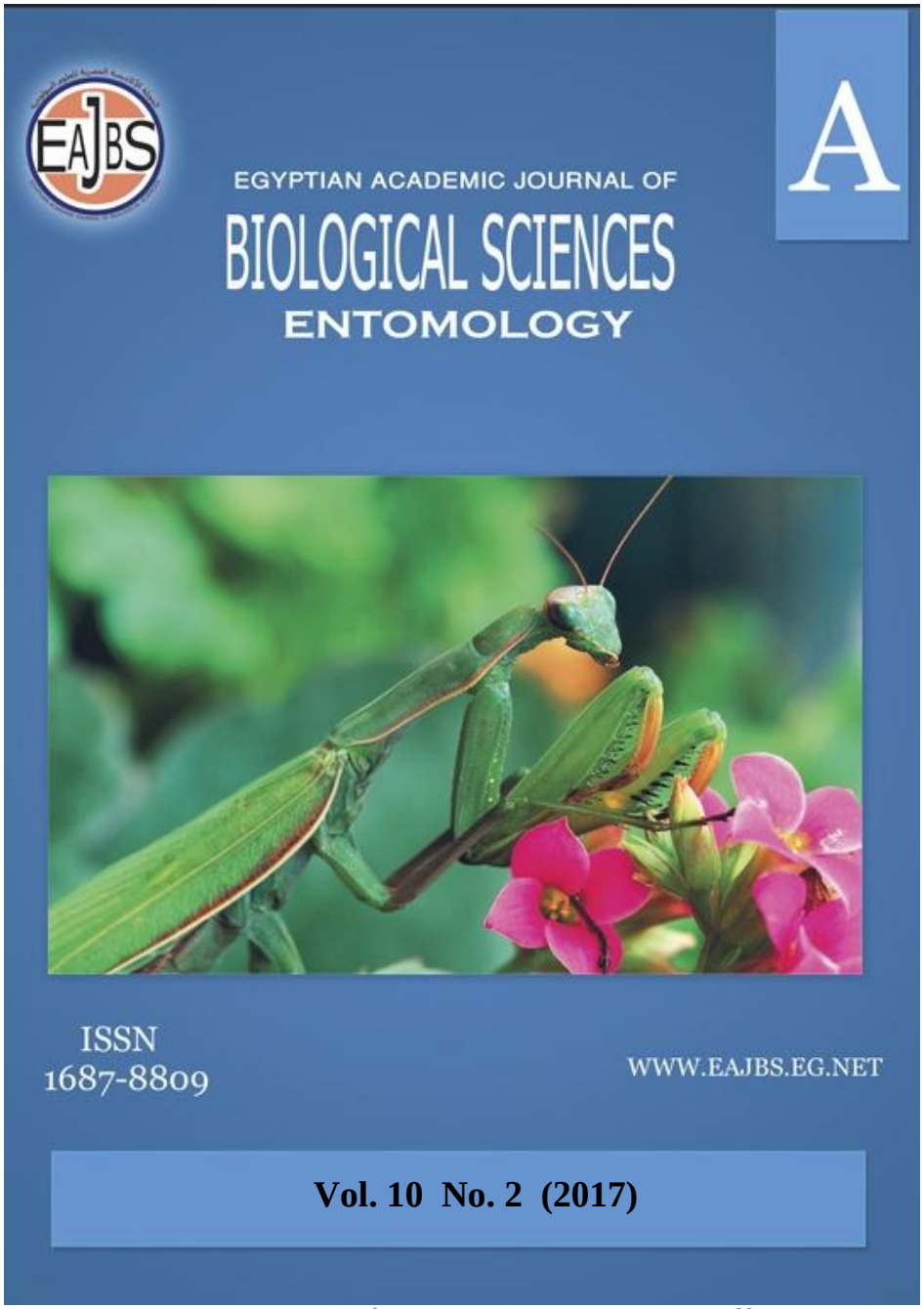

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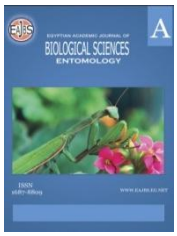

Characterizing the Circadian Locomotor Activity of *Drosophila melanogaster yellow white* Mutants under Different Temperatures

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ABSTRACT

The circadian clock is an innate timing mechanism that regulates behavior and physiology of most organisms. It anticipates fluctuations in daily environmental conditions and synchronizes biological activities accordingly to maximize ecological fitness. Light is its major environmental entraining cue, but temperature also plays a prominent role. Mutations affecting clock control helped understanding clock mechanisms and their neurobiological and genetic backgrounds. The objective of this study is to investigate the effect of the *yellow white* (*y w*) mutation in *Drosophila melanogaster* fruit flies on the circadian regulation of locomotor activity under different temperature conditions. This mutation causes impaired melanisation, eye pigmentation loss, disturbed neurotransmitter distributions and levels, plus some behavioral alterations. These changes are expected to affect circadian regulation. Results revealed that *y w* mutant flies retained a functional circadian clock, although with some loss of robustness and plasticity to temperature fluctuations. Total daytime and the lights-on activity was higher in *y w* mutants under high temperature and lower than wildtype *CS* under lower temperature, while nighttime activity was always lower and lights-off activity was lower at lower temperature. Most importantly, *y w* mutant flies didn't shift their morning and evening activity peaks sufficiently away from the light phase, in addition to having shorter siesta at all temperatures tested, compared to wildtype. In conclusion, the *y w* mutation seemed to disturb circadian control on locomotor activity, which exposes *y w* flies to higher levels of environmental risk by being active for longer time under high temperature usually correlated with light phase.

