importance in the protection of the natural population's health. Therefore, more studies should be done to clarify the effect mechanisms.

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Assessing effects of sex, mating status, and a white-eye mutation in a co-isogenic background, on circadian locomotor activity in *Drosophila melanogaster.*

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Introduction

A co-isogenic strain of *Drosophila melanogaster* was used to determine how white eye color, sex, and mating status affect circadian locomotor activity. This approach allowed us to test the effects of a white eye mutation against a constant genetic background. Changing the eye pigmentation may alter the input to the circadian clock in the brain driving the circadian locomotor rhythm. We also asked whether mating experience changes locomotor activity by comparing virgin

and non-virgin flies. It is possible that mating is associated with hormonal or other changes that may alter circadian locomotor activity, and fertile females who lay eggs that produce larvae inside the activity monitors may affect the sensitivity of the monitors compared to virgin females. Finally, we examined sex differences and interactions among all three treatments (white eye mutation, mating status, and sex).

Materials and Methods

A spontaneous white eye mutation in recombinant inbred *roo* line 22 (Nuzhdin *et al*., 1997) failed to complement the white eye mutation on the X chromosome (map position 1.5, Lindsley and Zimm, 1992; 43 white-eyed males and 30 white-eyed females were produced from a cross between four *roo* line 22 white-eyed females and four white-eyed strain w^{1118} males) and showed a recessive sex-linked pattern when 12 white-eyed *roo*-line 22 females were crossed with 12 red-eyed *roo* line 22 males (116 red-eyed females plus 139 white-eyed males, plus one red-eyed male which we can not account for). Purebred white and red-eyed inbred lines were derived in our lab. Virgin and non virgin red and white-eyed flies of both sexes, aged 1 to 3 days old (n = 16 per treatment group, 128 total) were placed in *Drosophila* Activity Monitors (Figure 1) on a 12 hours of light and 12 hours of dark cycle (12:12 LD) for 3 days with light levels at approximately 800 lux, and for 10 days in constant dark (DD). Activity counts were recorded in a 10 minute bins. Twelve flies did not survive in the activity monitors, and eight flies with activity counts exceeding two standard deviations from their treatment group mean were removed as statistical outliers. Actogram plots of activity histograms were produced for each fly (see sample actogram, Figure 2). The effects of genotype, mating status, and sex on mean activity level in 12:12 LD and DD and free-running circadian period in DD (estimated with Chi-square periodogram) and interactions were analyzed using analysis of variance (SAS, Carey, North Carolina USA).

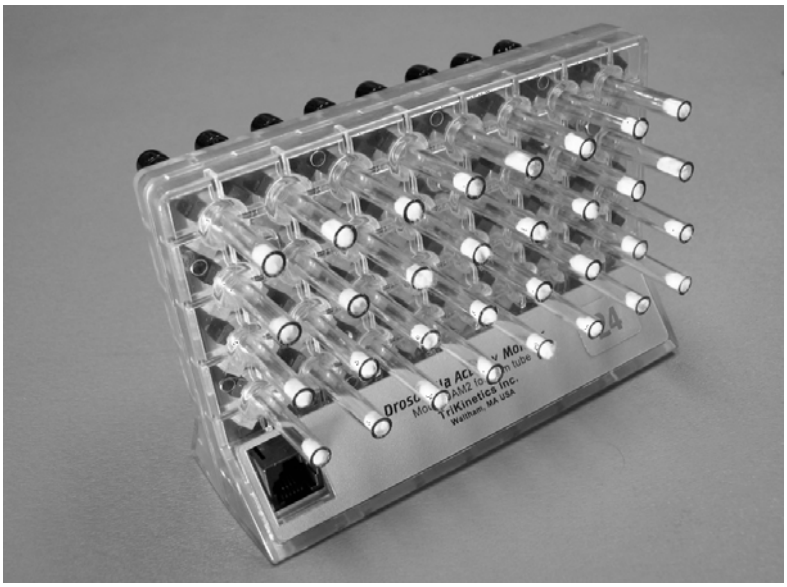

Figure 1. *Drosophila* activity monitor.

