invasive species *Z. indianus* and *D. malerkotliana* and will be used for selecting candidate TEs for more detailed studies.

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References: Biémont, C., and G. Cizeron 1999, Genetica 105: 43-62; Capy, P., and P. Gilbert 2004, 120: 5-16; Castro, F.L., and V.L.S. Valente 2001, Dros. Inf. Serv. 84: 15-17; Cizeron, G., F. Lemeunier, C. Loevenbruck, A. Brehm, and C. Biémont 1998, 15: 1589-1599; de Almeida, L.M., and C.M.A. Carareto 2005, Mol. Phylogenet. Evol. 35: 583-594; de Setta, N., E.L. Loreto, and C.M.A. Carareto 2007, J. Mol. Evol. doi: 10.1007/s00239-007-9051-7; García Guerreiro, M.P., and A. Fontdevila 2001, Genet. Res. 77: 227-238; Gõni, B., M.E. Martinez, G. Techera, and P. Fresia 2002, Dros. Inf. Serv. 85: 75-80; Heredia, F., E.L. Loreto, and V.L. Valente 2004, Mol. Biol. Evol. 21: 1831-1842; Kato, C.M., L.V. Foureaux, R.A. Cesar, and M.P. Torres 2004, Ciênc. Agrotec. 28: 454-455; Labrador, M., M.C. Seleme, and A. Fontdevila 1998, Mol. Biol. Evol. 15: 1532-1547; Val, F.C., and F.M. Sene 1980, Papéis Avulsos de Zoologia 33: 293-298; van der Linde, K., G.J. Steck, K. Hibbard, J.S. Birdsley, L.M. Alonso, and D. Houle 2006, Fla. Entomol. 89: 402-404; Vieira, C., D. Lepetit, S. Dumont, and C. Biémont 1999, Mol. Biol. Evol. 16: 1251-1255; Vilela, C.R., 1999, Dros. Inf. Serv. 82: 37-39.

Effects of Phloxine B and Hematoporphyrin IX on immature stages of *Drosophila melanogaster.*

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Introduction

Photosensitizers such as xanthene derivatives (*e.g*., phloxine B) and porphyrins (*e.g*., hematoporphyrin IX) are endowed with photoinsecticidal properties. Xanthene derivatives showed acute photo-toxicity against several dipterans (Ben Amor and Jori, 2000, Berni *et al*., 2002, 2003). Pujol-Lereis (2006) compared the photo-toxic effect of phloxine B (PhB) during the postembryonic development of *D. melanogaster* (*Dm*), *Haematobia irritans*, and *Ceratitis capitata*, and determined that *Dm* is affected during larval development. Low concentrations of hematoporphyrin (HP) rapidly decrease adult survival rates of *Ceratitis capitata*, *Bactrocera (Dacus) oleae*, and *Stomoxys calcitrans* (Ben Amor *et al.,* 1998, 2000).

Photosensitizer molecules react upon absorption of visible radiation with the subsequent formation of reactive oxygen species, mediating signaling cascades which either fortify antioxidant defenses of cells or switch to apoptotic death if oxidative pressure is too great (Girotti, 1998). There are two mechanisms by which the photosensitizer can react with biomolecules: type I reactions produce highly reactive oxygen species (*e.g*., the superoxide and the peroxide anions) which usually activate enzymatic antioxidant defense, and type II reactions result in the formation of singlet oxygen, leading mainly to lipid peroxidation. Studies of the effects in immature stages were carried

out in order to understand which phototoxic pathway is triggered and which main cellular and sub-cellular targets are affected.

Methods

Wild type (strain Oregon-R-C) and antioxidant enzyme mutant strains of *D. melanogaster* were obtained from Bloomington *Drosophila* Stock Center (Bloomington, IN). Larvae were reared in Formula 4.24 Instant *Drosophila* Medium (Carolina Biological Supply, Ca). Batches of 30 newly hatched larvae I of *Drosophila* were placed on the surface of the larval media with or without 0.1 mM PhB disodium salt (D&C Red Nº 28, Warner Jenkinson, St Louis, MO) and 0.1 mM hematoporphyrin IX (Sigma-Aldrich Inc., St Louis, MO). Cultures were maintained in the dark in a Conviron chamber CMP 3244 (Canadá), at 23°C, 50-60% RH, and exposed to light (5000 lux) during the wandering period. After pupariation, insects were maintained in the dark until imago ecdysis. Separate cultures of wt *Drosophila* were maintained in the dark throughout the entire experiment.

For semi-quantitative PCR analysis of antioxidant enzymes, stage III wandering larvae were transferred to 1% agar plaques and exposed to 1 hour of light. RNA was extracted with TRIzol Reagent (Life Technologies, GIBCO-BRL). cDNA was synthesized following the SuperScript Pre-amplification System for First Strand cDNA Synthesis (BRL).