INTRODUCTION

Animal behavior and physiology is now known to be largely regulated by the circadian clock. An endogenous timing system composed of an oscillator that generates a 24-h rhythm, a photoreceptor that synchronizes the clock to the environmental light-dark (LD) cycles, and an output system that relays the timing information to overt output behaviors and physiological functions. They have been found to exist from bacteria to humans (Saunders, 2002; Dunlap *et al.*, 2004; Tomioka and Matsumoto, 2010). Although light is the most important environmental cue to entrain the circadian clock in most animals, temperature fluctuations also play a major role in entrainment (Aschoff, 1981; Edmund, 1988; Tomioka and Yoshii, 2006). Temperature can change the free-running period, the phase of the rhythm, and is able to entrain the circadian rhythm in many animals such as lizards and insects

(Lankinen and Riihimaa, 1997; Tomioka *et al.*, 1998; Yoshii *et al.*, 2002).

Circadian clocks help the organisms to anticipate the change in environmental conditions, thus increasing their ecological fitness. Temperature involvement in circadian regulation is especially important in maximizing the adaptability to selective pressures along latitudinal and altitudinal gradients (Hut *et al.*, 2014).

Drosophila is the model of choice for circadian research as it has extensively been used in other behavioral studies. Analysis of *Drosophila* mutants made it possible to genetically dissect behaviors ranging from leg shaking, courtship, and associative learning to memory, including circadian regulation (Boynton and Tully, 1992; Allada and Chung, 2010). Screens for mutants with altered free-running periods, in constant darkness (DD), led to groundbreaking results of identifying the first clock gene, *period* (*per*) (Konopka and Benzer, 1971). Studying other clock mutants led to the discovery of other canonical clock genes including *timeless* (*tim*) (Sehgal *et al.*, 1994), *Clock* (*Clk*) (Allada *et al.*, 1998), *cycle* (*cyc*) (Rutila *et al.*, 1998), and many others.

The *yellow* and *white Drosophila* mutation is widely used in genetic backgrounds in behavioral research. The *yellow* (*y*) gene, located on the X chromosome, affects adult melanisation that occurs at day 4 of puparium, resulting in an altered yellow pigmentation of the adult cuticle and its derivative structures. The mutant yellowish pigmentation is clearly distinguishable from the dark brown of wild type (Lindsley and Grell, 1972). The *white* (*w*) gene, on the other hand, encodes a transmembrane transporter protein involved in the uptake of essential precursor amino acids in the synthesis of eye pigments (Sullivan *et al.*, 1974; Summers *et al.*, 1982; Ewart and Howells, 1998). *white* mutation results in white-eyed flies with impaired vision and disturbed neurotransmitter levels (Borycz *et al.*, 2008; Krstic *et al.*, 2013). The altered pigmentation and neurophysiology in the *y w* mutants is expected to have a considerable effect on the light input pathways to the clock, which is the main clock-entraining environmental cue (Hassaneen, 2015; Yoshii *et al.*, 2015).

The mutant *y w D. melanogaster* flies are used frequently in genetic and neurological studies of behavior to control for potential polygenic variability; however, very little is known about their circadian behavior. Therefore, this study aims to investigate the effect of temperature on their circadian clock and to determine their adaptability for living at different latitudes where different temperatures prevail. Results are also expected to expand our understanding of the clock in general and its role in different ecological niches.

MATERIALS AND METHODS

Experimental Animals

Two fly lines were used in the experiments. The wild-type *Canton S* (*CS*) line, originally isolated from a wild strain near Canton, Ohio, in 1930 (Bridges and Brehme, 1944), was used as control. On the other hand, the mutant *yellow white* (*y w) D. melanogaster* flies were the experimental group. Both lines were obtained from the University of California, San Diego *Drosophila* Species Stock Center (DSSC). Adult males at the age of 4-7 days after eclosion were collected under CO_2 anesthesia and used in the experiments. All flies were reared on standard cornmeal/agar medium with yeast (0.85% agar, 2.2% sugar beet syrup, 8% malt extract, 3.3% yeast, 1% soy flour, 8% cornmeal, and 0.3% propionic acid) in conventional 2.8×9 cm food vials at 25°C and kept on a light-dark cycle of 12:12 hours (LD 12:12) in a humidity and

temperature-controlled climate chamber (Schlichting *et al.*, 2014). They were transferred to fresh food vials every week during light phase without anesthesia.

Locomotor Activity Recordings

Locomotor activity of male flies was recorded individually in glass tubes (5 mm in diameter × 65 mm long) with an air penetrable porous plug at one end and agar/sugar food (2% agar and 4% sucrose) on the other end for the *Drosophila* Activity Monitor (DAM2; TriKinetics Inc., Waltham, MA). This system consists of activity monitors that can simultaneously record the activity of 32 individual flies, an interface device, and a software for computerized data collections. An IR light beam crosses each tube and is detected by a photodetector on the other side. The software automatically generates text files in which the number of beam crosses, indicating the level of locomotor activity, is saved in a consecutive 1-min time span resolution for each individual fly. This assay is currently the most common behavioral assay in flies (Schlichting and Helfrich-Förster, 2015). The light intensity used in all experiments at the animal's level was 100 Lux (19 $\mu W/cm^2$). Lights-on and -off were controlled by a controller software (Trikinetics, Waltham, MA). Behaviors were recorded in a programmable incubator (MIR-154, Panasonic Biomedical, NL) at 10°, 18°, 20°, and 28°C under a light-dark cycle of 12 hours light and 12 hours dark (LD 12:12) for at least 9 days. Flies became adapted to the LD cycles in the recording monitors and their activity rhythm becomes stable within 1 day, therefore, the first two days of recordings were excluded to make sure that only data of stable rhythm is included in the analysis. Eight groups, 32 flies each, were used in the experiments. The actual number of flies survived and used in the analysis for each group is shown in (Fig. 1).

