Conclusions

Strains established in this project are highly recommended for developing similar projects. As those combinations are unusual, students will not be able to easily find expected results online, which contributes to the development of their own observation, data collection, and analysis, and awakens their curiosity, which may increase their interest in the challenging scientific activities.

Acknowledgments: We are grateful to the basic Genetics team and enrolled freshmen in 2012, 2013, and 2014, which included 369 Biological Sciences students, four additional Professors (Drs. L. Mori, L.E.S. Netto, D.S. Sheepmaker, and T.T. Torres), monitor students of 2012 (C.R.R. Arantes, B.L.B. Argiona, V.Q. Pretti, and S.C. Vaz), 2013 (J.A. Bachur, C.S. Faria, and P. Perre), and 2014 (C.I.S. Costa, C. Kaid, I.L. Teixeira, and T.I. Yassumoto), two technicians (C.E. Lopes and F. Flauzino) for numerous reasons, Dr. Gerhard Bächli for gently sending us a copy of Mentzel (1684)'s paper, and Dr. Maxi Polihronakis Richmond for kindly reviewing the English version.

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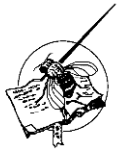

Using DGRP sequenced genomes to map heterozygous modifier effects on cell death in *Bar* eye of *Drosophila.*

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The *Drosophila* Genetic Reference Panel (DGRP) lines developed by Trudy MacKay and her colleagues (Mackay *et al*., 2012) offer a powerful resource for analyzing multi-gene influences on development, behavior, and physiology of *Drosophila melanogaster*. Rather than trying to isolate genes that influence a trait of interest using chromosomal substitutions, recombination mapping, or other approach, mapping of relevant loci begins with known genomes. By correlating specific trait expressions with the extensive database of SNPs for each sequenced line in the DGRP set, regions of the genome that consistently associate with a targeted phenotypic expression can be identified and explored in additional detail. But many of the traits our group is interested in studying require an additional element. We want to know about genes that act as modifiers of a mutation's expression, such as wing vein length mutations like *plexus*, with extra vein fragments, and *veinlet* with wing vein gaps in *Drosophila* (*e.g*., Thompson, 1974, 1975a, 1975b). A

major mutation exposes a variable range of expression due to secondary modifier loci in the genetic background. These modifier loci must be directly or indirectly relevant to the targeted pathway of the major mutation.

But one cannot introgress a targeted mutation into a sequenced strain without destroying the sequenced background by recombination and segregation. Some experimental designs may benefit from that approach. But drawing upon the exceptional resource offered by the DGRP lines, there is a middle road. In this pilot study, we test a limited set of the more than 200 currently available DGRP sequenced lines to assess their heterozygous modifier effects on mutant expression of *Bar*, a dominant cell death eye mutation (small duplication) with variable expression. Specifically, we are screening for genomic regions that influence cell death by measuring the number of eye facets in *Bar* eyes on different heterozygous sequenced genome backgrounds. If successful, the next phase will be to explore whether the same quantitative modifier loci are involved in phenotypic expression of other cell death-related traits such as mutations that cause notches in *Drosophila* wings.

This pilot experiment used five of the DGRP lines, obtained from the Bloomington *Drosophila* Stock Center (# 25174, 25175, 25177, 25179, and 25180). Virgin females were collected from the *Basc* strain, which carries *Bar*, white-apricot, and scute mutations along with inversions that make it an effective X-chromosome balancer. *Basc* females were mated to males from a DGRP strain yielding F1 males that carried *Bar* and were heterozygous for one of the sequenced genomes. Heads were removed and bisected so the eyes pointed up when mounted on an electron microscope plug. Samples were prepared using the protocol in Thompson *et al*. (2009) and were viewed and photographed with a Zeiss Neon 40EsB electron microscope. The number of successful mounts varied somewhat from one group to another. Future experiments will attempt to standardize the data sample size.

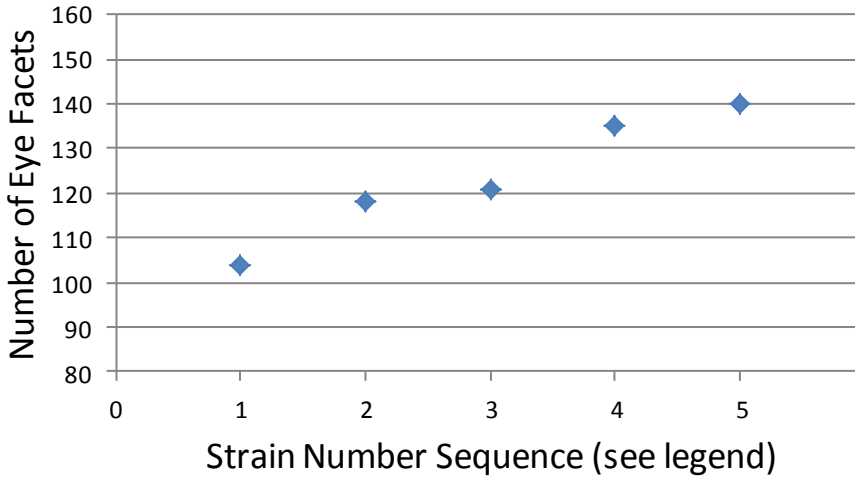

Figure 1. Average facet counts for five representative DGRP strains heterozygous for *Bar* (standard deviations are too small to show). 1, strain 25175; 2, strain 25177; 3, strain 25180; 4, strain 25179; 5, strain 25174.

