this area and that more collections are needed. Therefore, the present study aggregates a basis for future modeling, conservation efforts, and emphasizes the need for a broader sampling.

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Chronic exposure to tunicamycin during development has little effect upon the eyes of *GMR-Gal4 UAS-lacZ* males.

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Introduction

Endoplasmic reticulum (ER)-stress is caused by the intracellular accumulation of proteins and is implicated in several degenerative disease states (Boyce and Yuan, 2006; Haeri and Knox, 2012). Although characterized as a source of cellular damage, the ER-stress response to mild insult (ER pre-conditioning) has been demonstrated to be protective in *Drosophila* through an autophagy-dependent process (Fouillet *et al.,* 2012). In part, this response was achieved through acute exposure by feeding flies - for only four hours - with a medium containing tunicamycin, an antibiotic that inhibits glycosylation. Although this approach proved quite successful, a set of conditions that allow for chronic exposure to produce a continual level of protection or damage is very desirable.

Several avenues of research into ER-stress in *Drosophila* may depend upon the use of the *UAS/Gal4* system (Brand and Perrimon, 1993) to express various transgenes under conditions of stress. Our laboratory has characterized apoptosis-dependent developmental defects caused by *GMR-Gal4*^{12} (Kramer and Staveley, 2003; unpublished) under conditions of elevated temperatures and increased gene-dosage. As a result, we investigated the possibility that induction of ER-stress by tunicamycin might induce toxic effects when coupled with normally non-detrimental levels of *Gal4* expression controlling a standard *lacZ* transgene.

Materials and Methods

Drosophila *media*

The standard cornmeal-yeast-molasses-agar medium in our laboratory is made with 65 g/L cornmeal, 10 g/L nutritional yeast, and 5.5 g/L agar in water, heated to form a slurry, then cooked by autoclave @ 30 minutes under standard conditions for liquids. This is supplemented with 50 ml/L fancy grade molasses after cooking and with 5 ml of 0.1 g/ml methyl paraben (methyl 4-hydroxybenzoate from Sigma Life Science Research: *www.sigma.com*) in 95% ethanol and 2.5 ml of propionic acid when cooled to 55 to 60°C prior to decanting into standard plastic shell vials. Once

solidified, the medium is stored at 4 to 6°C and warmed to room temperature for use.

To induce an ER-stress response, tunicamycin (BioShop Canada Inc.: *www.bioshopcanada.com*) was dissolved in 95% ethanol to produce a 0.1 mg/ml stock solution and was added to the standard medium to the concentrations of 0.1 mg/L, 0.01 mg/L, and 0.001 mg/L just prior to decanting the media into vials.

Drosophila *stocks and culture*

The *GMR-Gal4*^{12} (Freeman, 1996) and *UAS-lacZ*$^{Bg4-1-2}$ (Brand and Perrimon, 1993) lines were obtained from the Bloomington Drosophila Stock Center at Indiana University. The *GMR-Gal4*^{12} line was originally selected from a collection of fifteen transgenic lines as one of two insertions that did not produce a "rough eye" phenotype as a heterozygote at 25°C but did drive the expression of *lacZ* behind the morphogenetic furrow (Freeman, 1996). Crosses between these parental lines are routinely used to express *lacZ* in the developing eye, in the "*Glass Multiple Reporter* (*GMR*)" pattern, as a benign control for the ectopic expression of transgenes of interest in the eye. As the *GMR-Gal4*^{12} transgene can produce a "rough eye" phenotype at 29°C (Kramer and Staveley, 2003), temperature must be well-controlled.

Due to poor success in preliminary crosses, a non-standard mating regimen was carried out: 1) three *GMR-Gal4* females and three *UAS-lacZ* males were initially mated upon standard media overnight and 2) then the adults were moved to the test vials (containing standard media supplemented with 0.1 mg/L, 0.01 mg/L, and 0.001 mg/L of tunicamycin plus control) for a period of six hours. To encourage oviposition, the vials were freshly "yeasted" with 5 to 10 grains of Fleischmann's "Instant Yeast" (*www.breadworld.com*) prior to introduction of the flies. Afterwards, the recovered mated adults were held on standard medium overnight and re-brooded twice. All incubations were carried out at 25°C.

