

## RESEARCH ARTICLE

# The sexually dimorphic behaviour of adult *Drosophila suzukii*: elevated female locomotor activity and loss of siesta is a post-mating response

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## ABSTRACT

The polyphagous *Drosophila suzukii* is a highly invasive species that causes extensive damage to a wide range of berry and stone fruit crops. A better understanding of its biology and especially its behaviour will aid the development of new control strategies. We investigated the locomotor behaviour of *D. suzukii* in a semi-natural environment resembling a typical summer in northern England and show that adult female *D. suzukii* are at least 4-fold more active during daylight hours than adult males. This result was reproduced in several laboratory environments and was shown to be a robust feature of mated, but not virgin, female flies. Both males and virgin females kept on a 12 h light:12 h dark (12LD) cycle and constant temperature displayed night-time inactivity (sleep) followed by weak activity in the morning, an afternoon period of quiescence (siesta) and then a prominent evening peak of activity. Both the siesta and the sharp evening peak at lights off were severely reduced in females after mating. Flies of either sex entrained in 12LD displayed a circadian pattern of activity in constant darkness confirming the importance of an endogenous clock in regulating adult activity. This response of females to mating is similar to that elicited in female *Drosophila melanogaster* by the male sex peptide (SP). We used mass spectrometry to identify a molecular ion ( $m/z$ , 5145) corresponding to the poly-hydroxylated SP of *D. suzukii* and to show that this molecule is transferred to the female reproductive tract during copulation. We propose that the siesta experienced by male and virgin female *D. suzukii* is an adaptation to avoid unnecessary exposure to the afternoon sun, but that mated females faced with the challenge of obtaining resources for egg production and finding oviposition sites take greater risks, and we suggest that the change in female behaviour is induced by the male SP.

**KEY WORDS:** Sex peptide, Fruit fly, Pest management, Sleep, Circadian rhythm

## INTRODUCTION

The spotted wing *Drosophila*, *Drosophila suzukii*, is an Asiatic pest that has recently attracted much attention with its emergence in both North America and Europe as a major pest of berry and stone fruit (Asplen et al., 2015; Calabria et al., 2012; Cini et al., 2014; Lee et al., 2011; Walsh et al., 2011). Unlike other fruit flies, *D. suzukii* lays eggs in ripening fruit, causing extensive damage leading to

serious economic losses (Bolda et al., 2010; Goodhue et al., 2011). This is facilitated by a modified ovipositor, which is larger and sharper than in other fruit flies (Atallah et al., 2014) and well adapted for puncturing the skin of ripening fruit during egg deposition. The speed at which *D. suzukii* populations have invaded and become established in Europe and North America is evidence of successful physiological and behavioural adaptation to new climatic environments either after passive dispersal through fruit imports or by adult migration (Asplen et al., 2015; Cini et al., 2014). The success and severity of the economic damage inflicted on the fruit industry has recently focused attention on various aspects of the biology of this pest which might inform the development of effective control strategies, especially those based on low-pesticide input and which are compatible with integrated pest management.

The chronobiology of *D. suzukii* is of particular interest as it is known that insects can display circadian variability in their susceptibility to chemical insecticides (Cole and Adkisson, 1964; Piechowicz et al., 2012; Shipp and Otton, 1976). It has been shown for both *D. suzukii* and the related *Drosophila melanogaster* that levels of enzymes involved in insecticide metabolism fluctuate with a daily rhythm, suggesting that variation in rates of metabolism during the day can influence toxicity to fruit flies (Hoooven et al., 2009; Hamby et al., 2013). A biological clock might also influence the temporal efficacy of an insecticide by controlling rhythmic locomotor activity of an insect pest, which might determine the timing and extent of contact between the insect and a sprayed surface. Much of our understanding of the mechanisms of insect biological clocks and what environmental timing cues are important for setting the clock come from studying *D. melanogaster*, a powerful genetic model (Hall, 2003; Peschel and Helfrich-Forster, 2011; Schiesari et al., 2011). Typically, such studies have been performed using a laboratory protocol of a 12 h light:12 h dark (12LD) cycle and constant temperature. Under these conditions, both male and female *D. melanogaster* display bimodal ‘morning’ and ‘evening’ peaks of walking activity with periods of sleep in-between (Helfrich-Forster, 2000). After the evening peak, a robust sleep-like state is evident for both sexes, whereas the extent and quality of the sleep period (the siesta) occurring between the morning and evening peaks can vary (Andretic and Shaw, 2005). This variability has been shown to be dependent on the age, sex and, in the case of females, mating status of the insects (Ho and Sehgal, 2005; Isaac et al., 2010; Koh et al., 2006). Some studies have compared circadian behaviour of *D. melanogaster* kept on the standard 12LD cycle with the behaviour of flies in either a semi-natural outdoor environment or an incubator capable of simulating semi-natural conditions by gradually changing both temperature and light levels (De et al., 2013; Kannan et al., 2012; Menegazzi et al., 2013, 2012; Vanin et al., 2012). They have highlighted differences between the daytime activity of adult *D. melanogaster* in these more

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natural conditions compared with the standard laboratory 12LD cycle and constant temperature conditions and, in particular, the unexpected appearance in some studies of an afternoon peak of activity in semi-natural environments (Menegazzi et al., 2012; Vanin et al., 2012). In contrast to the extensive study of locomotor rhythms in *D. melanogaster*, there has only been one report describing the daily pattern of locomotor activity of *D. suzukii* (Hamby et al., 2013). This study by Hamby et al. (2013) looked at the ambulation of virgin adults in a changing environment that approximated to local ‘summer’ and ‘winter’ conditions in North America. It showed that most of the activity of male and female flies occurred during daylight hours. Both sexes displayed peaks of activity at dusk, which also extended into the period of total darkness. Over 24 h, males appeared to be more active than females.

