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Developmental homeostasis reflected in symmetry of cell death in the *Bar* eye of *Drosophila*.

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One of our recent interests is the identification of genetic modifiers of cell death in compound eye facets using the sequenced strains of *Drosophila* developed by Mackay and her colleagues (Mackay *et al*., 2012; DGRP strains available from the Bloomington *Drosophila* Stock Center; for earlier pilot data see Thompson *et al*., 2015). But these experiments also provide an opportunity to explore a somewhat unrelated phenomenon: the degree of symmetry in the extent of developmentally-patterned cell death. Using *Drosophila* eyes carrying the mutation *Bar*, deviations from symmetry are a potential measure of developmental homeostasis – the compensations required to generate a symmetrically bilateral body plan. Cell death in *Bar* eyes is clearly variable, with phenotypes in our experiments ranging from less than 50 to over 300 facets per eye. Our hypothesis is that, in spite of this tendency to vary, there is developmental coordination within an individual that tends toward symmetrical expression in facet number.

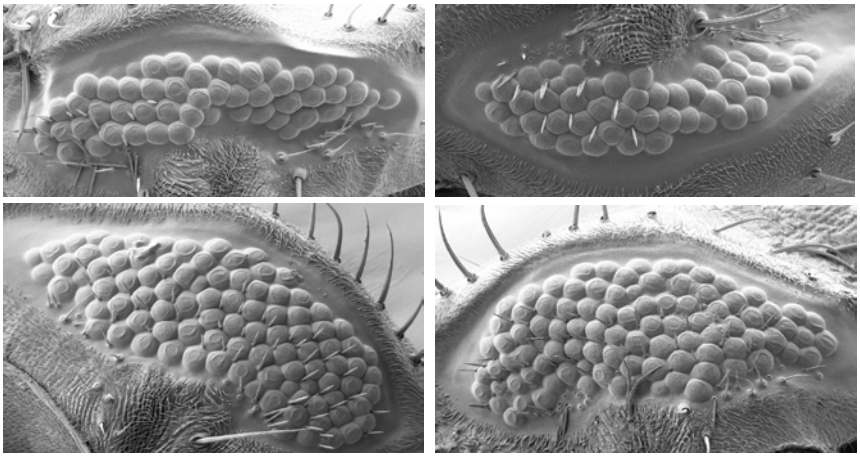

Figure 1. Representative pairs of *Bar* eyes from an F_1 male from #25745 (top row) and a male from # 25185 (lower row).

We have chosen two DGRP strains that yield quite different numbers of facets when heterozygous in males carrying the sex-linked mutation *Bar*. The average facet number from strain #25745 is 71 ± 13 (n = 80), while for #25185 it is 169 ± 44 (n = 62). We are able to get such precise facet numbers by using the Zeiss NEON 40EsB scanning electron microscope to image individual eyes (Figure 1). Inbred females carrying the balancer *Basc* with the dominant sex-linked *Bar* eye mutation were mated to males from each of two of the DGRP lines that had shown quite different F_1 facet numbers in earlier crosses. F_1 males carry the *Bar* mutation and are heterozygous for eye facet number modifiers from a sequenced DGRP line. Individual heads were removed with a razor blade and bisected between the eyes. Pairs of eyes were mounted on SEM plugs, air dried for several days, and then coated with gold-palladium in a Hummer 6 sputter coater. High resolution images were taken of each eye at an average magnification of 350×. Facet numbers were then counted in duplicate by several participants. This is clearly excessive replication, but, for this step in our project, it allowed each participant to become directly involved

in measurements and have their results compared to those of others. Variation associated with individual counters was not significant, and the analyses presented here were done on the average of each eye's counts.

