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Effect of *yellow white* Mutation on the Circadian Locomotor Activity of the Fruit Fly *Drosophila melanogaster*: A Comparison to *Canton S* Wild-Type

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ABSTRACT

Circadian clocks regulate the physiology and behavior of most animals. *Drosophila melanogaster* mutants helped more than any other animal in understanding these timing mechanisms. This study aims to investigate the effect of the *yellow white* (*y w)* mutation on circadian behavior of *D. melanogaster* flies under rhythmic cycles of light-dark (LD), constant dark (DD) and continuous light (LL) conditions. These mutant flies suffer from disturbances in eye and body pigmentation in addition to altered neural development and behavioral control, making them good candidates for investigating the function of the circadian clock. The obtained results indicated that *y w* mutants are rhythmic in LD conditions, with significantly higher daytime activity compared to control wild-type *Canton S* (*CS*) flies. In constant conditions, they free run in DD with a significantly shorter periodicity (Tau) (τ=23.82, SD=0.44) compared to (τ=24.59, SD=0.39) in *CS*, indicating a faster endogenous clock in DD, while they were arrhythmic in LL as expected, similar to *CS* flies. Mutant *y w* flies also exhibited a crepuscular activity pattern with two peaks of activity. The *y w* flies' morning peak was significantly delayed by about 2.5 hours, but ending at the same time in both groups. The evening peak was significantly advanced by about 1.7 hours. This resulted in a significant shortening of midday siesta by about 1.5 hours compared to *CS* flies. Collectively, these changes resulted in a significant shift of activity towards the light-phase in the mutant *y w* flies. Moreover, morning anticipation of these flies was slightly reduced and did not integrate smoothly into the morning peak. It is possible to conclude that the altered pigmentation and neuronal development in *y w* mutants has a profound effect on circadian organization of locomotor activity. These results provide new insights for a better understanding of behaviors studied in the *y w* genetic background.

Key words: *yellow white* mutation, circadian, locomotor, *Drosophila melanogaster.*

INTRODUCTION

Most organisms possess a timing regulator known as the "Circadian clock". It synchronizes their physiology and behavior to the daily cycles of light and temperature resulting from the revolution of the earth around its own axis. This endogenous mechanism consists of an oscillator that generates the 24-h oscillation, a photoreceptor that entrains (=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. These clocks were found to exist from bacteria to humans (Saunders, 2002; Dunlap *et al.*, 2004; Tomioka and Matsumoto, 2010). Those timing mechanisms provide the organisms with the ability to anticipate environmental changes, and consequently optimizing their ecological fitness (Hughes *et al.*, 2015).

The fruit fly, *Drosophila*, has been extensively used as the primary model organism in most labs specialized in circadian research. Its mutants helped dissecting the circadian machinery and discovering its core components and their roles. By screening mutants with altered free-running periods in constant darkness (DD), the first clock gene, *period* (*per*) was identified by Konopka and Benzer (1971). Investigating other clock mutants made it possible to discover other core clock genes including, *timeless* (*tim*) (Sehgal *et al.*, 1994), *Clock* (*Clk*) (Allada *et al.*, 1998), *cycle* (*cyc*) (Rutila *et al.*, 1998), in addition to many others (Hardin, 2011).

The *yellow* and *white* are famous *Drosophila* mutations that are frequently used in genetic backgrounds for developing other mutations. The *yellow* (*y*) gene is located at the tip of the X chromosome. It is involved in pattern-specific melanin pigmentation of the cuticle of the adult *D. melanogaster* flies and the mouth parts of its larvae. These parts become yellow pigmented in mutant phenotypes. It may have a role in other neural functions (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. Their vision is impaired, especially at high light intensities (Krstic *et al.*, 2013) and their photoreceptors receive about 19 times more light than those of wild-type flies with altered electroretinograms (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 have reduced levels and altered distributions of

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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).

This study aims to elucidate the circadian clock properties of the *y w* mutant *D. melanogaster* flies under standard LD 12:12 cycles and their free running profiles under DD conditions compared to the control wild-type *Canton S* (*CS*) flies. Abnormal eye and body pigmentation in addition to reduced levels and altered distributions of clock-related neurotransmitters especially serotonin is expected to affect the circadian clock, because the eyes are the main input pathway and the neurotransmitters play a major role in the output of the timing signals to overt rhythms. Since the *y w* mutants are used frequently in genetic and neurological studies of behavior although very little is known about their circadian regulation, the results are expected to provide a reference circadian locomotor activity profile for the *y w* mutation in those flies, and how altered eye and body pigmentation could possibly affect their circadian expression.