In the present study, we monitored the locomotor activity of adult *D. suzukii* in several different light/dark and temperature cycles for comparison with previous studies on *D. melanogaster* and the work of Hamby et al. (2013) on *D. suzukii*. Of particular interest was the question of whether male and female *D. suzukii* display high levels of afternoon activity when monitored in semi-natural and simulated natural environments as was found for *D. melanogaster*. *Drosophila melanogaster* females respond to mating by increasing their afternoon activity with consequential reduction in siesta sleep, a response triggered by the male accessory gland sex peptide (SP<sub>Dm</sub>) (Isaac et al., 2010). As SP<sub>Ds</sub> has been isolated from male accessory glands of *D. suzukii* (Schmidt et al., 1993), we considered the possibility that the locomotor behaviour of female *D. suzukii* is also influenced by the transfer of SP<sub>Ds</sub>.

## MATERIALS AND METHODS

### Insects

*Drosophila suzukii* (an Italian strain) were maintained on a standard *Drosophila* diet (oatmeal, 7.5%; molasses, 5%; agar, 8.4%; yeast, 8.4%; methyl paraben, 0.35% in water) at 25°C on a 12LD cycle. Insects were sexed as newly (<4 h) eclosed adults from stock bottles. Organically grown blueberries were bought from a local supermarket in Leeds, UK.

### Locomotor activity

Virgin adults (3–4 days old) were placed in small groups of 10 or 20, either single sex or equal numbers of males and females, in vials (80×20 mm) containing standard oatmeal/molasses/agar diet for 3 days. After 3 days, flies in the mixed sex vials were separated into ‘mated’ males and ‘mated’ females. Both virgin and ‘mated’ flies were then lightly anaesthetised using CO<sub>2</sub> and placed in single-sex groups of five in glass vials (100×16 mm) plugged at one end with 2% agar containing either 5% sucrose or 50% blueberry pulp and at the other end with cotton wool. Tubes were placed in an activity monitor (LAM16 Locomotor Activity Monitor, Trikinetics Inc., Waltham, MA, USA) that uses infrared beams to detect movement as flies move along the glass tube. The number of beam breaks occurring in 5 min time bins was recorded for each tube and the data were processed using the DAMFileScan application (Trikinetics Inc.) and Microsoft Excel. At the end of some experiments, individual ‘mated’ females were allowed to lay eggs on oatmeal/molasses/agar diet, revealing that >90% of the females laid fertile eggs and had therefore successfully mated. Quiescence, defined as 5 min time bins with zero beam breaks, was analysed using the BeFly sleep analysis tool provided by Dr Ed Green, Department of Genetics, University of Leicester, UK. Experiments were conducted using a Sanyo incubator (MIR-253) with a daylight fluorescent tube (Aquadaylight from Aqualine, Bradford, UK) as the light source controlled in an on–off manner by a programmable electronic timer. For studies in semi-natural light conditions, monitors were placed in a secure room (North Yorkshire, UK; latitude 54.0582N and longitude 0.7976W) with illumination by natural light from an east-facing skylight. This experiment started on 1 August 2013 and the weather was a mixture of bright sunlight and fast-moving clouds. For a laboratory-simulated natural environment, a controlled programmable

environment incubator (Memmert HPP 110, Camlab, Cambridge, UK) was used with variable LED illumination and humidity set at 75%. The programmed variation of temperature and light levels was confirmed using a DEEM *Drosophila* Environment Monitor (Trikinetics Inc.).

### Peptide extraction

Peptides were extracted from the male accessory gland and the female uterus by placing five dissected tissues into 10 µl of ice-cold acidic methanol (87% methanol, 5% glacial acetic acid). The extraction medium was removed after centrifugation (4°C, 12,000 g for 20 min) and the supernatant was stored at –20°C until required for analysis. Females were separated from males after 10 min of copulatory activity and dissected immediately to provide inseminated uteri.

### Mass spectrometry

Mass spectra were acquired using a Voyager DE STR matrix-assisted laser desorption ionisation mass spectrometer (MALDI-TOF MS; Applied Biosystems, Warrington, UK) in linear positive mode, using a 20 kV acceleration voltage, over the mass range *m/z* 1000–10,000 Da. Samples were mixed 1 µl:1 µl with sinapinic acid (10 mg ml<sup>-1</sup> in 30% acetonitrile, 0.1% trifluoroacetic acid); 1 µl was then pipetted onto a MALDI sample plate and air dried. External calibration was conducted using a calibration mixture (Applied Biosystems) containing angiotensin I, adrenocorticotrophic hormone [CLIP (corticotropin-like intermediate peptide) fragment 1–17, 18–39 and 7–38] and bovine insulin. Results are the mean of three independent MS measurements for each sample and all masses are shown as average masses [M+H]<sup>+</sup>.