Pairs of oligonucleotide primers were designed for amplification of *Actin* (5′ AAGCGTGGTATCCTCACCC 3′ and 5′ TCCTCCTCCTCCTCCAGC 3′), *Catalase* (*Cat*) (5′ CATGTTCTGGGACTTCCTC 3′ and 5′ GTC TGATCCGTCGAATCC 3′), *Glutathione S-transferase* (*Gst*) (5′GTATTTGGTGGAGAAGTACG 3′ and 5′ ATTGTCTTTGGAGACAGGC 3′), Mn *Superoxide Dismutase* (Mn*Sod*) (5′ CGACACCACCAAGCTGATTCAG 3′) and Cu, Zn *Superoxide dismutase* (Zn/Cu*Sod*) (5′ CCAAGATCATCGGCATCGG 3′ and 5′ TGACAACACCAATGGCTGC 3′). PCR reactions (50 µl) were carried out with 1 µl of each primer, 2.5 mM $MgCl_2$, 0.2 mM dNTP, 2 units Taq polymerase (Promega, WI). Amplification conditions were: 94ºC for 5 min, 25 cycles of 94ºC × 30 sec, annealing temperature × 30 sec, and 72ºC × 1 min, followed by a final extension at 72ºC for 5 min. Annealing temperatures were: 47ºC for *Gst,* 52ºC for *Actin*, *Cat* and Zn/Cu*Sod*, and 54ºC for Mn*Sod*. All PCR reactions were performed with a PCR*Express* Thermo Hybaid thermal cycler. PCR products were run through a 1.2 % agarose gel and band intensities relative to *Actin* were analyzed with ImageJ program.

Results and Discussion

The survival data of wt *Drosophila* when HP was added to the larval media showed that the photosensitizer was not toxic when the insects were maintained in the dark throughout development (Table 1). Instead, wt *Drosophila* treated with HP and exposed to light reduced the survival from 80% to 47.5%. Further mortality was not registered in wt after pupariation (not shown). We determined that the 50% lethal concentration (LC_{50}) of HP of wt *Drosophila* from egg eclosion to imago ecdysis was 0.11 mM.

Surprisingly, Catalase (*Cat*) and Glutathione S-transferase (*Gst*) mutant strains were not affected by the addition of HP to the larval media (Table 1). Survival of Zn/Cu Superoxide dismutase (Zn/Cu*Sod*) mutant strain showed an important survival reduction from larvae I to white pre-pupa when treated with HP and exposed to light (65% of the larvae could pupariate without HP whereas only 15% with HP). The percent of adult ecdysis of Zn/Cu*Sod* mutant strain was 7.5%, indicating that additional mortality occurred during the stages within the puparium (not shown).

Table 1. Percentage survival of wt and antioxidant enzyme mutant strains of *D. melanogaster* reared with 0.1mM HP. Each value represents the average of 3 replicas.

D. melanogaster mutant strains	LI to white pre-pupa survival (mean % ± sem)		
	0 mM HP	0.1 mM HP	Δ
Dark conditions			
wt Oregon-R-C	74.4 ± 17.1	72.5 ± 8.7	-1.9
Light during wandering			
wt Oregon-R-C	80 ± 10	47.5 ± 2.5	- 32.5
Sod 4018	65 ±10	15.0 ± 3	- 50.0
Cat 17939	70 ± 2	75.0 ± 5	+ 5.0
Gst 14114	40 ± 5	25.0 ± 10	- 15.0

Table 2. Survival decrease of wt and antioxidant enzyme mutant strains of *D. melanogaster* reared with 0.1 mM PhB. Each value represents the average of 3 replicas.

D. melanogaster mutant strains	Decrease in survival (mean %)*	
	LI to white pre-pupa	LI to adult ecdysis
Light during wandering		
wt oregon-R-C	26.2	65.3
Sod 4018	0.0	32.3
Cat 17939	11.0	43.8
Gst 14114	8.0	20.7

(*)Maximum standard deviation in the experiments was ± 12.0 %

Table 3. Transcription levels of antioxidant enzymes of wt *Drosophila* reared with PhB or HP.

	Intensity (mean ± se)		
	0mM	0.1mM PhB	0.1mM HP
Zn, Cu Sod	0.88 ± 0.35	0.84 ± 0.34	1.29 ± 0.28
MnSod	1.03 ± 0.32	0.86 ± 0.7	0.68 ± 0.14
Cat	1.16 ± 0.04	1.08 ± 0.13	1.01 ± 0.3
Gst	0.98 ± 0.1	0.90 ± 0.84	0.80 ± 0.10

Intensity: arbitrary units (Image J program) determined as relative to actin levels.

As shown with HP, 0.1 mM PhB was not toxic to wt *Drosophila* maintained in the dark throughout development (73.3% survival). Significant light-dependent toxicity was observed in wt both at pupariation (26.2% reduction) and at adult ecdysis (65.3%, Table 2). The 50% lethal concentration (LC_{50}) for PhB during the postembryonic development of wt *Drosophila* was 0.06 mM. No additional mortality due to PhB activation was observed in antioxidant enzyme mutant strains (Table 2).

