University of Groningen

Lifespan is unaffected by size and direction of daily phase shifts in *Nasonia*, a hymenopteran insect with strong circadian light resetting

Floessner, Theresa S. E.; Boekelman, Floor E.; Druiven, Stella J. M.; de Jong, Maartje; Rigter, Pomme M. F.; Beersma, Domien G. M.; Hut, Roelof A.

Published in:
Journal of Insect Physiology

DOI:
[10.1016/j.jinsphys.2019.103896](https://doi.org/10.1016/j.jinsphys.2019.103896)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2019

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Floessner, T. S. E., Boekelman, F. E., Druiven, S. J. M., de Jong, M., Rigter, P. M. F., Beersma, D. G. M., & Hut, R. A. (2019). Lifespan is unaffected by size and direction of daily phase shifts in *Nasonia*, a hymenopteran insect with strong circadian light resetting. *Journal of Insect Physiology*, 117, [103896]. <https://doi.org/10.1016/j.jinsphys.2019.103896>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Lifespan is unaffected by size and direction of daily phase shifts in *Nasonia*, a hymenopteran insect with strong circadian light resetting

Theresa S.E. Floessner, Floor E. Boekelman, Stella J.M. Druiven¹, Maartje de Jong, Pomme M.F. Rigter, Domien G.M. Beersma, Roelof A. Hut*

Chronobiology Unit, Neurobiology Expertise Group, Groningen Institute for Evolutionary Life Sciences, University of Groningen, the Netherlands

ARTICLE INFO

Keywords:

Circadian clock
Longevity
Lifespan
Nasonia
Range of entrainment
Phase shift
Photoperiod

ABSTRACT

Most organisms have an endogenous circadian clock with a period length of approximately 24 h that enables adaptation, synchronization and anticipation to environmental cycles. The circadian system (circa = about or around, diem = a day) may provide evolutionary benefits when entrained to the 24-h light-dark cycle. The more the internal circadian period (τ) deviates from the external light-dark cycle, the larger the daily phase shifts need to be to synchronize to the environment. In some species, large daily phase shifts reduce survival rate. Here we tested this 'resonance fitness hypothesis' on the diurnal wasp *Nasonia vitripennis*, which exhibits a large latitudinal cline in free-running period with longer circadian period lengths in the north than in the south. Longevity was measured in northern and southern wasps placed into light-dark cycles (T-cycles) with periods ranging from 20 h to 28 h. Further, locomotor activity was recorded to estimate range and phase angle of entrainment under these various T-cycles. A light pulse induced phase response curve (PRC) was measured in both lines to understand entrainment results. We expected a concave survival curve with highest longevity at $T = \tau$ and a reduction in longevity the further τ deviates from T ($\tau/T < > 1$). Our results do not support this resonance fitness hypothesis. We did not observe a reduction in longevity when τ deviates from T. Our results may be understood by the strong circadian light resetting mechanism (type 0 PRC) to single light pulses that we measured in *Nasonia*, resulting in: (1) the broad range of entrainment, (2) the wide natural variation in circadian free-running period, and (3) the lack of reduced survival when τ/T ratio's deviates from 1. Together this indicates that circadian adaption to latitude may lead to changes in circadian period and light response, without negative influences on survival.

1. Introduction

The circadian system regulates temporal organisation of physiological and behavioural processes. Cyanobacteria, fungi, plants, insects and vertebrates have an internal circadian system that oscillates with a period length of about 24 h (τ , circadian free-running period). The fact that circadian systems are present in many taxa indicates that synchronization to the environmental 24-h light-dark cycle is an evolutionary adaptation. The circadian period usually deviates slightly from 24 h, which may be a way to optimize phase (Floessner and Hut, 2017; Hut et al., 2013; Pittendrigh and Daan, 1976). Larger deviations of the circadian period from 24 h may carry fitness costs by reducing intrinsic survival rates due to increased daily phase shifts required for entrainment to 24 h (Pittendrigh and Bruce, 1959). Understanding possible fitness costs for daily phase shifts becomes particularly relevant for

modern societies where increasing occurrence of jetlag, social jetlag and shift work form health threats (Chen et al., 2010; Folkard and Lombardi, 2006; Gan et al., 2015; Levandovski et al., 2011; Plano et al., 2017; Roenneberg et al., 2012; Rosa, 1995; Su et al., 2008; Wittmann et al., 2006).

The circadian resonance hypothesis, originally proposed by Pittendrigh and Minis (1972), states that organisms with a circadian clock resonating with the environmental cycle have better internal synchrony and longer life span. Phrasing it differently, organisms that need larger daily phase shifts for circadian entrainment may have reduced fitness. Between mammalian species it was found that species with larger deviation of the internal circadian period from 24-h have indeed a shorter lifespan, suggesting a fitness costs of large daily phase shifts enabling circadian 24 h entrainment (Wyse et al. 2010). This 'resonance fitness hypothesis' is thought to apply for entrainment to

* Corresponding author.

E-mail address: r.a.hut@rug.nl (R.A. Hut).

¹ Current address: Department of Psychiatry, University Medical Center Groningen, University of Groningen, the Netherlands.

Fig. 1. Circadian resonance fitness hypothesis: Survival as a function of T-cycle. Highest survival is reached when the circadian period is in resonance with the external cycle. A) When $T > \tau_{DD}$, the system needs daily phase delays to entrain to longer environmental T-cycles, when $T < \tau_{DD}$, the system needs daily phase advances (daily phase shift = $\tau - T$). The more T deviates from τ , the larger the entraining daily phase shifts have to be. B) Survival decreases when the circadian system has to entrain to the environmental cycle by large daily delays ($T \gg \tau$) or advances ($T \ll \tau$).

external light-dark cycles (LD cycle) with a range of different period lengths (T; *Zeitgeber* period) within the range of entrainment (Floessner and Hut, 2017). Using different T-cycle durations, it can be tested whether survival is reduced the further τ deviates from T (τ/T ratio deviating from 1) for both, daily delays, where the internal circadian period (τ) is shorter than the environmental period (T), and daily advances, where $\tau > T$. As a result, the survival curve as a function of the *Zeitgeber* period is expected to have a concave shape (inverted U-shaped; Fig. 1).