MATERIALS AND METHODS

Experimental Animals

Adult male *D. melanogaster* flies at the age of 4-7 days after eclosion were collected under CO_2 anesthesia and used in the experiments. The wild-type *Canton S* (*CS*) strain that was isolated from a wild strain near Canton, Ohio, in 1930 (Bridges and Brehme, 1944) used as a control and the *yellow white* (*y w*) mutants were obtained from the University of California San Diego *Drosophila* Species Stock Center (DSSC). 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 1.2×9 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

Flies were placed individually in glass recording tubes (5 mm × 65 mm) with an air penetrable porous plug at one end and agar/sugar food (2% agar and 4% sucrose) on the other end inside a *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 software for computerized data collections. An IR light beam crosses the tube and is detected by a photodetector on the other side. The software automatically generates text files in which the number of beam crosses is saved in 1-min time span for each individual fly. This assay is currently the most common behavioral assay in flies (Schlichting and Helfrich-Förster, 2015). The monitors were placed in a light box with white LEDs (Lumitronix LED-Technik GmbH, Jungingen, Germany) set at its roof to emit light down the tubes through neutral density filters (Lee Filters Worldwide, Andover, UK) for fine-tuning of light intensity. 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 climate-controlled chamber at 20°C under two different light conditions. In the first eight days, a rectangular light-dark cycle of 12 h light and 12 h dark (LD 12:12) then followed by a week in complete dark (DD) or a week in continuous light (LL). 32 *CS* and 31 *y w* flies survived in (LD12:12 then DD) while 30 *CS* and 32 *y w* survived in (LD 12:12 then LL) for the entire experiment and were used to analyze behavior.

Data analysis

Raw data collected in Microsoft Excel 2010 (Microsoft Corp., Redmond, WA) 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), a freely available package for data analysis in life sciences.

Flies became adapted to the LD cycles in the recording monitors and their activity rhythm stabilized within 1 day, therefore, the first two days of recordings were excluded to make sure that only data of stable rhythm are included in the analysis. 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 entrained (LD) and/or constant conditions (DD and LL) and to measure their periods "Tau" (τ) under the free-running intrinsic day at 0.05 level of significance. Average day activity of all flies of each line were calculated and plotted over the number of entrained days for stably entrained flies. Average daily locomotor activity profiles of individually tested flies were plotted as 30-min bins, each represents the sum of activity within 30 minutes, and the phase of morning peak (M), siesta (S) and evening peak (E) was determined manually. 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 increased gradually around the lights-on and –off, 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. The morning anticipation index (AI) was calculated by the sum of activity within the last 3 hours of night phase divided by the sum of activity within the last 6 hours of night phase (Sheeba *et al.*, 2010). All data were analyzed and plotted using Microsoft Excel (2010) and SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY).

RESULTS

Locomotor activity rhythms in (LD 12:12)

The general locomotor activity profiles of mutant *y w D. melanogaster* flies under (LD 12:12) conditions and a temperature of 20°C were very similar to that of control wild-type *CS* flies (Fig. 1). However, Student *t*-test revealed that, daytime activity during the light phase (t_{61}=10.61, p<0.0001) and the total day activity (t_{61}=7.13, p<0.0001) is significantly higher in mutant *y w* flies, but nighttime activity during the dark phase is not significantly different (t_{61}=1.71, p=0.093; NS) between the two fly lines (Fig. 2).

Double-plotted actograms of representative flies displaying locomotor activity rhythms under (LD 12:12) are shown in (Fig. 4; the first six days). Chi-square periodogram analysis confirmed the entrainment to the (LD 12:12) cycles with a periodicity of 24 h in both *CS* and *y w* flies; representative results are shown in Figure (6 A and D).

Intrinsic clock free-running in constant conditions

To investigate the effect of *y w* mutation on the state of the endogenous clock, locomotor activity profiles were recorded after releasing the flies in constant conditions of complete dark (DD) or continuous light (LL) for one week after 8 days in (LD 12:12). In DD, *y w* flies showed a functional internal clock that free run, although with a significantly shorter periodicity denoting a higher speed clock (τ=23.82, SD=0.44, n=31) compared to the control wild-type *CS* (τ=24.59, SD=0.39, n=32); a Student *t*-test confirmed this observation (t_{61}=7.30, p<0.001) (Fig. 3). Double-plotted actograms of representative flies displaying free running in DD are shown in (Fig. 4). Representative free-running periods in DD conditions were calculated by a Chi-Square periodogram analysis (Fig. 6 B and E). In LL, both *y w* (n=32) and *CS* (n=30) were 100% arrhythmic according to the periodogram analysis (Fig. 6 C and F), in which the periodicity of all flies failed to exceed the significance level of 0.05. Double-plotted actograms of representative flies displaying arrhythmic locomotor activity in LL are shown in Figure (5).

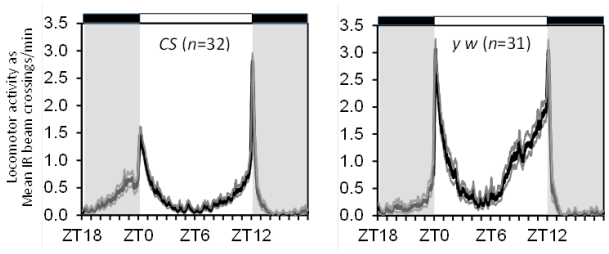

Figure (1): Average circadian locomotor activity rhythms of control wild-type *CS* and *y w* mutant *D. melanogaster* flies under light-Dark cycles of (LD 12:12) and a temperature of 20°C for 6 days. *Zeitgeber* time 0 (ZT0) and 12 (ZT12) indicates the beginning of the light phase and the dark phase and coincides with 6:00 AM and 18:00 PM Egypt Standard Time, respectively. 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. Black and white bars above the figures indicate dark and light phases of the LD cycle, respectively. Grey shadings inside the graphs also represent dark phases of the LD cycle.

