

recognized *Drosophilid* larvae in these material pieces. In addition, we got pitaya dulce fruits (*S. thurberi*) with *Drosophilid* larvae at the San Antonio town (23.800903, -10.109672). So, we took fruits and rotten material samples to the lab. I anticipated that these larvae could be *D. spenceri* and *D. mojavensis*.

Once in the lab, I saw with surprise that pupae had a reddish-brown color without clear horns. When adults emerged, I positively identified the species as *Z. indianus*. Surprisingly, *Z. indianus* didn't arrive to the banana baits. This note is the first report of *Z. indianus* using cacti fruits and rotten cactus tissue. So, I suspect *Z. indianus* could potentially detoxify some of the alkaloids present in the tribe Pachycereeae and if it is not competing, at least *Z. indianus* could potentially disturb some *Drosophilidae* desert populations.

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Effect of Cyclophosphamide on *hsp70* expression in transgenic *Drosophila melanogaster* (*hsp-70-lacZ*) Bg⁹.

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Abstract

In the present study the effect of 0.0035, 0.025, 0.050, 0.10, and 1 µl/ml of cyclophosphamide (CP) was studied on the 3rd instar larvae of transgenic *Drosophila melanogaster* (*hsp70-lacZ*) Bg⁹ for 6, 24, and 48 hr durations. The treatment of 0.0025 µl/ml of CP did not induce significantly the activity of *hsp70* as compared to control. The treatments of 0.025, 0.050, 0.10, and 1 µl/ml of CP induced a significant increase in the activity of *hsp70* for the different duration of exposure. The results of the present study suggest that the doses of 0.025, 0.050, 0.10, and 1 µl/ml are cytotoxic in the 3rd instar larvae of transgenic *Drosophila melanogaster* (*hsp70-lacZ*) Bg⁹.

Introduction

Cyclophosphamide is an alkylating agent (Ren *et al.*, 1998). It is used as a chemotherapeutic agent to treat various forms of leukemia (Shanafelt *et al.*, 2007) and tumors (Young *et al.*, 2006). It

is also used to treat rheumatoid arthritis (Scott *et al.*, 1984), Wegner's granulomatosis (Hoftman *et al.*, 1990). All living organisms under stressful conditions respond by synthesizing heat shock proteins (HSPs) (Nover, 1994, 1991). HSPs function as a molecular chaperone that prevents cellular damage (Bennet and Waters, 2000). In recent years, *hsp70* has been considered to be one of the candidate genes for predicting cytotoxicity against environmental chemicals (Bierkens, 2000; Mukhopadhyay *et al.*, 2002, 2003). In the present study, the toxicity of Cyclophosphamide was investigated by the quantification of *hsp70* expression in the 3rd instar larvae of transgenic *Drosophila melanogaster* (*hsp70-lacZ*) Bg⁹, for the different doses and hours of exposure.

Materials and Methods

Fly strain

A transgenic *Drosophila melanogaster* line that expresses bacterial β -galactosidase as a response to stress was used in the present study (Lis *et al.*, 1983). The flies and larvae were cultured on the standard *Drosophila* food containing agar, cornmeal, sugar, and yeast (Nazir *et al.*, 2003).

ONPG assay

Cyclophosphamide at 0.0025, 0.025, 0.050, 0.10, and 1 μ l/ml of food concentrations were established. The third instar larvae were allowed to feed on them for different time intervals, *i.e.* 6, 24, and 48 hr. The expression of *hsp70* was measured by soluble o-nitrophenyl- β -D-galactopyranoside (ONPG) assay (Nazir *et al.*, 2003; Lakhotia and Singh, 1989). Briefly, after washing in phosphate buffer, the larvae were taken in a microcentrifuge tube (20 larvae/tube, 5 replicates/group), permeabilized for 10 min in acetone, and incubated overnight at 37°C in 600 μ l of ONPG staining buffer. Following incubation, the reaction was stopped by adding 300 μ l of Na₂CO₃. The extent of reaction was quantified by measuring the absorbance at 420 nm using Systronics UV/VIS spectrophotometer 118 (India).

Statistical analysis

Statistical analysis was carried out by student's "t" test using Commercial Software Statistica Ssoft Inc (2007).

Table 1. β -galactosidase activity measured in transgenic *Drosophila melanogaster* (*hsp70-lacZ*) Bg⁹ third instar larvae exposed to different concentrations of Cyclophosphamide for various time intervals.