Data Analysis

Raw data collected in Microsoft Excel 2010 were used to draw double-plotted actograms using ActogramJ (http://actogramj.neurofly.de/) (Schmid *et al.*, 2011), a plugin for ImageJ open-source software (http://rsb.info.nih.gov/ij), and a freely available software for data analysis in life sciences. Activity patterns of individual flies were analyzed by Chi-square periodogram analysis (Sokolove and Bushell, 1978) to determine whether the flies showed significant rhythmicity in their behavior under the experimental conditions at a significance level of 0.05. Average daily activity profiles of stably entrained flies of each line were calculated and plotted in 30-min blocks (not shown), each block represents the sum of activity in 30 minutes, then the phase of morning peak (M), siesta (S), and evening peak (E) was determined by checking individual activity profiles. This enabled reliable determination of peak onsets and offsets, because raw data at the high resolution of 1-min interval are often noisy. The M and E peaks of activity were considered to start when the activity increases gradually around the transition from light to dark and from dark to light, respectively, until reaching a peak then coming down again to a base line activity.

The siesta (S) is the steady activity at the base line in the middle of the light phase between the M and E peaks, which equals (E activity onset – M activity offset). To determine the timing of the peaks, the average day activity was smoothed by a moving average of 11 extending five time points before and after each time point. Consequently, randomly occurring spikes are reduced and the real maximum of the fly's activity can be determined. All data were analyzed and plotted using Microsoft Excel 2010 (Microsoft Corp., Redmond, WA) and SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY). The statistical tests used in data analysis were the Student *t*-test and the Analysis of Variance (ANOVA) followed by post hoc Tukey test.

RESULTS

Locomotor Activity Profiles under Different Temperatures

The general locomotor activity profile of the two fly lines in (LD12:12) under different temperatures showed many similarities (Figures 1 and 2). The *y w* flies expressed a crepuscular pattern with two peaks of activity around the transitions from dark to light and from light to dark, respectively, comparable to the morning (M) and evening (E) activity peaks known in *D. melanogaster*, with a resting mid-day siesta (S) in the middle.

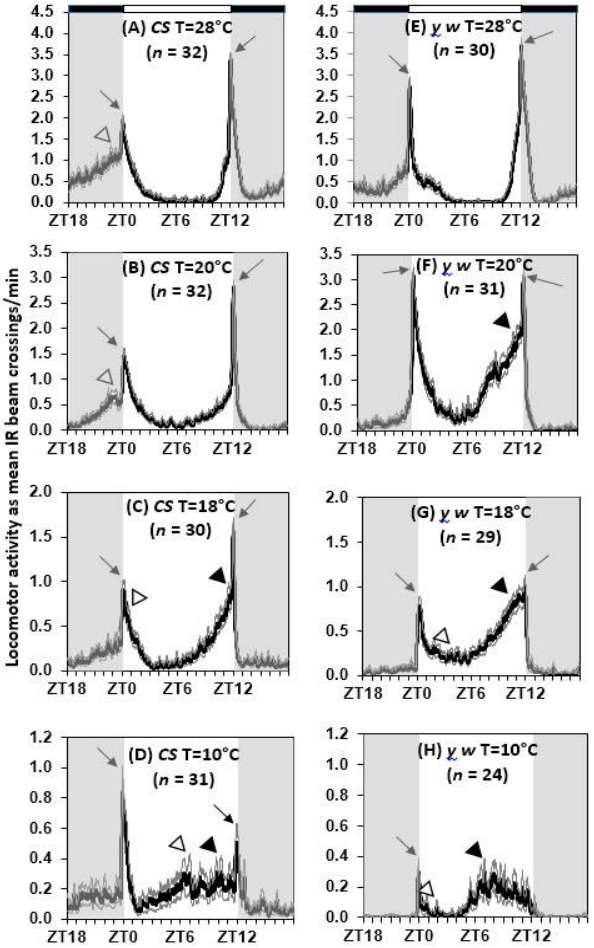

Fig. 1: Average circadian locomotor activity profiles of control wild-type *CS* and *y w* mutant *D. melanogaster* flies under light-dark cycles of (LD 12:12) and temperatures of 10°C, 18°C, 20°C, and 28°C. Locomotor activity is represented on the y-axis as the mean infrared beam crossings per minute by a thick black line; the (Mean), with (Standard Error of Mean) represented by two thin lines above and below the mean. *Zeitgeber* time 0 (ZT0) indicates the beginning of the light phase and coincides with 6:00 am Egypt Standard Time. Black and white bars above the figures indicate dark and light phases of the circadian LD cycle, respectively. Grey shadings inside the graphs also represents dark phases of the LD cycle. Arrows indicate lights-on and lights-off activity peaks, while white and black arrowheads indicate the mathematically calculated maximum activity in the morning and evening activity peaks. *n* is the sample size.

