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**Effect of *hsp83* activation on cell death as quantified using phenotypic variation of Bar eye in *Drosophila melanogaster*.**

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Quantifying the phenotypic variation in a trait is a sensitive way to assess the role a genetic or environmental factor has on a targeted developmental process. Thompson *et al.* (2009) used this approach to evaluate heat shock effects on cell death. Scanning electron micrographs allowed us to measure phenotypic changes very precisely. The current study draws upon that same experimental design to measure the influence of heat shock protein 83 (*hsp83*) on the expression of cell death in the *Drosophila* eye.

The Experimental Genetics and Cell Biology Lab (ZOO 4970) course used this genetic system to study the effect of an *hsp*-defective allele on cell death under normal room temperature (control) or heat shock (37°C) conditions. The hypothesis was that a significant increase in cell death would occur in the *hsp*-defective genotype when stressed by elevated temperature. The normal heat shock system, represented by a dominantly-marked balancer chromosome, would have significantly reduced cell death.

The *hsp83* mutation was balanced over the dominant Tubby, which causes a shortening of the body (Bloomington Stock Center #5696, w\*; *Hsp83*<sup>e6D</sup>/TM6B, *Tb*<sup>1</sup>). By crossing Bar females with males from this stock, F1 flies that are either *hsp83* or Tubby are easily distinguishable as 3<sup>rd</sup> instar larvae. There were four treatments: third instar larvae (*hsp83* or Tubby) treated for 40 minutes at 37°C and the same two genotypes raised at room temperature. The 37°C exposure activates the heat shock activity, although it is defective in the *hsp83* strain.

Treatment was done by selecting F1 larvae of each genotype and placing them in 1.5 ml microfuge tubes containing 0.5 ml of yeast-glucose medium. These were then placed in baggies and either submerged in a 37°C water bath for 40 minutes or left on the lab bench for the same period. Tubes were then uncapped and inserted into a normal food tube, where the flies were allowed to pupate and eclose. Heads were removed from Bar-eyed males eclosing from each of the four conditions. In addition to touring the Electron Microscope Facility and developing this experimental plan, the students benefitted from the microdissection practice needed for successful mounting of

heads on SEM plugs, a process that requires care but which can be learned quickly. Each heads was cut in half with a razor blade so the eyes could be mounted flat for ready visualization in the SEM. Preparations were dried and sputter-coated as described in Thompson *et al.* (2009). Eyes were viewed and photographed with a Zeiss DSM-960A scanning electron microscope. This yields images that allow very accurate counting of facet number (Figure 1).

