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# Genetics Education

## Innovations in Teaching and Learning Genetics

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## Genomewide Clonal Analysis of Lethal Mutations in the *Drosophila melanogaster* Eye: Comparison of the X Chromosome and Autosomes

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ABSTRACT

Using a large consortium of undergraduate students in an organized program at the University of California, Los Angeles (UCLA), we have undertaken a functional genomic screen in the Drosophila eye. In addition to the educational value of discovery-based learning, this article presents the first comprehensive genomewide analysis of essential genes involved in eye development. The data reveal the surprising result that the X chromosome has almost twice the frequency of essential genes involved in eye development as that found on the autosomes.

GENOMEWIDE *in vivo* functional analysis is critical for our ability to understand the role played by large numbers of uncharacterized genes identified with the sequencing of multiple genomes. A whole-genome functional analysis in Drosophila that overcomes the problem of organismic lethality of essential genes is realistic with the use of the FLP/FRT system (Xu and Rubin 1993), but is time- and labor-intensive. Through the creation of a unique set of discovery-based science education programs for undergraduate students at the University of California, Los Angeles (UCLA), we have performed a screen in the Drosophila eye by making FLP/FRT clones in 2100 lines bearing mutations throughout the fly genome. By so doing, we distributed the difficulty inherent in such a five-generation screen to the large numbers of students involved, and concurrently provided them with a unique educational experience in genetics. Previously, we introduced the educational goals of our program in a community forum article, which included preliminary and representative results for a subset of the autosomal mutants in this study (Chen *et al.* 2005). Here we present details of our educational program and the complete scientific data from mutants on the X chromosome and the two autosomes, providing the most complete genomewide functional analysis for genes involved in eye development to date. Through this substantial effort, we have generated a large population of FRT recombinant lines that are publicly available and an online database for the complete dissemination of our data. The analysis of these lethal mutations identifies the surprising finding that the X chromosome has a disproportionately large percentage of genes essential for viability that are involved in eye development compared to the autosomes.

## PEDAGOGICAL METHODS AND OUTCOMES

In each 10-week academic quarter, up to 30 undergraduates from different departments (the majority first and second year students) were enrolled in an elective lower-division class named Life Sciences 10 Honors (LS10H). The class consisted of a research laboratory, a computer laboratory, and a series of lectures. The only prerequisite for LS10H was high school advanced-placement-level biology. The course required 90 min