Supporting the resonance fitness hypothesis, early studies found that longevity of flies is reduced the further T-cycles deviate from 24 h (Aschoff et al., 1971; Pittendrigh and Minis, 1972). Furthermore, *Drosophila* flies with mutations of the period gene (*per*) causing different free-running periods (*per^L* $\tau \approx 29$ h and *per^T* $\tau \approx 16$ h), have about 10–20% reduction in life span under $T = 24$ h compared to wild type animals (Klarsfeld and Rouyer, 1998). However, not all results of their study are in line with the resonance fitness hypothesis, since survival of wild-type and *Per^L* flies does not differ significantly between $T = 24$ h and $T = 16$ h. A thorough and supportive test of the resonance fitness hypothesis was performed in Cyanobacteria. In mixed bacterial solutions with wild type and *kaiC* mutants cyanobacteria strains, the strain with a free-running period closest to the environmental period length

outcompeted the strains with a circadian period deviating from the environmental period (Ouyang et al., 1998). The impact of repeated phase shifts on health was tested with a chronic jetlag protocol in mice (Davidson et al., 2006). It was shown that phase shifts of 6-h advances or delays applied every 7 days cause reduced life span. The authors argue that internal desynchrony between central and peripheral clocks may cause shorter life span. Remarkably, in Davidson et al., 2006 phase advances seemed more detrimental than phase delays, possibly because the phase response curve in mice shows stronger delays than advances. This suggests that entrainment to an advancing jetlag protocol might be less feasible for the mouse circadian system than entrainment to a delaying protocol. In addition, mice carrying the τ -mutation (casein kinase 1 ϵ mutation) with circadian periods much shorter than 24 h (Meng et al., 2008) had reduced survival and reproduction under semi-natural conditions (Spoelstra et al., 2016). Thus, fitness effects of light synchronization have been observed in bacteria, insects and mammals, although the range of tested species is restricted. In addition, some of these studies were performed with clock gene mutations that may carry pleiotropic effects besides a clock phenotype. The mammalian τ -mutation, for example, has reduced fitness in mice under natural conditions (Spoelstra et al., 2016) but also causes an upregulation of metabolism by about 20%, (Meng et al., 2008; Oklejewicz et al., 1997). We tested the circadian resonance hypothesis in the parasitic jewel wasp *Nasonia vitripennis* (Hymenoptera), which has a broad global and latitudinal distribution and is expected to exhibit large natural variation in circadian period length and circadian light sensitivity. We used a range of light-dark cycle periods and photoperiod combinations to gain better insight in the potential evolutionary trade-off between survival and circadian entrainment phase shifts.

2. Materials & methods

2.1. Experimental lines

All experiments were performed with two *Nasonia vitripennis* iso-female lines, one originating from Oulu, Finland (65.01°N; northern line) and the other from Corsica, France (42.04°N southern line; Paolucci et al., 2013). These lines were reared in a temperature and humidity controlled climate chamber ($20 \pm 1^\circ\text{C}$, 50–55% RH), under a daily light-dark cycle of 16 h of light and 8 h of darkness (LD 16:8). The wasps used during these experiments were offspring from individually housed females, supplied with *Calliphora* spp. pupae as hosts.

2.2. Longevity assessment

Three to five days after emerging from their host puparia, males and mated females were separated and transferred to the experimental setup to assess longevity. Wasps were kept in polystyrene tubes (6×1 cm) in groups of 10, with food (0.5 ml, 30% sucrose, 1.5% agar, 0.15% nipagin) and placed in ventilated light-tight boxes ($23 \times 14 \times 32$ cm) in a climate controlled room at 18°C ($\pm 1^\circ\text{C}$). Each box was equipped with one LED light source (Neutral White 4000 K PowerStar, Berkshire, UK) providing $2.1 \cdot 10^{15}$ photons $\cdot\text{cm}^{-2}\cdot\text{s}^{-1}$ at the level of the animals (Supplementary Material Fig. 1) as measured with a broad-band spectrometer (SpecBos 1211, JETI Technische Instrumente, GmbH, Germany, Jena, Germany). Different groups of wasps were exposed to a range of continuous light-dark cycles with different T-cycles, generated by changing either the light or the dark phase duration (Table 1). Throughout the entire assessment period, the light-tight boxes were opened each day during the light phase and dead animals were counted. To assess differences in mean longevity, a Kaplan-Meier survival analysis was performed in SPSS (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY, USA: IBM Corp.). Differences in the shapes of the survival curves were tested by a Tarone-Ware test. Average survival differences between sexes, strains and conditions were tested with a general linear model regression

Table 1

Applied external light-dark cycles. The duration of the light-dark cycle (T-cycle) was changed either by changing the duration of the light phase while keeping the dark phase constant at 8 h ($\Delta L:D$), or by changing the duration of the dark phase, while keeping light phase duration constant at 16 h (L: ΔD). Number (n) of animals per light condition.

	LD-cycle (h)	T-cycle (h)	female		male	
			North (n)	south (n)	north (n)	south (n)
$\Delta L:D$	12:8	20	99	100	101	92
$\Delta L:D$	14:8	22	99	100	100	103
$\Delta L:D$	16:8	24	100	101	101	100
$\Delta L:D$	18:8	26	99	99	100	100
$\Delta L:D$	20:8	28	100	99	96	100
L: ΔD	16:4	20	20	40	38	29
L: ΔD	16:6	22	20	40	50	30
L: ΔD	16:8	24	20	40	50	20
L: ΔD	16:10	26	20	40	50	20
L: ΔD	16:12	28	20	40	50	19

analysis (Statistix 8, Analytical Software, USA), including a quadratic τ/T term to test for the expected concave relationship between τ/T ratio and longevity (Fig. 1).

