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RESEARCH ARTICLE

Phenotypic plasticity in the invasive pest *Drosophila suzukii*: activity rhythms and gene expression in response to temperature

Christophe Plantamp*, Hélène Henri, Thibault Andrieux, Corinne Régis, Gladys Mialdea, Stéphane Dray, Patricia Gibert and Emmanuel Desouhant

ABSTRACT

Phenotypic plasticity may contribute to the invasive success of an alien species in a new environment. A highly plastic species may survive and reproduce in more diverse environments, thereby supporting establishment and colonization. We focused on plasticity in the circadian rhythm of activity, which can favour species coexistence in invasion, for the invasive species *Drosophila suzukii*, which is expected to be a weaker direct competitor than other *Drosophila* species of the resident community. We compared the circadian rhythms of the locomotor activity in adults and the expression of clock genes in response to temperature in the invasive *D. suzukii* and the resident *Drosophila melanogaster*. We showed that *D. suzukii* is active in a narrower range of temperatures than *D. melanogaster* and that the activities of the two species overlap during the day, regardless of the temperature. Both species are diurnal and exhibit rhythmic activity at dawn and dusk, with a much lower activity at dawn for *D. suzukii* females. Our results show that the *timeless* and *clock* genes are good candidates to explain the plastic response that is observed in relation to temperature. Overall, our results suggest that thermal phenotypic plasticity in *D. suzukii* activity is not sufficient to explain the invasive success of *D. suzukii* and call for testing other hypotheses, such as the release of competitors and/or predators.

KEY WORDS: Circadian rhythm, Clock genes, *Drosophila melanogaster*, *Drosophila suzukii*, Functional principal component analysis, Invasion biology

INTRODUCTION

Phenotypic plasticity has been proposed to play an important role in successful invasions of alien species (Kolar and Lodge, 2001; Shea and Chesson, 2002; Stachowicz and Tilman, 2005; Catford et al., 2009). Highly plastic species may survive and reproduce in a broader range of ecological conditions, thus decreasing the niche overlaps with resident species. Previous studies in birds and mammals found that behavioural plasticity is higher in successful invasive species (Sol and Lefebvre, 2000; Sol et al., 2008). However, the general role of phenotypic plasticity in invasion success remains controversial, which was revealed by different meta-analyses that compared native and invasive plant species (Davidson et al., 2011; Godoy et al., 2011; Palacio-López and Gianoli, 2011). The study of Davidson et al. (2011) concluded that invasive species have higher plasticity, while in other studies, no differences in the level of plasticity were found. These contradictory conclusions could be explained by the fact that phenotypic plasticity may be costly or transient. Therefore, after a rapid initial increase, the phenotypic plasticity may be followed by genetic assimilation that leads to a decrease of its level (Lande, 2015).

Successful invasion through the coexistence of competitive species may rely on differences in circadian period lengths (Daido, 2001; Vaze and Sharma, 2013). For instance, Fleury et al. (2000) showed that within a parasitoid wasp community, the coexistence of weaker parasitoid competitors with other competitors is favoured by their activity during a greater range of the daytime. Being active during a broader period allows this parasitoid species to lay its eggs earlier in the day so that its offspring can exploit the resource before the arrival of stronger competitors. Exploiting a resource longer – and thus earlier – in the day would thus be advantageous to a less competitive alien species. Temperature is one of the main environmental factors that controls the activity period in ectotherms and, thus, the coexistence of competitive species. The thermal tolerance range that is exhibited by invasive species is generally wider than for non-invasive species (reviewed by Kelley, 2014). Moreover, because the circadian rhythm of activity changes in relation to temperature (Brunner and Diernfellner, 2006; Chen et al., 2007; Low et al., 2008; Bartok et al., 2013; Montelli et al., 2015), fully understanding the influence of temperature and the circadian rhythm on the success of an invasive species requires investigating their combined effects. The circadian rhythm of activity and its sensitivity to temperature depend on endogenous time-keeping signals. The molecular mechanisms underlying these behaviours are well known in model species, such as the fly *Drosophila melanogaster* or the filamentous fungus *Neurospora crassa* (Dunlap, 1999; Edery, 2000; Allada and Chung, 2010). For instance, in neurons of *D. melanogaster*, levels of expression of core pacemaker genes are strongly correlated with levels of locomotor activity (Stanewsky, 2002; Stoleru et al., 2004; Grima et al., 2004). The three main genes that are involved in the pacemaker function are *period*, *timeless* and *clock*, with the first two being temperature sensitive (Majercak et al., 1999; Boothroyd et al., 2007; Kyriacou et al., 2008; Montelli et al., 2015).

Here, we compared phenotypic plasticity across temperatures of the circadian rhythm of activity between an invasive species, *Drosophila suzukii*, the spotted wing *Drosophila*, and a resident one, *D. melanogaster*. Coming from Asia, *D. suzukii* successfully invaded three continents over the span of a few years (Cini et al., 2012; Deprá et al., 2014; Asplen et al., 2015) and is considered to be a major pest. The role of phenotypic plasticity in its invasion is currently being considered (Fraimout et al., 2018; Stockton et al., 2018). Unlike other *Drosophila* species, *D. suzukii* is able to oviposit eggs in ripening, healthy fruits (Lee et al., 2011) using its

Université de Lyon, 69000 Lyon, Université Claude Bernard Lyon 1, CNRS, VetAgro Sup, INRIA, Laboratoire de Biométrie et Biologie Evolutive, UMR 5558, 43 Bd du 11 Novembre 1918, 69622 Villeurbanne Cedex, France.

*Author for correspondence (christophe.plantamp@anses.fr)

C.P., 0000-0003-2554-359X; S.D., 0000-0003-0153-1105; P.G., 0000-0002-9461-6820; E.D., 0000-0003-0317-4463

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serrated ovipositor (Atallah et al., 2014). However, adult emergence was observed in the field from the same rotting fruits as other *Drosophila* species (Dancau et al., 2017; Hennig and Mazzi, 2018; Mitsui et al., 2006, 2010; Poyet et al., 2014; Shaw et al., 2018a; Stemberger, 2016), thus revealing conditions of sympatry. Moreover, laboratory studies suggest that *D. suzukii* is a weak competitor compared with other European *Drosophila* species (e.g. longer development time and lower fecundity than *D. melanogaster*: Emiljanowicz et al., 2014; Lin et al., 2014a; Tochen et al., 2014; Kinjo et al., 2014; Asplen et al., 2015).

In our study, we adopted a multi-level approach to the circadian rhythm of locomotor activity, associating behaviour and molecular measurements, with three main objectives. First, we aimed at characterizing the circadian rhythm of the locomotor activity of *D. suzukii* in both sexes and in two populations. Second, we compared the phenotypic plasticity of the circadian rhythm of the locomotor activity between *D. suzukii* and *D. melanogaster* at different constant temperatures. We expected differentiated periods of activity during the day between the two species depending on specific temperatures (as predicted by Kelley, 2014) and that the invasive *D. suzukii* should exhibit a higher level of phenotypic plasticity than that of *D. melanogaster*. Third, to explore the phenotypic plasticity response at molecular level, we investigated the potential molecular mechanisms underpinning the circadian activity and quantified the expression of two candidate genes, *timeless* and *clock*, during the daytime.

MATERIALS AND METHODS

Fly strains and rearing

Drosophila suzukii (Matsumura 1931) is described as a temperate species, and its thermal tolerance for development in different populations has been predicted to range from 7 to 32°C (Tochen et al., 2014; Asplen et al., 2015). Complete development from egg to adult can be obtained for *D. melanogaster* Meigen 1830 between 12 and 32°C (David et al., 1983).

We used 'Ly' strains of *D. melanogaster* and *D. suzukii*, which were founded from 20 wild females captured in 2009 and 2012, respectively, near Lyon in Sainte Foy-Lès-Lyon (France; 45°44′ 23.98″N, 4°47′26.79″E). We also used 'Ba' strains of *D. suzukii*, which were founded from 8 isofemale lines captured in 2012 in Barcelona (Spain; 41°25′48.0″N, 2°07′48.0″E). The strains were maintained on a standard *Drosophila* medium (David and Clavel, 1965) in a climate-controlled room kept at 21±1°C, with a relative humidity of 54±8% and a 12 h:12 h light:dark cycle.

In all experiments, adults were sexed after a short ether anaesthesia under a binocular microscope 48 h after emergence. Then, flies were put individually in Petri dishes (5.5 cm diameter) with moistened paper (water+5% sucrose) for 24 h to allow them to recover from anaesthesia. The locomotor activity of these 72 h-old insects in Petri dishes was then monitored (see below).