## RESULTS

### The locomotor behaviour of *D. suzukii* in a semi-natural summer environment is sexually dimorphic

Young male and female flies were kept together in equal numbers for a period of 3 days, to allow sufficient time for mating to occur, before being separated and placed into 16 mm diameter tubes for recording locomotor activity using a Trikinetics LAM16. The daily activity profiles of male and mated female *D. suzukii* in semi-natural conditions were compared by placing the LAM beneath an east-facing skylight to provide natural daily fluctuations of light (maximum 700 lx) and temperature, ranging between 19 and 26°C with a minimum at around dawn and a maximum occurring in the late afternoon. The light:dark cycle approximated to 16 h light:8 h dark (16L8D) with sunrise at around 05.00 h and sunset at around 21.00 h. In comparison to males, females displayed greatly elevated (442±77%, mean±s.e.m. for six consecutive days) daily locomotor behaviour (Fig. 1). Female activity began at around 50 min before dawn light had reached the 1 lx sensitivity threshold of the environmental monitor (Fig. 1A). The maximum afternoon activity of females occurred between 18:00 h and 21:00 h over the 6 days of recording; this activity declined rapidly to 50% by the time light levels had dropped to around 15 lx. This highly significant sexually dimorphic behaviour observed for *D. suzukii* was markedly different from the findings of a previous study by Hamby et al. (2013), where males were found to be more active than females under summer-like conditions. The main difference between these two studies was the mating status of the insects; Hamby et al. (2013) used virgin adults as opposed to the mated flies of the present study.

### Increased activity of females is a post-mating response that disrupts the siesta

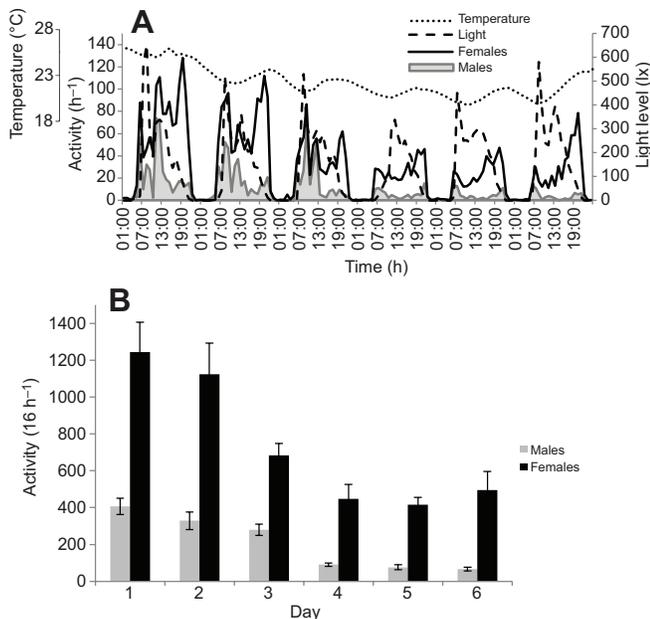
The activity of virgin and mated *D. suzukii* of both sexes was compared under laboratory conditions of constant 25°C temperature and a rectangular 12LD cycle with the lights-on level set to 800 lx. In these conditions, virgin and mated males displayed rhythmic morning and evening peaks of activity separated by

extended periods of quiescence with few either quantitative or qualitative differences between the behaviour of the two groups (Fig. 2). Males increased their activity during the late afternoon period leading up to a strong and sharp evening peak, occurring around 20 min after lights off. Switching from a LD regime to constant darkness (DD) resulted in the replacement of the prominent evening peak with a general increase in male activity throughout the subjective day, but with most ambulation occurring just prior to the time of expected lights off.

