For other studies, the use of dust is preferable, since it is less time-consuming and a more conventional marking technique for insects (Hagler and Jackson, 2001).

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Effect of Fly Nap® on ovipositing and fertility in *Basc* mutant and wild type *Drosophila melanogaster*.

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Abstract

The objective of this study is to determine whether wild and *Basc* mutant genotypes of *Drosophila melanogaster* treated with the anesthetic Fly Nap® displayed significant differences in their egg deposition and subsequent egg fertility when compared to etherized wild type and *Basc* mutants. Some mutant genotypes are more sensitive to environmental insult than are the wild type (Nguyen *et al.*, 1979). We were particularly curious to note any significant effects of treatment with Fly Nap® on the *Basc* mutant as compared to the wild genotype. Flies were kept in egg-laying chambers at 25ºC, and ovipositing was quantified for each treatment group by counting the number of eggs deposited on agar at post-treatment intervals of 16, 24, 40, 48, 64, and 72 hours. Ovipositing of *Basc* mutants exposed to Fly Nap® was significantly ($p < 0.05$) lower than etherized *Basc* during the first 24 hours post-treatment. After 40 hours the most significant ($p < 0.01$) difference appeared between *Basc* mutants and wild type flies both treated with Fly Nap®. Wild type flies treated with Fly Nap®, while initially displaying lower egg deposition than etherized wild flies, recovered to control levels after 64 hours. The ovipositing of *Basc* mutants treated with Fly Nap® remained significantly ($p < 0.01$) lower than that of similarly anesthetized wild types from 40 hours post-

treatment through the remainder of the post-treatment time course. Fertility was assessed by taking 25 eggs from each group at post-treatment intervals, then counting the number of flies that had fully eclosed as adults. This adult to egg ratio was expressed as percent viability, with Fly Nap®-treated *Basc* mutants showing significantly ($p < 0.01$) lower egg viability than wild type flies treated concurrently with Fly Nap®. These results indicate that ovipositing of the *Basc* mutant, while characteristically lower than the wild type, is even further reduced after exposure to Fly Nap® anesthetic, with an accompanying significant reduction in viability in offspring.

Introduction

Certain mutants of *Drosophila melanogaste*r have shown higher sensitivity to certain environmentally-introduced chemicals than wild-type strains. Delayed maturation, decreased fertility and longevity, and significantly reduced mating success are just some of the detrimental effects of such chemicals. It is especially problematic when this sensitivity is found to include certain anesthetics, which are commonly employed in laboratory and educational settings (Tinklenberg *et al*., 1991; Walcourt and Nash, 2000; Barron, 2000; Weber *et al*., 2009). These are often used for the simplicity and speed in which they render test subjects more easily manipulated (Greenspan, 1997). Such responses to anesthetic can significantly affect behavior to such a degree that test scoring of flies is questionable at best. The detrimental effects of carbon dioxide, chilling, and even rough handling (*e.g*., aspiration) are well documented (Van Kijken *et al*., 1977; Kaiser, 1995; Nilson *et al*., 2006).

During semester-long experiments in which undergraduate genetics students measured changes in gene frequencies among two populations of *Drosophila*, wild-type and *Basc* mutants, we observed a significant reduction in the stabilization frequency of the Bar-eyed mutant alleles after replacement of ether anesthetic with Fly Nap®. *Basc* gene frequencies consistently stabilized at between 0.32 and 0.34 when populations were anesthetized with ether, but dropped to 0.10 or lower after switching to Fly Nap®. While most anesthetics have been shown to cause a reversible loss of mobility and coordination in wild-type and mutant flies (Champion De Crespigny and Wedell, 2008), it is our hypothesis that Fly Nap® causes a more prolonged course of such deficits. In *Basc*, an outbred mutant, that is already known to have comparatively lower fitness (*i.e*., reduced fertility and viability) (Volkova and Vorobjova, 2005), such detrimental effects may prove irreversible.

The objective of this study is to measure ovipositing between two treatment groups. Each treatment group is composed of wild-type and *Basc* mutants. One group was anesthetized with ether and the other with Fly Nap®. A random sample of eggs deposited by each treatment group was sampled and the number of eclosed adults was used as an indicator of fertility and offspring viability. As both strains have been drawn from populations selected to respond to ether anesthetization, the ether-treated group is essentially functioning as a control group.

Materials and Methods

Treatment of Flies

Two samples were taken from each stock population of wild-type and *Basc* mutants, and each placed in 1 in. × 4 in. clear plastic vials. Each treatment group received one sample of wild-type and one sample of *Basc* mutant flies. The first treatment group was anesthetized with ether, and each sample strain sexed. Fifty females were removed from each treated strain and placed in a separate

plastic vial. Five males from each strain were also added to the appropriate vial of like females. The etherized wild-type were designated "ether wild" (EW) and the etherized *Basc* mutants were designated "ether *Basc*" (EB). The second treatment group was anesthetized with Fly Nap®, and each sample sexed and segregated similarly to the first group. Fly Nap®-anesthetized wild-type were designated "Nap wild" (NW) and *Basc* mutants designated "Nap *Basc*" (NB). Each vial of treated flies was inserted and mounted in an egg-laying chamber so that flies could remain isolated from chamber media until they recovered sufficient mobility to enter the chamber. The chambers were then kept at 25°C.