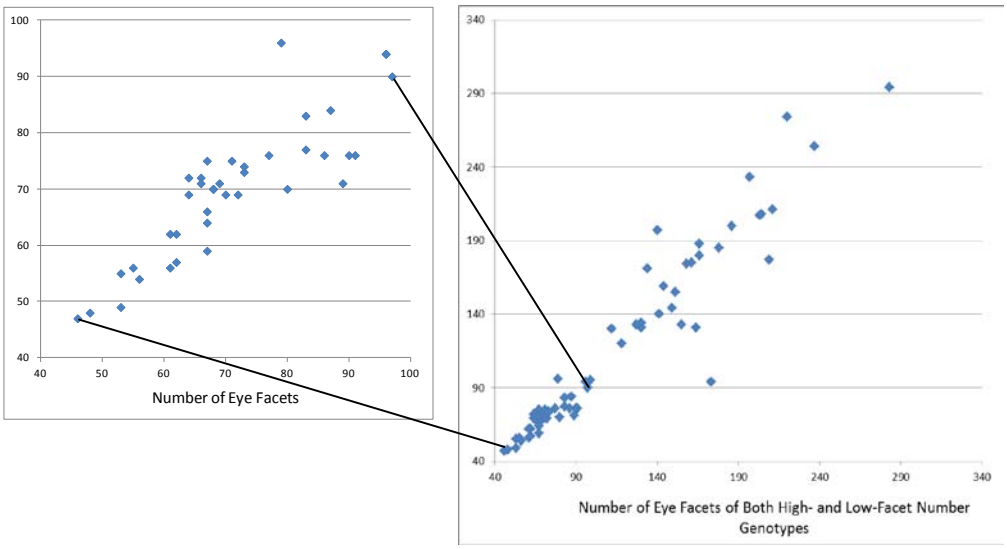

Figure 2. Facet numbers from matings with strain #25745 (left graph), showing how symmetrical the expression is, even over a range of phenotypes in which facet number approximately doubles. The graph at the right shows data from both genetic backgrounds, with the lines indicating where the first set of data are located on the second graph.

Even though there was the expected difference in the degree of cell death expressed by the two heterozygous sequenced genotypes, the symmetry of expression was remarkable. The pairs of eyes in Figure 1 are representative. To quantify this more clearly, Figure 2 plots the two eyes (randomly as "left" and "right"). The tightness of fit to a slope of 1.0 (actually, 1.06 in these data) for this wide range of facet numbers is due to close symmetry of cell death levels in individual pairs of eyes. Within-fly developmental homeostasis for cell death occurs between sides of the fly. This is perhaps especially surprising, since many key aspects of Dipteran development show cell autonomy, as seen for example in the distinct sexual dimorphism in male-female mosaics, *i.e.*, gynandromorphs.

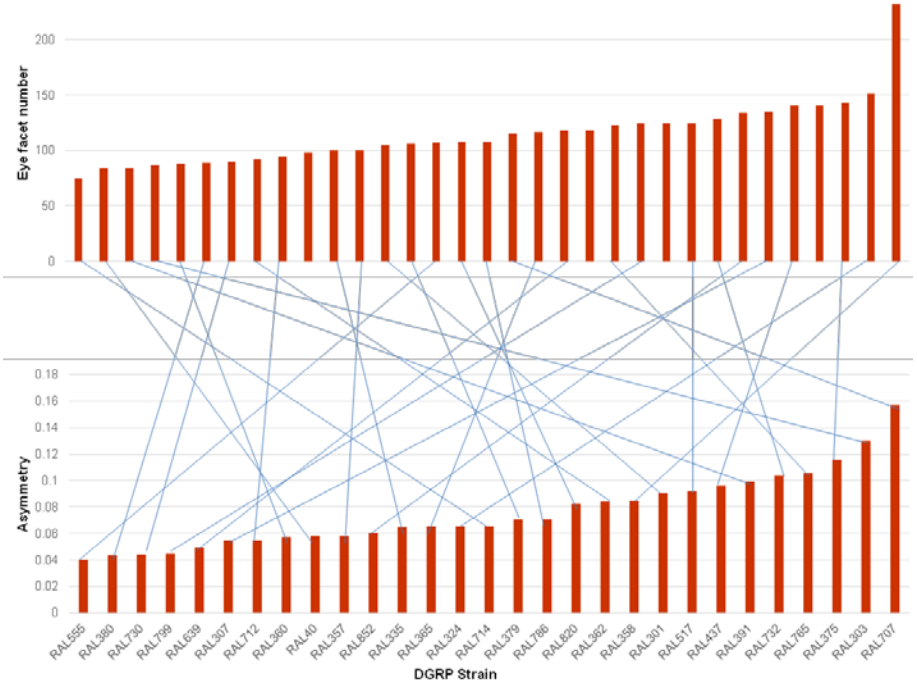

Figure 3. Lack of correlation between DGRP backgrounds affecting *Bar* eye facet number (top) and fluctuating asymmetry (FA) below.

One early prediction was that the degree of symmetry would be inversely correlated with the number of facets. There would be more randomness in eye facet counts as the number of facets increased. Symmetry might happen by chance if the number of facets is low, but symmetry would decline if facet number was higher. This is a reasonable prediction given the fact that, even within the same genotype, there is fly-to-fly variation in *Bar* gene expression. Indeed, there is some hint of that in these data, with more spread as facet number increases (Figure 3). But, overall, these data show that symmetry is not directly correlated with facet number when fluctuating asymmetry (FA) is compared to average facet number for a large series of DGRP F_1 data sets. To quantify deviations from symmetry within each strain background, we calculated fluctuating asymmetry (FA) = |one side – the other side|/(the sum of the two sides * 0.5). Developmental homeostasis, reflected in a high level of cell death bilateral symmetry, must be a process that over-rides the cell autonomous expression seen in other aspects of *Drosophila* development.

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