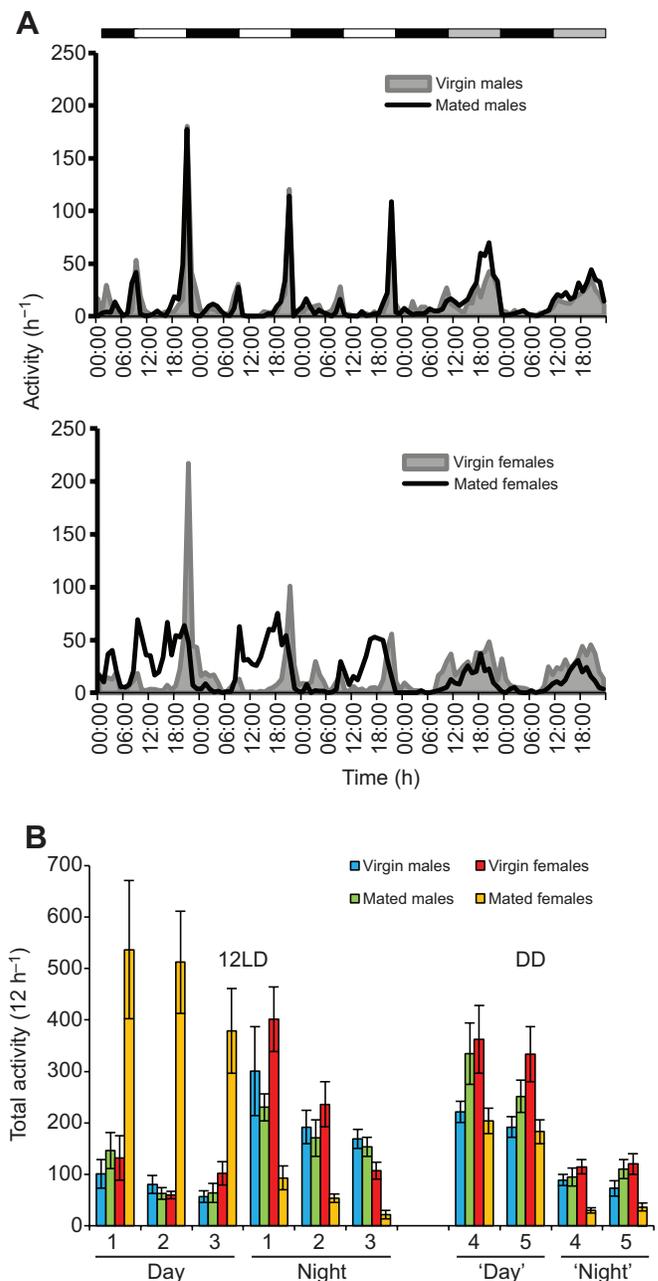
In the 12LD cycle, virgin females, but not mated females, behaved in a similar manner to the males, with a prominent evening activity that peaked just after lights off and which disappeared in DD conditions (Fig. 2A). Mated females responded to lights on with a sharp rise in activity that diminished after 1 h, but remained relatively high all morning before rising to a broad peak during the afternoon. The average increase in lights-on activity relative to virgins was  $540 \pm 125\%$  (mean  $\pm$  s.e.m.) for the 3 days of LD, whereas night-time activity was reduced by  $78 \pm 1\%$  (mean  $\pm$  s.e.m.; Fig. 2B). The large drop of activity in the dark was primarily the result of the absence after entering the dark phase of the sharp spike in activity, which was so prominent in virgin females and males. In DD conditions, most of the mated female activity was restricted to the subjective day and was reduced by around 45% in comparison to the daytime activity of the LD regime. There appeared to be a general trend amongst all groups of flies of a progressive reduction in daily activity during the 12LD cycle.

When the activity data were expressed as quiescence/sleep (5 min periods of inactivity) using parameters established for studying sleep in *D. melanogaster* (Ho and Sehgal, 2005), the increase in

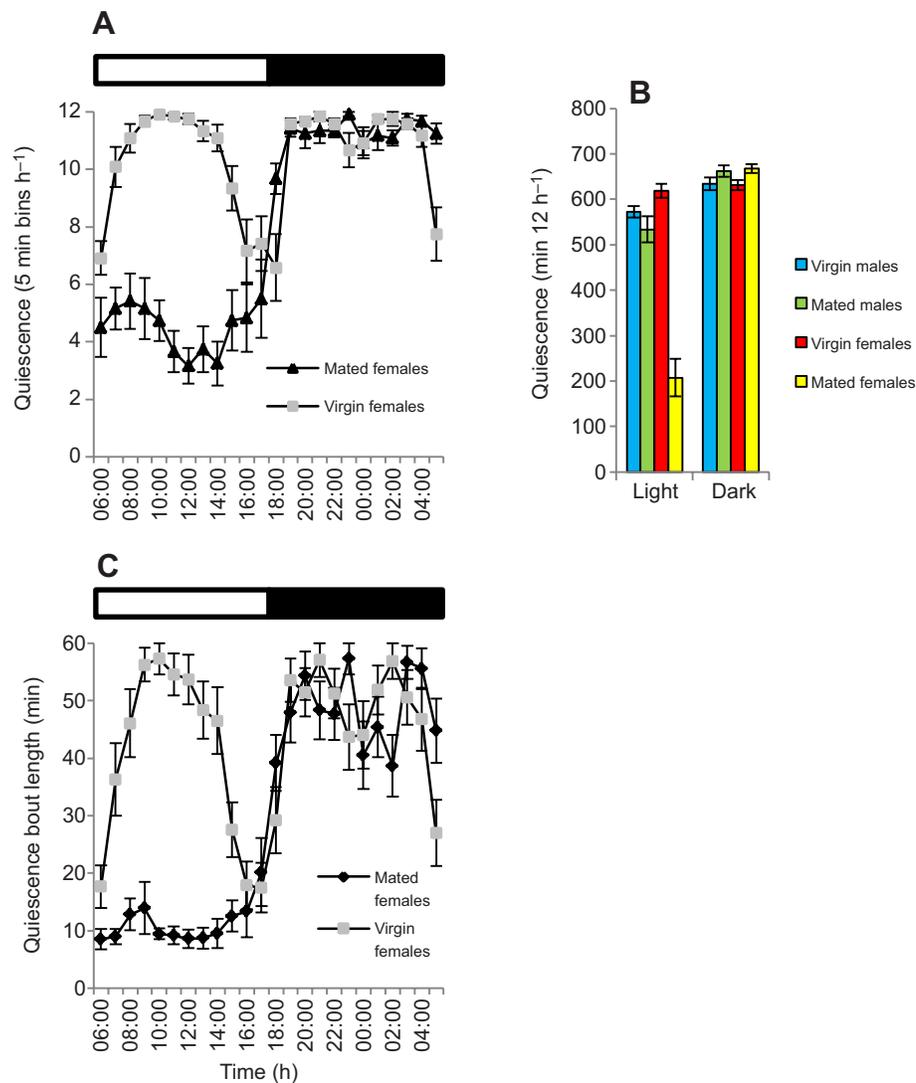
female activity after mating resulted in  $>50\%$  loss of siesta quiescence, but had no significant effect on night-time sleep (Fig. 3A,B). Much of the remaining daytime quiescence of mated females was characterised by a large reduction in the bout length of the inactivity (Fig. 3C).