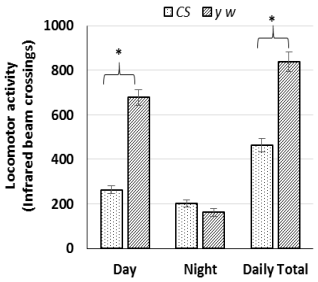

Figure (2): Comparing locomotor activity during daytime, nighttime and the total daily activity between control wild-type *CS* and mutant *y w D. melanogaster* flies under laboratory conditions of (LD 12:12, 20°C). Daytime and total day activity is significantly higher in mutant *y w* flies, but nighttime activity is not significantly different. Locomotor activity is expressed in count of infrared beam crossings in the "TriKinetics Drosophila Monitoring System". Error bars represent standard error of mean; data in the graph is (Mean±SE). * Indicates a significant difference (t-test, p<0.05).

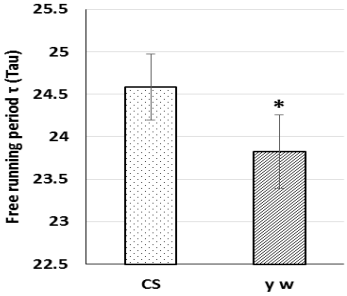

Figure (3): The free running period τ (Tau) in total dark (DD) of mutant *y w Drosophila melanogaster* flies (n=31) is significantly shorter than that of the control wild-type *CS* flies (n=32). Error bars represent standard deviation of mean; data in the graph is (Mean±SD). * Indicates a significant shortening of the free-running period in the *y w* flies compared to control CS (t-test, p<0.05).

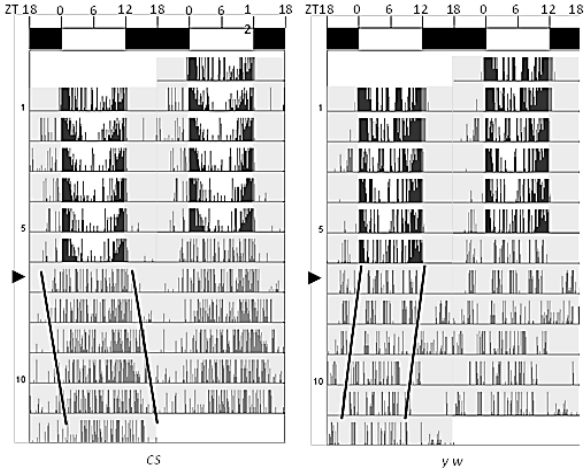

Figure (4): Double-plotted representative actograms for the circadian locomotor activity rhythms of control wild-type *CS* (*n*=32) and mutant *y w* (*n*=31) *Drosophila melanogaster* flies recorded under (LD 12:12) for the first 6 days followed by free-running in total dark (DD) from day 7 to day 12. Black and white bars above the figures indicate the dark and light phases respectively. Shaded areas inside the figures also indicate dark phases. The black arrowhead at day 7 represents the start of release into total dark (DD) free-running conditions. *Zeitgeber* times (ZT) are indicated on top of the figures, with ZT0 and ZT12 marking the beginning of the light and dark phase respectively for the first 6 days in (LD 12:12). The two parallel oblique lines in the DD phase indicate longer free running period τ in *CS* compared to *y w* flies.

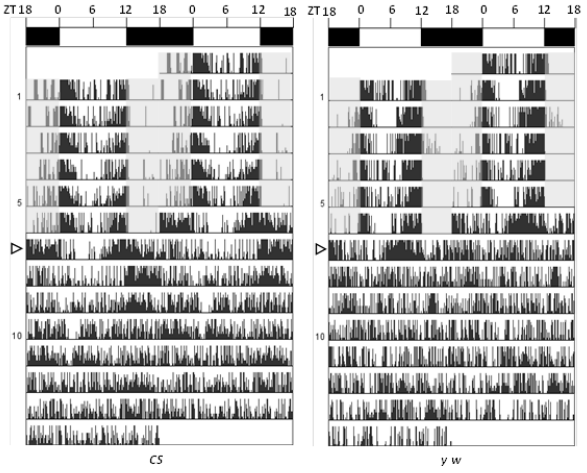

Figure (5): Double-plotted representative actograms for the circadian locomotor activity rhythms of control wild-type *CS* (*n*=30) and mutant *y w* (*n*=32) *D. melanogaster* flies recorded in (LD 12:12) for the first 6 days followed by continuous light from day 7 to day 14. Black and white bars above the figures indicate the dark and light phases, respectively. Shaded areas inside the figures also indicate dark phases. The white arrowhead at day 7 represents the start of release into continuous light (LL) conditions, in which both fly lines were arrhythmic. *Zeitgeber* times (ZT) are indicated on top of the figures, with ZT0 and ZT12 marking the beginning of the light and dark phase respectively for the first 6 days in (LD 12:12).

