sample preparation and scanning electron microscopy, (b) capture digital images using the software, (c) process the image using the Photoshop or imaging software, and (d) develop a series of image portfolios to present their results.

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Heat shock effects upon cell death in *Bar* eye quantified by scanning electron microscopy.

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Bar (*B*) is a well-known sex-linked dominant mutation that arose spontaneously in *Drosophila melanogaster* as a tandem duplication in cytological location 16A1-2 (Tice, 1914; Lindsley and Zimm, 1992). The vertical bar-eye phenotype is due to cell death, especially in the anterior region of the eye disc (Fristrom, 1969), or disruptions in the pattern of mitosis. But the extent of cell death can be influenced genetically (*e.g.*, variegated position effect; Brosseau, 1960) and by environmental conditions like temperature (*e.g.*, developmental temperature and log facet number are inversely proportional; Hersh, 1930) and chemical treatments (*e.g.*, being raised on media supplemented with acetamide, lactamide, cytosine, and other chemicals; Fristrom, 1972; and references in Lindsley and Zimm, 1992). Given its sensitivity to modifying factors, the severity of *Bar* eye cell death can be a model for quantifying experimental influences on development. But for this model system to be sensitive enough to detect comparatively small effects, eye facet (ommatidium) number must be measured very accurately. In spring 2009, the Experimental Genetics and Cell Biology Lab course taught in the Department of Zoology at the University of Oklahoma undertook to test experimental design options and the feasibility of using scanning electron microscopy of *Drosophila Bar* eyes to evaluate the effect on cell death by an experimental treatment, exposure to heat shock that activates chaperone proteins of the stress response. Additional data were

collected and analyzed later in the year. The system worked very well and can be modified to allow quantification of an almost unlimited array of physical and chemical treatments that might influence cell death or mitotic cycles during development.

The choice of a brief exposure to high temperature to activate heat shock proteins fitted this experiment into our on-going interest in genotype × stress interactions and allowed the class to design an original experiment after gaining basic information about *Drosophila* breeding programs and cellular stress responses. The initial hypothesis was that a brief heat shock exposure of third instar *Drosophila* larvae would reduce cell death in *Bar* eyes by elevating the protection of precursor cells during eye formation. The heat shock response increases the activity of heat shock proteins, which include chaperone proteins that help repair protein damage as part of the organism's stress response pathways. Chaperone proteins help refold proteins damaged by environmental stress conditions to improve cellular function, although they have also been linked to cell death (Gething, 1997).

Experiments were done with the *Basc* strain of *Drosophila melanogaster* (In(1)sc^{S1L} sc^{8R} + S, a balancer chromosome with multiple inversions carrying *white-apricot*, w^a, and *Bar*, *B*; Lindsley and Grell, 1992). Third instar larvae were collected from culture bottles raised on standard cornmeal medium at 25 ± 1°C. Groups of ten larvae were placed in 2 ml microfuge tubes containing about 0.5 ml of medium to maintain humidity. Tubes for heat shock were then submerged in the wells of a tempblock heater at 37°C for 40 minutes (Ashburner, 1989). Tubes for control samples remained on the lab bench at room temperature for the same period. Caps were then removed from each microfuge tube, and open tubes were inserted into 8 dram shell vials containing standard cornmeal medium. The third instar larvae could emerge from the small microfuge tube and pupate in the larger space from which adults were collected the following week.

Specimen preparation was simplified by the fact that *Drosophila* compound eyes will desiccate for scanning electron microscope (SEM) examination without the need for alcohol dehydration or critical point drying. Although minor deformations sometimes occur, their severity is generally small enough to counter the cost of the added time required for more sophisticated preparation when the data to be gathered are as simple as in this experiment. First, a head is dissected from the body with a single-edge razor blade, and the head is bisected near the midline between the compound eyes. Each half is then transferred with sharp forceps onto double-sided adhesive surface cut to fit an aluminum specimen plug (the size of the plug is determined by the specific SEM microscope that will be used). The two head halves are placed very near each other to facilitate identification of pairs. But no attempt was made to keep track of which was the left and which was the right hand side, since we did not plan to explore sidedness biases, if any. A small spot of silver paste helps ground each specimen to the plug surface. After drying in a desiccator for several days, plugs were sputter-coated with gold-palladium in a Hummer 6 sputter coater. Specimens were viewed with the Zeiss DSM-960A SEM at 10 kV, and digital images were taken at 350×. Sample images are shown in Figure 1. Eye facet counts were made directly from the digital images.

As expected with images of this quality, the variation due to repeatability error of counts among students was very low (average standard deviation among eight replicate counts of a representative sample of 33 images = 0.79; average number of facets for these 33 eyes = 73.96; repeatability factor from ANOVA is not significant). Some sources of variation are illustrated in Figure 2, which shows two eyes that have minor distortions due to processing or eye development. If an eye was damaged during dissection or mounting so that counts of facets were ambiguous, it was omitted from the data set. The remaining single eyes contributed to the data about average expression in treated *versus* control conditions, but they were not used in measures of symmetry (FA, fluctuating asymmetry).