Monitoring locomotor activity rhythm

The monitoring setup was composed of an infrared-sensitive camera hanging in the middle of a closed, square, opaque box (75×50×50 cm) that monitored up to 48 insects simultaneously using an infrared light floor (infrared LED). Individuals in the Petri dishes were placed according to a matrix of 6 rows×8 columns. The circadian rhythm was stimulated within the box using white LEDs with a 12 h:12 h light:dark cycle. The position of each fly in the matrix was randomly determined to avoid any confounding effects. Individual movements were quantified using VideoTrack Software v3.22 (Viewpoint Life Sciences). Data consisted of the movement quantity integrated every 10 min for each individual. The movement was estimated based on the number of pixels that changed over time and, thus, was correlated to the locomotor activity of the individuals. Each individual (and, hence, its movement quantity) was characterized by its position, species, sex, population and/or temperature.

Movement data can be biased by the deformation of pictures due to the distance to the camera axis and the fact that more movement (in pixels) is recorded with large individuals than with small ones. Thus, movements were corrected for position and individual size. To remove any effect due to position (i.e. distance to the central camera axis), the data were divided by the maximum value of the time series per position (maximum movement quantity for one individual at a given position). To control for the effect of body size on movement quantity, the length of the right wing (i.e. the distance between the r-m vein and the end of the R4+5 vein at the tip of the wing; Alexander et al., 1981) was measured on all flies after monitoring. Wing length is classically used as a proxy for size in *Drosophila* spp. (David et al., 1994), because it is correlated to thorax length (Tantawy and Rakha, 1964). To account for this size effect in our measurements, each time series was divided by the wing length of the individual being monitored. This experimental setup and the data adjustment procedure were used in all measurements of the rhythm of locomotor activity.

Experiment 1: effect of sex on the circadian rhythm of the locomotor activity (CRLA)

There were three goals for this experiment. First, we aimed to validate our experimental design by testing whether it allowed us to find similar results to those of previous studies on *D. melanogaster* (Allada and Chung, 2010). Second, we investigated the potential temporal niche differentiation between sexes in the two species by testing whether males and females express different patterns of CRLA under a 12 h:12 h light:dark cycle. We expected that males could show mating activity at different times during the day, avoiding courtship interference between species. We also expected that *D. suzukii* females might forage earlier in the day than *D. melanogaster* females in order to feed or to oviposit first. The light regime corresponds to autumnal equinox in September, when the two species are very abundant (Fleury et al., 2009; Asplen et al., 2015). The monitoring of activity was carried out at 19.5±0.5°C (relative humidity, RH, 70±10%) over 4 days.

Third, we tested whether the CRLA varied between two geographically separated populations of *D. suzukii*: strain Ly, trapped in a continental climate near Lyon, and strain Ba, trapped in a Mediterranean climate around Barcelona. We expected local adaptation to the different climates to result in less activity in the afternoon at the hottest time of the day in Barcelona than in Lyon. Individuals were monitored at 20±0.5°C with a RH of 66±5% over 2 days with a 12 h:12 h light:dark cycle. Four series of 9 individuals per sex and population were replicated so that a total of 36 males and 36 females were monitored per population.

Experiment 2: effects of temperature on plasticity of the CRLA and on expression of clock genes

Plasticity of the CRLA of *D. suzukii* and *D. melanogaster* in response to temperature

We aimed to (i) determine the thermal width of the locomotor activity of *D. suzukii* and *D. melanogaster*, (ii) test whether they were active at different times during the day depending on specific temperatures, and (iii) assess the plasticity of the CRLA of the two species in response to temperature. We expected that the invasive

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D. suzukii would be active over a larger thermal width than *D. melanogaster*, especially at cold temperatures as *D. suzukii* is more cold resistant (Stephens et al., 2015).

The locomotor activity of four different groups of females of each species (Ly strains) was recorded at four constant temperatures (10±1, 17±0.5, 24±1 and 30±1°C) for two consecutive days (12 h:12 h light:dark cycle, RH 78±10%). For each species and each temperature, a total of 48 different individuals were monitored in two replicates. In each temperature replicate, the two species were recorded at the same time.

Transcription kinetics of candidate clock genes for *D. suzukii* and *D. melanogaster* in response to temperature

Our goal was to investigate the thermal width of *D. suzukii* and *D. melanogaster* on a small and complementary molecular scale, and, thus, the plasticity of gene expression in response to temperature. We used a candidate-gene approach to target *timeless* and *clock* genes in *D. suzukii*. We chose these genes because they are known to be correlated with the circadian rhythm of locomotor activity in *D. melanogaster*. The transcription kinetics of *clock* and *timeless* were studied in female *D. suzukii* and *D. melanogaster* (Ly strains) at six different times during the day (04:00 h, 08:00 h, 12:00 h, 16:00 h, 20:00 h, 00:00 h) and at four temperatures (10, 17, 24, 30°C). Females were conditioned in the same way as those that were used in measurements of locomotor activity. They were put individually in Petri dishes with paper moistened with water+5% sucrose in the temperature conditions described above (10±1, 17±0.5, 24±1 and 30±1°C) in a climate-controlled chamber (SANYO, MLR-351H) at RH 77±5%. The experiment was replicated three times with different individuals. In total, 60 females were randomly sampled per time of the day, temperature and species.

All samples were stored dry at −80°C. Four pools of 15 female heads were established per modality for mRNA extraction and quantification. A circadian pacemaker driving locomotor activity is localized in neurons of the head of the fly (for review, see Allada and Chung, 2010). Total RNA was extracted using an RNeasy mini kit (Qiagen) following manufacturer's instructions but including a TURBO DNase treatment on-column (Ambion). cDNA synthesis was carried out with a SuperScript III First-Strand Synthesis System kit (Invitrogen) with random hexamers. mRNA (cDNA) levels were measured via real-time quantitative PCR (qPCR) using SsoFast EvaGreen SuperMix (Bio-Rad) on a CFX 96 Bio-Rad machine. mRNA levels from *timeless* and *clock* genes were normalized to those of the housekeeping gene *rpL32* (Rakshit et al., 2012). Raw Cq values were calculated using CFX Manager Software v3.1 (applying the regression method). The mean Cq values from duplicate or triplicate (when possible) wells were the values used for further analysis. The temperature of the hybridization step was optimized for each primer pair for efficient PCR (sequences and temperatures are given in Table S1). The specificity of amplification was systematically checked with melting curves.

Statistical analyses

We applied functional principal component analysis (FPCA; Ramsay and Silverman, 2006) to summarize the variation in circadian movement among individuals and treatments (species, population, sex and temperature). FPCA is a multivariate method that explicitly accounts for the chronology of observations by considering the whole curves of circadian activity as statistical units. This ensures that, unlike standard PCA, the main variations that are identified by FPCA are constrained to temporal trends (i.e. curves) and are not due to a few non-subsequent values with a characteristic level of activity. FPCA produces eigenvalues (representing the variation explained by each dimension), and individuals were positioned on the factorial map by their scores on the principal components to summarize the main similarities among their circadian activity (more details on FPCA are given in Figs S1–S3). We display groups on the output of FPCA using convex hulls so that the effects of treatment (e.g. temperature) can be easily observed by comparing the distance between the barycentre of each group. We computed and tested (by a permutation procedure with 1000 repetitions) the percentage of variation in the first two principal component scores that were explained by the experimental factors ('species' or 'population' and 'sex' in experiment 1; 'species' and 'temperature' in experiment 2, CRLA plasticity) to evaluate which source of variation contributed the most to differences in circadian movements. The level of CRLA plasticity at different temperatures was calculated from the individual means on the entire time series of movement quantity, corrected by size and normalized to the maximum of each time series.

Unlike locomotor activity, the mRNA of different individuals was quantified for each time point. The normalized transcription kinetics of the genes were analysed using a generalized additive mixed model (GAMM; Wood, 2006) with a gamma distribution and log link function, with species, temperature and their interaction as factors and time of day as a quantitative variable smoothed by cubic regression splines. To consider the autocorrelation between sequential time points, we used autoregressive moving average (ARMA) models. We selected the best models based on the AIC method between several GAMM with different ARMA (p, q) models, by testing different combinations of p (autoregressive terms) and q (moving-average terms) coefficients varying between 0 and 3 (Zuur et al., 2009). Models with the lowest Akaike's information criterion (AIC) were a GAMM with an ARMA (3,2) for the *timeless* gene and a GAMM with an ARMA (1,1) for the *clock* gene.

R software (version 3.1.3; http://www.R-project.org/) was used to perform the statistical analyses. Packages fda 2.4.4, ade4 1.7-2 and adegraphics 1.0-4 were used for the FPCA analysis and the associated graphical representations, and mgcv 1.8-15 was used for the GAMM analysis.