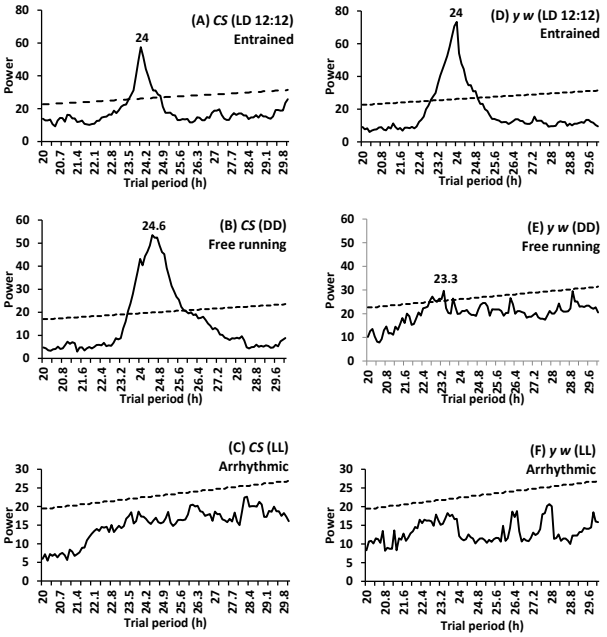

Figure (6): Representative Chi-Square Periodogram analysis of control wild-type *CS* and mutant *y w D. melanogaster* flies at 20° C in LD 12:12 (A and D), total dark "DD" (B and E), continuous light "LL" (C and F). Flies were entrained to the 24-hour cycle in LD 12:12, free-running in DD with a periodicity of (24.59±0.39, *n*=32) and (23.82±0.44, *n*=31) for *CS* and *y w* flies, respectively, while they were arrhythmic in LL. The dashed lines in the periodograms indicate the 0.05 level of significance.

Peak analysis

Analysis revealed that both *CS* and *y w* flies have a normal crepuscular activity rhythm that is bimodal with two peaks of activity. The morning peak (M) starts before the light phase and gradually increases until reaching a maximum then gradually decreases to a base line activity. M is followed by a siesta (S), which is the base line activity during the afternoon between the offset of the morning peak and onset of the evening peak (E) that starts with a continuous gradual increase in activity after the afternoon siesta and offsets around lights-off. The two fly lines appeared similar in their general activity pattern although some differences appeared in the onset, offset and duration of each activity phase (Figures. 7 and 8).

In the following, the locomotor activity of 32 *CS* and 31 *y w* flies was analyzed using Student *t*-test and reported as (Mean ±SD). It revealed that the M peak of *CS* flies in average starts (3.24±0.95) hours before lights-on, while the *y w* flies start only (1±0.34) hour before lights-on (t_{61}=9.73, p<0.0001) (Fig. 7a). On the other hand, the M peak offsets nearly at the same time after lights-on for both *CS* (2.66±0.97) and *y w* (2.69±1.95) flies (t_{61}=1.16, p=0.25; NS). The length of the M peak is significantly longer in *CS* (5.89±1.4) hours, while it is only (3.69±1.16) Hours in *y w* flies, (t_{61}=6.88, p<0.0001) (Fig. 7c).

The maximum activity recorded in a 30-min bin during the M peak was also significantly earlier in *CS* (18.75±9.31) minutes before lights-on, while in *y w* it occurs (6.56±15.58) minutes after lights-on, (t_{61}=2.65, p<0.01) (Fig. 7b).

For the midday siesta (S), the two fly lines started to rest nearly at the same time; *CS* started (3.22±0.81) hours after lights-on, while *y w* started theirs (3.28±1.87) hours after lights-on; (t_{61}=1.76, p=0.08; NS). At the offset of S, they differed again, the *y w* flies ended their rest significantly earlier (6.23±2.39) hours after lights-on, while *CS* needed about 1.5 hours more rest to end their S at (7.73±1.22) hours after lights-on; (t_{61}=4.15, p<0.0001) (Fig. 7d). Consequently, the S length for *CS* flies was significantly longer (4.52±1.54) hours than that of *y w* flies (2.95±1.40) hours; (t_{61}=4.06, p<0.0001) (Fig. 7e).

For the E peak, the *CS* flies started (3.61±1.11) hours before lights-off, while the *y w* flies started about 1.7 hours earlier at (5.30±2.48) hours before lights-off; (t_{61}=5.04, p<0.0001) (Fig. 7f). The two fly lines ended their E peak after lights-off, with the *y w* flies being earlier only at (18.75±34.44) minutes after lights-off, while the E of *CS* lasted significantly longer, about double, that time at (39.38±37.69) minutes after lights-off; (t_{61}=3.07, p<0.01) (Fig. 7h). The maximum activity in a 30-min bin during the E peak occurred before the lights-off in both fly lines, although significantly earlier in the *y w* flies at (1.66±3.22) hours before lights-off, while it was much closer to the lights-off in *CS* flies at (14.06±22.84) minutes before lights-off. The length of the E peak for the *CS* flies was (4.27±1.30) hours, that is about 45-min shorter than in *y w* flies (4.98±1.33) hours, a shortening that was found significant (t_{61}=3.02, p<0.01) (Fig. 7i).