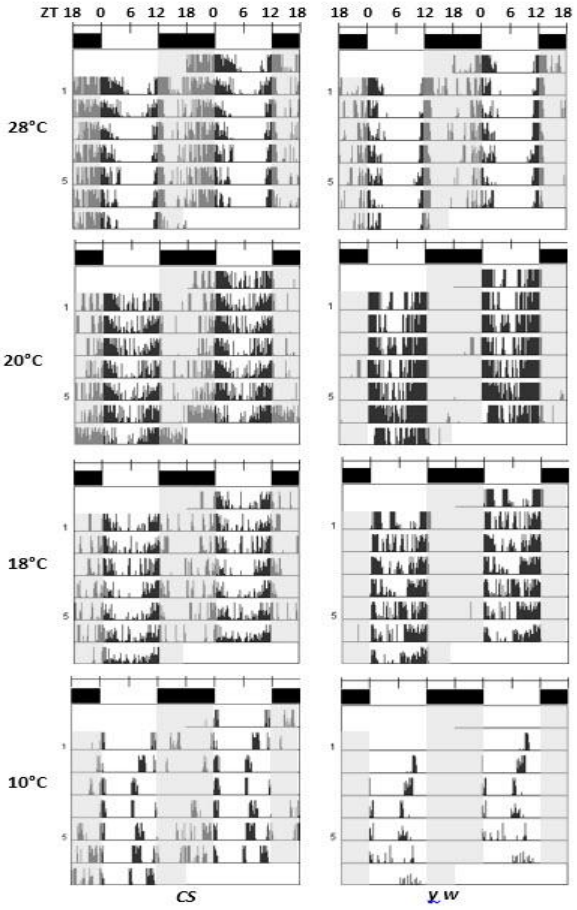

Fig. 2: Representative double-plotted actograms for the circadian locomotor activity rhythms of control wild-type *CS* and mutant *y w D. melanogaster* flies recorded under (LD 12:12) and 10°C, 18°C, 20°C, and 28°C for 7 days. Black and white bars above the figures indicate the dark and light phases, respectively. Shaded areas inside the figures also indicate dark phases. *Zeitgeber* times (ZT) are indicated on top of the figures, with ZT0 and ZT12 marking the beginning of the light and dark phase, respectively.

The activity reached a maximum once per activity peak. Circadian locomotor activity profiles calculated from the average locomotor activity per 1 min for 7 days is showed in (Fig. 1), while a representative actogram of a fly from each group is shown in (Fig. 2).

Average Daytime, Nighttime, and Total Daily Locomotor Activity

The control *CS D. melanogaster* flies maintained a nearly stable daytime locomotor activity level over temperatures ranging from 18°C to 28°C, only dropping significantly at 10°C (ANOVA, $F_{3,121}$= 9.74, p<0.0001). However, the daytime activity of *y w* flies increased significantly at 18°C then again at 20°C but decreased significantly at 28°C to reach the same level at 18°C (ANOVA, $F_{3,112}$=112.81, p<0.0001) (Fig. 3A). Comparing the two fly lines at each temperature step using *t*-test revealed that the *y w* flies had a significantly higher daytime locomotor activity level at 18°C (t_{57}=3.63, p<0.001), 20°C (t_{61}=5.31, p<0.0001), and 28°C (t_{60}=0.91, p<0.05), but significantly lower at 10°C (t_{55}=11.96, p<0.001) (Fig. 3A). On the other hand, the nighttime activity of both fly lines increased significantly at 20°C and 28°C for *CS* (ANOVA, $F_{3,121}$= 97.21, p<0.0001) and for the *y w* flies (ANOVA, $F_{3,112}$= 91.46, p<0.0001), respectively, (Fig. 3B). Comparing the two fly lines at each temperature step using *t*-test revealed that the *y w* flies had a significantly lower nighttime activity at 10°C (t_{55}=7.65, p<0.001), 18°C (t_{57}=6.83, p<0.001), and 28°C (t_{60}=3.59, p<0.001), but not significantly different at 20°C (t_{61}=1.68, NS) (Fig. 3B).

The average total daily locomotor activity of control *CS* flies increased significantly at 20°C and 28°C but was nearly stable at the low 10°C and 18°C° (ANOVA, $F_{3,121}$= 59.4, p<0.0001). However, in the mutant *y w* flies, it increased significantly at 18°C then again at 20°C, but decreased significantly at 28°C (ANOVA, $F_{3,112}$= 103.35, p<0.0001) (Fig. 3C).

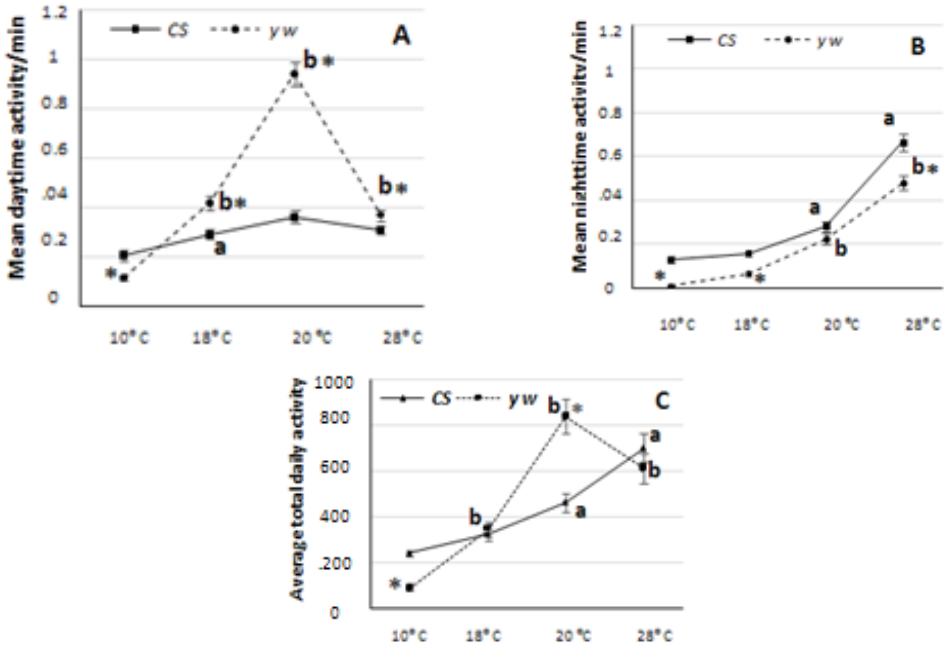

Figure 3: Effect of temperature on locomotor activity in daytime "light phase" (A), nighttime "dark phase" (B), and average total daily activity (C) of control wild-type *CS* and mutant *y w D. melanogaster* flies. Mean number of IR crossings/min across the 12 hours duration of daytime (A), nighttime (B), or the 24h (C) activity of 7 days is represented with standard error of mean. Sample size *n* for each fly group is as in Figure 1. a and b Indicate a significant difference between the locomotor activity level at a specific temperature step and the one before it within the *CS* or *y w* fly lines, respectively, using ANOVA, while *denotes a significant difference between the activity level of *CS* and *y w* flies at each temperature step using *t*-test, both at (p<0.05).

