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.

Methods

All *D. melanogaster* strains were from Bloomington Stock Center, CS; *tan*^{1} (*t* ^{1}); *ebony*^{1} (*e*^{1}). Cultures were kept at 23ºC and 16/8 light-dark cycle on standard corn meal yeast agar medium.

Three experiments were carried out intended to study embryo and larva survival and adult fertility on *tan* or *ebony* mutants. To collect eggs, around 150 adults aged 1 week were placed in bottles with agar plates and yeast as described in Wieschaus and Nusslein-Volhard (1986). The agar plates (with yeast) were placed at 8 pm and eggs were collected overnight. Twenty or thirty eggs were removed and placed on new agar plates, and the number of hatched eggs counted after 48 hours. Alternatively, a piece of agar with 30 eggs was placed in a vial with a fixed amount of corn meal yeast agar medium. The number of pupae and imagoes was recorded daily for two weeks.

In order to detect anomalies in fertility and egg-laying behavior, five virgin females and three males were placed in vials for eight days. Afterwards adults were removed and the number of pupae and new imagoes were recorded twice a day over a period of three weeks. To prevent further egg laying, new imagoes were discarded after each counting event.

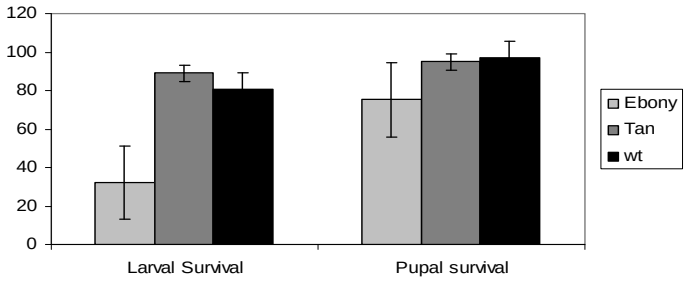

Figure 1. Larval and pupal survival in tan, ebony and wt. Larval survival is calculated as the percentage of larvae reaching the pupa stage. Pupal survival is calculated as the percentage of pupae reaching the imago stage.

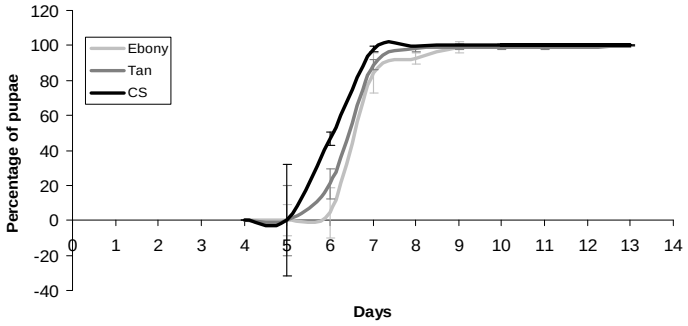

Figure 2. Percentage of pupae formed was calculated as the amount of pupae formed relative to the maximum of pupae observed for each strain.

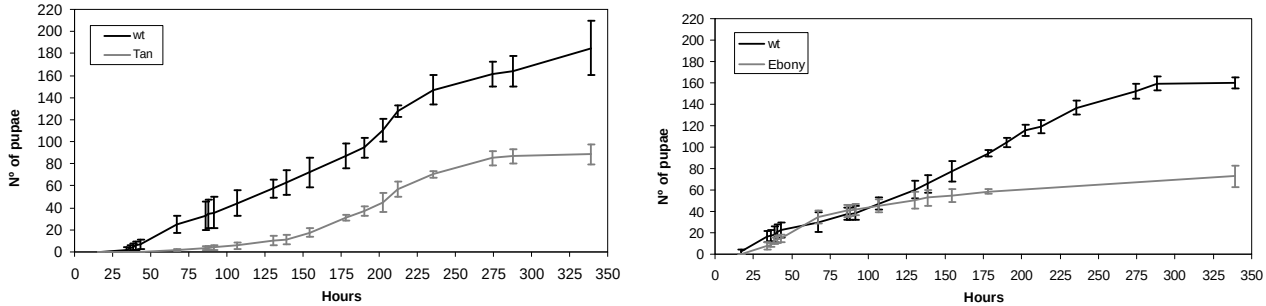

Figure 3. Number of pupae observed through three weeks of experiment for tan (left) and ebony (right).