RESULTS

Experiment 1: effect of sex on the CRLA

The CRLA under a 12 h:12 h light:dark cycle was significantly different between the species×sex treatments (permutation test, P=0.001, Fig. 1). The females of each species had their own patterns of activity and behaved differently from their conspecific males. *Drosophila melanogaster* females were uniformly active during the day, whereas *D. suzukii* females were mostly active at dusk. In contrast, the males of the two species had similar patterns of activity, with one peak of activity at dawn and one at dusk (Fig. 1).

The first FPCA axis (associated with the amount of activity, Fig. S1) explained 60.3% of the variation, with lower coordinates for *D. suzukii* than for *D. melanogaster*, which indicated that the locomotor activity of *D. suzukii* was lower than that of *D. melanogaster* (up to 20% less active). The activity of males and females of a given species overlapped throughout the whole day, as shown by the low variability (13.2%) of the locomotor activity, which is explained by the second axis of the FPCA (Fig. S1). The daytime range of activity of *D. suzukii* was similar to that of *D. melanogaster*; the range for *D. melanogaster* females was even slightly wider than that of *D. suzukii* females.

Comparison of the CRLA between the two populations (Ly versus Ba) of *D. suzukii* showed no significant difference at 20°C (permutation test, P=0.36; Fig. 2). The interaction between

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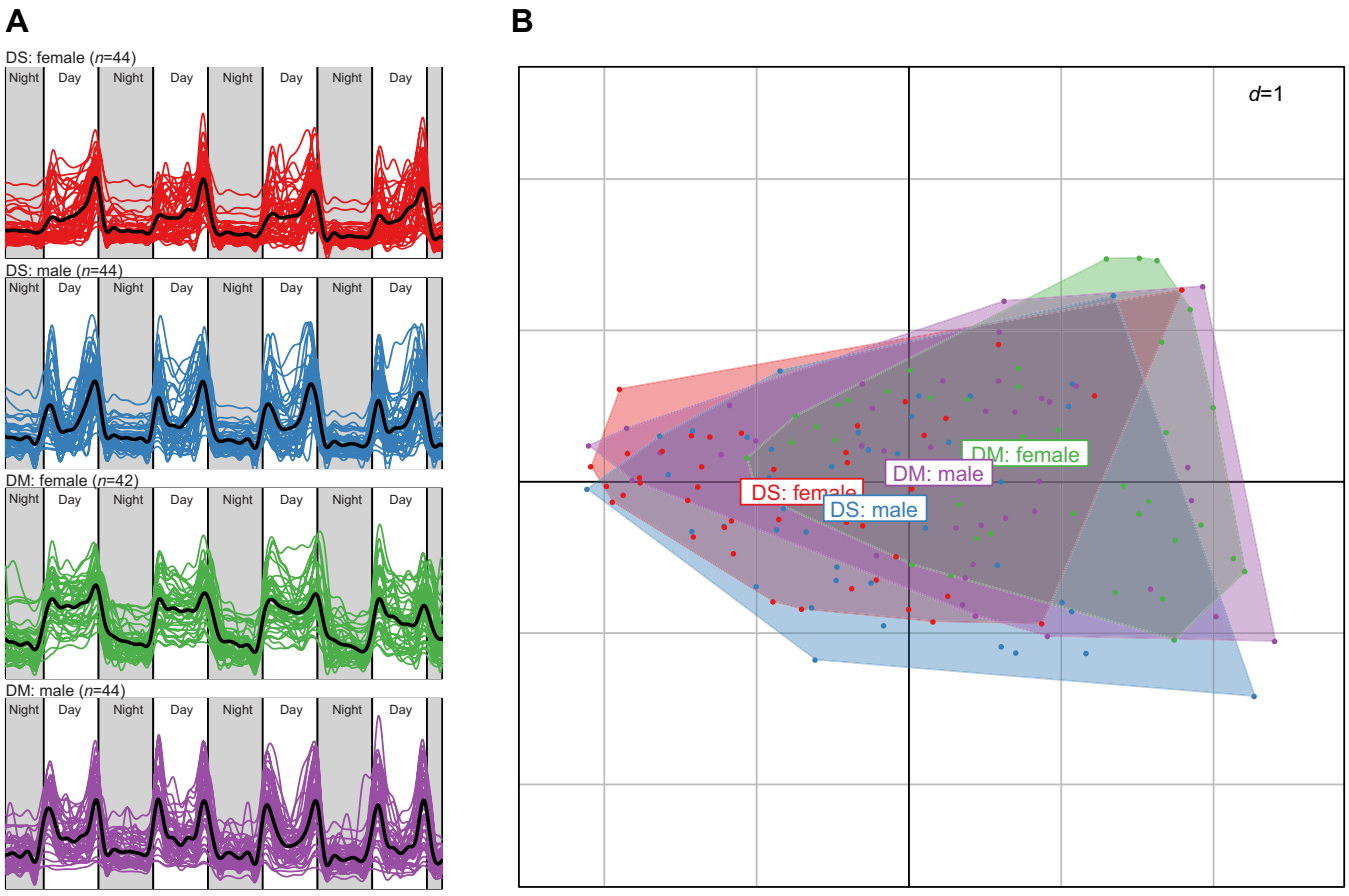

Fig. 1. Circadian rhythm of locomotor activity (CRLA) of female and male *Drosophila melanogaster* and *Drosophila suzukii*. Data were obtained at 20°C over 4 days under a 12 h:12 h light:dark cycle (experiment 1). (A) Time series of circadian movement for the two species [from top to bottom: *D. suzukii* (DS) females and males and *D. melanogaster* (DM) females and males] corrected by size and normalized to the maximum of each time series (pixels/maximum of series per µm). Data for individuals are represented in colour and the group average is represented in black. (B) Results for the first two axes of functional principal component analysis (FPCA) on the normalized circadian movement for females and males of *D. suzukii* and *D. melanogaster*. The length of the side of grid squares, *d*, is equal to 1. The axes explained 60.3% and 13.2% of the variability, respectively. Individuals are represented by points and treatments/groups are represented by convex hulls. The curves that were associated with the harmonics of the first and second axes of the FPCA are shown in Fig. S1. Sample sizes are specified in A.

population and sex was also not significant (permutation test, P=0.18). As described above, sex had a significant effect irrespective of the population of origin (permutation test, P=0.048): males had two peaks of activity at dawn and dusk and were more active at dawn than were females. Males were, on average, more active than females, and this observation was also confirmed by their opposite positions on the first axis of the FPCA, which is explained by the amount of activity (Fig. S2).

Experiment 2: effects of temperature on plasticity of the CRLA and on expression of clock genes

Plasticity of CRLA in *D. suzukii* and *D. melanogaster* in response to temperature

The CRLA was significantly affected by the interaction between temperature and species (permutation test, P<0.001). The pattern of the locomotor activity rhythm differed between species when the temperature increased (Fig. 3). At 10°C, an almost uniform activity pattern was observed, without any peak during the day for both species; at 17, 24 and 30°C, different patterns were observed for the two species. At 17 and 24°C, *D. suzukii* females were active mainly at dusk while *D. melanogaster* females were active during the whole day, with two peaks of activity at dawn and at dusk. At 24°C, we saw a slight shift in the activity towards night in *D. suzukii*. The amount of locomotor activity increased with temperature, except at 30°C for *D. suzukii*, where the locomotor activity was low. For *D. melanogaster*, the activity pattern changed at 30°C, with a long inactive period at midday, a high level of activity at dawn and at dusk, and a shift of activity towards the night (Fig. 3). The groups that were formed by the points in the FPCA confirmed the results, where the first axis (67.8% of the total variation) was associated with the amount of locomotor activity and the second axis (11.4%) was associated with the change of pattern: the midday inactive period and the shift of activity towards the night (Fig. S3).

The thermal plasticity of *D. suzukii* was lower than that of *D. melanogaster*, as indicated by the reaction norm of the CRLA according to temperature (Fig. 4). This conclusion was also supported by the smaller distances between convex hull centres and the narrower distribution of individuals (Fig. 3). The change of shape of the activity pattern at 30°C for *D. melanogaster* is illustrated by the strong positive scores of this treatment on the second axis of the FPCA (Fig. 3). Whatever the temperature, the daytime range of CRLA of *D. melanogaster* and of *D. suzukii* overlapped.