2.3. Entrainment assay & determination of circadian free-running period (τ) and phase

Locomotor activity of individual wasps was recorded to observe their ability to entrain to the different T-cycles and photoperiods (DAMS, Drosophila Activity Monitoring System; TriKinetics, Waltham, USA). Furthermore, we determined τ in constant darkness after entrainment to LD 16:8 in $2.1 \cdot 10^{15}$ photons $\cdot$ cm $^{-2}$ $\cdot$ s $^{-1}$. Three to five day old mated females and males from the northern and southern lines were individually placed in activity tubes (6.5 $\times$ 0.5 cm) filled one quarter with agar food (30% sucrose, 1.5% agar, 0.15% nipagin) and closed with an air permeable plug. Tubes were placed in 32-slot recording monitors, with each slot containing an infrared light beam. The

monitors were placed into the light-tight boxes (see above) at 18 °C (± 1 °C) with the following light regimes: T-20 h (LD 16:4), T-24 h (LD 16:8), and T-28 h (LD 16:12). The activity of each individual wasp was recorded as the number of light beam interruptions per minute by the DAMSystem. Actograms were created with the ImageJ plugin software ActogramJ (Schmid et al., 2011). Average activity profiles were calculated using data from day 10 to 15 for each individual. Following this, the data of each experimental group and condition were smoothed using a moving average of 11 bins and normalized to the highest value as described in Schlichting and Helfrich-Forster, 2015. Circadian free-running period of northern and southern females and males were determined in Microsoft Excel by using a χ^2 -periodogram analysis (Sokolove and Bushnell, 1978) as described in Hermann et al., 2012.

To determine phase-period relationship, phase determination was conducted in ChronoShop (Spoelstra et al., 2004) by determine centre of gravity per individual, averaging of five consecutive cycles of stable entrainment (around day 10–15) and further averaging within the experimental group.

2.4. Phase response curve

To understand the light resetting capacity of the circadian system, a circadian phase response curve was established to single light pulses at different phases of the circadian cycle for both sexes from the northern and southern line. We applied the Aschoff type II PRC protocol (Aschoff, 1965), using LD 16:8 entrainment followed by free-run in continuous darkness (DD), where the light stimulus was applied on the third day of release in DD. Each group received a single 8-h light pulse of $2.1 \cdot 10^{15}$ photons $\cdot$ cm $^{-2}$ $\cdot$ s $^{-1}$ at twelve different time points separated by 2 h across the circadian cycle. The phase shift was quantified by recording the free-running activity rhythm in constant darkness after the light pulse. The phase of the light pulse was calculated relative to the light dark cycle, while applying free-run phase correction in DD before the light pulse was applied. Individual phase shifts were determined with ChronoShop software methodology (Spoelstra et al., 2004) using circular centre of gravity.

Fig. 2. Survival curve for females from northern and southern lines in five different T-cycles. Different curves indicate proportion surviving wasps over time (in days of 24 h) LD cycle length (T) equals 20 h (black), 22 h (blue), 24 h (green), 26 h (orange), 28 h (magenta). A Kaplan-Meier survival analysis was performed and a Taron-Ware test was applied using SPSS. A) $\Delta L:D$ northern line: $p < 0.01$, $\chi^2 = 97.77$; $\Delta L:D$ southern line: $p < 0.01$, $\chi^2 = 57.91$; B) L: ΔD northern line: $p = 0.08$, $\chi^2 = 8.49$; L: ΔD southern line: $p = 0.14$, $\chi^2 = 6.87$. During the light phase survival has been assessed of each individual.

Fig. 3. Survival curve for males from northern and southern lines in five different T-cycles. Different curves indicate proportion surviving wasps over time (in days of 24 h) LD cycle length (T) equals 20 h (black), 22 h (blue), 24 h (green), 26 h (orange), 28 h (magenta). A Kaplan-Meier survival analysis was performed and a Taron-Ware test was applied using SPSS. A) Δ L:D northern line: $p < 0.01$, $\chi^2 = 87.39$; Δ L:D southern line: $p < 0.01$, $\chi^2 = 111.35$; B) L:ΔD northern line: $p < 0.01$, $\chi^2 = 14.93$; L:ΔD southern line: $p = 0.05$, $\chi^2 = 9.57$.

3. Results

3.1. Survival curves

Overall, females survived longer than males (mean survival females 16.6 days (SEM 0.71), males 10.7 days (SEM 1.03); $T = 4.75$, $df = 38$, $p < 0.0001$). Females lived longer in L:ΔD than Δ L:D conditions (Fig. 2, $F_{1,17} = 13.07$, $p < 0.002$), both in the northern and southern strain (Fig. 2, $F_{1,17} = 0.02$, $p < 0.91$). Females under LD cycles with different periods showed significant differences in survival only when the duration of the light phase was changed to modify the period of the LD cycle (Fig. 2A). Survival seemed independent of the duration of the dark phase (Fig. 2B).

Males also lived longer in L:ΔD than in Δ L:D conditions (Fig. 3, $F_{1,17} = 105.37$, $p < 0.0001$), while males from the southern strain had higher survival than males from the northern strain (Fig. 3, mean south 11.6 (SEM 1.8) days, mean north 9.8 (SEM 1.1) days; $F_{1,17} = 4.91$, $p < 0.05$). Survival rate differed between T-cycles in both Δ L:D and L:ΔD experiments (Fig. 3A, B).