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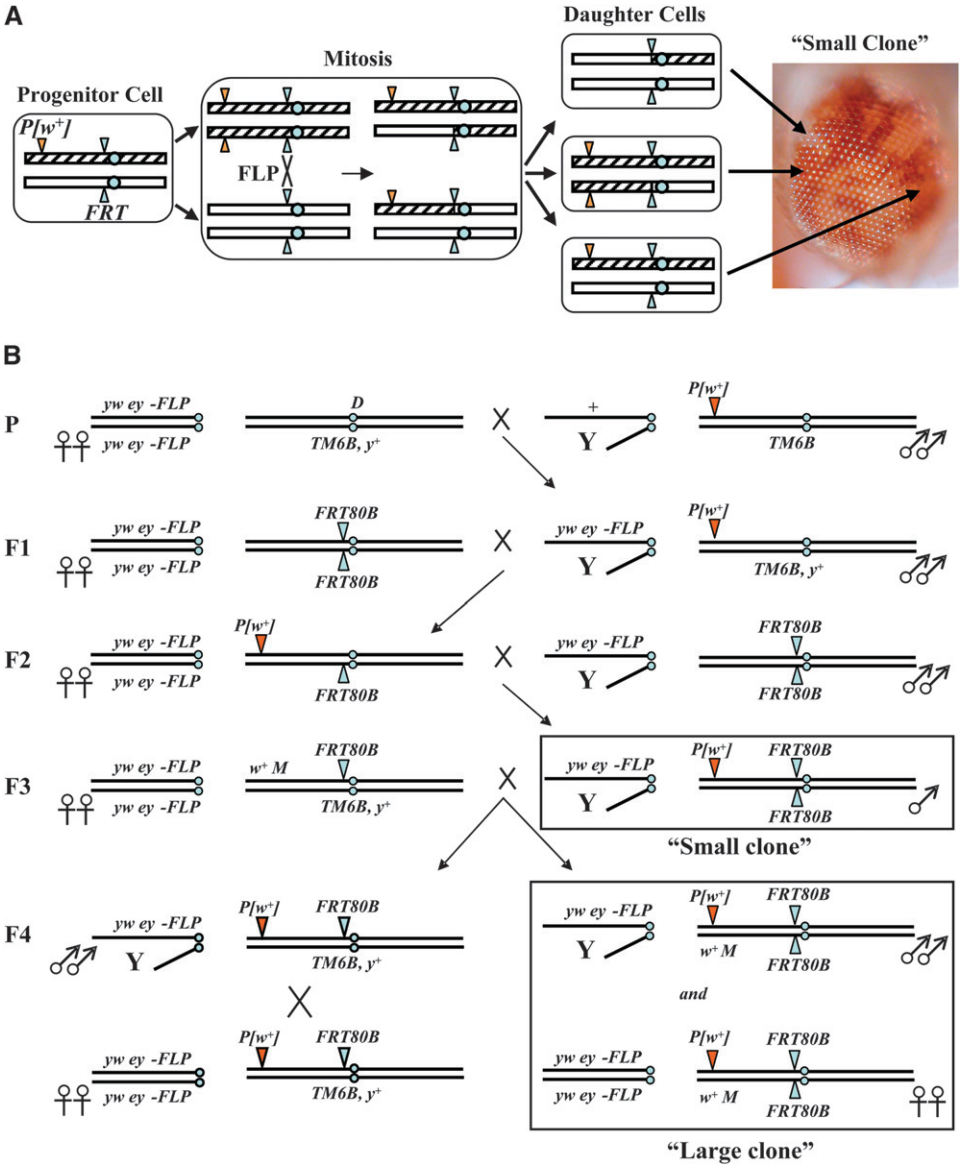


FIGURE 1.—FLP/FRT system and crossing scheme. (A) Chromosomes bearing a lethal mutation ($P[w^+]$) can be made homozygous through FLP-mediated mitotic recombination at FRT sites in the chromosome. The resulting daughter cells are of three potential genetic lineages: homozygous mutant, homozygous wild type, or heterozygous. The students identified FRT recombinant flies using the *mini-white* ($w^+$) gene, a pigmentation marker located in the transposon, by observing mosaic eyes. (B) By using the eye-specific enhancer *eyeless* (*ey*) to drive the expression of FLP, the students were able to create homozygous mutant tissue of lethal mutations specifically in the eye in the third generation. These recombinants were then crossed to a stock bearing a *Minute* ($w^+M$) or cell-lethal mutation on its FRT chromosome over a balancer chromosome, which generates a balanced stock of FRT recombinant flies and siblings that have eyes that are mostly homozygous mutant due to the *Minute* mutation. The scheme shown is specific for the 3L chromosome arm; however, other chromosome arms use this same core scheme. For all of the crossing schemes used in our project, please see our website at http://www.BruinFly.ucla.edu.

each of lecture and computer lab, along with 9 hr of laboratory research per week. Six of these hours were scheduled and 3 hr were unscheduled. The research laboratory was open during the week for students to come in and work during their free time. Lectures were delivered both in a classroom setting and inside the laboratory and were designed to be interactive. The ultimate goal of the didactic component was to emphasize "learning through hearing" as in a scientific seminar setting and to develop in the student the ability to create links between ideas and concepts. Students wrote a National Institutes of Health (NIH)-style grant proposal for their midterm, while their final paper was a research report written in the format of a publishable scientific manuscript.

During the first week of the laboratory, students set up their first crosses and learned basic Drosophila genetic techniques, including sexing males and females, collecting virgins to set up crosses, scoring adult genetic markers, and basic microscopy. As their projects progressed, they began to learn more complex genetic concepts based on their new crosses. For example, in the $F_2$ cross (Figure 1), they learned how natural meiotic recombination can be used to genetically engineer flies and to map mutations with respect to genetic markers. Specifically, they calculated the recombination distance between each unique transposon-induced mutation and the FRT site (a fixed marker). The most important central and difficult concept, inducing FLP-mediated mitotic recombination, was introduced by the $F_3$ cross. Students learned the difference between artificially induced mitotic recombination, which occurs in the somatic cells of $F_3$ progeny, and natural meiotic recombination, which takes place in the female germline. By the $F_4$ generation, most students had gained an appreciation for using mitotic recombination to bypass the lethality caused by

homozygosity of their assigned mutations and to reveal the roles of the respective genes in eye development.