Transcription kinetics of candidate clock genes for *D. suzukii* and *D. melanogaster* in response to temperature

The interaction between species and temperature had a significant effect on the transcription of *timeless* ($F_{3,143}$=41.71, P<0.001; Fig. 5) but not on transcription of the *clock* gene ($F_{3,145}$=2.39,

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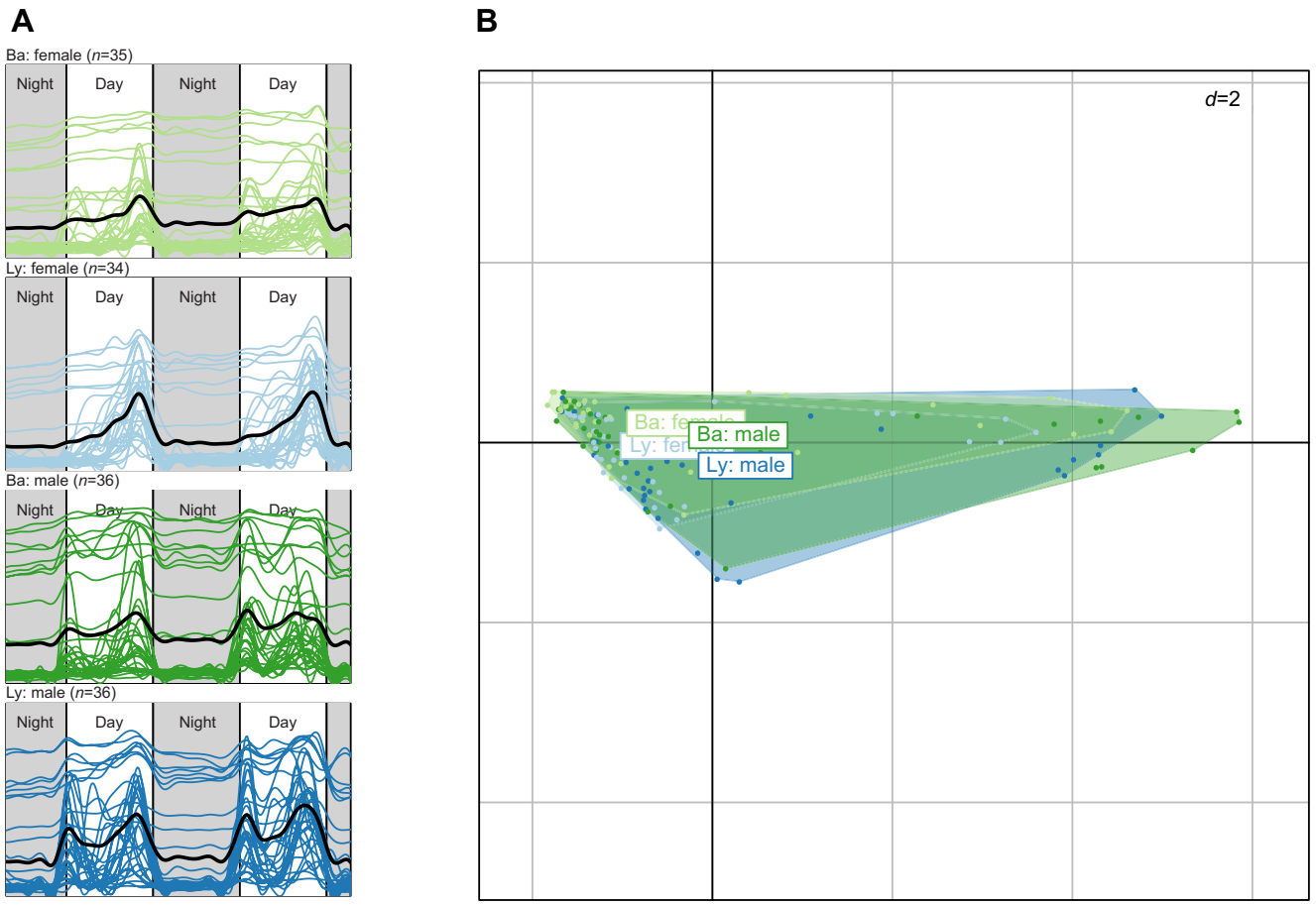

Fig. 2. CRLA of female and male *D. suzukii* from two populations. Data were obtained from flies from the Lyon ('Ly') and Barcelona ('Ba') strains at 20°C over 2 days under a 12 h:12 h light:dark cycle (experiment 1). (A) Time series of circadian movement (from top to bottom: females of Ba and Ly strain and males of Ba and Ly strain) corrected by size and normalized to the maximum of each time series (pixels/maximum of series per µm). Data for individuals are represented in colour and the group average is represented in black. (B) Results for the first two axes of FPCA on the normalized circadian movement quantity of females and males of the two populations. The length of the side of grid squares, *d*, is equal to 2. The axes explained 89.6% and 3.9% of the variability, respectively. Individuals are represented by points and treatments/groups are represented by convex hulls. The curves that were associated with the harmonics of the first and the second axes of the FPCA are shown in Fig. S2. Sample sizes are specified in A.

P=0.071; Fig. 6). For the latter, we detected only the additive effect of species and temperature ($F_{3,145}$=52.87, P<0.001 and $F_{1,145}$=30.06, P<0.001, respectively).

The transcription level of *timeless* in the two fly species showed a significant non-linear variation at all temperatures. At 10°C, the level of fluctuation was lower than that at the other temperatures for both species (Fig. 5). At 17°C, a peak in the transcription level was observed at dusk (20:00 h) and a trough was observed at dawn (08:00 h). At 24 and 30°C, the peak and the trough shifted by 4 h (00:00 h and 12:00 h, respectively, as illustrated by the grey bars in Fig. 5) and were more pronounced in *D. melanogaster* than in *D. suzukii*. Temperature has a weak effect on the average level of transcription in both species. Nevertheless, the transcription kinetics were more plastic in relation to temperature for *D. melanogaster*: this species showed pronounced fluctuations at 24 and 30°C during the day.

In *D. suzukii*, the transcription level of the *clock* gene showed a significant non-linear fluctuation only at 17 and 24°C, whereas in *D. melanogaster*, it fluctuated significantly at 17, 24 and 30 (Fig. 6). The *clock* transcription level was globally in antiphase with that of *timeless*, and it varied with a non-linear trend, with a trough around dusk (20:00 h) and a peak at dawn (08:00 h) at 17 and 30°C for *D. melanogaster* and at 24°C for *D. suzukii*. A difference in this pattern appeared in *D. suzukii* at 17°C and in *D. melanogaster* at 24°C, with a shift of 4 h (a trough at 00:00 h and a peak at 12:00 h, as illustrated by the grey bars in Fig. 6). We observed that the average level of transcription and the extent of fluctuations increased with temperature, mainly for *D. melanogaster*.

DISCUSSION

We investigated the CRLA at different temperatures in two species of *Drosophila*: *D. melanogaster* and the invasive *D. suzukii*. We found that the CRLA of the two species overlap during the day, regardless of the temperature: adults are diurnal and exhibit a rhythmic activity pattern at dawn and dusk for both males and females. However, the activity patterns also show slight differences between the two species, with *D. suzukii* females mainly having a peak of activity at dusk and being less active at dawn than *D. melanogaster* females. In *D. suzukii*, the observed CRLA and the influence of temperature on its pattern are well explained by the circadian regulatory genes *timeless* and *clock*. We also showed that, unexpectedly for an invasive species, the thermal range of activity of *D. suzukii* is narrower than that of *D. melanogaster*, and the CRLA is less plastic in *D. suzukii*.

The CRLA of *D. melanogaster* at different temperatures has already been described (Helfrich-Förster, 2000; Fujii et al., 2007). Our results in *D. melanogaster* agree with the previous studies, so