Comparing the two fly lines at each temperature step using *t*-test revealed that the low 10°C suppressed the total daily locomotor activity of *y w* flies more significantly than *CS* (t_{55}=6.29, p<0.001), while the 20°C caused a significant increase in their activity compared to *CS* (t_{61}=7.13, p<0.001). However at 18°C and the high 28°C, that activity level was nearly the same in the two fly lines (t_{57}=0.85, NS) and (t_{60}=1.89, NS), respectively (Fig. 3C).

Lights-on and Lights-off Locomotor Activity Peaks

Lights-on and lights-off peaks reflect the startle response of flies to the abrupt transition in the rectangular artificial light-dark cycle from dark to light and from light to dark, respectively (Fig 1). At the higher temperature of 28°C, The M peak is fused to the lights-on peak and the E peak is fused to the lights-off peak (Figs, 1A and 1E) in the two fly lines. As the temperature decreases, the M and E peaks migrate away from the lights-on and lights-off peak as in (Figs. 1D and 1H). The lights-on activity peak of the *CS* flies increased significantly only at 20°C and 28°C, (ANOVA, $F_{3,121}$= 25.72, p<0.0001), while the *y w* flies increased significantly at 18°C, 20°C, and 28°C (ANOVA, $F_{3,112}$= 173.38, p<0.0001) (Fig. 4A). On the other hand, the lights-off activity peak increased in both *CS* and *y w* lines at 18°C, 20°C, and 28°C (ANOVA, $F_{3,121}$= 172.97, p<0.0001) and (ANOVA, $F_{3,112}$= 250, 70, p<0.0001), respectively (Fig. 4B). Comparing the two lines at every temperature using *t*-test revealed a pattern in lights-on peak similar to mean daytime activity (Figs. 3A and 4A), also in lights-off peak and mean night-time activity (Figs. 3B and 4B); however, that of *y w* flies was significantly suppressed only at 10°C and 18°C, but not at the higher temperatures.

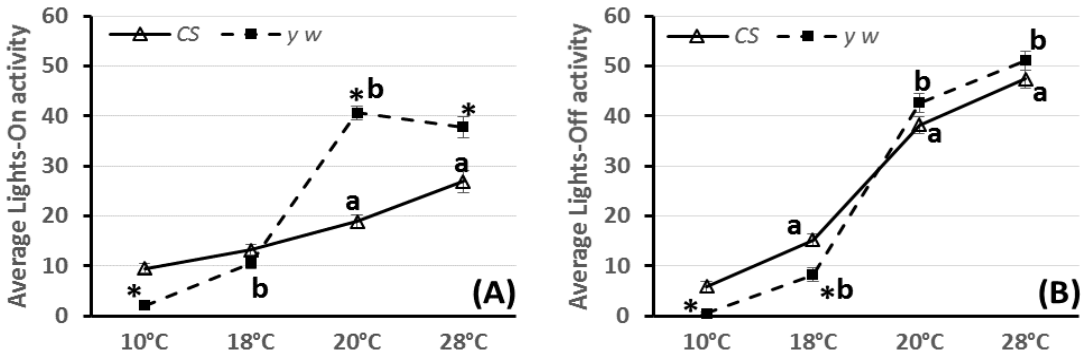

Fig. 4: Effect of temperature on lights-on (A) and lights-off (B) locomotor activity peaks of control wild-type *CS* and mutant *y w D. melanogaster* flies under 10°C, 18°C, 20°C, and 28°C and (LD 12:12) for 7 days. Data represent the average activity of 15 minutes after lights-on and lights-off (Mean ± standard error of mean). Sample size details are in Figure 1. * Indicates a significant difference between *CS* and *y w* flies at the same temperature using *t*-test, while a and b denotes a significant difference between the activity level at a temperature step and the step before it within the same fly line of *CS* and *y w*, respectively, using ANOVA, both at (p<0.05).

Temperature-Adaptive Activity Timing

Although the general locomotor activity profile of the two fly lines under different temperatures showed many similarities (Figs. 1 and 2), differences can be found by analyzing profile segments. These differences were manifested in the timing of start and end of each activity peak, and consequently affected their duration (Fig. 5).

The start of the morning activity peak (M) in wildtype *CS* flies advanced significantly with every temperature increase (ANOVA, $F_{3,121}$= 168.53, p<0.0001),

while in *y w* flies it only advanced significantly at the highest temperature of 28°C (ANOVA, $F_{3,111}$= 49.89, p<0.0001), but started at the same time at 10°C, 18°C, and 20°C. Comparing the two lines at each temperature step revealed that *y w* flies started their M peak significantly earlier than wildtype *CS* at 10°C (t_{55}=3.63, p<0.001) and significantly later at 18°C (t_{56}=5.28, p<0.001), 20°C (t_{61}=9.73, p<0.001), and 28°C (t_{60}=4.79, p<0.001). On the other hand, the end of M peak in wildtype *CS* flies delayed significantly only at the lowest temperature of 10°C (ANOVA, $F_{3,121}$= 17.01, p<0.0001), but ended nearly at the same time at 18°C, 20°C, and 28°C. However, the *y w* files ended their M peak significantly earlier at 10°C then delayed significantly at 18°C (ANOVA, $F_{3,111}$= 30.05, p<0.0001) with flies at 20°C and 28°C ending nearly at the same time.