The transcription levels of the four antioxidant enzymes corresponding to the above tested mutants (Zn/Cu*Sod*, *Cat*, *Gst*, and Mn*Sod*) were estimated by semi-quantitative PCR, followed by gel electrophoresis, for larvae reared with or without 0.1 mM PhB or HP (Table 3). In agreement with the above results no significant increase in transcription of Mn*Sod*, *Cat*, and *Gst* antioxidant enzymes was registered with HP nor with PhB (Table 3). Therefore, Type I reactions do not seem to prevail in our experimental model with photosensitizers.

However, survival was significantly reduced in mutant strain 4018 when HP was added to the diet. This strain presents mutation of Zn/Cu*Sod* enzyme. Moreover, although not statistically significant, the transcription level of Zn/Cu*Sod* seems to be enhanced when wt *Drosophila* was reared with HP. These experiments must be repeated in order to attain statistical significance.

From these results we can conclude that in our experimental conditions, Type II reactions are likely implied in photo-toxicity. Preliminary studies in the medfly *Ceratitis capitata* showed 2-fold increase in the level of weak chemoluminiscence associated to lipid peroxidation, when treated with comparable concentrations of PhB and HP and exposed to light (data not shown). Further studies are on the way to confirm the existence of rapid lipid peroxidation (type II mechanism).

References: Ben Amor, T., L. Bartolotto, and G. Jori 1998, Photochemistry and Photobiology 68: 314-318; Ben Amor, T., and G. Jori 2000, Insect Biochem. Mol. Biol. 30: 915-925**;** Berni, J., 2002, PhD Dissertation, University of Buenos Aires; Berni, J., A. Rabossi, and L.A. Quesada-Allué 2003, J. Econ. Entomol. 96: 662-668**;** Girotti, A.W., 1998, J. of Lipid Research. 39: 1529-1542; Pujol-Lereis, L.M., A. Rabossi, A. Filiberti, C.E. Argaraña, and L.A. Quesada-Allué 2006, Dros. Inf. Serv. 89: 82-84.

The *white*$^{-}$ mutation provides a growth advantage for mosaic clones in *D. melanogaster* and *D. simulans*.

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In this short communication we demonstrate that a *white* mutation can provide a positive bias to a mosaic clone homozygous for a recessive marker linked with *white*$^{-}$ compared to its twin clone homozygous for another marker linked with *white*$^{+}$. The effect is not very strong and requires for its observation a large sample or a strong mutagenic treatment generating enough clones. At the same time, the effect remains persistent and statistically significant in two fly species (*D. simulans* and *D. melanogaster*), with different alleles of *white* and mosaic clone markers and with different mutagenic agents applied. The protein White is a part of ATP-dependent nucleotide and amino acid transporters, particularly for guanine and tryptophan, the precursors for eye pigments (Evart *et al*., 1994). The gene *white* is also known to affect courtship behavior and response to anesthesia (Svetec *et al*., 2005; Campbell and Nash, 2001). No function relevant to cell growth has been reported for this gene by now.

Genes. y^2 – *D. simulans yellow*^{2}, *yellow* chaetae, normal wings, the mutation of spontaneous origin in the *vermilion* strain; *sn* – *D. simulans singed*, "singed" chaetae, homozygous females are sterile, radiation-induced mutation in the *vermilion* strain; *v* – *D. simulans vermilion*, vermillion, brilliant red eye color; nH^{+} – an autonomous *D. simulans* transposable element located in X-chromosome, the genetic instability H^{+}-factor, n represents the number of H^{+} copies in the X chromosome; *w* – *D. simulans white*, white eye color; *wy* – unspecified *D. simulans wavy* allele, grey wavy wings, the mutation was originated spontaneously in the *sn v* strain; *y* – *D. melanogaster* unspecified *yellow* allele; *w* – *D. melanogaster* unspecified loss-of-function *white* allele; w^{1118} – *D. melanogaster* loss-of-function *white* allele, homozygotes have white eyes with only 1% red pigment (compared to 100% pigment in wild type); sn^3 – *D. melanogaster singed*^{3} allele, homozygous viable and fertile.

Stocks and Crosses. *Drosophila simulans*: (1) y^2, H^{+}, (2) *sn v*, H^{+} / $\overset{\wedge}{XX}$, (3) y^2 *w*, (4) *sn v wy*, $2H^{+}$/ $\overset{\wedge}{XX}$. *Drosophila melanogaster*: (1a) *y*, (2a) *w* sn^3, (3a) *y* w^{1118}; (4a) sn^{MR2}. The following crosses were performed to obtain *y* + +/+ + *sn*, *y w* +/+ + *sn* and *y* + +/+ *w sn* heterozygotes: (I) ♀ y^2, H^{+} × ♂ *sn v*, H^{+} /*Y* ; (II) ♀ y^2 *w* × ♂ *sn v wy*, $2H^{+}$ /*Y* (*D. simulans*); (III) ♀ *y* × ♂ *w* sn^3 ; (IV) ♀ *y* w^{1118} × ♂ sn^{MR2} (*D. melanogaster*).

Mutagenesis. To generate mosaic clones by somatic recombination, we used the following procedure. The F_1 48-hrs old larvae of the cross (III) were treated with 0.3 ml per vial 0.5% water solution of phosphemide (CAS # 882-58-6). Analogously, the larvae from the cross (IV) were treated