Treatments Cyclophosphamide (μ l/ml)	After 6 hr O.D. (Mean \pm SE)	After 24 hr O.D. (Mean \pm SE)	After 48 hr O.D. (Mean \pm SE)
0.0025	0.2103 \pm 0.0032	0.2036 \pm 0.0041	0.1898 \pm 0.0082
0.025	0.2433 \pm 0.0043*	0.2639 \pm 0.0011*	0.2723 \pm 0.0054*
0.050	0.2593 \pm 0.0071	0.2763 \pm 0.0018*	0.2844 \pm 0.0063*
0.10	0.2634 \pm 0.0023	0.3012 \pm 0.0073*	0.2930 \pm 0.0034*
1.0	0.1132 \pm 0.0011	0.1034 \pm 0.0017	-
Untreated	0.2011 \pm 0.0039	0.2112 \pm 0.0040	0.1994 \pm 0.0050

*P < 0.05 compared to control; O.D. = Optical Density; SE = Standard Error.

Results

The treatment of 0.0025 $\mu\text{l/ml}$ of CP did not show any significant increase in the β -galactosidase activity for various time intervals as compared to untreated (Table 1). The treatment of 0.025 $\mu\text{l/ml}$ of CP did not show any significant increase in the β -galactosidase activity for 6 hr of exposure but showed a significant increase in the β -galactosidase activity for 24 and 48 hr of duration of exposure (Table 1). The exposure of 3rd instar larvae to 0.050 and 0.10 $\mu\text{l/ml}$ of CP showed a dose dependent significant increase for each exposure (Table 1). The treatment of 1 $\mu\text{l/ml}$ of CP results in a decrease in the activity of β -galactosidase for 6 and 24 hr of duration (Table 1). After 48 hr of exposure to 1 $\mu\text{l/ml}$ of CP, the ONPG was not performed due to the mortality of larvae.

Discussion

The results of the present study reveal that the CP is not cytotoxic at 0.0025 $\mu\text{l/ml}$. The metabolite of CP is phosphoramidate mustard (Hales, 1982). It forms DNA cross links between and within DNA strands of guanine N-7 positions that lead to the cell death (Benson *et al.*, 1988). The higher doses of CP are associated with cytotoxicity (Hales, 1982). Although having protective roles in living systems, HSPs are being exploited by toxicologists (Bierkens, 2000; Mukhopadhyay *et al.*, 2002, 2003; Nazir *et al.*, 2003). Now-a-days, the use of animals for toxicological evaluations has become the fundamental concern for scientists, not only because of protests from animal rights organizations but also because of difficulty in interpreting data due to intra-species variation and exorbitant costs (Benford *et al.*, 2000). This has led researchers to encourage the use of alternative animals in toxicological evaluations. *Drosophila* is a well established animal model for genetics, developmental and molecular biologists. In the past years a significant contribution has been made by successfully employing transgenic *D. melanogaster* as an alternative animal model for toxicological research (Mukhopadhyay *et al.*, 2002). Although there is no comparative data, the studies by Hirsch *et al.* (2003) indicate that flies and humans have similar dose response relationships with lead. At the highest tested dose, *i.e.* 1 $\mu\text{l/ml}$ of CP, the decrease in the activity of *hsp70* expression is due to the tissue damage at this dose, which is also evident by the mortality of the larvae after 48 hr of exposure. HSPs are formed in response to stressors like LPO, DNA damage, osmotic imbalance, protein misfolding, membrane perturbation, metals, heat shock, and so forth (Nazir *et al.*, 2003). A dose dependent increase in the activity of β -galactosidase clearly demonstrates the dose dependent toxic effects of CP in transgenic *Drosophila melanogaster* (*hsp70 lacZ*) Bg⁹ and give support to the utility of *hsp70* expression as a bioindicator of exposure to the environmental chemicals.

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Inconsistent associations between recombination rate and codon bias across *Drosophila* species.

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Abstract

A positive association between recombination rate and codon bias has been observed at broad and fine scales in *Drosophila* species. However, this relationship is complicated by other genomic features that correlate with codon bias. No prior studies have evaluated the relationship between recombination rate and codon bias across multiple species within this genus. Utilizing published recombination maps along with complete genome sequences, we contrasted recombination rate to codon bias and intronic GC across four *Drosophila* species. We did not observe a consistent significant correlation between recombination rate and codon bias across the species examined. Indeed, we did not observe even the previously reported trend of higher codon bias in regions of high recombination, though we saw some evidence this pattern may be affected by differences between centromeric/telomeric regions and central regions. More fine-scale recombination data from more *Drosophila* species is necessary for a comprehensive picture of the relationship between recombination rate and codon bias.

Introduction

Rates of recombination vary across *Drosophila* genomes at broad and fine scales and have been shown to be correlated with codon usage bias (Cirulli *et al.*, 2007; Comeron *et al.*, 1999; Hey and Kliman, 2002; Kliman and Hey, 1993; Marais *et al.*, 2001; Singh *et al.*, 2005; Stevison and Noor, 2010). Specifically, regions of high recombination tend to have disproportionately high usage of G- or C-ending codons relative to regions of low recombination. The relationship of recombination rate to codon bias is complicated by other features that also correlate with codon bias