3.2. Mean longevity and Zeitgeber period

Mean longevity values were analysed in a single ANOVA model (Fig. 4), which shows that survival is affected by strain ($p < 0.007$), sex ($p < 0.0001$), protocol (Δ L:D or L:ΔD, $p < 0.0001$), Zeitgeber period (T-cycle, $p < 0.015$), and the interaction terms strain-protocol ($p < 0.011$) and sex-protocol ($p < 0.002$). Although Zeitgeber period duration influences survival in this analysis (Fig. 4), the resonance fitness hypothesis predicts a concave dependency of survival on T or the τ/T ratio (Fig. 1). We calculated τ/T ratios using circadian free-running periods measured in each line and sex under continuous darkness after LD entrainment. Free-running circadian periods were found to be: 26.3 h (± 1.6 h) for northern females, 24.8 h (± 1.0 h) for northern males, 25.0 h (± 0.3 h) for southern females, 24.2 h (± 0.3 h) for southern males (Table 2). A concave relationship between survival and T-cycle duration is not immediately clear from the data (Fig. 4) and requires statistical testing by adding τ/T and $(\tau/T)^2$ as linear terms in a

sample size weighted regression model that includes strain, sex, protocol (Δ L:D or L:ΔD) and their mutual interaction terms. In a backward step wise regression, the interaction terms of τ/T and $(\tau/T)^2$ with protocol ($p > 0.29$ and $p > 0.27$) and strain ($p > 0.13$ and $p > 0.15$), were found to be not significant and were therefore dropped from the model. Also, the interaction between strain and sex was not significant and dropped from the model ($p > 0.19$). Hence, there was no statistical evidence in the data supporting a concave relationship between τ/T ratio and survival.

3.3. Activity patterns and phase angle of entrainment

To determine whether the wasp lines are able to entrain to the different T-cycles, locomotor activity was recorded and actograms were generated for T-20 h (LD 16:4), T-24 h (LD 16:8), and T-28 h (LD 16:12) (Fig. 5). Activity was recorded in 1-minute activity bins and double plotted in actograms. All experimental groups did entrain to all T-cycles. Consistent with data from Bertossa et al. (2013) the wasps showed high locomotor activity throughout the light phase and nearly no activity during the dark phase. Females were active during almost the entire light phase, whereas males were active during a smaller portion of the light phase. The northern line showed overall higher activity levels than the southern line. Although a rhythmic activity pattern is evident at T-20 h in females of both lines, there is also some activity separated from the main activity bout and some individuals showed a second activity peak (Fig. 5A & Fig. 6A). Overall, the wasps are clearly able to entrain to the different light cycles.

The actogram data were analysed by generating activity profiles under the three different light conditions. 1-minute activity bins were averaged for each individual over five consecutive days and individual average profiles were again averaged within each experimental group and condition (Fig. 6). Standard error of the mean is based on variation between individuals. As described above, females show locomotor activity throughout almost the entire light phase whereas males compress their activity in half of the light phase in all T-cycles. Overall, northern females show higher activity levels than southern females. In T-20 h, females from both lines exhibit an increase in activity with lights-on

Fig. 4. Mean longevity in different T-cycles per light-dark approach A) Δ L:D and B) L:ΔD for males and females; northern line (blue) and southern line (orange). Mean longevity was calculated from survival data; bars represent standard error.

Table 2

Free-running period and percentage of rhythmic animals of the northern and southern females and males; τ was determined after an entrainment period in LD 16:8 in constant darkness.

	female		male	
	north	South	north	South
n	16	15	13	9
$\tau \pm$ SD	263. $\pm$ 1.6	25.0 $\pm$ 0.3	24.8 $\pm$ 1.0	24.2 $\pm$ 0.3
rhythmic%	100	100	69	100

and a short dip in activity at the end of the first half of the light phase (Fig. 6). In the middle of the light phase, activity increased again and remained for the rest of the light phase. At T-24 h and T-28 h, northern females showed activity onset slightly after lights-on whereas southern females got active right away with lights-on. At T-20 h, activity levels of males were highest in the middle of the light phase whereas in T-24 h and T-28 h activity peaked in the first half of light phase. Furthermore, activity in northern males gradually increased after lights-on, peaked in the first half of the light phase and from there decrease gradually again. They showed a small second activity increase right before lights-off whereas in the southern line high activity started immediately after lights-on, followed by a further increase of activity near the end of the dark phase.

Taken together, both in the actograms and in the activity profiles a coherent diurnal activity pattern indicates normal circadian entrainment under all conditions and strains (Fig. 5 & Fig. 6). The northern line showed higher activity levels than the southern line and females are more active than males. Females were active nearly the entire light phase, while males are mainly active in the first half of the light phase (in T-24 h and T-28 h) or in the middle of the light phase (in T-20 h). In

both males and females (when considering the major activity bout) we find evidence for a phase-period rule (Pittendrigh and Daan, 1976) with earlier phase angle of entrainment in longer T-cycles (Fig. 7). Phase was determined by taking the average centre of gravity over five cycles of stable entrainment (Fig. 6) within each individual. Phase angle of entrainment was negatively correlated with T-cycle duration with different slopes for strain, but not for sex within a strain. Overall males had an earlier phase angle of entrainment than females (Fig. 7; for full statistics see figure text).

The different levels of the phase-period regression analysis can be understood when we compare free-running circadian periods between lines and between males and females (Table 2). Indeed, in both lines males have shorter free-running periods than females, which would lead to a predicted earlier phase of entrainment. In addition, the southern line displays shorter free-running periods, which indeed would also lead to earlier phase angle of entrainment (Fig. 7).

The steeper slope of the phase-period relationship in the southern line, however, needs to be explained from differences in circadian phase resetting response to light. Oscillator theory would predict that stronger phase resetting would yield more shallow phase-T-cycle period relationships because a strong circadian light response would increase the range of entrainment. To answer the above relationship we evaluated circadian light responses by measuring a PRC in both lines and sexes.