Counting of Eggs

Eggs were harvested from egg-laying chambers maintained at 25°C, by inserting a petri-dish containing nutrient agar tinted with blue food dye. The agar surface was "roughed" and a thin layer of yeast slurry applied down the center of the plate. Flies were allowed to oviposit on agar medium overnight. After 16 hours post-treatment, the oviposited agar was removed and a new petri-dish inserted. Flies were then allowed to oviposit for eight hours during the day. This "recharging" of the egg-laying chambers was carried out for a total of three days, with petri dishes collected and deposited eggs counted at 16, 24, 40, 48, 64, and 72 hours post-treatment.

Removal of Eggs for Viability Testing

At each time point indicated above, 25 eggs were randomly sampled from each agar plate using a metal probe and transferred to plastic vials containing standard cornmeal food agar. Vials were then kept at 25°C. Eggs were allowed to develop until they eclosed after eight to ten days. Only eggs that began their developmental cycle (*i.e.*, developed into instar larvae) were scored as fertile, and all eclosed adult flies were scored and viability expressed as the percentage of full-term adults (including moribund) arising from the original 25 eggs seeded per vial.

Results

Etherized wild-type deposited significantly *($p < 0.05$) more eggs during initial 16 hours post-treatment (Figure 1). There remained significantly **($p < 0.01$) lower egg deposition between Fly Nap®-treated and etherized flies throughout the 48 hour post-treatment interval. Significant ($p < 0.05$) differences remained between etherized wild-type and *Basc* during this period, as well. At 64 hours, differences between wild-type treated with Fly Nap® and ether controls were no longer observed, while Fly Nap®-treated *Basc* deposition remained significantly **($p < 0.01$) lower than for both etherized strains. At 72 hours post-treatment, Fly Nap®-treated *Basc* ovipositing remained significantly *($p < 0.05$) lower than all other treatment conditions.

Offspring viability was quantified as the number of fully-developed adult flies arising from 25 eggs randomly sampled from each treatment condition and expressed as percent viability. *Basc* mutants from both treatment groups exhibited significantly *($p < 0.05$) lower offspring viability than wild-type (Figure 2).

Discussion

In this study, we provide evidence to support the hypothesis that anesthetization with Fly Nap® causes significant differences in post-treatment behaviors, such as ovipositing, and that these changes are more prolonged in the already fitness-stressed *Basc* mutant. Ovipositing in flies treated

with Fly Nap® is reduced by 79% (avg.) in wild-type and 92.4% (avg.) in *Basc* for up to 48 hours post-treatment, when compared with ether controls. After 72 hours post-treatment, ovipositing of wild-type treated with Fly Nap® recovered to within 16% of etherized wild-type. Fly Nap®-treated *Basc* ovipositing remains markedly lower (3.8% of etherized *Basc*) than all other treatment groups.