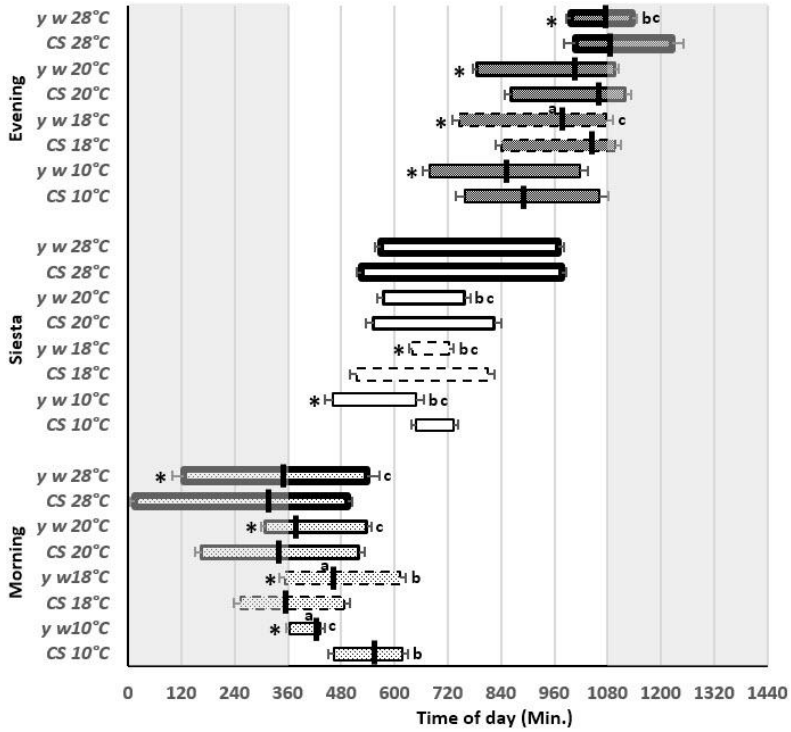

Fig. 5: Effect of temperature on timing of locomotor activity peaks of control wild-type *CS* and mutant *y w D. melanogaster* flies. Horizontal bars represent the average morning, mid-day siesta, and evening activity peaks for about 30 flies/group for 7 days. Start, end, and duration of these peaks has been compared within each group (Morning, mid-day siesta, and evening) using One-Way ANOVA followed by Tukey test. The "max" activity was also compared in morning and evening peaks. Significant differences between *CS* and *y w* within each group (Morning, siesta, and evening) at the same temperature is highlighted using (*) for the start, (a) for the maximum activity of 15 minutes, (b) for end, and (c) for the length of each activity peak. Data are represented as (Mean ± Standard error of mean). Error bars at the start and end of activity bars are for the duration of activity peaks.

Comparing the end of M peak in the two lines at each temperature step revealed that *y w* flies ended their M peak significantly earlier than wildtype *CS* at 10°C (t_{55}=5.97, p<0.001), significantly later at 18°C (t_{56}=7.99, p<0.001), and 28°C (t_{60}=3.51, p<0.001), but ending nearly at the same time at 20°C (t_{61}=1.17, NS). The duration of M increased significantly with temperature in both wildtype *CS* (ANOVA, $F_{3,121}$= 114.21, p<0.0001) and *y w* flies (ANOVA, $F_{3,111}$= 65.81, p<0.0001) except for *y w* flies at 20°C (Figure 5). The length of the M peak in *y w* flies was shorter than *CS* at 10°C (t_{55}=5.37, p<0.0001), 20°C (t_{61}=6.88, p<0.001), and 28°C (t_{60}=2.26, p<0.03), but they were similar at 18°C (t_{56}=1.24, NS). The locomotor activity reached a maximum that usually lasts for about 15 minutes in average within

the M peak (Fig. 1); from hereafter it will be denoted as MAX. The M peak's MAX of the wildtype *CS* flies was significantly delayed at 10°C (ANOVA, $F_{3,121}$= 49.29, p<0.0001), while it occurred nearly at the same time, close to lights-on, at 18°C, 20°C, and 28°C. The M peak's MAX of the *y w* flies also occurred close to the lights-on at 20°C and 28°C, but it was significantly delayed at 10°C and 18°C (ANOVA, $F_{3,111}$= 14.56, p<0.0001). Comparing M's MAX between the two lines shoed that *y w* flies are significantly delayed compared to *CS* at 18°C (t_{56}=5.80, p<0.001), 20°C (t_{61}=2.66, p<0.01), and 28°C (t_{60}=2.52, p<0.01), but significantly advanced at 10°C (t_{55}=5.06, p<0.001) (Fig. 5).

For the midday siesta S, *CS* flies started to rest nearly at the same time at 18°C, 20°C, and 28°C, but significantly delayed at 10°C (ANOVA, $F_{3,121}$= 18.22, p<0.0001). However, the *y w*' S started nearly at the same time at 20°C and 28°C, but significantly later at 18°C and significantly earlier at 10°C (ANOVA, $F_{3,111}$= 36.82, p<0.0001). At 20°C, *CS* and *y w* flies started to rest nearly at the same time (t_{61}=1.76, NS), while S start of *y w* flies was significantly delayed at 18°C (t_{56}=7.99, p<0.001) and 28°C (t_{60}=3.51, p<0.001) and significantly advanced at 10°C (t_{55}=6.20, p<0.001). On the other hand, the end of S was significantly delayed with increasing temperature within both *CS* (ANOVA, $F_{3,121}$= 56.04, p<0.0001) and *y w* lines (ANOVA, $F_{3,111}$= 200.93, p<0.0001), except for wildtype *CS* at 20°C that ended nearly a the same time as at 18°C. *y w* flies ended their S significantly earlier than wildtype *CS* at 10°C (t_{55}=3.36, p<0.001), 18°C (t_{56}=5.33, p<0.001), and 20°C (t_{61}=4.16, p<0.001), but at 28°C both lines ended S at the same time (t_{60}=0.91, NS). Moreover, the duration of S increased significantly with temperature increase in both wildtype *CS* (ANOVA, $F_{3,121}$= 127.58, p<0.0001) and *y w* flies (ANOVA, $F_{3,111}$= 103.18, p<0.0001), except for *CS* at 18°C and 20°C which had the same length, and also *y w* at 10°C and 20°C. The duration of S was significantly shorter in *y w* flies than wildtype *CS* at 18°C (t_{56}=11.49, p<0.001), 20°C (t_{61}=4.06, p<0.001), and 28°C (t_{60}=3.39, p<0.001), but significantly longer at 10°C (t_{55}=5.33, p<0.001) (Fig. 5).