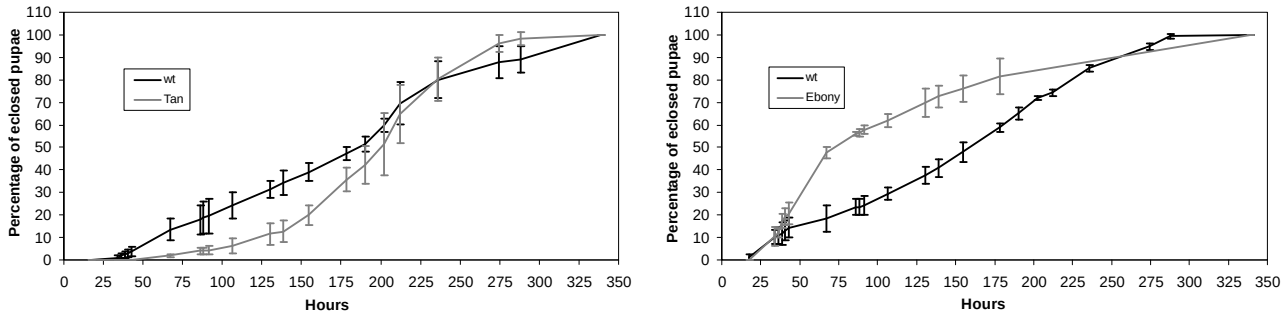

Figure 4. Percentage of pupae formed was calculated as the amount of pupae formed relative to the maximum of pupae observed for tan (left) and ebony (right).

Results

As shown in Figure 1, there was a significant difference in larvae survival among the strains. There was a great rate of mortality in e^1 (around 70%), whereas in t^1 and wt strains around 90% of the larvae survive until the pupa stage. We did not find differences on embryo survival among these strains (data not shown). No differences were observed in the number of eclosed pupae (nº of imagoes/nº of pupae) among the strains. The developmental times were similar in larvae (Figure 2) and pupae (data not shown) in the three strains.

When grown at high densities, t^1 larvae showed similar mortality rate to that of e^1. Figure 3 shows that the amount of larvae reaching pupae stage was reduced roughly to 50% with respect to wild type. This effect was not observed at low population densities (with three females left laying eggs for four days instead of five females eight days), suggesting that this effect is mainly due to larval density and not to a reduced fertility.

Surprisingly, the dynamics of the growth curve were different in the three strains. While wt showed a steady, almost linear, increase in the number of pupae (Figure 4), t^1 and e^1 presented opposite and more curved increments. *ebony* showed a hyperbolic curve, with an early high slope which decreases over time. On the other hand, t^1 presented a sigmoid-shape curve; it showed an initial low slope, a rapid increment in the middle of the experiment and a low slope at the end. This strain showed a delay in the development. The first pupae appeared almost 48-hour later than wt. The delay in the appearance of the first t^1 pupae was apparently not due to a delay in egg-laying or ovary maturation since the delay was not observed at low densities.

The results regarding *tan* are similar to that observed by other authors (Neckameyer, 1996; Simon *et al*., 2009), who reported that flies with reduced levels of dopamine show elevated larval mortality at high population density and delayed development. What remains to be determined is the cause of the high larval mortality observed in e^1. We hypothesize that this high mortality is a consequence of the altered dopamine or NBAD levels in ebony. It is worth pointing out that t^1, supposedly having low dopamine levels and high NBAD levels, showed a delayed development, while e^1, which presumably has high dopamine levels and low NBAD levels, presented a faster development than *wt*.

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Genetic variability analysis of two natural populations of *Drosophila antonietae* (Diptera; Drosophilidae).

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Abstract

Drosophila antonietae belongs to the *D. buzzatti* cluster (*D. repleta* group), and its populations are always associated with the cactus *Cereus hildmaniannus*. It differs from the other *D. buzzatii* cluster species by showing a higher genetic homogeneity among its populations for different markers despite the wide geographic distribution of this species. Gene flow restriction by distance, along with a relatively recent fragmentation maintenance of ancient polymorphism, has been used as the most likely explanations for this pattern. All previous analyses were, however, realized with populations located in the high and low portions of the Parana-Uruguay rivers basin. No population located in the middle portion was analyzed so far. Thus, this work aimed to analyze two *D. antonietae* populations located in the middle portion of Parana-Uruguay rivers basin, Rio do Poço-Guarapuava/PR and Cantagalo/PR, not yet sampled, using 10 allozymic loci, to try to correlate our findings with previously published data for this species. Our results showed that the allozymic genetic variabilities of both *D. antonietae* populations are in agreement with that expected for the species, and the most likely factors responsible for the high within population diversity (Cantagalo – Ho = 0.2337; Rio do Poço – Ho = 0.3425) and low among population differentiation (D = 0.0307; I = 0.9698; Fst = 0.0081) must be gene flow restricted by distance with recent fragmentation and maintenance of ancient polymorphism.

Introduction

Studies using different species of the *Drosophila buzzatii* cluster (*D. repleta* group) showed that they constitute an interesting biological system for evolutionary research as they present polymorphisms, polytypism, ecological specificities and different levels of among population