Results

The white eye mutation and mating status had no significant effect on free-running circadian period (tauDD) (Figure 3), mean activity level in the 12:12 LD cycle(XLD) (Figure 4), or mean activity level in constant dark (XDD) (Figure 5). Sex differences were observed only for mean activity level in constant dark (Figure 5) with female activity levels significantly higher than males ($F[1,107]$, $p < 0.04$). There were no significant interactions between treatments for any variable.

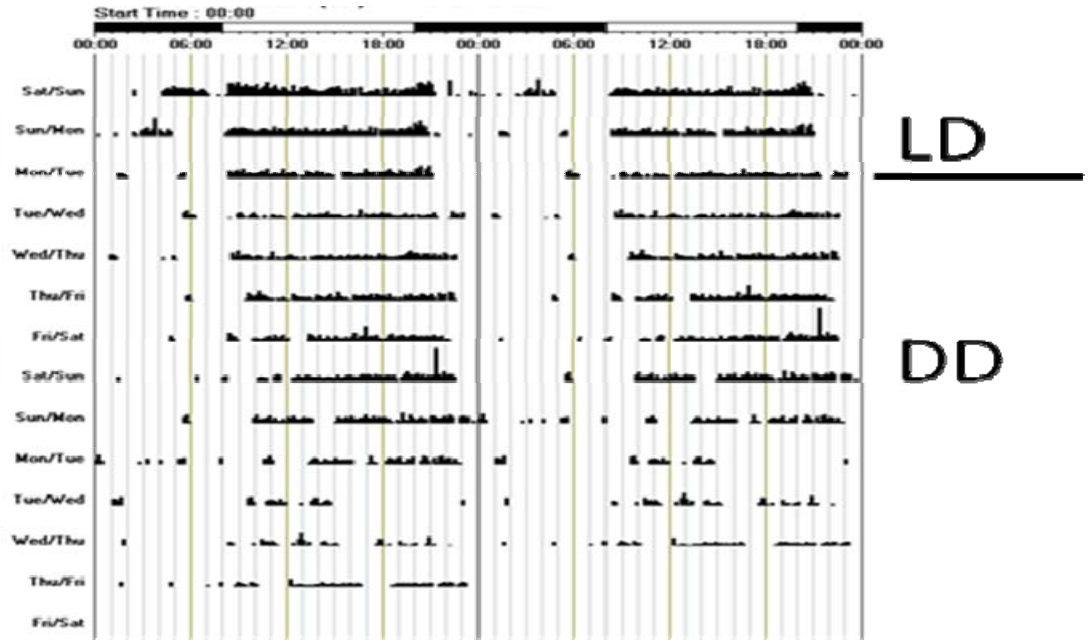

Figure 2. Representative actogram showing three days of activity in 12:12 LD (LD) followed by 10 days in constant dark (DD.) The Y axis shows consecutive days and the X axis is double-plotted, showing forty eight hours of continuous activity with the second day re-plotted as the first day on the next line.

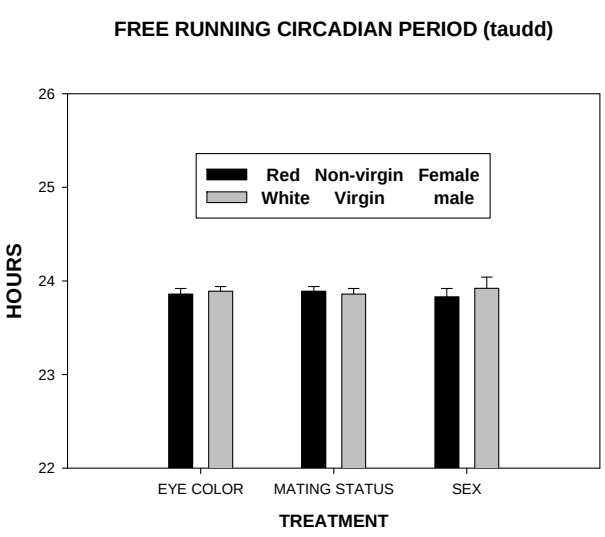

Figure 3. Eye color, mating status and sex had no significant effect on free-running circadian period (tauDD). There were no significant interactions between treatments.