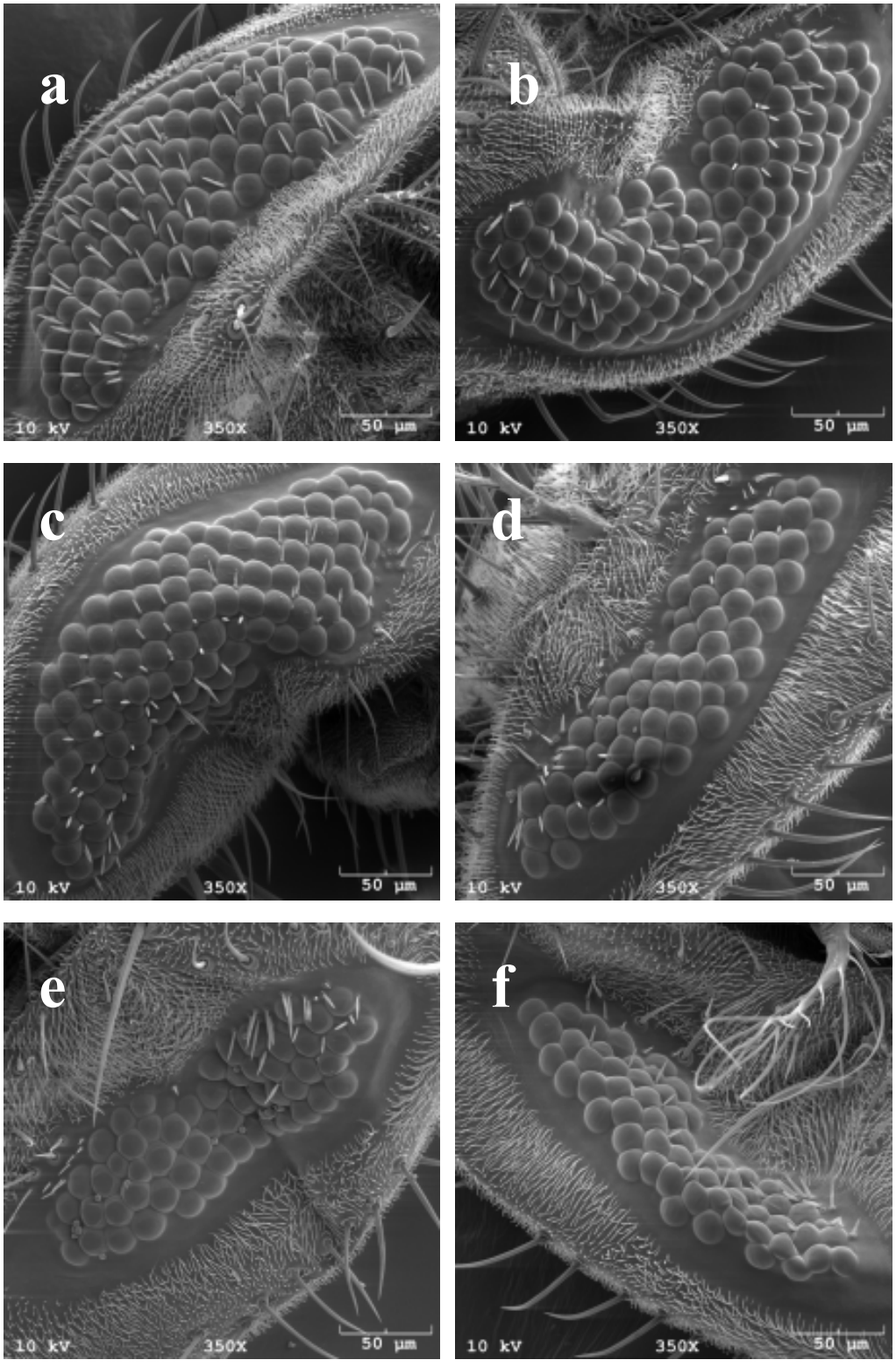

Figure 1. SEM images of *Bar* eye. a, b, Control left and right of the same fly showing deviations from symmetry; c, Control, compared to d, Heat Shock, to illustrate differences in facet number; f, g, Heat Shock to illustrate variation in facet number and appearance. All images are 350×.

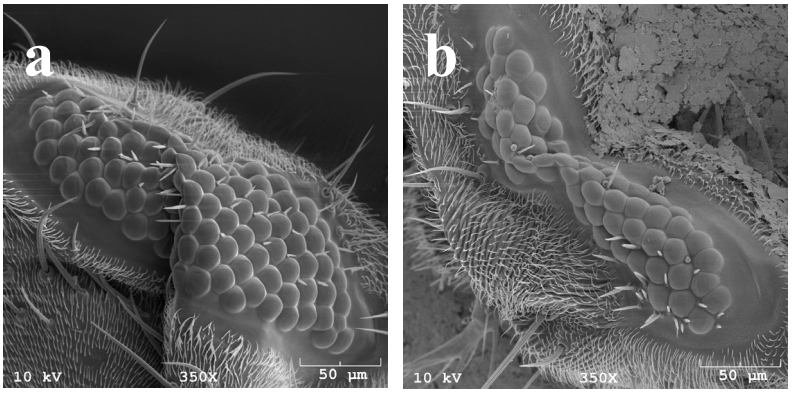

Figure 2. *Bar* eye images to illustrate some of the complexities in counting facet numbers accurately. a, slight fold in eye after processing; b, folding of tissue due to developmental variation or processing of sample.