**Fig. 1. Sexually dimorphic locomotor rhythms of *Drosophila sukuzii* in a semi-natural environment.** Male and female flies were given the freedom to copulate over a period of 3 days before being separated and placed in a LAM16 locomotor activity monitor for 6 days. (A) Locomotor activity is expressed as the mean number of beam breaks per hour ( $N=12$ ). Daily fluctuations in temperature and light levels were also monitored. (B) The daylight period was from the time that the environmental monitors first registered light (05:00 h) until light levels fell below 1 lx (21:00 h) and therefore total daylight activity was expressed as the number of beam breaks per 16 h (mean  $\pm$  s.e.m.,  $N=11$ ). The variance between mated males and females for each of the 6 days was highly significant (maximum  $P=0.019$ , independent sample  $t$ -test).



**Fig. 2. Elevated activity is a post-mating response in female *D. sukuzii*.** (A) Activity profiles of male and female *D. sukuzii* maintained on a 12 h light:12 h dark (12LD) cycle. Lights off, black bars; lights on, white bars; subjective day, grey bars. (B) Total activity during the lights-on (day) and lights-off (night) periods for three consecutive days in 12LD followed by 3 days in constant dark (DD) expressed as mean ( $\pm$  s.e.m.) number of beam breaks per 12 h ( $N=8$ ). 'Day' and 'night' refer to subjective periods during DD. During daylight, the activity of the mated female was significantly higher than that of the other groups (maximum  $P=0.005$ , one-way ANOVA followed by a Bonferroni *post hoc* test) for all 3 days. Night-time activity of the mated females was significantly lower on night 1 and night 2 compared with that of the other groups (maximum  $P=0.003$ , one-way ANOVA followed by a Bonferroni *post hoc* test).



**Fig. 3. Mating disrupts daytime quiescence (siesta) of female *D. sukukii*.** (A,B) Mating results in significant loss of daytime quiescence for females compared with males and virgin females (maximum  $P=0.000$ , one-way ANOVA followed by a Bonferroni *post hoc* test) and a severe reduction in the length of the daytime sleep bout (C) of mated females. Mating has little effect on the siesta and night-time sleep of males. White and black bars in A and C indicate daytime and night-time, respectively. Quiescence or sleep is expressed as either the number of 5 min periods of no activity per hour (A) or the number of 5 min periods of no activity per 12 h of light and dark (B) (mean values for two consecutive days  $\pm$  s.e.m.,  $N=16$ ).

### Locomotor behaviour of *D. sukukii* in simulated natural summer conditions

We were interested to see whether the marked difference in the activity of virgin and mated female *D. sukukii* would also occur when fly behaviour was recorded under simulated natural conditions in the laboratory as this would allow greater control of environmental conditions. The first step increase in light levels of our incubator was from 0 to 100 lx and occurred at 05:00 h with incremental changes until a peak of 1000 lx was attained at 12:00 h, before declining back to 0 lx at 21:00 h. The temperature cycled between 22 and 30°C with the minimum and maximum temperatures occurring at 05:00 h and 13:00 h, respectively (Fig. 4). In this simulated natural LD cycle, both virgin and mated males as well as virgin females experienced an extended siesta period of 6–8 h that coincided with both high light and high temperature levels. Locomotor activity increased in the late afternoon, reaching a maximum at 21:00–22:00 h (26.6–26.0°C and 105–0 lx; Fig. 4A). In contrast, there was a large increase (>400%) in daytime (05:00 h and 21:00 h) activity for mated females during the first 3 days of recording (Fig. 4B), recapitulating the previously seen reduction of the siesta in mated females in the 12LD cycle. This elevated afternoon activity peaked earlier than the activity of males and virgin females with a maximum between

18:00 h (28.5°C and 440 lx) and 20:00 h (27°C and 170 lx; Fig. 4A). A parallel study was conducted using the same environmental parameters, but with the sucrose food replaced with blueberry pulp, which allowed eggs to be laid. Again, mated females displayed elevated daytime activity (~300%) during the first 2 days of the recording. The difference in activity between virgin and mated females was, however, not significant on the third day (Fig. S1). When we repeated these experiments using the same light cycle but with the temperature cycling between 27 and 35°C, all flies died within 24 h.