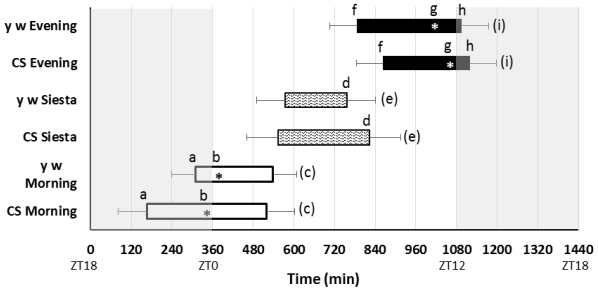

Figure (7): Analysis of locomotor activity peaks of control wild-type *CS* and mutant *y w D. melanogaster* flies under LD 12:12 and 20°C for 6 days. The floating bars display the average onset, duration and offset of "Morning peak" (M), midday siesta (S) and evening peak (E) analyzed on a 30-min resolution scale for *CS* (n=32) and *y w* (n=31) flies. Error bars at the start and end of each period represents standard deviation (SD) of the duration of each activity period. * Inside floating bars indicate the maximum activity of a 30-minute bin (Note that Lights-on and –off peaks are not considered here). Similar letters (a,b,d,f,g,h) indicate a significant difference between the start, maximum and end points of each activity phase of the two fly lines, while letters in parentheses (c,e,i) indicate a significant difference in the length of each activity period. On the X-axis, the 24-h day is represented in minutes at a 120-min resolution with key *Zeitgeber* times ZT0 and ZT12 indicating the start of the light and dark phase of the (LD 12:12) cycle, respectively. The dark phase is further indicated by shading inside the main chart area.

Morning anticipation

Calculating the morning anticipation index (AI) using "Harrisingh's individual index" method (Harrisingh *et al.*, 2007) showed that the AI of *CS* (0.77±0.11) and *y w* flies (0.73±0.23) is not significantly different; (t_{61}=0.89, p=0.38; NS) (Fig. 8A). However, calculating the ratio between the activities of 3 hours after and before lights-on revealed a lower homogeneity of the M peak and higher skew towards the light phase in the *y w* flies compared to *CS*. Nevertheless, this ratio for the *CS* (2.07±2.4) and that of *y w* (17.04±44.20) was also not significantly different (t_{61}=1.92, p=0.06; NS) (Fig. 8B).

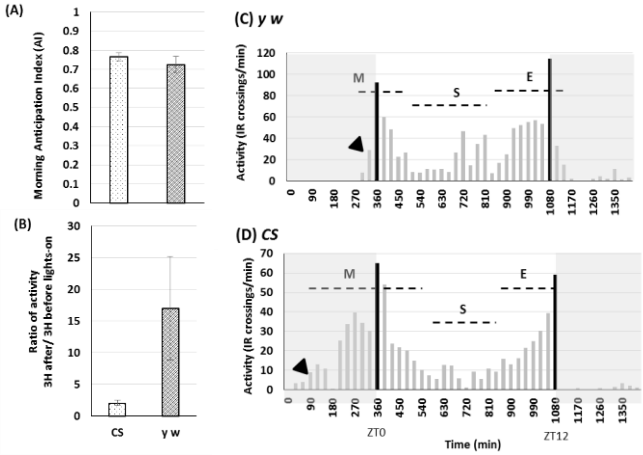

Figure (8): Morning anticipation (AI) and morning (M), siesta (S) and evening (E) activity phases of the control wild-type *CS* and mutant *y w D. melanogaster* flies under (LD 12:12) and temperature of 20°C. **(A)** Shows non-significant difference in AI between the two lines when calculated by Harrisingh's *et al.* (2007) individual index method. **(B)** The ratio of 3 hours of activity after/3 hours before lights-on. **(C&D)** are representative activity profiles from the two fly lines at a resolution of 30-min bins and illustrates the onset and offset of the M, S and E (dashed lines) in addition to the morning anticipation (black arrow head). Key *Zeitgeber* times; ZT0 marks the start of light phase at 360-min, while ZT12 marks the start of the dark phase at 1080-min from the beginning of the day. Gray shading also highlights the dark phase. The two dark 30-min activity columns at 360 and 1080 minutes is the activity at lights-on and lights-off, respectively.

DISCUSSION

Many animals show two activity bouts, one in the morning (M) and the other in the evening (E). This pattern of behavioral organization evolved primarily to survive the seasonal environmental changes that affect organisms. Circadian clocks optimize animal activities under long and hot summer days to avoid the high temperature of midday by shifting the activity to the morning and late evening or even the night. While in

short cold days in spring and autumn, it is favorable for the animals to be active during the warmer intervals of the day, by bringing the two peaks closer together and reducing the midday siesta (Yoshii *et al.*, 2012). A dual oscillator model was proposed to explain this regulatory mechanism in which the timing of the M and E activity peaks is controlled by M and E clock neurons in the fly's brain (Helfrich-Förster, 2014). In this study, the effect of *y w* mutation in the fruit fly *D. melanogaster* on the circadian locomotor activity under rhythmic and free-running conditions was investigated.