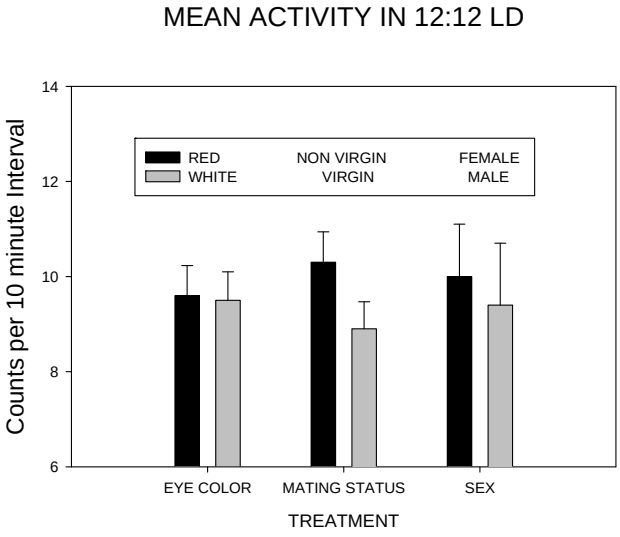

Figure 4. Eye color, mating status and sex had no significant effect on mean activity counts in the 12:12 LD cycle (XLD). There were no significant interactions between treatments.

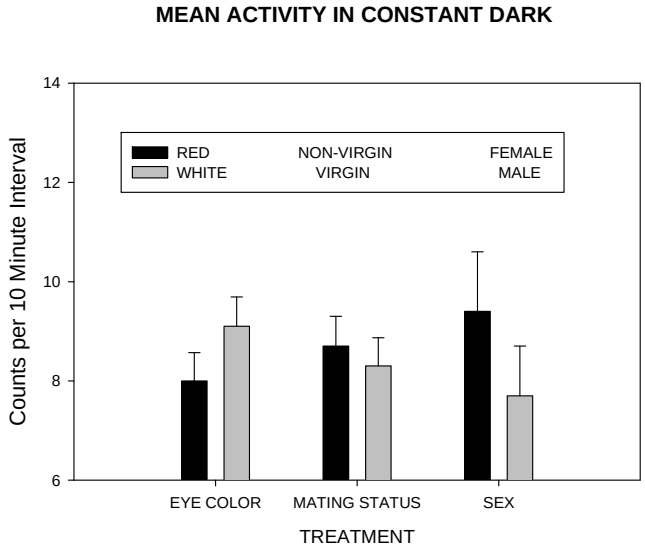

Figure 5. Eye color and mating status had no significant effect on mean activity counts in constant darkness (XDD). Female activity in DD was significantly higher in females ($P < 0.04$). There were no significant interactions between treatments.