### The *D. sukukii* sex peptide is transferred from the male to the female during copulation

We have shown previously that an increase in afternoon activity and loss of siesta in mated female *D. melanogaster* is triggered by the male SP<sub>Dm</sub>, which is transferred to the female in the seminal fluid. An orthologous SP<sub>Ds</sub> has been isolated from the male accessory glands of *D. sukukii* and shown to be a 41 amino acid peptide (Fig. 5A) by N-terminal sequencing and translation of cloned SP<sub>Ds</sub> cDNA (Schmidt et al., 1993). We used MALDI-MS to analyse the peptides in the male accessory glands of *D. sukukii* and identified a mass ion of  $m/z$  5145, which corresponds to the SP<sub>Ds</sub> containing a disulphide link between two cysteines and three 4-hydroxyproline

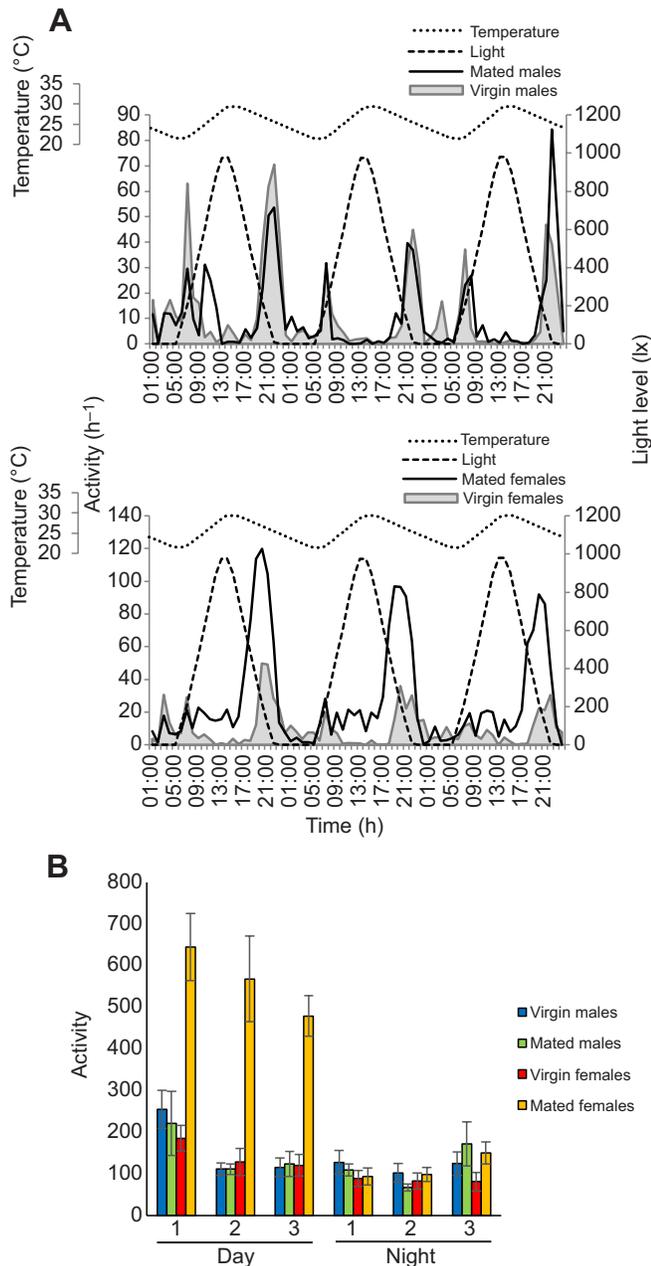
residues (Fig. 5B). This molecular ion was absent from extracts of the female reproductive tract of virgin females (Fig. 5C), but present in the same tissues taken from copulating females that were separated from their male partner 10 min after initiation of copulation, which normally lasts around 25 min (Fig. 5D).

## DISCUSSION

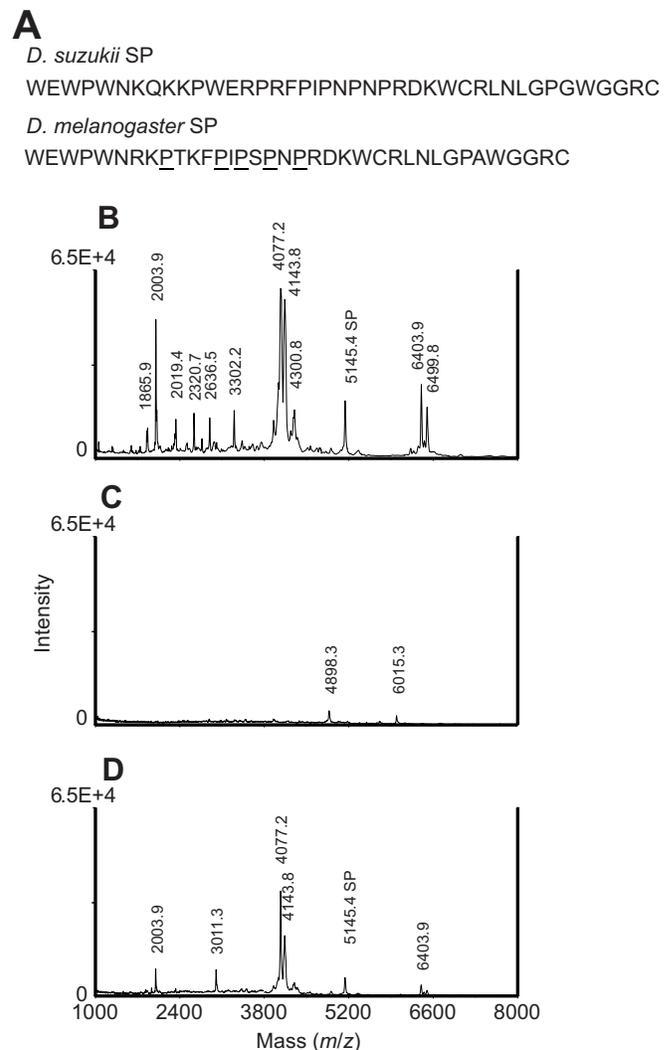
In this study, we used LAM16 monitors (Trikinetics Inc.) to record the locomotor activity of *D. sukuzii* under various environmental