Each quarter, ~5–10 students who completed LS10H were selected to participate in an advanced series of three upper-division classes called Life Sciences 100 Honors (LS100H) A/B/C. Students in the advanced classes developed individual projects on the basis of their findings in the introductory LS10H class. In addition to working on individual projects, these advanced students also verified the data of the LS10H students. A number of these advanced students presented their individual projects at local and regional meetings and are coauthors on other publications.

## RESEARCH METHODS AND OUTCOMES

The Drosophila eye is composed of ~800 individual light-sensing units called ommatidia. The precise hexagonal arrangement of the ommatidia allows for the detection of even minor perturbations in eye development. Clonal analysis using eye pigmentation as a marker can be used with relative simplicity to differentiate between mutant and normal tissue in the adult. As the eye is dispensable for organismic viability and reproduction, it represents an ideal system to study the role of essential genes in a postembryonic developmental process.

Several thousand transposon-induced mutations have been generated through the combined effort of the Drosophila community, Exelixis (Thibault *et al.* 2004), and the Drosophila Genome Disruption Project (Bellen *et al.* 2004). We obtained lethal transposon insertion stocks from public stock centers and through the five-generation series of crosses discussed above; the transposon mutations were meiotically recombined onto an FRT-containing chromosome (Figure 1). From the progeny of the second cross, the students identified initial FRT recombinant flies, which were then crossed to a stock bearing a *Minute* or cell-lethal mutation on its FRT chromosome. Concurrently, a chromosome that contains a construct expressing flippase under the control of the eyeless enhancer was introduced. This ultimately generated a balanced stock of FRT recombinant flies, as well as siblings that have eyes that are mostly homozygous mutant. The students documented this "large clone" eye phenotype with light micrographs (Nikon E600, equipped with a Nikon Coolpix 4500 camera) and natural scanning electron micrographs (Hitachi 2460N scanning electron microscope) and uploaded the data onto a template for the online database. The use of natural SEM does not require any special preparations of the fly before photography. The students developed bioinformatic skills as they performed BLAST analysis of their transposon stocks and identified the gene(s) affected by the insertion, using currently available FlyBase data (Grumbling and Strelets 2006). Determination of the gene disrupted by the transposon is based on the most proximal gene identified in the *Drosophila melanogaster* genome 5.1 release. We have performed this work for 2100 individual lines, documenting the phenotypes for each (supplemental Table S1 at http://www.genetics.org/supplemental/).

**TABLE 1**

**Numbers of recombinants created and unique genes identified for each chromosome arm**

| Chromosome arm (source) | Recombinants | Unique genes identified |
|---|---|---|
| X (Bloomington) | 339 | 151 |
| 2L (Bloomington) | 496 | 367 |
| 2R (Bloomington) | 419 | 321 |
| 3L (Bloomington) | 139 | 113 |
| 3L (Szeged) | 206 | 8 |
| 3R (Bloomington) | 111 | 88 |
| 3R (Szeged) | 390 | 12 |
| Total | 2100 | 1060 |

The source for each stock is noted. From 2382 stocks originally obtained, 2100 were successfully recombined with FRT.

Examination of the genes disrupted revealed that a large proportion of available mutant stocks are allelic. This is particularly true for older curated stocks, especially for the X chromosome, where there were 16 genes that had 5–10 alleles represented. Although all 2100 stocks were analyzed for their eye phenotype, to avoid redundancy, the analysis in this article focuses only on unique genes identified from all of the FRT recombinant stocks characterized. From these stocks, 1060 unique genes that had molecular information were identified using publicly available data (Table 1). In cases of allelic stocks with different phenotypes, the allele with the strongest mutant phenotype is included. Supplemental Table S2 (http://www.genetics.org/supplemental/) is a list of all the unique disrupted gene stocks used in this article's analysis. It includes the cytological location of the transposon insertion, the large clone eye phenotype, and the primary gene identified, based on current FlyBase data (Grumbling and Strelets 2006). Additionally, pictures of the mosaic eyes, descriptions of the phenotypes, and more can be found in the online database at http://www.BruinFly.ucla.edu.

