest.

372 **Figure legends**

373 **Figure 1. A.** Power of activity rhythm, **B.** period of circadian rhythm, and **C.** total

374 locomotor activities by sex when measured at 25^0C and 29^0C respectively. The error bars

375 represent SEM.

376

377

378

379

380

381

382

383

384

385

386

387

388

389

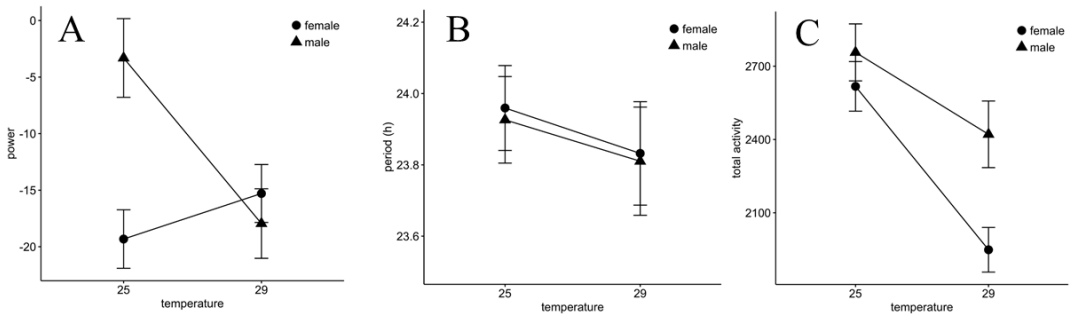

A
female
male
power
0
-5
-10
-15
-20
25
29
temperature
B
female
male
period (h)
24.2
24.0
23.8
23.6
25
29
temperature
C
female
male
total activity
2700
2400
2100
25
29
temperature