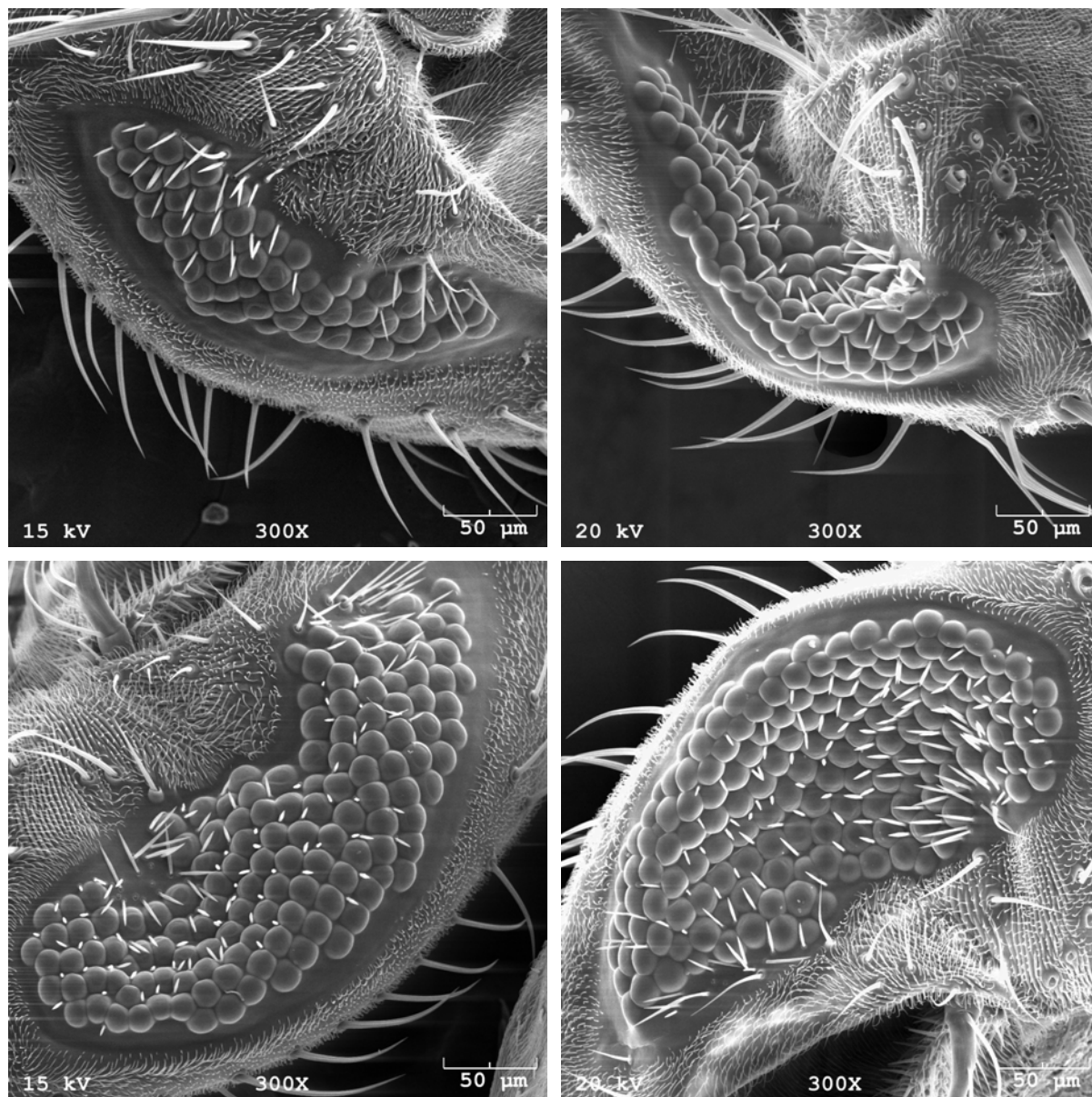


Figure 1. Bar eyes from four different genotypes and treatments: Top left, *hsp83* mutant at 37°C; bottom left, control at 37°C; top right, *hsp83* mutant at room temperature; bottom right, control at room temperature.

Representative eyes of each genotype and treatment are shown in Figure 1. The challenge posed by shape of some eyes is shown in Figure 2. Although replicate numbers differed slightly due to genotype survival, the main source of sample variation was in the successful mounting of heads

and eyes. Often it is difficult to know if a good quality image can be produced until the plug is dried, sputter-coated, and examined in the SEM. Since this was a classroom experiment with defined time available, the opportunity to repeat treatments was limited. But the project still showed the students the steps that would be needed to produce a larger dataset and provided an estimate of the data yield for one treatment cycle.

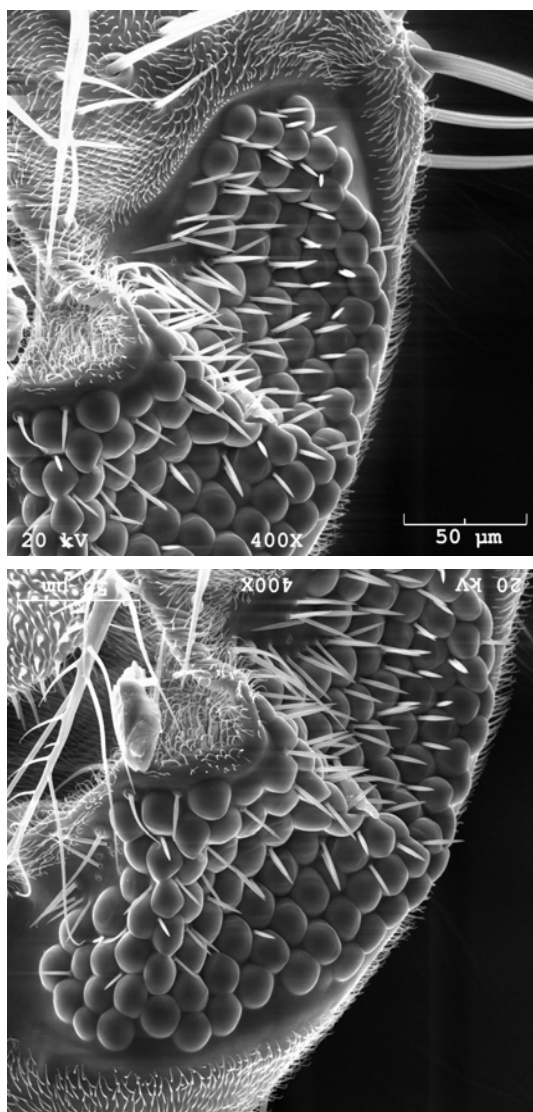


Figure 2. Bar eye showing the challenge to accurate counts caused by folds in the eye surface that can occur during specimen drying or microdissection. Note that the lower image is rotated 180°.

For 37°C treatments and averaging over the replicated counts by eight students, there were significantly fewer eye facets in the Bar-eyed flies with *hsp83* background genotypes ( $84.47 \pm 1.46$ ) than with normal *hsp83* activity ( $116.64 \pm 3.67$ ). From five replication counts of the genotypes raised at room temperature, Bar-eyed flies with *hsp83* background genotypes had fewer facets ( $84.14 \pm 0.30$ ) than did those with normal *hsp83* activity ( $146.24 \pm 1.05$ ). These differences can be evaluated more in-depth with a larger number of samples and an appropriate ANOVA, but this study at least points to an initial conclusion. Cell death in the Bar eye of *Drosophila* is greatest when *hsp83* activity is defective. Even when “activated” at 37°C, the phenotype is the same for *hsp83* deficient samples.

Taking both eyes from most heads also allowed us to measure developmental homeostasis as reflected in symmetry, or more precisely the deviations from symmetry (fluctuating asymmetry (FA) =  $|L - R| / (L + R)/2$ ). In this case, the data do not show a significant trend (FA: 37°C treatments for 7 heads, *hsp83* =  $0.114 \pm 0.078$ ; control for 6 heads =  $0.090 \pm 0.087$ ; room temperature treatments, *hsp83* for 9 heads =  $0.062 \pm 0.049$ ; control sample too small). The sample sizes are clearly too small for any conclusion, but the observations so far can help students consider new alternative hypotheses.

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