Data from 24 control and 45 heat-shock treated eyes show that a 40-minute heat shock causes a significant reduction in the number of facets (control, mean = 101.79 ± 18.88; heat shock, mean = 77.78 ± 21.29; from t-test, $P < 0.001$). This is the opposite of the prediction from our initial hypothesis. Although we know that increased temperature during development is correlated with a reduction in eye facet number in *Bar* (Hersh, 1930), we were surprised that this effect can be traced to just a 40-minute exposure to high temperature during an approximately 10 day developmental life cycle.

Fluctuating asymmetry (FA), a measure of symmetrical development, can be interpreted as reflecting developmental stability (Markow, 1994; Møller and Swaddle, 1997; Polak, 2003). Unregulated responses to environmental perturbations are expected to increase asymmetry. In our experiment, we predicted that activating an elevated stress response should improve developmental regulation and make expression, *i.e.*, eye facet number in this case, more similar within matched pairs of compound eyes. One way to quantify symmetrical expression is:

$$FA = |L - R| / [(L + R) \times 0.5],$$

which scales the difference between the two sides of the trait by their average. Although there is a lot of variation in facet number among eyes, the degree of symmetry is surprisingly high (Figure 3; slope = 0.75). The pooled data from control and heat-shock samples yielded a correlation coefficient between eyes of 0.84 ($r^2 = 0.71$).

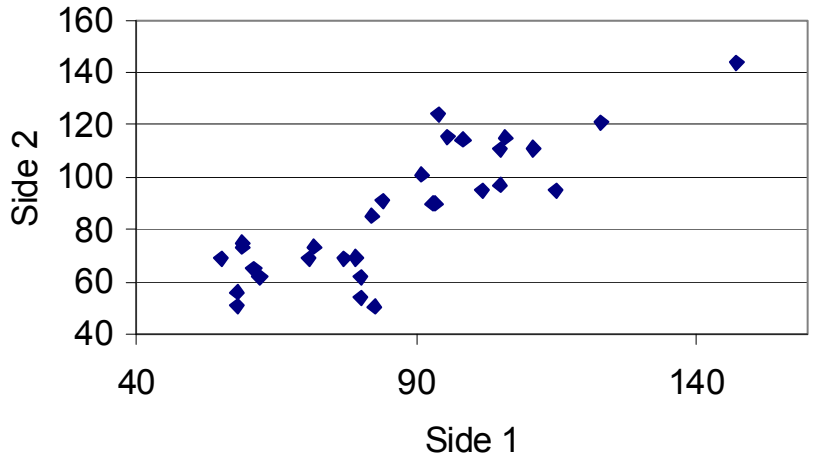

Figure 3. Symmetry of facet number of the two sides of *Bar* eye, combined control and heat shock.

But when two treatments are considered separately, the average FA is slightly higher after heat shock ($FA_{heat\ shock} = 0.1490 \pm 0.1375$, n = 19 pairs; $FA_{control} = 0.0833 \pm 0.0544$, n = 11 pairs), but the difference is not significant ($t = 2.20$, $P = 0.14$).

Heat shock proteins influence cell death (apoptotic) pathways and can either prevent or increase cell death (Fristrom, 1972). Heat shock protein 60D, for example, is involved in ommatidial apoptosis in *Drosophila* (Arya *et al*., 2007). Our data show a significant effect of heat shock on eye facet number that seems hard to account for as a simple developmental temperature influence. A more reasonable hypothesis is that the heat-shock-induced chaperone proteins assist in the maintenance of proteins involved in cell death promotion cascades. Alternatively, Vazquez *et al*. (1993) reported an overall reduction in transcription in *Drosophila* cells following heat shock for 15 minutes at 37ºC. Core histone protein transcription was suppressed at severe temperature exposure, and H1 histone and the 5C actin gene transcription was suppressed at all heat shock temperatures. RNA polymerase II, which accounts for up to 75% of all transcriptional activity in these cells, showed a five-fold reduction in activity after 37ºC heat shock. This suggests that there is priority given to proper packaging and protection of the cell's DNA. Furthermore, Westwood and Steinhardt (1989) showed that protein degradation rates increase in heat shocked *Drosophila* cells, primarily through the ubiquitone signaling pathway. Thus, the heat shock environment coincides with a number of cellularly debilitating activities that can retard overall cell proliferation and growth while the chaperone proteins and other elements of the stress response work to repair and protect protein function and keep the cell alive. In that light, the trend observed in our data encourages further exploration that can form the basis of future student-designed experiments using the *Bar* eye experimental model.

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