Biometric analysis of the Drosophila *eye*

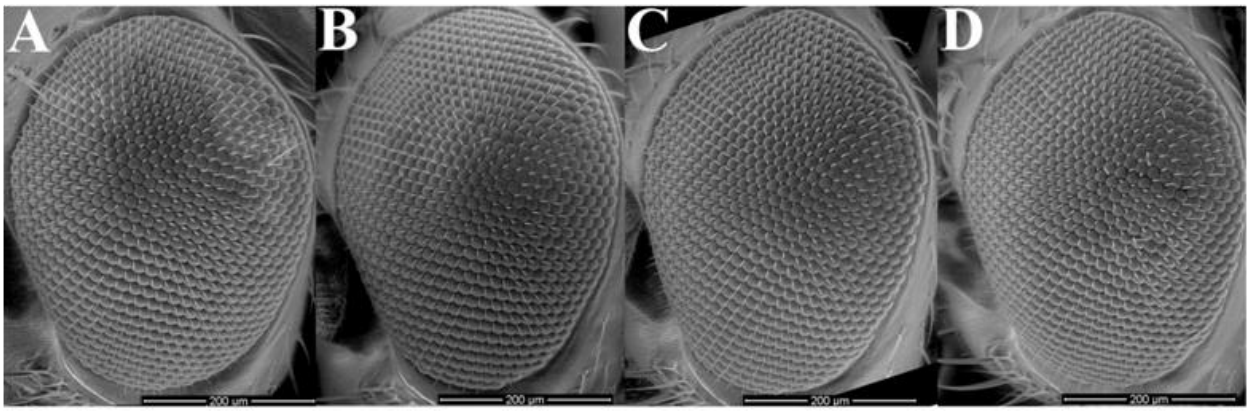
A cohort of critical class males were collected and aged for three to five days on the test medium at 25°C. They were frozen, stored at -80°C, and then mounted on aluminum studs and desiccated for at least 24 hours. Micrographs were taken with the FEI Quanta 400 Environmental SEM at a magnification of 543× and a horizontal field width of 550 μm. Micrographs were analyzed using NIH ImageJ software (Abramoff *et al.*, 2004). For each cross five images were analysed and the mean number of ommatidia and bristles were determined and standard error of the mean was calculated.

Results and Discussion

In *Drosophila melanogaster*, eye development is tightly controlled during the organization of the ommatidial array (reviewed by Cagan, 2009). Expression of the benign *lacZ* gene in the developing eye under the control the transgenic driver *GMR-Gal4* can act as control for the expression of genes of interest under any of a number of given circumstances. Here we demonstrate that our attempts to challenge flies to ER-stress via chronic exposure to tunicamycin does not alter eye development under these conditions very much.

As can be observed by analysis of scanning electron micrographs (Figure 1), the mean number of ommatidia was 724.2 (SEM = 6.47) and the mean number of interommatidial bristles was 599.4 (SEM = 9.99) when *GMR-Gal4/UAS-lacZ* males develop on our standard medium. Supplementation of the media with tunicamycin at the concentrations of 0.001 mg/L, 0.01 mg/L, or

0.1 mg/L does alter the number of ommatidia. However, there is a slight decrease in bristle number in the two treatments of 0.01 mg/L and 0.1 mg/L of tunicamycin. As a consequence, we conclude that experiments that utilise the *GMR-Gal4*^{12} transgene to drive expression in the eye will not be compromised greatly by interactions between tunicamycin and *Gal4*-induced toxicity.



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Tunicamycin (mg/L)	Ommatidia (n = 5)	Bristles (n = 5)
0 mg/L	724.2 (SEM 6.47)	599.4 (SEM 9.99)
0.001 mg/L	718.0 (SEM 4.56)	608.2 (SEM 7.50)
0.01 mg/L	719.6 (SEM 8.87)	561.4 (SEM 8.23)
0.1 mg/L	727.3 (SEM 6.92)	563.4 (SEM 12.4)

Figure 1. Tunicamycin does not greatly influence the eye development of *GMR-Gal4 UAS-lacZ* flies. Scanning electron micrographs of the eyes of *GMR-Gal4/UAS-lacZ* males that have developed upon (A) control medium or in the presence of tunicamycin at concentrations of (B) 0.001 mg/L, (C) 0.01 mg/L, and (D) 0.1 mg/L at 25°C. (E) The number of bristles reveals a very slight decrease at the two highest concentrations of tunicamycin. SEM = standard error of the mean.

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