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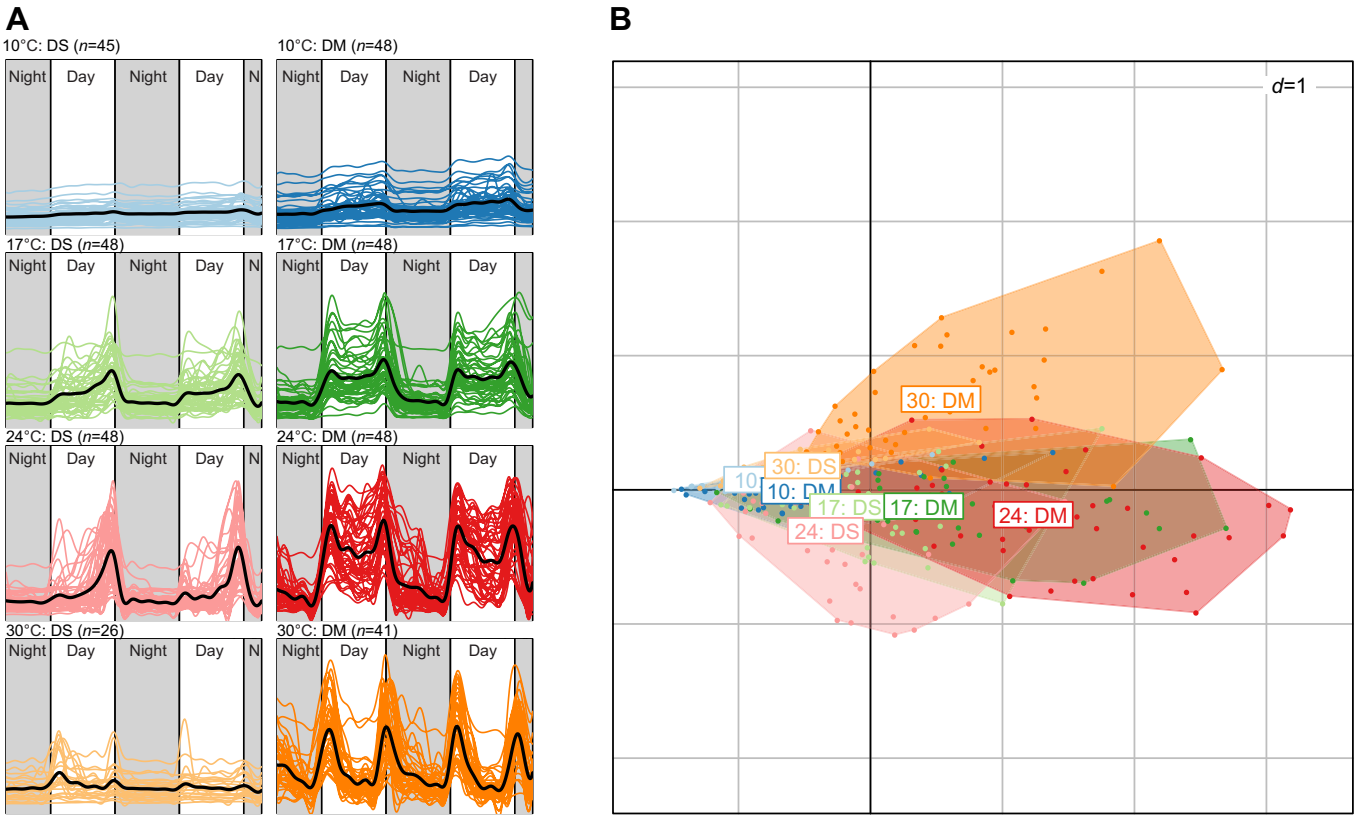

Fig. 3. CRLA of female *D. melanogaster* and *D. suzukii* at different temperatures. Data were obtained at four temperatures over 2 days with a 12 h:12 h light: dark cycle (experiment 2). (A) Time series of the circadian movement of *D. suzukii* (left) and *D. melanogaster* (right) females at four temperatures (from top to bottom: 10, 17, 24, 30°C) corrected by size and normalized to the maximum of each time series (pixels/maximum of series per µm). Data for individuals are represented in colour and the group average is represented in black. (B) Results for the first two axes of FPCA on the normalized circadian movement quantity of *D. suzukii* and *D. melanogaster* at the four temperatures. The length of the side of grid squares, *d*, is equal to 1. The axes explained 67.8% and 11.4% of the variability, respectively. Individuals are represented by points and treatments/groups are represented by convex hulls. The curves that were associated with the harmonics of the first and the second axes of the FPCA are shown in Fig. S3. Sample sizes are specified in A.

we are confident in our experimental setup. Males of the two species have similar activity patterns, while *D. suzukii* females are less active than *D. melanogaster* females at dawn (Figs 1 and 3). Our results seem to be generalizable, as the CRLA that was observed in *D. suzukii* was similar for individuals of the two populations, whatever their climatic origin (Fig. 2). In the recordings, few individuals exhibited a high and constant locomotor activity. This could be due to consistent inter-individual differences in terms of activity, also called personality (e.g. Gomes et al., 2019) or to experimental bias through camera parallax effect. Regardless, our statistical analysis provides conservative results as these individuals increase the variability of our data.

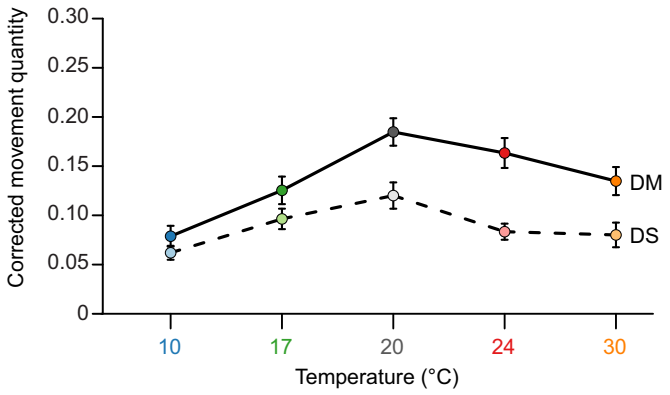

Fig. 4. Reaction norm of the CRLA between *D. suzukii* and *D. melanogaster* at different temperatures. Data for *D. suzukii* (DS) and *D. melanogaster* (DM) were obtained at 10, 17, 20, 24 and 30°C. Levels of plasticity are represented as means±2 s.d. of individual means calculated on the entire time series of movement quantity corrected by size and normalized to the maximum of each time series (pixels/maximum of series per µm), i.e. the coloured curves of Fig. 3A and the females curves of Fig. 1A. Data for 20°C were calculated based on the time series of the first 2 days of females in experiment 1.

Our results contrast with the prediction of the daily difference of activity between the two species, although we know that *D. suzukii* and *D. melanogaster* can be in sympatry on the same fruits (Dancau et al., 2017; Hennig and Mazzi, 2018; Mitsui et al., 2006, 2010; Poyet et al., 2014; Shaw et al., 2018a; Stemberger, 2016). Unexpectedly, we did not find any thermal conditions under which *D. suzukii* was active at a different time from *D. melanogaster* (Fig. 3): the CRLA patterns of the two species overlap. However, *D. suzukii* and *D. melanogaster* showed different responses to temperature (Figs 3 and 4). At 10°C, the two species have a low and similar activity pattern. At 30°C, *D. suzukii* is much less active, while *D. melanogaster* is still active but with a long midday inactive period and a shift towards night-time activity. This result is in accordance with the 'midday siesta' that was described by Majercak et al. (1999) and Low et al. (2008). This interspecific difference of the CRLA in females could be related to other behavioural differences that were observed in these two species:

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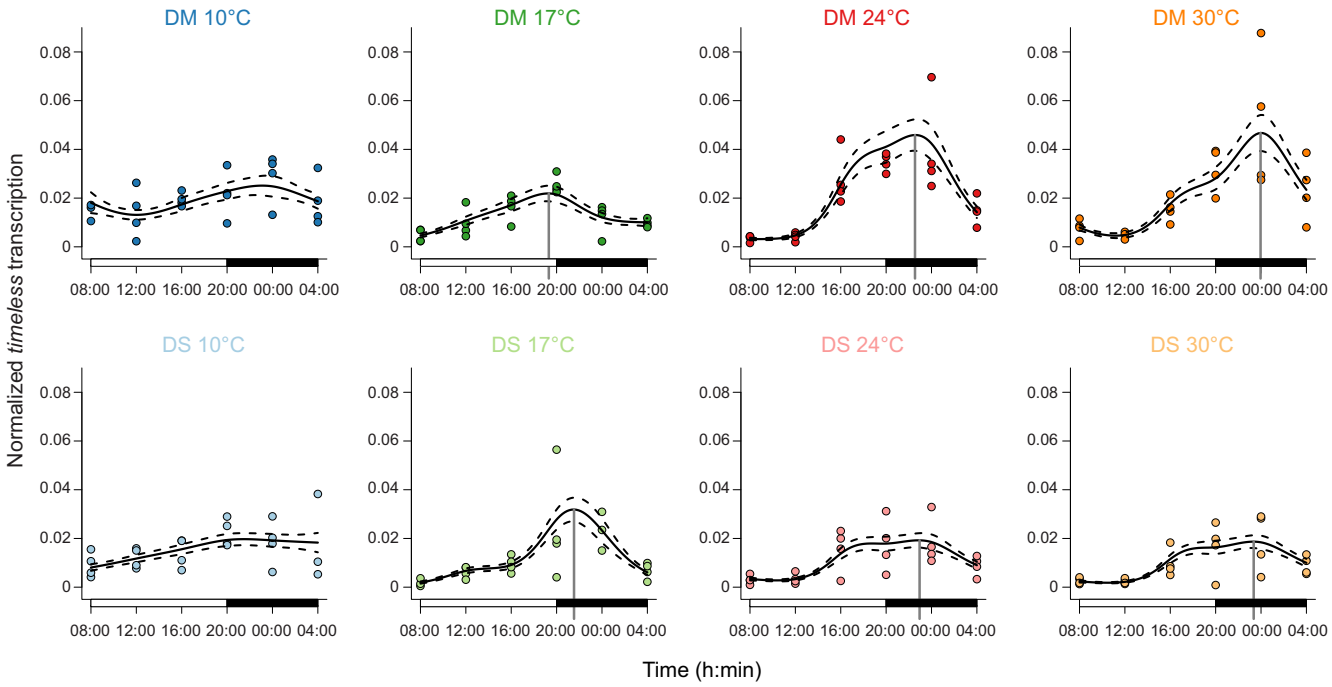