Under rhythmic cycles of 12 hours light: 12 hours dark (LD 12:12), *y w* flies maintained robust rhythmicity and exhibited a generally similar circadian activity pattern to *CS* flies. However, their activity was significantly higher during daytime (light phase), as their M peak delayed and E peak advanced compared to wild-type *CS* (Figs. 1, 2 and 7). The *y w* mutation was also found to affect the speed of the endogenous clock. The release of flies into constant dark conditions resulted in a free running with a periodicity that is significantly shorter than the control wild-type *CS* flies (Figures 3 and 4). On the other hand, in continuous light (LL), *y w* flies were arrhythmic similar to *CS* flies (Figures 5 and 6). This arrhythmicity is because the blue-light photopigment CRYPTOCHROME (CRY) interacts in a light-dependent manner with the TIMELESS (TIM) protein, leading to its permanent degradation under LL and consequently to the arrest of the clock (Busza *et al.*, 2004; Helfrich-Förster, 2014).

Analysis of onsets, offsets and durations of M and E activity peaks and the resting midday siesta revealed significant effects of *y w* mutation compared to control wild-type *CS* flies. The *y w* flies started their M activity later than *CS*, shifted their maximum activity even after lights-on, but ended their M peak at the same time as *CS*. These changes led to a shorter M peak that is significantly delayed and shifted towards the light phase (Fig. 7a,b,c and 8). The midday siesta starting nearly at the same time in both *y w* and *CS*, but ended significantly earlier in the *y w* flies, leaving the *CS* with a significantly longer siesta (Figures 7 (d and e) and 8). Having their siesta ended earlier, the *y w* flies also started, reached a maximum and ended their E activity peak significantly earlier than *CS* (Figures 7 (f, g and h) and 8). Therefore, the whole E peak was significantly shifted towards the light phase, similar to the M peak that was delayed to shift also towards the light phase. Moreover, the length of the E peak in *y w* flies was significantly longer than in *CS* flies (Figures 7i and 8). Therefore, the *y w* flies appears more diurnal, having M delayed and E advanced to occur mostly in the light phase with a shorter siesta in midday. Results suggest that the disturbed pigmentation and disturbed neural control due to the *y w* mutation led the *y w* flies to shift their activity to the light phase probably to compensate for their poor eyesight (Campbell and Nash, 2001).

The morning anticipation is usually considered an indicator for clock-controlled activities. The results indicate that it has not been significantly affected by the *y w* mutation. Using Harrisingh's *et al.* method to calculate the anticipation index (AI) revealed no significant difference between *CS* and *y w* flies. However, the actograms and average activity profiles showed a much reduced anticipation in *y w* flies (Fig. 8 (C and D)). The reason for that is the highly suppressed activity of *y w* flies at the 3 hours after midnight. So, comparing the low activity in the 3 hours before lights-on with the 3 hours before them, in which the activity is highly suppressed and the *y w* flies barely move, caused them to appear with a good morning anticipation, which is slightly deceptive. However, comparing the homogeneity and logical gradual increase then decrease after reaching a maximum activity that smoothly builds the M peaks reveals that they are much more nicely built in *CS* compared to *y w* flies. To quantify that, the ratio between the 3 hours of activity after lights-on to the 3 hours before lights-on was calculated. In *y w* flies it was about 17 times higher compared to only 2 times higher in *CS* flies (Fig. 8B), making the M activity peaks of *y w* flies much skewed towards the light phase, with the morning anticipation appearing tiny compared to the activity after lights-on (Figures 1 and 8 (C and D)). In other words, the morning anticipation of *y w* flies looks unnatural and does not integrate smoothly into the M peak compared to *CS* flies. So, although *y w* mutation does not significantly obliterate morning anticipation, it severely reduced it.

It could be concluded that the altered pigmentation and neuronal development in *y w* mutants has a profound effect on circadian organization of locomotor activity of *D. melanogaster*. These results provide new insights for a better understanding of behaviors studied in the *y w* genetic background. Moreover, prospective similar studies on pests would optimize insecticide and pest management applications with respect to more precise timing of pest activity, leading to economically efficient protocols and reduced toxicity to the environment.

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REFERENCES

ALLADA, R., WHITE, N.E., SO, W.V. AND HALL, J.C. 1998. A mutant *Drosophila* homolog of mammalian *Clock* disrupts circadian rhythms and transcription of *period* and *timeless*. Cell **93**: 791-804.

BIESSMANN, H. 1985. Molecular analysis of the *yel-*

low gene (*y*) region of *Drosophila melanogaster*. Proceedings of the National Academy of Sciences of the United States of America **82**: 7369-7373.

BORYCZ, J., BORYCZ, J.A., KUBOW, A., LLOYD, V. AND MEINERTZHAGEN, I.A. 2008. *Drosophila* ABC transporter mutants *white*, *brown* and *scarlet* have altered contents and distribution of biogenic amines in the brain. The Journal of Experimental Biology **211**(Pt 21): 3454-3466.

BRIDGES, C.B. AND BREHME, K.S. 1944. The mutants of *Drosophila melanogaster*. Washington, DC, Carnegie Institution of Washington.