3.4. Phase response curve

Phase response curves (PRCs) were measured to determine possible differences in circadian light response between the different lines. Light pulses of 8-h applied to different groups of animals at different times of day caused phase shifts of various magnitude, depending on the internal phase at which the pulses were applied. In both lines and both sexes the light pulses applied around ZT0, ZT24 respectively, caused

Fig. 5. Actograms of individual wasps, females and males, from northern and southern lines in L:ΔD A) T-20 h (LD 16:4), B) T-24 h (LD 16:8) and C) T-28 h (LD 16:12). Activity was recorded in 1-minute activity bins and double plotted in actograms (each line in the figure represents 48 h = 2 days, where the first line represents day 1 and 2, the second line day 2 and 3 and so forth). Animals appear to be active during the light phase. In T-20 h (LD 16:4) females showed two activity bouts, in T-24 h (LD 16:8) and T-28 h (LD 16:12) females and males, as well as in T-24 h (LD 16:8), expressed a unimodal activity pattern during the light phase. Females are active longer than males; northern animals have higher activity level than the southern ones.

large phase shifts of several hours (Fig. 8). The phase shifts were reduced towards the dead zone between ZT8 and ZT12, depending on line and sex. Because maximal phase shifts can reach up to 12-h advance or delay, these PRCs are also called type 0 or strong resetting PRCs. Although a similar shaped curve in all experimental groups is seen, individual variation is larger in the northern than in the southern line. Nevertheless, the large phase shifts indicate a complete phase resetting of the circadian system, with minor deviations from a straight line.

4. Discussion

The resonance fitness hypothesis predicts that fitness increases with decreasing difference between the internal circadian period and the environmental Zeitgeber period (Fig. 1; Pittendrigh and Minis 1972). We tested this hypothesis with two *Nasonia vitripennis* wild-type isofemale strains originating from northern and southern Europe and exhibiting a wide natural variation in circadian free-running period (Table 2). Our results do not support the resonance fitness hypothesis as longevity did neither show a concave relationship with Zeitgeber period (T) nor with

Fig. 6. Activity profiles of females and males, from northern and southern lines in L:ΔD A) T-20 h (LD 16:4), B) T-24 h (LD 16:8) and C) T-28 h (LD 16:12). For each experimental group and condition, activity data were averaged and normalized by its highest level. Wasps were predominantly active during the light phase; females were active during the entire light phase, males were more active only during part of the light phase. In general, the northern line has higher activity levels than the southern line.

Fig. 7. Phase-period relationship. Regression analysis on the centre of gravity of locomotor activity showing that the slope of the phase-T-cycle relationship differs between lines and sexes. The dependent variable is phase calculated as the individual centre of gravity (in hours after lights on) and the independent variables are T-cycle duration (T-cycle, 20, 24, 28 h), strain (south = 0, north = 1), sex (female = 0, male = 1). Regression parameters include: constant (28.44), T-cycle (-0.88 , $p < 0.0001$), strain (-5.62 , $p = 0.017$), sex (-2.72 , $p < 0.0001$), T-cycle*strain (0.37 , $p = 0.0002$), strain*sex (-1.63 , $p = 0.013$). T-cycle*sex and T-cycle*sex*strain were found to be not significant and were dropped from the model (full model $R^2 = 0.85$, $F_{5,80} = 93.68$, $p < 0.0001$).

τ/T ratio. These findings differ from earlier reports from cyanobacteria (Ouyang et al., 1998), *Drosophila* (Pittendrigh and Minis, 1972), blow flies (Aschoff et al., 1971) and mice (Spoelstra et al., 2016). The difference between the aforementioned studies and our current study may partly be explained by the studies in cyanobacteria (Ouyang et al., 1998) and mice (Spoelstra et al., 2016) that show successfully the importance of a functional circadian clock in competitive settings. Ouyang et al. (1998) challenged the cyanobacteria lines directly by mixing lines with different free-running periods together in one solution. Likewise, Spoelstra et al. (2016) mixed mice with genetically different circadian periods in a mixed population. In both conditions, reproduction was included and food was initially not limited. However, in a growing population, resources may eventually become limited. Additionally, differences in phase angle of entrainment, especially in cyanobacteria, may result in reduced availability of light at the right time of the circadian cycle as an energy resource for survival and reproduction. In both, the cyanobacteria and the mice studies, limited resources may therefore expose an existing trade-off between parental survival and reproductive effort as a basis for fitness reduction the more τ/T ratio deviates from 1. *Nasonia* wasps in our setup did not compete for food since food was plenty in a relatively large vial with only 10 wasps present. Additionally, our study only focussed on a single fitness component, namely individual longevity, whereas the studies supporting the resonance fitness hypothesis included reproduction. Females were not offered pupae, which effectively blocked reproductive attempts. Additionally, T-cycle experiments can be seen as chronic jetlag experiments. Under chronic jetlag conditions, individually housed mice showed compromised health and reduced longevity, indicating that the mechanism underlying the resonance fitness hypothesis is not only decreasing reproduction, but also individual longevity (Davidson et al., 2006; Karatsoreos et al., 2011).

To explain why *N. vitripennis* survival did not decrease with

Fig. 8. Phase response curve (PRC) of A) females from northern (n = 6–8) and southern lines (n = 7–8) and B) males from northern (n = 4–6) and southern lines (n = 5–11). Phase shift (hours) is plotted against ZT of mid point of light pulse (hours). Open circles indicate individual phase shifts, connected solid circles indicate circular averages for each time point. Different groups of animals received an 8-h light pulse at different times of day after an entrainment phase and dark adaption phase around ZT0, ZT24 respectively, phase shifts of about 12-h occur in females and males from northern and southern lines, which vanish gradually towards the dead zone. Due to large phase shifts, these kinds of PRCs are called type 0 and display a total resetting of the circadian system induced by the strength of the light stimulus.

increasing difference between τ and T , we considered the effect of τ/T ratio on the phase angle of entrainment. Extreme τ/T ratios may either fall outside of the range of entrainment or, when falling within the range of entrainment, may cause strongly deviating phase angle of entrainment (Floessner and Hut, 2017). Both lack of entrainment and altered phase angle of entrainment may have deleterious effects on longevity. Strong circadian light resetting - type 0 - will result in broadening the range of entrainment as well as reducing the effect of τ/T ratio on the phase angle of entrainment (Floessner and Hut, 2017). Indeed, we find that our *Nasonia* lines have strong - type 0 - circadian light resetting, using identical light sources as in the longevity study (Fig. 8). In fact, the PRCs show a rather linear trend, which explains the shallow relationship between τ/T ratio and phase angle of entrainment (Fig. 7).