Fig. 5. Circadian rhythm of *timeless* gene transcription in female *D. melanogaster* and *D. suzukii* at different temperatures. Transcription levels for *D. melanogaster* (DM; upper panels) and *D. suzukii* (DS; lower panels) were obtained at 10, 17, 24 and 30°C over 20 h under a 12 h:12 h light:dark cycle (experiment 2). Solid lines are the regression from a generalized additive mixed model (GAMM) with an autoregressive moving average (ARMA; 3,2) and dashed lines are the 95% confidence interval. Measurements were normalized based on the transcription of the *rpL32* housekeeping gene. The vertical grey bars represent the mode of the curves. Each point represents data from a pool of 15 female heads.

feeding, oviposition and copulation rhythms tend to be more frequent at dusk in *D. suzukii* than in *D. melanogaster* (Ferguson et al., 2015; Lin et al., 2014b; Hamby et al., 2013, 2016; Shaw et al., 2018b). In *D. suzukii*, olfaction and gustation may follow a different circadian rhythm from that of *D. melanogaster* (Krishnan et al., 1999; Chatterjee et al., 2010).

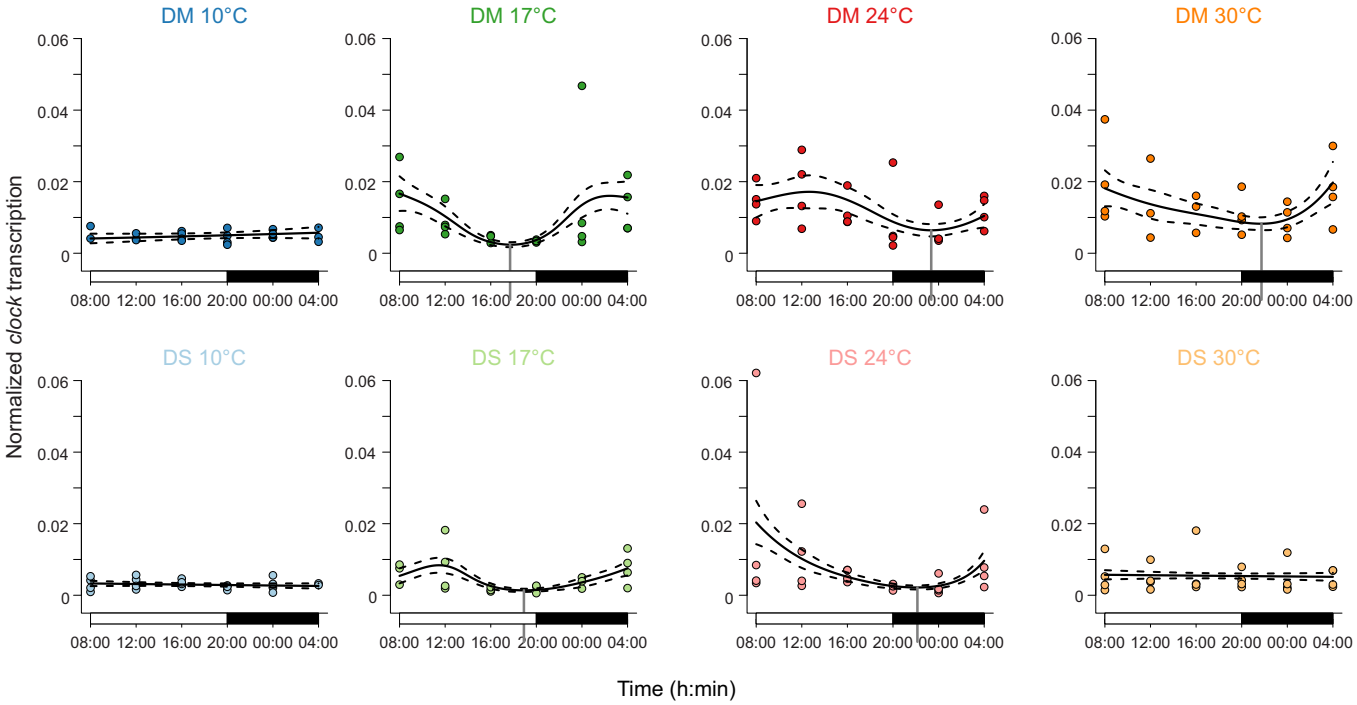

Fig. 6. Circadian rhythm of *clock* gene transcription in female *D. melanogaster* and *D. suzukii* at different temperatures. Transcription levels for *D. melanogaster* (DM; upper panels) and *D. suzukii* (DS; lower panels) were obtained at 10, 17, 24 and 30°C over 20 h under a 12 h:12 h light:dark cycle (experiment 2). Solid lines are the regression from GAMM with an ARMA (1,1) and dashed lines are the 95% confidence interval. Measurements were normalized based on the transcription of the *rpL32* housekeeping gene. The vertical grey bars represent the mode of the curves. Each point represents data from a pool of 15 female heads.

Our integrative approach also provides information concerning potential genes that are involved in CRLA and their sensitivity to temperature. Temperature does not affect the average level of transcription of the two genes, but it mostly affects the pattern. In the same way as the CRLA, the transcription levels of *timeless* and *clock* genes in *D. suzukii* varied less across temperature than in *D. melanogaster* (Figs 5 and 6). The transcription peak of *timeless* shifted slightly at night with increasing temperature in both species (from 20:00 h to 00:00 h; Fig. 5), which confirmed a thermally sensitive expression of *timeless*, as previously shown in *D. melanogaster* (Majercak et al., 1999; Boothroyd et al., 2007; Montelli et al., 2015). It is likely that the pattern of transcription kinetics explains the variation in activity pattern. This shift is positively correlated with the shift that was observed in *D. melanogaster* for activity at night at 30°C, and with the small shift of activity in the evening in *D. suzukii* at 24°C (Fig. 3). Temperature also has an effect on *clock* gene transcription in both species, with a change in circadian gene transcription at 17, 24 and 30°C in *D. melanogaster* but only at 24°C in *D. suzukii* (Fig. 6). The trough in the level of *clock* transcription shifted slightly at night with increasing temperature in both species (from approximately 20:00 h to 00:00 h, Fig. 6). We noticed that *timeless* and *clock* are in antiphase in both species, as already observed in *D. melanogaster* (Bae et al., 1998; for review see Dunlap, 1999). As in *D. melanogaster*, this antiphase transcription is likely to be due to the transcriptional/translational feedback loops between *period*, *timeless* and *clock* (Lee et al., 1998; Glossop et al., 1999; Cyran et al., 2003), and the light-induced degradation of TIM proteins that occurs at dawn (Emery et al., 1998; Stanewsky et al., 1998). On the whole, our results strongly suggest that *timeless* and *clock* genes are involved in the pacemaker mechanism of *D. suzukii*. The low level of activity at dawn in *D. suzukii* might be explained by a moderate influence of the 'morning oscillator' and a low expression of the neuropeptide PDF (Stoleru et al., 2004). Further investigations should focus on the kinetics of the expression of the core gene *period* and should examine whether some introns are thermally sensitive in *D. suzukii*, such as *dmpi8* intron in *D. melanogaster* (Majercak et al., 1999; Montelli et al., 2015). After analysis of *clock* and *timeless* transcription, because of drying and deterioration of the cDNA, the samples were unfortunately unusable for investigating transcription of the *period* gene properly.