conditions. The LAM16 employs multiple infrared beams and detectors held in a thin board to detect insect movement as beam breaks. The monitor has been designed differently from the commonly used DAM2 Trikinetics monitors to reduce the risk of shadows being cast by the plastic housing. Such shadows might attract flies to the infrared beams as they seek shade from strong light and possibly lead to inaccurate reporting of activity (De et al., 2013).

Our initial objective was to record the activity profiles of mated male and female *D. sukuzii* in a natural summer environment. Conducting such an experiment outdoors was technically problematic as natural infrared radiation resulted in spurious counts being recorded by the activity monitors. We therefore resorted to placing our monitors beneath a skylight window in a building located away from any polluting artificial light. In these semi-natural summer conditions, the activity of the mated females greatly exceeded that of mated males, with the most pronounced difference in behaviour occurring during the late afternoon when



**Fig. 4. Activity of virgin and mated *D. sukuzii* in a simulated summer environment.** (A) Locomotor activity for three consecutive days expressed as the mean number of beam breaks per hour ( $N=8$ ). Daily fluctuations in temperature and light levels are indicated. (B) Total day (16 h) and night-time (8 h) activity for males and females expressed as the mean  $\pm$  s.e.m. ( $N=8$ ). The daytime activity of mated females was significantly different from that of all other groups ( $P=0.000$ ) and there was no discernible statistical difference between any of the groups during the night-time (minimum  $P=0.912$ ) using one-way ANOVA, followed by a Bonferroni *post hoc* test.



**Fig. 5. Mass spectra of peptides present in reproductive tissues of *D. sukuzii*.** Peptides were extracted from five reproductive tissues of *D. sukuzii*. (A) The amino acid sequence of male sex peptide from *D. sukuzii* (SP<sub>Ds</sub>) aligned with SP<sub>Dm</sub> of *D. melanogaster*. P marks the position of 4-hydroxyproline residues in SP<sub>Dm</sub>. The position of the 4-hydroxyprolines in SP<sub>Ds</sub> has not been determined. (B–D) Mass spectra from the male accessory gland (B), the uterus of virgin females (C) and the uterus of mid-copulation females (D).

females were consistently more active than males. Although the intensity of the activity waned with time, the sex difference was sustained for at least 6 days. The only previous study of locomotor activity of adult *D. sukukii* was conducted under ‘summer’ conditions where a temperature cycle of between 12.2 and 22.2°C simulated the gradual natural gradients of Watsonville, CA, USA (Hamby et al., 2013). Changes in light intensity, however, were restricted to two undefined levels, with the lower intensity occurring in the morning (dawn) and late afternoon (dusk). This study, using virgin males and females, showed that activity was largely restricted to daylight hours, with peaks occurring at ‘dawn’ and ‘dusk’, and indicated that males were more active than females, which is opposite to what was observed in the present study.

As we had shown previously that mating increased daytime activity of female *D. melanogaster*, we suspected that the reason for the high female activity relative to male activity seen with *D. sukukii* in our semi-natural conditions resulted from a similar post-mating response (Isaac et al., 2010). This was confirmed when we looked at the activity profiles of mated male and female *D. sukukii* using standard incubator conditions of a 12LD cycle and constant temperature. Here, we observed for virgin and mated males, as well as virgin females, a small peak of morning activity and a late afternoon rise in activity leading to a prominent evening peak. There was a period of quiescence that extended for several hours in the early to middle of the afternoon and was reminiscent of the siesta sleep of *D. melanogaster*. In contrast to male and virgin female *D. sukukii*, mated females were active throughout the daytime, with the evening peak now occurring much earlier in the afternoon. The sharp peak of activity that occurred just after lights off in the 12LD conditions appears to be a startle effect from the sudden switch to complete darkness. This reaction, which was seen in males and virgin females, was much reduced or even blocked in mated females, perhaps resulting from the dampening down of the startle response by SP signalling pathways. The induced activity in mated *D. sukukii* females gave rise to a large reduction in daytime quiescence, but not night-time sleep. Furthermore, the mated females could not sustain a daytime sleep-like state for periods much beyond 10 min. Rhythmic activity was maintained when both male and female flies, irrespective of mating status, were placed in continuous dark (DD) after 12LD entrainment, as expected for behaviour under the control of an internal clock. It was very noticeable, however, that the activity was now spread across the whole of the subjective lights-on period, suggesting that afternoon light suppresses activity, reflecting a reluctance to be active in the middle of the day to protect against the afternoon sun. In constant darkness, the evening peak of activity occurred earlier in the subjective day as a result of the disappearance of the prominent lights-off peak, consistent with this being a startle response to the sudden switching off of the light.