We propose that a process similar to chronic jetlag causes longevity reduction when τ/T ratios deviate from 1 by causing internal circadian disruption. Strong circadian light resetting of a central pacemaker and tight coupling of that pacemaker to peripheral oscillators may overcome negative fitness effects of circadian disruption under entrainment when τ/T deviates from 1. Individual longevity appears to be affected in cyanobacteria when τ/T strongly deviates from 1 because a functional circadian organisation is directly involved in glycogen accumulation and energy availability (Pattanayak et al., 2014). Also, in *Drosophila*, different light input pathways (Cry dependent and opsin dependent; Stanewsky et al., 1998; Yasuyama and Meinertzhagen 1999, Emery et al., 2000, Helfrich-Förster et al., 2001, Rieger et al., 2003, Klarsfeld, 2004, Veleri et al., 2007, Ruijter et al., 2009, Saint-Charles et al., 2016) with different levels of weak circadian resetting in peripheral tissue clocks may disrupt internal circadian organisation when τ/T deviates from 1. In mice, a flexible coupling between the light entrained SCN to peripheral tissues may be responsible for longevity effects of internal circadian disruption through effects on metabolism and immune function (Brager et al., 2013; Castanon-Cervantes et al., 2010; Phillips et al., 2015). While the SCN in mice is likely to show strong phase angle of entrainment effects when τ/T deviates from 1, peripheral clocks may be more directly coupled to the effects of light (Van Der Veen et al., 2017), resulting in strong internal desynchronization the more T differs from τ . The circadian light resetting of *Nasonia* differs from *Drosophila* in that the Hymenopteran (mammal-like) CRY is not light sensitive (Yuan et al., 2007). It is therefore likely that *Nasonia* exclusively entrains to light through an opsins driven neural circuitry that perhaps entrains a central clock like in mammals. Strong light resetting of such a central clock mechanism could result in a wide range of entrainment with relatively stable phase angles. As a result, *Nasonia* may remain predominantly diurnally active even when τ/T deviates from 1. This stability of entrained phase will reduce internal disruption even when τ deviates strongly from 24 h.

We suggest that the lack of reduced longevity in *Nasonia* under a wide range of τ/T ratios, is a direct result of adaptation in circadian organisation and light resetting. Consequently, the increase in female τ from 25.0 h in the south to 26.3 h in the north can be seen as a possible adaption to latitude without negative effects on longevity. The negative consequences of τ deviating from 24 h are possibly compensated by increased circadian light sensitivity, resulting in strong circadian resetting, stable circadian entrainment, and maintenance of internal circadian coherence. This stability in circadian entrainment and internal circadian coherence could be critical for maintaining longevity.

Declaration of Competing Interest

We have no competing interests.

Acknowledgements

We thank Prof. dr. L. Beukeboom for the *Nasonia vitripennis* lines and Anna Rensink and Dr. Sylvia Paolucci for their support in handling

and rearing. We kindly thank Dr. Taishi Yoshii for providing ImageJ based circadian activity profile software. This work was supported by the Marie Curie Initial Training Network programme INsecTIME [Grant number PITN-GA-2012-316790].

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jinsphys.2019.103896>.