The start of evening peak E delayed significantly with increasing temperature in both wildtype *CS* (ANOVA, $F_{3,121}$= 60.80, p<0.0001) and *y w* flies (ANOVA, $F_{3,111}$= 186.30, p<0.0001), except wildtype *CS* at 18°C and 20°C which started nearly at the same time. The *y w* flies started their E peak earlier than wildtype *CS* at 10°C (t_{55}=3.17, p<0.002), 18°C (t_{56}=5.50, p<0.001), and 20°C (t_{61}=5.06, p<0.001), but nearly at the same time at 28°C (t_{60}=7.13, NS). In wildtype *CS*, E peak ended nearly at the same time at 10°C, 18°C, and 20°C, but was delayed significantly only at 28°C (ANOVA, $F_{3,121}$= 24.75, p<0.0001), while in *y w* flies, E end delayed significantly with increasing temperature (ANOVA, $F_{3,111}$= 26.89, p<0.0001), except at 20°C which ended nearly at the same time as at 18°C. *y w* flies always ended their E peak significantly earlier than wildtype *CS* at 10° (t_{55}=2.46, p<0.001), 18°C (t_{56}=2.01, p<0.05), 20°C (t_{61}=3.07, p<0.003), and 28°C (t_{60}=3.44, p<0.001). Increasing temperature didn't affect the duration of E peak within wildtype *CS* (ANOVA, $F_{3,121}$= 3.25, NS) but caused a significant shortening within *y w* flies only at 28°C (ANOVA, $F_{3,111}$= 49.28, p<0.0001). However, the duration of E peak was significantly shorter in wildtype *CS* at 18°C (t_{56}=3.62, p<0.001) and 20°C (t_{61}=3.02, p<0.05), but significantly longer at 28°C (t_{60}=2.84, p<0.05) and nearly having the same length as *y w* at 10°C (t_{55}=1.27, NS). The E MAX in wildtype *CS* occurred nearly at the same time at 18°, 20°, and 28°C close to the lights-off, but was significantly advanced at 10°C (ANOVA, $F_{3,121}$= 41.75, p<0.0001), while in *y w* it occurred nearly at the same time at 18°C and 20°C, but was significantly advanced at 10°C and significantly delayed at 28°C (ANOVA, $F_{3,111}$= 60.93, p<0.0001). Comparing the two lines

showed that E MAX occurred nearly at the same time at 10°C (t_{55}=1.11, NS) and at 28°C (t_{60}=0.76, NS), but *y w* E MAX was significantly advanced compared to wildtype *CS* at 18°C (t_{56}=4.60, p<0.001) and 20°C (t_{61}=3.94, p<0.001) (Fig. 5).

DISCUSSION

The *y w* mutation in *D. melanogaster* flies is suggested to have neural implications and possible behavioral alterations. Mutant *y* males are at a mating disadvantage possibly through altered levels of neuroactive catecholamines that are synthesized via dopa-like melanin (Biessmann, 1985). This suggestion is supported by the fact that its transcript level undergoes significant changes during development, being highest in late embryos prior to hatching compared to much lower levels in larval instars and adults, even higher than in pupae when melanisation of the adult cuticle occurs. This prominent elevation at hatching cannot be explained solely via melanisation, since pigmentation is repeated at every molt (Biessmann, 1985). The *white* (*w*) gene, on the other hand, encodes a transmembrane ABC transporter protein involved in the uptake of guanine and tryptophan, which are indispensable precursors in the synthesis of red (drosopterins) and brown (ommochrome xanthommatin) *Drosophila* pigments (Sullivan *et al.*, 1974; Summers *et al.*, 1982; Ewart and Howells, 1998). The absence of pigments in the eye of flies without a functional *w* gene results in ommatidia without optical insulation. Hence, the vision of such white-eyed flies is impaired, especially at high light intensities (Krstic *et al.*, 2013). Their photoreceptors receive about 19 times more light than those of wild-type flies and their electroretinograms are abnormal (Wu and Wong, 1977). Since guanine is further required for the synthesis of dopamine and serotonin, also tryptophan is a precursor of serotonin (Coleman and Neckameyer, 2005), *w* mutants show much reduced levels and altered distributions of these neurotransmitters (Borycz *et al.*, 2008). Accordingly, mutations affecting the *w* function have an impact on the neural control of various behaviors, independent of proper eyesight (Campbell and Nash, 2001). Regarding their circadian locomotion, *y w* mutants were found rhythmic in LD conditions and free-running in constant dark (DD) conditions with a shorter periodicity of (τ=23.82) and a preserved subjective morning and evening activity peaks (Hassaneen, 2015).