It has recently been shown that adult *D. melanogaster* display a mid-afternoon (A) peak of activity, but only when the afternoon temperatures reach above 30°C (Das et al., 2015; Green et al., 2015). This thermo-sensitive A peak was seen in simulated natural conditions in a controlled environment incubator where temperatures fluctuated from a low of 17 or 25°C to a peak of 32 or 35°C in the afternoon. When the variable light and temperature were out of phase, the A peak coincided with the highest temperature and not peak light levels (Das et al., 2015). Furthermore, the A peak requires a functioning neuronal temperature-sensitive transient receptor potential ion channel (TrpA1) (Das et al., 2015; Green et al., 2015). The afternoon peak of activity recorded for *D. sukukii*, including that of the mated females, occurred several hours after the hottest period and

therefore is not equivalent to the temperature-sensitive A peak of *D. melanogaster*. We attempted to monitor the activity of *D. sukukii* with temperatures cycling between 27 and 35°C, but did not succeed because of the high mortality that occurred, reflecting the fact that this species is poorly adapted to hot climates (Tochen et al., 2014).

These behavioural responses by the female *D. sukukii* to mating are very similar to what has been observed previously in *D. melanogaster* (OregonR, Dahomey and, to a lesser extent, CantonS strains) (Isaac et al., 2010). For this species, the loss of daytime quiescence is elicited by the transfer in the seminal fluid of SP<sub>Dm</sub>, a 36 amino acid pheromone peptide made by the male accessory gland, which comprises three domains with distinct functions. SP<sub>Dm</sub> elicits a wide variety of female post-mating responses that include rejection of male advances, increase in oviposition, activation of immune defences, elevated food intake and altered food preference, in addition to the effect on sleep (Avila et al., 2011). The biological activity resides in the C-terminal region, which is separated from the N-terminal domain by a proline-rich peptide sequence (Kubli, 2003). The N-terminal tryptophan-rich region tethers SP<sub>Dm</sub> to the sperm exterior and this anchor is eventually cleaved by a peptidase, resulting in the slow release of SP<sub>Dm</sub> from sperm stored in the female (Liu and Kubli, 2003; Peng et al., 2005). This process is the likely mechanism underpinning the long-term effects of SP<sub>Dm</sub> on female behaviour and physiology that can last up to 1 week. Unusually for a signalling peptide, five of the prolines in the central region of SP<sub>Dm</sub> are hydroxylated to 4-hydroxyproline and these modifications are considered important for eliciting an immune response in the mated female (Domanitskaya et al., 2007). The orthologous SP<sub>Ds</sub> of *D. sukukii* has the same domains as SP<sub>Dm</sub>, although there are five additional amino acids in the central proline-rich region. Some of the functionality of SP<sub>Ds</sub> is also conserved as the synthetic peptide elicits oviposition and loss of male receptivity when injected into female *D. sukukii* as well as female *D. melanogaster* (Schmidt et al., 1993). Unlike SP<sub>Dm</sub>, however, there is no knowledge from amino acid analysis of any post-translational modifications of the amino acid side-chains, e.g. prolyl hydroxylation. Mass spectrometry detected a molecular ion ( $m/z$  5145) corresponding to the predicted SP<sub>Ds</sub> with three 4-hydroxyproline residues, although these post-translational modifications have yet to be confirmed by chemical analysis. The detection of the  $m/z$  5145 molecular ion in the reproductive tract of the mated females provides evidence of its transfer from the male, and leads us to conclude that SP<sub>Ds</sub> in the ejaculate is probably responsible for the behavioural changes we observed in mated female *D. sukukii*. The presence of the tryptophan-rich domain in SP<sub>Ds</sub> implies that the peptide binds to sperm in the female’s sperm storage organs and that the slow-release mechanism described for SP<sub>Dm</sub> is also conserved, which would explain the long-term post-mating response observed in our studies (Liu and Kubli, 2003).

The siesta observed for male and virgin female *D. sukukii* is probably a defensive adaptation for conserving energy, protection from predators and avoiding exposure and loss of water during hot summer afternoons. A recent study has shown that *D. sukukii* mate in the morning and evening of a 16L8D day and are seldom seen copulating in the afternoon, which is consistent with our observation of a siesta for virgin males and virgin females (Lin et al., 2014). Mated females, however, are expected to adopt a higher risk lifestyle for the acquisition of nutrients to support egg production, for finding suitable egg-laying sites and for reducing competition through increased dispersal. When mated *D. sukukii* females were encouraged to lay

eggs by replacing the sucrose in the activity glass tubes with blueberry pulp, the elevated activity declined towards virgin female levels within 2 days, suggesting that the post-mating response can be attenuated on access to suitable food and egg-laying substrate. We propose that the switch in the afternoon behaviour of the female is triggered by the transfer of SP<sub>Ds</sub> from the male during copulation and the impact of the peptide on sensory neurons in behaviour-modifying pathways.

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#### Competing interests

The authors declare no competing or financial interests.

#### Author contributions

C.T.J.F., T.L.O. and R.E.I. designed and carried out the activity experiments and N. A. performed mass spectrometry. All authors provided critical interpretation of the data and all contributed to the writing of the paper.

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#### Supplementary information

Supplementary information available online at <http://jeb.biologists.org/lookup/suppl/doi:10.1242/jeb.125468/-/DC1>

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