Figure 1

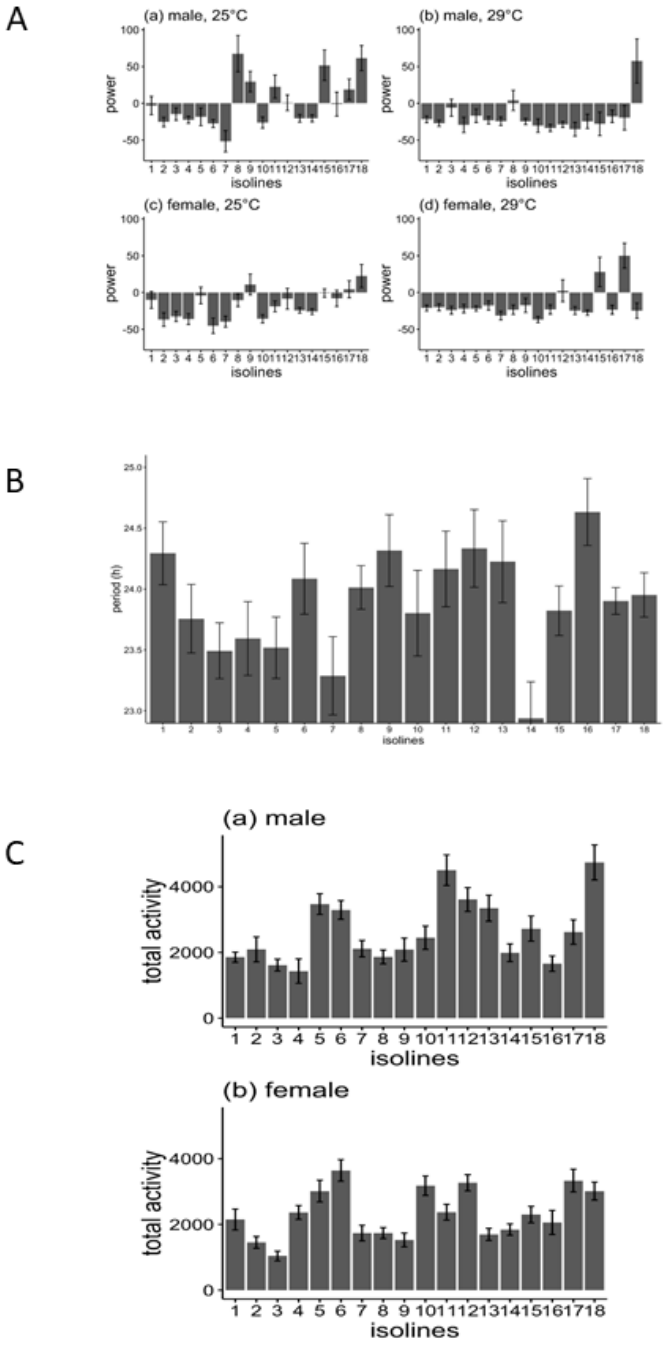
Legends for supplementary materials

S1 Figure A: The three-way interaction between temperature, sex and genotype (isoline) affecting activity rhythm power. This shows that the effects of temperature depend on sex and on genotype, such that for some genotypes males are more active at 29ºC and for others females are for example (S2 Fig. shows the sex-by-temperature element of this interaction). B: The period of activity for different genotypes (isolines). Genotype was the only variable that explained significant variation in activity period. C: The interaction between sex and genotype (isoline) affecting total activity. On average males tend to be more active but this varies across genotypes, so that in isoline 10 for example, females are more active. In the all graphs, the error bars represent SEM.

S2 Figure The three-way interaction between temperature, sex and genotype (isoline) affecting total activity. This shows that the effects of temperature depend on sex and on genotype. The error bars represent SEM.

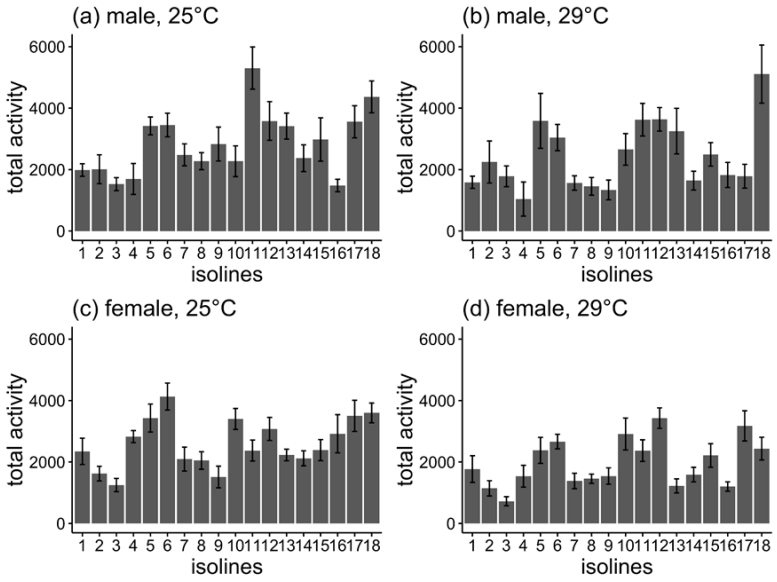
S3 Figure Examples of activity rhythm figures of males and females under two temperatures, 25ºC and 29ºC.