Discussion

Fruit flies are a valuable model system for the analysis of circadian biological clock function (Takahashi *et al*., 2008). *Drosophila* circadian clocks in the brain are entrained to the light-dark cycle through multiple photoreceptor pathways, and all these pathways receive light that is filtered through pteridine (red) and ommochrome (brown) eye color pigments (Ashmore and Sehgal, 2003; Hofbauer, 2010). Our results suggest that these pigments, removed by the white eye mutation (Lindsley and Zimm, 1992) do not play a significant role in determining circadian clock period, or mean levels of activity in either a light-dark cycle or constant darkness. Since our results are based on an isogenic strain comparison, the white eye mutation is not confounded with any other genetic differences between the strains. On the other hand, our results are specific to this particular strain and may not generalize to different genetic backgrounds. Strain-dependence of sex differences in mean levels of circadian locomotor activity have been documented, for example, among different wild-type strains (Helfrich-Förster, 2000). We also observed, independently of eye color, that mating status did not alter mean activity level or circadian period in males and females. Helfrich-Förster (2000) also found no effect of mating status in females for mean activity level, phase of peak activity and circadian period in two wild type strains, and observed an effect of mating status only on mean activity in LD for a third. This suggests that endocrinological, neural, or behavioral changes that might be induced from mating in *Drosophila* do not involve circadian clock mechanisms (*e.g*., circadian period) driving locomotor behavior, but may have strain-dependent effects on mean activity level depending on genetic background. It is probably not necessary, therefore, to isolate virgin flies for analysis in *Drosophila* Activity Monitors, even though mated females may lay eggs that hatch and release larvae in the apparatus during an experiment. We observed a sex difference in mean activity in constant dark with females displaying more activity than males, but no difference in the 12:12 LD cycle. This is consistent with a tendency for females to display higher mean activity levels than males (Helfrich-Förster, 2000) in two out of three wild-type strains. We observed no significant interactions among pigmentation, sex, and mating status for any variable. Overall, our results suggest that the absence of eye pigmentation in *Drosophila* and mating status differences do not significantly alter mean circadian locomotor activity levels or free-running circadian clock period. Our results, however, must be qualified in several ways. The most important is the trade-off inherent in the use of

co-isogenic strains, which cleanly isolate effects of a single genetic locus against an isogenic background, but also necessarily limit results to that one inbred genotype. Other qualifications include the possibility that larger sample sizes may reveal more subtle effects that we were not able to detect with 16 flies per treatment group, and that analysis of additional variables may reveal significant effects (*e.g*., sleep, amplitude, phase). Finally, our use of relatively high light levels (approximately 800 lux) might have obscured effects that may emerge at lower levels of illuminance where differences in pigmentation may be more meaningful if circadian response mechanisms are below a saturation threshold. It is necessary to conduct further analyses of circadian behavior in *Drosophila* pigmentation mutants before drawing more definitive conclusions about the role of photoreceptor pigmentation and their genetic loci in mediating circadian behavior.

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Embryo and larval survival in *Drosophila melanogaster* pigmentation mutants *tan* and *ebony*.

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Introduction

Drosophila melanogaster genes *ebony* and *tan* are responsible for the synthesis and hydrolysis of N-β-alanylderivatives of biogenic amines, such as N-β-alanyldopamine (NBAD) and N-β-alanylhistamine (carcinine) (Wright, 1987; Hovemann *et al*., 1998; True *et al*., 2005). These are pleiotropic genes expressed in epidermis and nervous tissue (Badaracco *et al*., 2009; Pérez *et al*., 2010). The activities of these proteins are required in epidermis during cuticular melanization regulating the pigmentation of the insect. In the nervous system these enzymes are necessary for the synthesis of carcinine (N-β-alanylhistamine) and posterior hydrolysis to histamine (and β-alanine), recycling and maintaining the levels of photoreceptor neurotransmitters (Wright, 1987; Borycz *et al*., 2002; True *et al*., 2005). Mutants of these genes show reciprocal pigmentation defects; *ebony* is darker than wt flies and *tan* is lighter. Flies lacking Ebony or Tan function, however, exhibit similar abnormalities in vision (Benzer, 1967; Inoue *et al*., 1988; True *et al*., 2005), and males display abnormal courtship behavior (Crossley and Zuill, 1970; Cook, 1980; Tomkins *et al*., 1982). Neurotransmitter levels are altered in *ebony* and *tan*; both mutants have reduced levels of histamine (Borycz *et al*., 2002). However, *ebony* shows an increased level of dopamine (Hodgetts, 1972), whereas *tan* shows reduced levels of this catecholamine (Konopka, 1972). Biogenic amines are important in reproduction. Mutants that have low levels of neurotransmitters show impaired reproduction or conditional viability (Neckameyer, 1996; Simon *et al*., 2009). In the present report we analyzed the fertility and viability of e^1 and t^1 in order to elucidate if the altered levels of neurotransmitters have a consequence in the reproduction of these mutants.