Results of this study showed that mutant *y w D. melanogaster* flies retained a functional circadian clock, since they expressed a crepuscular bimodal rhythm that preserved the general profile of the wildtype (Allada and Chung, 2010) even with reduced plasticity and robustness (Figures 1, 2, and 5). Total activity of *y w* flies increased significantly with temperature compared to wildtype *CS* during daytime (Light phase) (Figure 3(A)), while became more significantly suppressed during nighttime (dark phase) (Figure 3(B)). Both fly lines expressed the same total daily activity level at mid-range 18°C, but *y w* were significantly less active at the low 10°C and significantly more active at 20°C (Figure 3(C)). Thus, it seems that the *y w* mutation appears to impair the fly's ability to cope with changing temperature causing them to respond radically to its change, while the intact circadian clock of the wildtype seemed more efficient in buffering temperature effects within physiological ranges. The radical startle response to lights-on (Figure 4(A)) and lights-off (Figure 4(B)) in mutant *y w* flies might be another evidence on the circadian clock buffering effect on behavioral responses of wildtype flies to temperature fluctuations.

The circadian clock is adaptive to maximize ecological fitness of animals. In cold short days of winter, it is favorable for the animals to be active during the

warmer parts of the day, while during the hot long summer days, it is better for them to shift their activity to the cooler morning and/or night (Yoshii *et al.*, 2012). In this study, with increasing temperature, wildtype *CS* flies advanced their morning activity towards the dark phase, lengthened their mid-day siesta, and delayed their night activity (Figure 5). Although in this experiment, temperature was fixed in both light- and dark-phases, the flies preferred to avoid light in high-temperature, while seek light in low temperature. It makes sense because the flies primarily use light as an entrainment agent to the LD cycle, but also they seemed as if they are avoiding the damaging effect of high temperature naturally linked with sunny light phase, while in low temperature they delayed their activity as if they are seeking the warm temperature naturally linked to the light phase. *y w* mutation; however, seemed to impair this adaptive function and the mutants responded poorly to temperature fluctuations. The mutant *y w* flies significantly shifted their M peak only at the highest 28°C and they were mostly shorter and more delayed than wildtype *CS*. For the mid-day siesta, wildtype *CS* rested mostly longer with increased temperature. The E peak of *y w* flies was also much significantly advanced and longer compared to wildtype *CS*. Collectively, the behavioral changes in *y w* mutant made most of their locomotor activity in the light phase compared to the wildtype *CS*. In addition, their responses to light changes were radical at the low and high temperature extremes. Which suggest that the mutation exposed them to higher levels of environmental risk.

In conclusion, the *y w* mutation appeared to affect the circadian regulation of locomotor activity. Mutant *y w* flies appeared more vulnerable to environmental risk factors and less adaptive to temperature fluctuations compared to wildtype *CS*. The results provide new insights for better understanding of behaviors studied in the *y w* genetic background. Further studies are required to investigate the primary causes and mechanisms behind these findings. Impaired melanisation, eye pigmentation loss, disturbed neurotransmitter distributions, and levels in *y w* mutation could all be contributing to disturbed circadian control.

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ARABIC SUMMERY

تحليل النشاط الحركي اليومي في طفرة دروسوفيلا ميلانوجاستر (الأصفر الأبيض) تحت تأثير درجات حرارة مختلفة

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الساعة البيولوجية هي آلية توقيت داخلية تنظم سلوك ووظائف أعضاء معظم الكائنات. حيث تتوقع التذبذب اليومي للظروف البيئية وتُزامِن أنشطة الكائن البيولوجية تِباعاً، لتحسين الكفاءة البيئية للكائن لأقصى حد ممكن. وعلى الرغم من أن الضوء هو عامل المزامنة الرئيسي للساعة البيولوجية إلا إن درجة الحرارة أيضاً تلعب دوراً بارزاً. وقد ساعدت الطفرات التي تؤثر على تنظيم الساعة للأنشطة الحيوية في فهم آليات عمل هذه الساعة وخلفياتها العصبية والوراثية. والهدف من هذه الدراسة هو تحليل تأثير طفرة (الأصفر الأبيض) في ذبابة الفاكهة دروسوفيلا ميلانوجاستر، على تنظيم نشاطها الحركي اليومي تحت درجات حرارة مختلفة. أما هذه الطفرة فتسبب خلل في تكوين الصبغيات وفقد صبغيات العين واضطراب في مستويات وتوزيع الناقلات العصبية بالإضافة لبعض التغيرات السلوكية. ومن المتوقع أن يؤثر هذا النوع من التغيرات على تنظيم الساعة البيولوجية. وقد أظهرت النتائج أن الساعة البيولوجية للذباب المُطفَّر مازالت تعمل، إلا أنها تفتقد بعض الكفاءة والمرونة وبخاصة لتغيرات درجة الحرارة. وقد وجد أن النشاط الحركي النهاري والاستجابة المباشرة للضوء كانتا أعلى في الذباب المُطفَّر عنه في الطرز البري بالمجموعة الضابطة في درجات الحرارة العالية وأقل في درجات الحرارة المنخفضة. في حين كان النشاط الحركي الليلي والاستجابة المباشرة للظلام أقل من الطرز البري في درجات الحرارة المنخفضة. والأهم من ذلك أن الذباب (الأصفر الأبيض) لم يستطع أن يزيح فترتي النشاط الصباحي والمسائي بشكل كافي بعيدا عن فترة الإضاءة كما يفعل ذباب المجموعة الضابطة، كما تميز بقصر فترة راحته عن الطرز البري بغض النظر عن درجة الحرارة. ويمكن القول إن الدراسة قد خلصت إلى أن طفرة (الأبيض الأصفر) قد أدت إلى اضطراب في الساعة البيولوجية المتحكمة في النشاط الحركي اليومي، الأمر الذي يعرض هذا النوع من الذباب لمستويات عالية من المخاطر البيئية، لكونها نشطة لفترة طويلة في فترة منتصف النهار التي تتميز عادة بدرجات حرارة مرتفعة والتي تكون مرتبطة بفترة الإضاءة.