References

- Aschoff, J., 1965. Response curves in circadian periodicity. *Circadian Clocks* (Ed. Aschoff, J.) 95–111.
- Aschoff, J., von Saint, P.U., Wever, R., 1971. Lifetime of flies under influence of time displacement. *Naturwissenschaften* 58, 574.
- Bertossa, R.C., van Dijk, J., Diao, W., Saunders, D.S., Beukeboom, L.W., Beersma, D.G.M., 2013. Circadian rhythms differ between sexes and closely related species of *Nasonia* wasps. *PLoS One* 8, 1–13. <https://doi.org/10.1371/journal.pone.0060167>.
- Brager, A.J., Ehlen, J.C., Castanon-Cervantes, O., Natarajan, D., Delisser, P., Davidson, A.J., Paul, K.N., 2013. Sleep loss and the inflammatory response in mice under chronic environmental circadian disruption. *PLoS One* 8. <https://doi.org/10.1371/journal.pone.0063752>.
- Castanon-Cervantes, O., Wu, M., Ehlen, J.C., Paul, K., Gamble, K.L., Johnson, R.L., Besing, R.C., Menaker, M., Gewirtz, A.T., Davidson, A.J., 2010. Dysregulation of inflammatory responses by chronic circadian disruption. *J. Immunol.* 185, 5796–5805. <https://doi.org/10.4049/jimmunol.1001026>.
- Chen, J.D., Lin, Y.C., Hsiao, S.T., 2010. Obesity and high blood pressure of 12-hour night shift female clean-room workers. *Chronobiol. Int.* 27, 334–344. <https://doi.org/10.3109/07420520903502242>.
- Davidson, A.J., Sellix, M.T., Daniel, J., Yamazaki, S., Menaker, M., Block, G.D., 2006. Chronic jet-lag increases mortality in aged mice. *Curr. Biol.* 16, R914–R916. <https://doi.org/10.1016/j.cub.2006.09.058>.
- Emery, P., Stanewsky, R., Hall, J.C., Rosbash, M., 2000. A unique circadian-rhythm photoreceptor. *Nature* 404, 456–457. <https://doi.org/10.1038/35006558>.
- Floessner, T., Hut, R.A., 2017. Basic principles underlying biological oscillations and their entrainment. In: Kumar, V. (Ed.), *Biological Timekeeping: Clocks, Rhythms and Behaviour*. Springer, India, pp. 47–58.
- Folkard, S., Lombardi, D.A., 2006. Modeling the impact of the components of long work hours on injuries and “accidents.” *Am. J. Ind. Med.* 953–963. <https://doi.org/10.1002/ajim.20307>.
- Gan, Y., Yang, C., Tong, X., Sun, H., Cong, Y., Yin, X., Li, L., Cao, S., Dong, X., Gong, Y., Shi, O., Deng, J., Bi, H., Lu, Z., 2015. Shift work and diabetes mellitus: a meta-analysis of observational studies. *Occup. Environ. Med.* 72, 72–78. <https://doi.org/10.1136/oemed-2014-102150>.
- Helfrich-Förster, C., Winter, C., Hofbauer, A., Hall, J.C., Stanewsky, R., 2001. The circadian clock of fruit flies is blind after elimination of all known photoreceptors. *Neuron* 30, 249–261. [https://doi.org/10.1016/S0896-6273\(01\)00277-X](https://doi.org/10.1016/S0896-6273(01)00277-X).
- Hermann, C., Yoshii, T., Dusik, V., Helfrich-Förster, C., 2012. Neuropeptide F immunoreactive clock neurons modify evening locomotor activity and free-running period in *Drosophila melanogaster*. *J. Comp. Neurol.* 520, 970–987. <https://doi.org/10.1002/cne.22742>.
- Hut, R.A., Paolucci, S., Dor, R., Kyriacou, C.P., Daan, S., 2013. Latitudinal clines: an evolutionary view on biological rhythms. *Proc. R. Soc. B Biol. Sci.* 280, 20130433. <https://doi.org/10.1098/rspb.2013.0433>.
- Karatsoreos, I.N., Bhagat, S., Bloss, E.B., Morrison, J.H., McEwen, B.S., 2011. Disruption of circadian clocks has ramifications for metabolism, brain, and behavior. *Proc. Natl. Acad. Sci. U.S.A.* <https://doi.org/10.1073/pnas.1018375108>.
- Klarsfeld, A., 2004. Novel features of cryptochrome-mediated photoreception in the brain circadian clock of *Drosophila*. *J. Neurosci.* 24, 1468–1477. <https://doi.org/10.1523/JNEUROSCI.3661-03.2004>.
- Klarsfeld, A., Rouyer, F., 1998. Effects of circadian mutations and LD periodicity on the life span of *Drosophila melanogaster*. *J. Biol. Rhythms* 13, 471–478. <https://doi.org/10.1177/074873098129000309>.
- Levandovski, R., Dantas, G., Fernandes, L.C., Caumo, W., Torres, I., Roenneberg, T., Hidalgo, M.P.L., Allebrandt, K.V., 2011. Depression scores associate with chronotype and social jetlag in a rural population. *Chronobiol. Int.* 28, 771–778. <https://doi.org/10.3109/07420528.2011.602445>.
- Meng, Q.J., Logunova, L., Maywood, E.S., Gallego, M., Lebiecki, J., Brown, T.M., Sládek, M., Semikhodskii, A.S., Glossop, N.R.J., Piggins, H.D., Chesham, J.E., Bechtold, D.A., Yoo, S.H., Takahashi, J.S., Virshup, D.M., Boot-Handford, R.P., Hastings, M.H., Loudon, A.S.I., 2008. Setting clock speed in mammals: the CK1 ϵ tau mutation in mice accelerates circadian pacemakers by selectively destabilizing PERIOD proteins. *Neuron* 58, 78–88. <https://doi.org/10.1016/j.neuron.2008.01.019>.
- Oklejewicz, M., Hut, R.A., Daan, S., Stirling, A.J., 1997. Metabolic rate changes proportionally to circadian frequency in tau mutant Syrian hamsters. *J. Biol. Rhythms* 12, 413–422. <https://doi.org/10.1177/074873049701200503>.
- Ouyang, Y., Andersson, C.R., Kondo, T., Golden, S.S., Johnson, C.H., 1998. Resonating circadian clocks enhance fitness in cyanobacteria. *Proc. Natl. Acad. Sci. U.S.A.* 95, 8660–8664.
- Paolucci, S., Van de Zande, L., Beukeboom, L.W., 2013. Adaptive latitudinal cline of photoperiodic diapause induction in the parasitoid *Nasonia vitripennis* in Europe. *J.*