Contrary to our expectation, the invasive species *D. suzukii* showed a lower thermal plasticity than *D. melanogaster* (Fig. 4). Low thermal plasticity in the CRLA has previously been observed in another *Drosophila* species, *Drosophila yakuba* (Low et al., 2008) but was explained by the low variability of the thermal conditions that were encountered by this Afro-tropical species. Such a hypothesis cannot be supported in *D. suzukii*, which is distributed widely and tolerates a large range of temperatures. A more plausible hypothesis is that the establishment and successful invasion of *D. suzukii* is due not to its thermal plasticity but to other factors, such as the 'enemy release hypothesis' (Keane and Crawley, 2002) or the 'vacant trophic niche' (MacArthur, 1970; Behmer and Joern, 2008). Indeed, *D. suzukii* is exposed to a low level of natural enemies in the areas it has invaded (Kacsoh and Schlenke, 2012; Chabert et al., 2012; Poyet et al., 2013, 2017), and it can exploit fruits at a stage of maturity where there are few competitors (Lee et al., 2011; Atallah et al., 2014; Keesey et al., 2015). Its main hosts, which are healthy fruits, seem to be exploited by few resident species, thus leaving an omnipresent trophic resource that is suitable for the survival, reproduction and dispersal of this alien species.

In conclusion, this study provides evidence that *D. suzukii* displays a CRLA and that circadian regulatory genes, which have already been identified in *D. melanogaster* (*timeless* and *clock*), could partly explain the variability in the behavioural response to temperature. Our results demonstrate that the ranges of CRLA of the two species overlap, which suggests that *D. suzukii* adults do not exploit a daily empty niche left by other *Drosophila* species. Even though we conducted the experiments in the laboratory, our results must be ecologically relevant, as field observations of the circadian rhythm of flight show the same pattern of activity, with *D. suzukii* flies being more active at dusk (Evans et al., 2017; Shaw et al., 2018a,b; Van Timmeren et al., 2017). Finally, our results do not support a role for thermal phenotypic plasticity in adult activity in the successful invasion of *D. suzukii*. It would be necessary to study this question on the seasonal scale to test whether *D. suzukii* exploits the fruit resource earlier in the year.

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Competing interests
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Author contributions
Conceptualization: C.P., P.G., E.D.; Methodology: C.P., H.H., S.D., E.D.; Software: H.H., S.D.; Validation: C.P..; Formal analysis: C.P., H.H., S.D., E.D.; Investigation: C.P., H.H., T.A., C.R., G.M.; Data curation: C.P., H.H., S.D.; Writing - original draft: C.P.; Writing - review & editing: C.P., S.D., P.G., E.D.; Visualization: C.P., S.D.; Supervision: P.G., E.D.; Project administration: P.G., E.D.; Funding acquisition: P.G., E.D.

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Supplemental Material 1: Transcription analysis protocol

Table S1: qPCR specifications and primers sequences

Species	Gene (exons)	5' primer	3' primer	Amplicon size	Hybridization temperature	Source
D. melanogaster	*rp49/rpL32*	GCCCAGCATACAG**C**CCCAAG	AAGCGGCGACGCACTCTGTT	133 pb	61°C	Rakshit et al. 2012
D. suzukii	*rp49/rpL32*	GCCCAGCATACAG**G**CCCAAG	AAGCGGCGACGCACTCTGTT	133 pb	61°C	-
D. melanogaster	*timeless* (E12-E13)	A**G**TTGGTCATGCGCAGCAAATG	TCCTT**T**TCGTACACAGATGCCA	448 bp	63°C	Grima et al. 2012
D. suzukii	*timeless*	A**A**TTGGTCATGCGCAGCAAATG	TCCTT**C**TCGTACACAGATGCCA	~459 bp	63°C	-
D. melanogaster	*clock*	**T**AATGAGGCCACCGA**T**CG	CTCCAGCAT**G**AGGTG**A**GT	97 pb	63°C	Rakshit et al. 2012
D. suzukii	*clock*	**C**AATGAGGCCACCGA**C**CG	CTCCAGCAT**C**AGGTG**G**GT	97 pb	63°C	-

Primers were designed on *D. melanogaster* genome and adapted for *D. suzukii* by BLAST on *D. suzukii*'s contigs (Ometto et al. 2013).

Supplemental Material 2: Harmonics (eigenfunctions) corresponding to the three FPCA

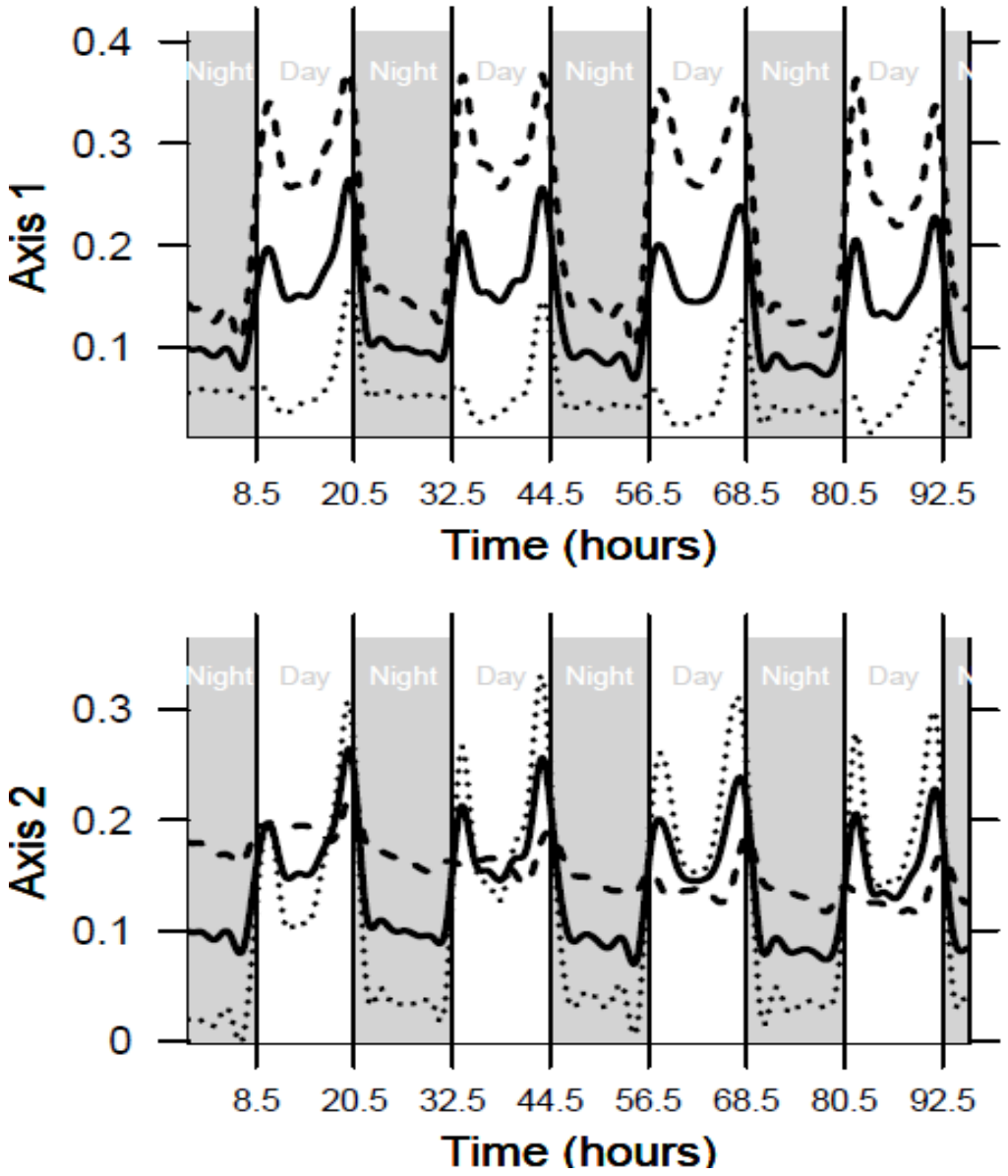

Fig. S1 Curves associated with harmonics (eigenfunctions) of the first (upper panel) and the second axis (lower panel) of the FPCA on the normalized circadian movement quantity of females and males of *D. melanogaster* and *D. suzukii* at 20°C over 4 days at LD 12 h:12 (experiment 1; results in Fig. 1). In the case of FPCA, principal components are defined by eigenfunctions, which are named harmonics that allow for the main differences in CRLA associated with each dimension to be identified. The full, dashed and point lines correspond to the average CRLA for all individuals on a given axis, the deviation for individuals located on the positive side of the axis and the deviation for individuals located on the negative side. On the first axis, in comparison to the average, individuals on the positive side are more active whereas individuals on the negative side are less active, especially at dawn. On the second axis, in comparison to the average, individuals on the positive side have a flat activity during the day, whereas individuals on the negative side have higher activity during the day, with higher peaks but lower activity at night.