BUSZA, A., EMERY-LE, M., ROSBASH, M. AND EMERY, P. 2004. Roles of the two *Drosophila* CRYPTOCHROME structural domains in circadian photoreception. Science **304**(5676): 1503-1506.

CAMPBELL, J.L. AND NASH, H.A. 2001. Volatile general anesthetics reveal a neurobiological role for the *white* and *brown* genes of *Drosophila melanogaster*. Journal of Neurobiology **49**(4): 339-349.

COLEMAN, C.M. AND NECKAMEYER, W.S. 2005. Serotonin synthesis by two distinct enzymes in *Drosophila melanogaster*. Archives of Insect Biochemistry and Physiology **59**(1): 12-31.

DUNLAP, J.C., LOROS, J. AND DECOURSEY, P.J., EDS. 2004. Chronobiology: Biological timekeeping. Sunderland, MA, Sinauer.

EWART, G.D. AND HOWELLS, A.J. 1998. ABC transporters involved in transport of eye pigment precursors in *Drosophila melanogaster*. In: Methods in Enzymology. Academic Press. **292:** pp 213-224.

HARDIN, P.E. 2011. Molecular genetic analysis of circadian timekeeping in *Drosophila*. Advances in Genetics **74**: 141-173.

HARRISINGH, M.C., WU, Y., LNENICKA, G.A. AND NITABACH, M.N. 2007. Intracellular Ca^{2+} regulates free-running circadian clock oscillation *in vivo*. Journal of Neuroscience **27**(46): 2489-2499.

HELFRICH-FÖRSTER, C. 2014. From neurogenetic studies in the fly brain to a concept in circadian biology. Journal of Neurogenetics **28**(3-4): 329-347.

HUGHES, S., JAGANNATH, A., HANKINS, M.W., FOSTER, R.G. AND PEIRSON, S.N. 2015. Photic regulation of clock systems. Methods in Enzymology **552**: 125-143.

KONOPKA, R.J. AND BENZER, S. 1971. Clock mutants of *Drosophila melanogaster*. Proceedings of the National Academy of Sciences of the United States of America **68**(9): 2112-2116.

KRSTIC, D., BOLL, W. AND NOLL, M. 2013. Influence of the *White* locus on the courtship behavior of *Drosophila* Males. PLoS ONE **8**(10): e77904.

RUTILA, J.E., SURI, V., LE, M., SO, W.V., ROSBASH, M. AND HALL, J.C. 1998. CYCLE is a second bHLH-PAS clock protein essential for circadian rhythmicity and transcription of *Drosophila period* and *timeless*. Cell **93**: 805-814.

SAUNDERS, D.S., ED. 2002. Insect clocks, Third Edition. Amsterdam, Elsevier.

SCHLICHTING, M., GREBLER, R., PESCHEL, N., YOSHII, T. AND HELFRICH-FÖRSTER, C. 2014. Moonlight detection by *Drosophila*'s endogenous clock depends on multiple photopigments in the compound eyes. Journal of Biological Rhythms **29**(2): 75-86.

SCHLICHTING, M. AND HELFRICH-FÖRSTER, C. 2015. Photic entrainment in *Drosophila* assessed by locomotor activity recordings. Methods in Enzymology **552**: 105-123.

SCHMID, B., HELFRICH-FÖRSTER, C. AND YOSHII, T. 2011. A new ImageJ Plug-in "ActogramJ" for chronobiological analyses. Journal of Biological Rhythms **26**(5): 464-467.

SEHGAL, A., PRICE, J.L., MAN, B. AND YOUNG, M.W. 1994. Loss of circadian behavioral rhythms and *per* RNA oscillations in the *Drosophila* mutant *timeless*. Science **263**(5153): 1603-1606.

SHEEBA, V., FOGLE, K.J. AND HOLMES, T.C. 2010. Persistence of morning anticipation behavior and high amplitude morning startle response following functional loss of small ventral lateral neurons in *Drosophila*. PLoS ONE **5**: e11628.

SOKOLOVE, P.G. AND BUSHELL, W.N. 1978. The Chi square periodogram: its utility for analysis of circadian rhythms. Journal of Theoretical Biology **72**: 131-160.

SULLIVAN, D.T., GRILLO, S.L. AND KITOS, R.J. 1974. Subcellular localization of the first three enzymes of the ommochrome synthetic pathway in *Drosophila melanogaster*. Journal of Experimental Zoology **188**(2): 225-233.

SUMMERS, K.M., HOWELLS, A.J. AND PYLIOTIS, N.A. 1982. Biology of eye pigmentation in insects. In: Advances in Insect Physiology. M. J. Berridge, J. E. T. and Wigglesworth, V. B. Academic Press. **16:** pp 119-166.

TOMIOKA, K. AND MATSUMOTO, A. 2010. A comparative view of insect circadian clock systems. Cellular and Molecular Life Sciences **67**: 1397-1406.

WU, C.-F. AND WONG, F. 1977. Frequency characteristics in the visual system of *Drosophila*: Genetic dissection of electroretinogram components. Journal of General Physiology 69(6): 705-724.