- Evol. Biol. 26, 705–718. <https://doi.org/10.1111/jeb.12113>.
- Pattanayak, G.K., Phong, C., Rust, M.J., 2014. Rhythms in energy storage control the ability of the cyanobacterial circadian clock to reset. *Curr. Biol.* 24, 1934–1938. <https://doi.org/10.1016/j.cub.2014.07.022>.
- Phillips, D.J., Savenkova, M.I., Karatsoreos, I.N., 2015. Environmental disruption of the circadian clock leads to altered sleep and immune responses in mouse. *Brain Behav. Immun.* <https://doi.org/10.1016/j.bbi.2014.12.008>.
- Pittendrigh, C.S., Bruce, V.G., 1959. Daily rhythms as coupled oscillator systems and their relation to thermoperiodism and photoperiodism. In: Withrow (Ed.), *Photoperiodism and Related Phenomena in Plants and Animals*. A.A.A.S., Washington, pp. 475–505.
- Pittendrigh, C.S., Daan, S., 1976. A functional analysis of circadian pacemakers in nocturnal rodents. IV. Entrainment: pacemaker as clock. *J. Comp. Physiol. A* 106, 291–331.
- Pittendrigh, C.S., Minis, D.H., 1972. Circadian systems: longevity as a function of circadian resonance in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. U.S.A.* 69, 1537–1539.
- Plano, S.A., Casiraghi, L.P., Moro, P.G., Paladino, N., Golombek, D.A., Chiesa, J.J., 2017. Circadian and metabolic effects of light: Implications in weight homeostasis and health. *Front. Neurol.* 8, 1–21. <https://doi.org/10.3389/fneur.2017.00558>.
- Rieger, D., Stanewsky, R., Helfrich-Förster, C., 2003. Cryptochrome, compound eyes, Hofbauer-Buchner eyelets, and ocelli play different roles in the entrainment and masking pathway of the locomotor activity rhythm in the fruit fly *Drosophila melanogaster*. *J. Biol. Rhythms* 18, 377–391. <https://doi.org/10.1177/0748730403256997>.
- Roenneberg, T., Allebrandt, K.V., Mellow, M., Vetter, C., 2012. Social jetlag and obesity. *Curr. Biol.* 22, 939–943. <https://doi.org/10.1016/j.cub.2012.03.038>.
- Rosa, R.R., 1995. Extended workshifts and excessive fatigue. *J. Sleep Res.* 4, 51–56. <https://doi.org/10.1111/j.1365-2869.1995.tb00227.x>.
- Ruijter, J.M., Ramakers, C., Hoogaars, W.M.H., Karlen, Y., Bakker, O., van den Hoff, M.J.B., Moorman, A.F.M., 2009. Amplification efficiency: linking baseline and bias in the analysis of quantitative PCR data. *Nucl. Acids Res.* 37. <https://doi.org/10.1093/nar/gkp045>.
- Saint-Charles, A., Michard-Vanhée, C., Alejevski, F., Chélot, E., Boivin, A., Rouyer, F., 2016. Four of the six *Drosophila* rhodopsin-expressing photoreceptors can mediate circadian entrainment in low light. *J. Comp. Neurol.* 524, 2828–2844. <https://doi.org/10.1002/cne.23994>.
- Schlichting, M., Helfrich-Förster, C., 2015. Photic entrainment in *Drosophila* assessed by locomotor activity recordings. *Methods Enzymol.* 552, 105–123. <https://doi.org/10.1016/bs.mie.2014.10.017>.
- Schmid, B., Helfrich-Förster, C., Yoshii, T., 2011. A new ImageJ plug-in “actogramJ” for chronobiological analyses. *J. Biol. Rhythms* 26, 464–467. <https://doi.org/10.1177/0748730411414264>.
- Sokolove, P.G., Bushell, W.N., 1978. The chi square periodogram: its utility for analysis of circadian rhythms. *J. Theor. Biol.* 72, 131–160. [https://doi.org/10.1016/0022-5193\(78\)90022-X](https://doi.org/10.1016/0022-5193(78)90022-X).
- Spoelstra, K., Albrecht, U., van der Horst, G.T.J., Brauer, V., Daan, S., 2004. Phase Responses to Light Pulses in Mice Lacking Functional *per* or *cry* Genes. *J. Biol. Rhythms* 19, 518–529. <https://doi.org/10.1177/0748730404268122>.
- Spoelstra, K., Wikelski, M., Daan, S., Loudon, A.S.I., Hau, M., 2016. Natural selection against a circadian clock gene mutation in mice. *Proc. Natl. Acad. Sci.* 113, 686–691. <https://doi.org/10.1073/pnas.1516442113>.
- Stanewsky, R., Kaneko, M., Emery, P., Beretta, B., Wager-Smith, K., Kay, S.A., Rosbash, M., Hall, J.C., 1998. The *cry(b)* mutation identifies cryptochrome as a circadian photoreceptor in *Drosophila*. *Cell* 95, 681–692. [https://doi.org/10.1016/S0092-8674\(00\)81638-4](https://doi.org/10.1016/S0092-8674(00)81638-4).
- Su, S. Bin, Lu, C.W., Kao, Y.Y., Guo, H.R., 2008. Effects of 12-hour rotating shifts on menstrual cycles of photoelectronic workers in Taiwan. *Chronobiol. Int.* 237–248. <https://doi.org/10.1080/07420520802106884>.
- Van Der Veen, D.R., Riede, S.J., Heideman, P.D., Hau, M., Van Der Vinne, V., Hut, R.A., 2017. Flexible clock systems: adjusting the temporal programme. *Philos. Trans. R. Soc. B Biol. Sci.* <https://doi.org/10.1098/rstb.2016.0254>.
- Veleri, S., Rieger, D., Helfrich-Förster, C., Stanewsky, R., 2007. Hofbauer-Buchner eyelet affects circadian photosensitivity and coordinates TIM and PER expression in *Drosophila* clock neurons. *J. Biol. Rhythms* 22, 29–42. <https://doi.org/10.1177/0748730406295754>.
- Wittmann, M., Dinich, J., Mellow, M., Roenneberg, T., 2006. Social jetlag: misalignment of biological and social time. *Chronobiol. Int.* 23, 497–509. <https://doi.org/10.1080/07420520500545979>.
- Wyse, C.A., Coogan, A.N., Selman, C., Hazlerigg, D.G., Speakman, J.R., 2010. Association between mammalian lifespan and circadian free-running period: the circadian resonance hypothesis revisited. *Biol. Lett.* 6, 696–698. <https://doi.org/10.1098/rsbl.2010.0152>.
- Yasuyama, K., Meinertzhagen, I.A., 1999. Extraretinal photoreceptors at the compound eye's posterior margin in *Drosophila melanogaster*. *J. Comp. Neurol.* 412, 193–202. [https://doi.org/10.1002/\(SICI\)1096-9861\(19990920\)412:2<193::AID-CNE1>3.0.CO;2-O](https://doi.org/10.1002/(SICI)1096-9861(19990920)412:2<193::AID-CNE1>3.0.CO;2-O).
- Yuan, Q., Metterville, D., Briscoe, A.D., Reppert, S.M., 2007. Insect cryptochromes: gene duplication and loss define diverse ways to construct insect circadian clocks. *Mol. Biol. Evol.* 24, 948–955. <https://doi.org/10.1093/molbev/msm011>.