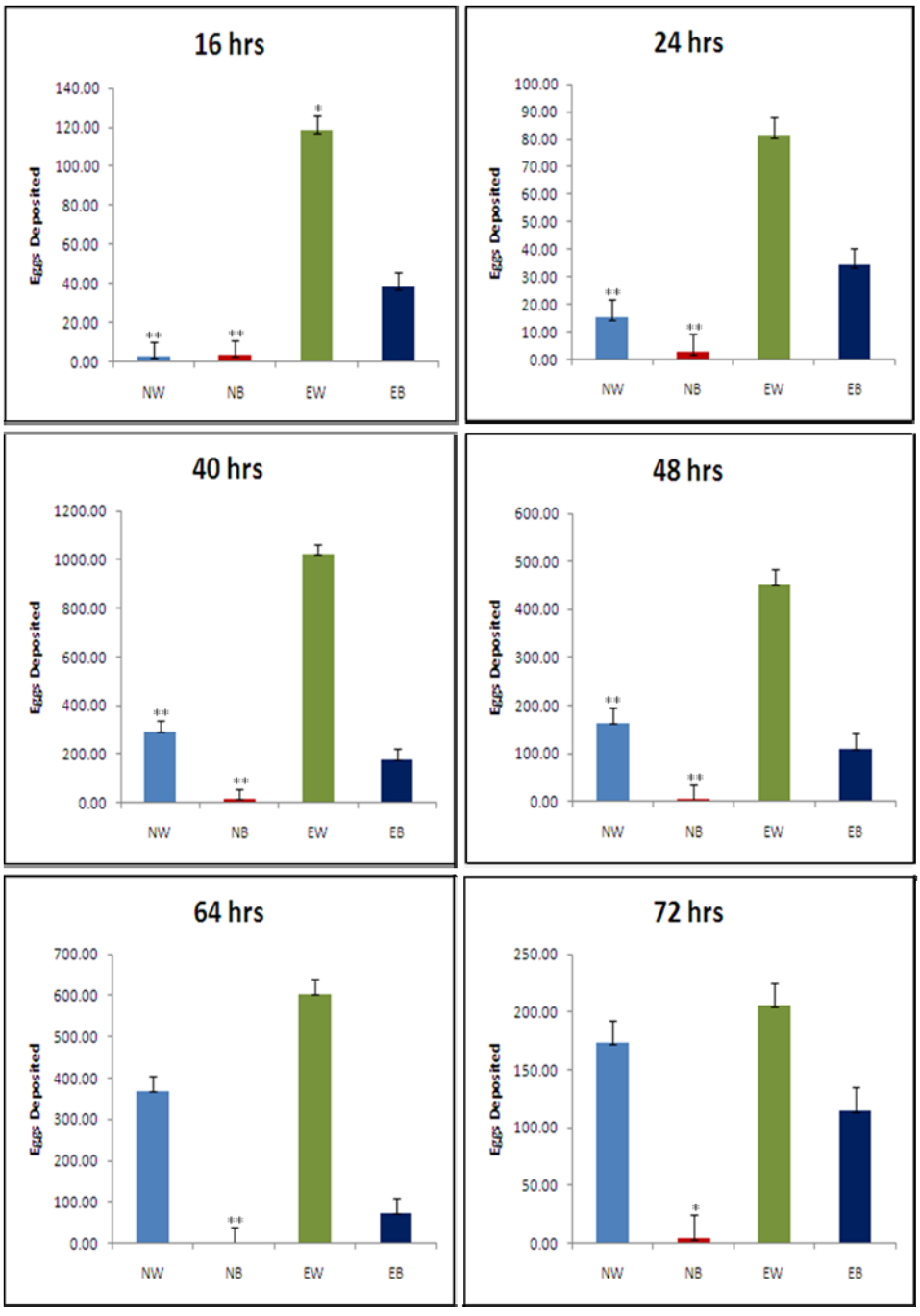

Figure 1. Effects on egg deposition of wild-type and *Basc* mutants anesthetized with Fly Nap® (NW and NB) or ether (EW and EB) at 16, 24, 40, 48, 64, and 72 hour intervals.

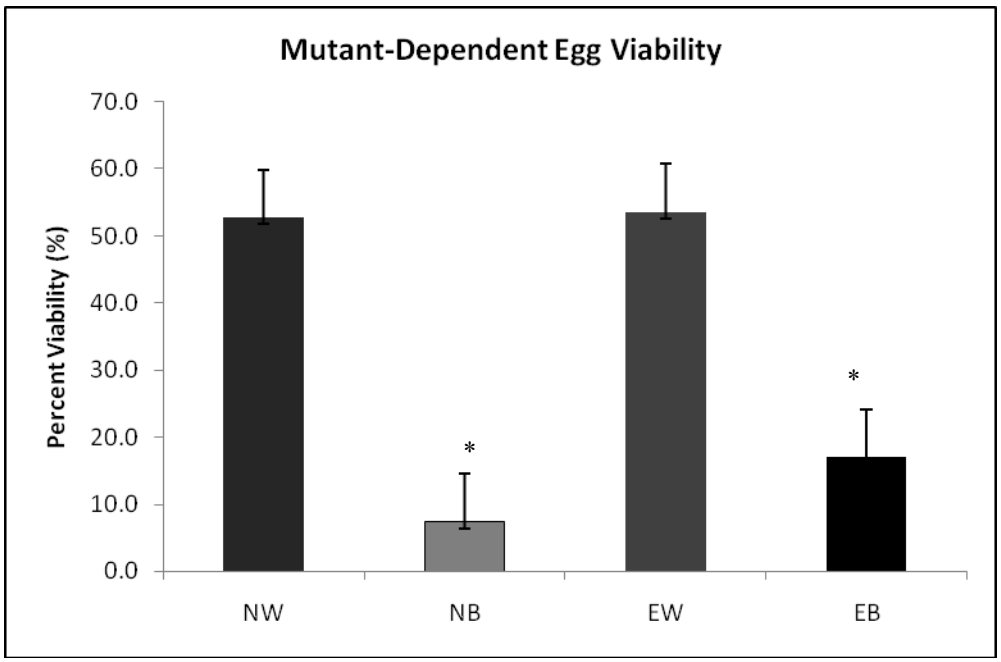

Figure 2. Viability of offspring from wild-type and *Basc* mutants treated with either Fly Nap® or ether anesthetic.

While the level of detrimental effects of Fly Nap® on post-treatment viability of *Basc* offspring remains unclear, mutants from both treatment groups displayed significantly lower ($p < 0.05$) viability than wild-type from either group. These results suggest that treatment with Fly Nap® results in a significant ($p < 0.05$) reduction in egg-laying in both wild-type and *Basc* mutant groups, and that the *Basc* mutants are more highly sensitive to such pertubations from which they fail to recover.

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Notes regarding the collection of African *Drosophila melanogaster.*

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