YOSHII, T., RIEGER, D. AND HELFRICH-FÖRSTER, C. 2012. Chapter 4 - Two clocks in the brain: An update of the morning and evening oscillator model in *Drosophila*. In: Progress in Brain Research. Andries Kalsbeek, M. M. T. R. and Russell, G. F. Elsevier. Volume 199: pp 59-82

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تأثير تطفر (الأصفر الأبيض) على روتين الأنشطة الحركية اليومية في ذبابة الفاكهة "دروسوفيلا ميلانوجاستر": مقارنة مع الطرز البري (كانتون إس)

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الملخص العربي

تقوم الساعة البيولوجية بتنظيم فسيولوجية وسلوك معظم الحيوانات. وقد تمت دراستها بشكل موسع في ذبابة الفاكهة "دروسوفيلا ميلانوجاستر" حيث ساعدت طفراتها، أكثر من أي حيوان آخر، على فهم الآليات الجزيئية، والعصبية والسلوكية لهذه المؤقتات الزمنية. وتهدف هذه الدراسة الي استكشاف تأثير طفرة (الأصفر الأبيض) على الأنشطة الحركية اليومية لذبابة الفاكهة تحت تأثير دورات الليل والنهار اليومية وكذلك في ظروف الإظلام (DD) والإضاءة (LL) المستمرة. ويعاني الذباب الحامل لهذه الطفرة من اضطراب في صبغيات الأعين والجسم بالإضافة إلى تغير في النمو العصبي والتحكم السلوكي، مما يجعلها مرشحا جيدا لدراسة وظائف الساعة البيولوجية. أظهرت النتائج أن الذباب المطفر حافظ على ايقاع نشاطه اليومي في ظروف دورات الاضاءة : الظلام (LD)، ولكن بمستوى نشاط حركي أعلى بشكل كبير من ذباب المجموعة الضابطة وخاصة خلال فترة الإضاءة. أما في ظروف الإظلام المتصل فقد أظهر الذباب المطفر إيقاعا سلوكيا حرا وإن كان بفترات أقصر من مثيلاتها في ذباب المجموعة الضابطة بقيمة (τ=23.82, SD=0.44) مقارنة بـ (τ=24.59, SD=0.39)، بما يدل على أن ساعتها البيولوجية الداخلية تسير بإيقاع أسرع من مثيلاتها في المجموعة الضابطة. في نفس الوقت، فقد الذباب المطفر انتظام ايقاعه في ظروف الإضاءة المستمرة، وإن كان ذلك متوقعا، كما هو معتاد في ذباب الفاكهة في ظروف الإضاءة المستمرة. وقد أظهرت النتائج أيضا أن النشاط الحركي للذباب المطفر تميز بوجود نوبتين، نوبة النشاط الصباحية (M) ونوبة النشاط المسائية (E) وبينهما فترة راحة خلال فترة الظهيرة (S). وبتحليل متوسط بداية ونهاية وطول كل نوبة من نوبات النشاط تبين أن الذباب المطفر يبدأ نوبة نشاطه الصباحي متأخرا بشكل كبير عن الذباب في المجوعة الضابطة وذلك بحوالي ساعتان ونصف تقريبا بينما ينهيان نشاطهما الصباحي معا، وكانت نوبة النشاط المسائي فيها مبكرة بشكل واضح، بحوالي ١.٧ ساعة. وقد أدي ذلك الى قصر نوعي في فترة راحتها خلال منتصف النهار لتكون أقصر بحوالي ١.٥ ساعة عن مثيلاتها في المجموعة الضابطة والتي قضت فترة راحة تصل إلى (4.52±1.54) ساعة. ويمكن تلخيص هذه التأثيرات بالقول بأن الذباب المطفر قد أزاح فترتي نشاطه تجاه مرحلة الإضاءة وأرتفع مستواه فيها بشكل كبير، بينما لم يتغير نشاطه في فترات الظلام بشكل ملحوظ، مقارنة بذباب المجموعة الضابطة. وقد أظهر التحليل الإحصائي أن الذباب المطفر قد أظهر انخفاض محدود في القدرة على توقع بدء فترة الإضاءة. ولكن ما يعزز الاعتقاد بأن هذا النوع من الطفرات قد أدى إلى تردي في قدرة الذباب على توقع بداية فترة النهار هو أن الزيادة في النشاط الحركي خلال الساعات الأخيرة في الظلام أقل بكثير من مستوى النشاط بعد بدء فترة الإضاءة، مقارنة بالمجموعة الضابطة. وقد خلصت الدراسة إلى أن لهذا النوع من الطفرات، بما تسببه من اضطراب صبغيات الأعين والجسم وفي نمو الجهاز العصبي، له تأثير نوعي على تنظيم الساعة البيولوجية للنشاط الحركي. وتقدم نتائج هذه الدراسة فرصة أكبر لفهم أعمق لسلوكيات أخري عند دراستها في هذه الخلفية الوراثية. كذلك تمثل الدراسة نموذجا مبشرا يمكن استخدامه لفهم أنماط نشاط الآفات، بما يمكن من تطبيق آليات مكافحتها بشكل اقتصادي وبأقل تأثير ملوث للبيئة.