420

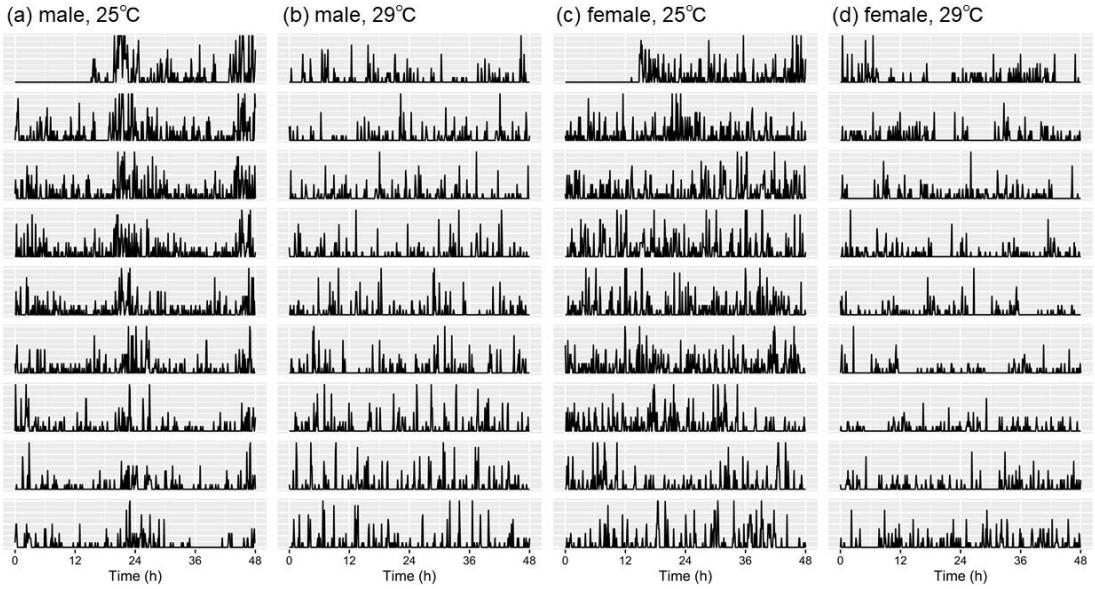


421

422 S1 Figure



S2 Figure



S3 Figure

Table 1. Post-hoc ANOVAs of the MANOVA outcomes testing effects on each activity trait (period and power of rhythm, and total activity) separately.

Traits	Variables	d. f.	Sum sq	Mean Sq	*F*	*P*
Power of rhythm	Sex	1	13358	13838	8.9481	**0.0029**
	Temperature	1	5794	5794	3.8811	**0.0492**
	Isoline	17	283308	16665	11.1636	< **0.0001**
	Sex*temperature	1	19396	19396	12.9927	**0.0003**
	Sex*isoline	17	82189	4835	3.2386	< **0.0001**
	Temperature*isoline	17	60760	3574	2.3942	**0.0013**
	Sex*temperature*isoline	17	60981	3587	2.4029	**0.0012**
	Residuals	793	1183807	1493		
Period of rhythm	Sex	1	15	15	0.0395	0.8425
	Temperature	1	316	316	0.8517	0.3564
	Isoline	17	14474	851	2.2961	**0.0021**
	Sex*temperature	1	0	0	0.0001	0.9911
	Sex*isoline	17	4405	259	0.6988	0.806
	Temperature*isoline	17	9671	569	1.5341	0.0763
	Sex*temperature*isoline	17	4885	287	0.775	0.7234
	Residuals	793	294043	371		
Total activity	Sex	1	18867003	18867003	9.1322	**0.0026**
	Temperature	1	54091677	54091677	26.182	< **0.0001**
	Isoline	17	4.97E+08	29211404	14.1392	< **0.0001**
	Sex*temperature	1	5260553	5260553	2.5463	0.1109
	Sex*isoline	17	1.35E+08	7952461	3.8492	< **0.0001**
	Temperature*isoline	17	24922413	1466024	0.7096	0.7949
	Sex*temperature*isoline	17	54409111	3200536	1.5492	0.0717
	Residuals	793	1.64E+09	2065983		

S1 Table. Sizes of samples of each isoine of *G. cornutus*.

Isoline	25°C		29°C	
	Male	Female	Male	Female
1	23	21	11	11
2	21	17	11	9
3	17	18	9	12
4	12	18	8	10
5	18	17	7	11
6	19	20	12	10
7	15	12	10	12
8	12	10	12	11
9	11	9	11	11
10	12	12	10	10
11	12	12	11	11
12	11	10	11	12
13	11	10	9	11
14	11	11	12	12
15	9	11	10	11

16	10	11	11	11
17	9	10	10	11
18	11	10	11	10

434

435

436

S2 Table MANOVA with period and power of circadian rhythm, and total activity

Variables	Df	Pillai	Approx. F	num Df	den DF	*P*
sex	1	0.0198	5.3128	3	791	**0.0013**
temperature	1	0.0339	9.2477	3	791	**<0.0001**
isoline	17	0.4575	8.3935	51	2379	**<0.0001**
sex*temperature	1	0.0222	5.9977	3	791	**0.0005**
sex*isoline	17	0.1513	2.4770	51	2379	**<0.0001**
temperature*isoline	17	0.0994	1.5986	51	2379	**0.0048**
sex*temperature*isoline	17	0.0926	1.4854	51	2379	**0.0150**
residuals	793					

437