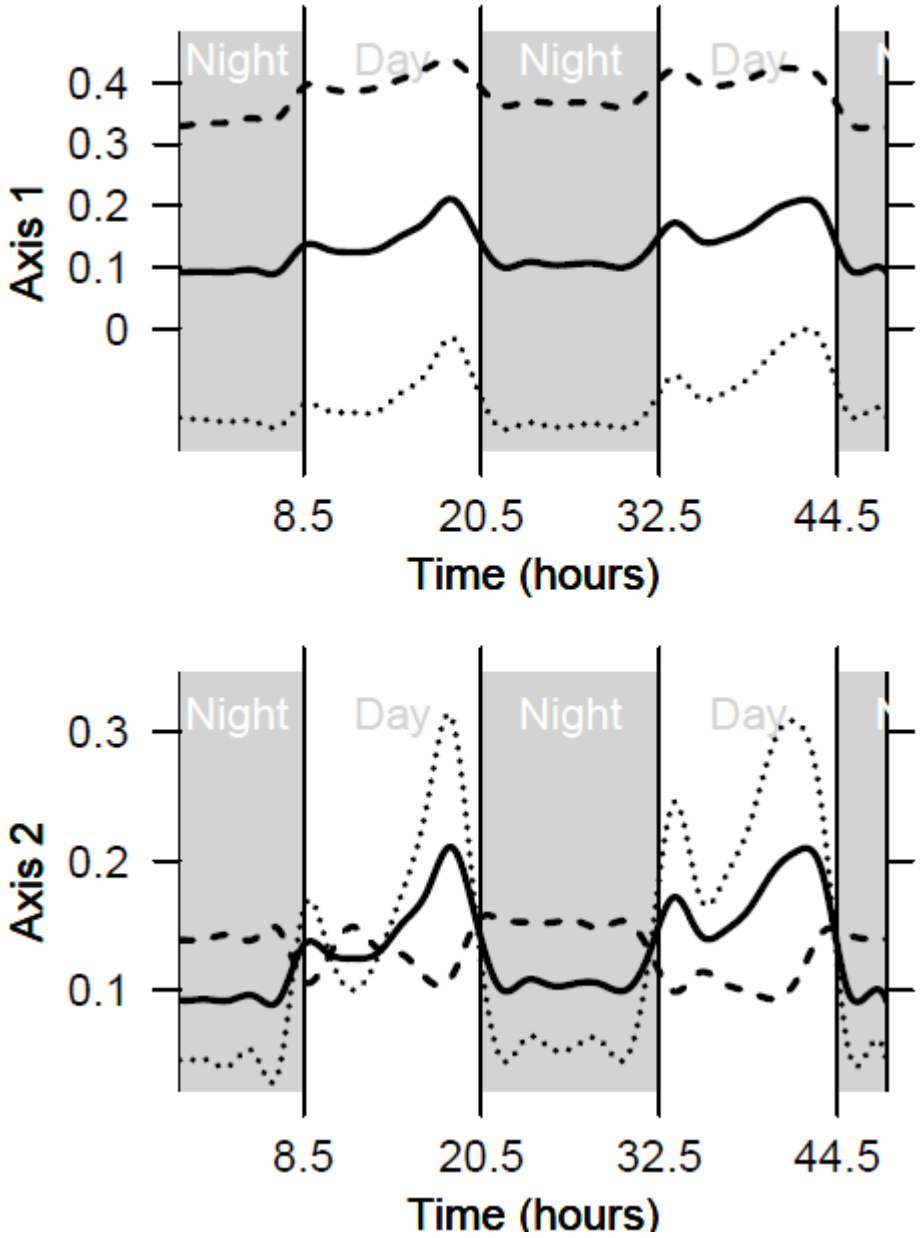

Fig. S2 Curves associated with harmonics (eigenfunctions) of the first (upper panel) and the second axis (lower panel) of the FPCA on the normalized circadian movement quantity of females and males of "Ba" and "Ly" populations of *D. suzukii* at 20°C over 2 days at LD 12 h:12 h (experiment 1; results in Fig. 2). The full, dashed and point lines correspond to the average CRLA for all individuals on a given axis, the deviation for individuals located on the positive side of the axis and the deviation for individuals located on the negative side. On the first axis, in comparison to the average, individuals on the positive side are more active at the opposite of individuals on the negative side that are less active. On the second axis, in comparison to the average, individuals on the positive side have lower peaks than those on the negative side, which have especially high peaks at dusk.

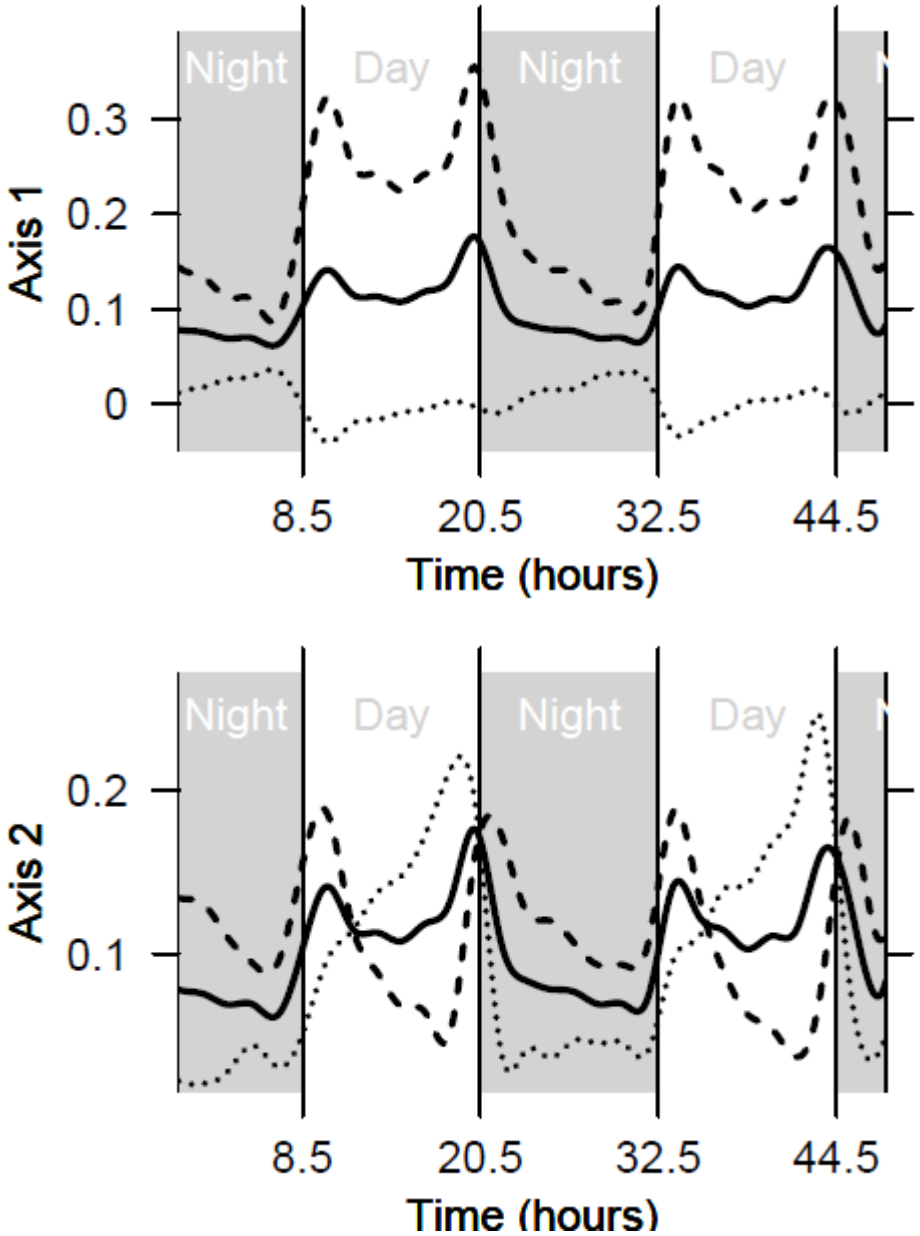

Fig. S3 Curves associated with harmonics (eigenfunctions) of the first (upper panel) and the second axis (lower panel) of the FPCA on the normalized circadian movement quantity of females of *D. melanogaster* and *D. suzukii* at 10, 17, 24 and 30°C over 2 days at LD 12 h:12 h (experiment 2-1; results in Fig. 3). The full, dashed and point lines correspond to the average CRLA for all individuals on a given axis, the deviation for individuals located on the positive side of the axis and the deviation for individuals located on the negative side, respectively. On the first axis, in comparison to the average, individuals on the positive side are more active during the day, which is opposite of individuals on the negative side that are less active. On the second axis, in comparison to the average, individuals on the positive side have a higher peak at dawn and a shift of activity at night, whereas individuals on the negative side have a higher diurnal activity with a higher peak at 7 pm.

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