Perhaps surprisingly (or luckily), even just these five representative sequenced genomes demonstrated significantly different influences on cell death in the *Drosophila* eye (Figure 1). For a sample subset of these data, photographs were scored by up to 15 students, and the replicate variation associated with repeatability among researchers was not significant. Differences were typically only one or two facets in an eye having perhaps 100. Against this "replicate repeatability", strain effects were easily identifiable (Figure 2; for a comparison of the two extremes in this small sample, mean ± sd for strain 25175 is 105.4 ± 17.4, n = 7; for strain 25174, 141.0 ± 7.7, n = 15, facets per eye). It is clear that even this small sample of DGRP genomes carries cell death modifiers that differ in their effects on *Bar* as heterozygotes.

A future addition to the analysis will be a measure of fluctuating symmetry (FA) when sufficient data are available from both eyes of an individual. FA is a standardized difference between the left and right sides of a trait that is expected to be symmetrical and for which deviations can be interpreted in terms of developmental stress. This will yield an insight into developmental homeostasis influencing cell death expression.

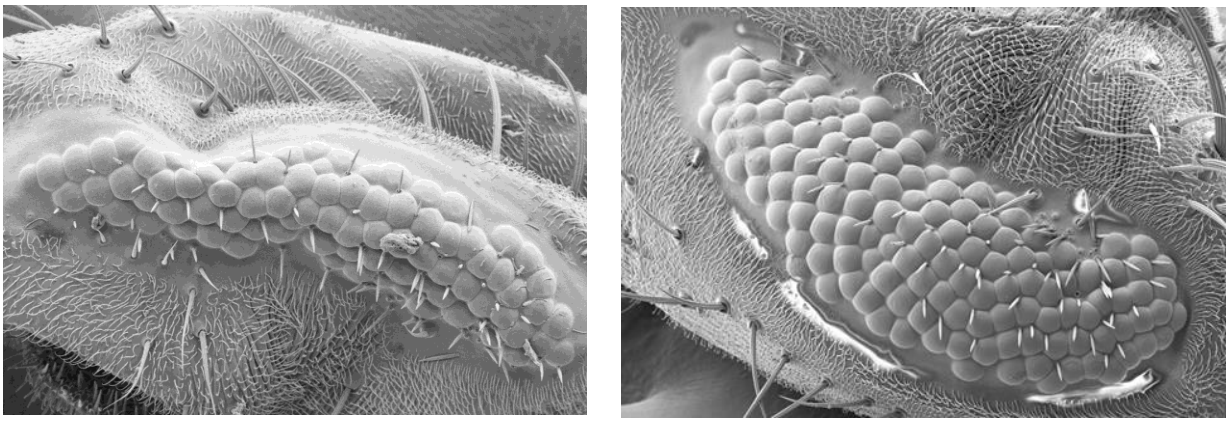

Figure 2. Representative images of *Bar* eyes heterozygous for a sequenced DGRP strain genotype: left, # 25175; right, # 25174.

Acknowledgments: This pilot study was done as an experiment designed by students in an advanced Biology lab course and was made possible through the advice and electron microscope supervision of Preston Larson and Gregory Strout, Samuel Roberts Noble Microscopy Laboratory at the University of Oklahoma.

References: Mackay, T.F.C., *et al*., 2012, Nature 482: 173-178; Thompson, J.N., jr., 1974, Heredity 33: 389-401; Thompson, J.N., jr., 1975a, Nature 258: 665-668; Thompson, J.N., jr., 1975b, Genetics 81: 387-402; Thompson, J.N., jr., C.N. Hallman, M.A. Anderson, T.R. Bradford, S.J. Lee, K.L. Meyer, S.J. Smith, A.S. Theppote, R.E. Woodson, S.D. Kinzie, and B. Safiejko-Mroczka, 2009, Dros. Inf. Serv. 92: 180-184.

Erratum

Erratum: Sterility in *D. pseudoobscura / D. p. bogotana* hybrid males. 1995, Dros. Inf. Serv. 76: 143.

Noor and Coyne (1995) reported that hybrid males from a cross between a recently (seven-generations prior) collected strain of *D. p. bogotana* and a stock of *D. pseudoobscura* were sterile. While the conclusion was correct, re-examination of records finds that two pieces were incorrectly described. First, the sample size was five rather than twenty (or no clear record was kept of additional dissections), and second, the hybrid males were tested for fertility 1 day after eclosion rather than 7. I (Noor) apologize for my errors in presentation.

Reference: Noor, M.A., and J.A. Coyne 1995, Dros.. Inf. Serv. 76: 143.