The large clone eye phenotypes are categorized into four broad categories: wild type, rough, cell lethal, and glossy. The rough phenotype is assigned to eyes in which the highly ordered hexagonal arrangement of the ommatidia is disrupted (Figure 2B). If the eye size is smaller, and/or the mutant tissue is not present, the phenotype is classified as cell lethal (Figure 2C). Finally, if the lens is not secreted properly, it gives a shiny appearance to the eye under light microscopic observation, which we call the glossy phenotype (Figure 2D). In cases where the phenotype is a mixture, the predominant phenotype is used for classification purposes in Table 2.

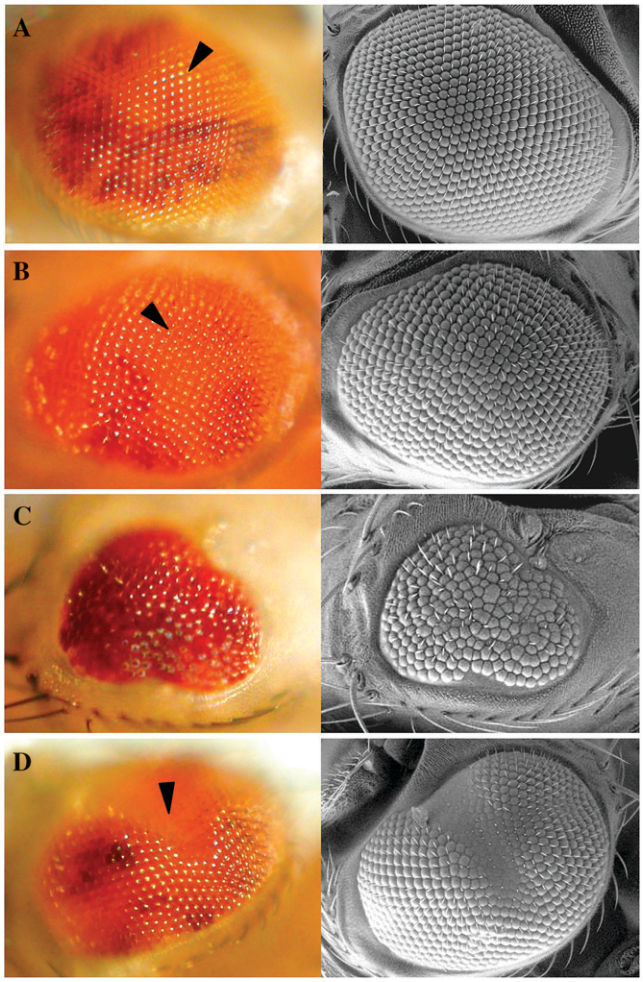


Figure 2.—Examples of eye phenotypes identified in the screen. All images show mosaic eyes with orange, homozygous mutant tissue (arrowheads) and red, heterozygous tissue. The right column is a scanning electron micrograph of the eye shown on the left. (A) An eye with a mutation in a gene that leads to no eye defects; note the perfect symmetrical arrangement of the repeating ommatidia. (B) A rough phenotype results when the mutant gene disrupts the regular pattern of the ommatidia. (C) The cell-lethal phenotype is assigned when the homozygous mutant (orange) tissue is lost or reduced, and the eye is smaller in size. (D) When the mutation leads to a lack of lens secretion, the eye takes on a glossy phenotype.

The overall percentage of genes essential for viability that gives a mutant eye phenotype on the X chromosome is 72% (Table 2). This finding is in agreement with the smaller scale X chromosome lethal mutation data reported earlier (Thaker and Kankel 1992). However, the autosomes have an average of 45% of their lethal mutations involved in eye development, indicating that the X chromosome has significantly more ($P < 0.0001$ by Fisher's exact test) lethal mutations than the autosomes that lead to a mutant eye phenotype (Table 2). The unique genes utilized in our study were mapped on all chromosomes (Figure 3). These positional data established that the larger numbers of essential genes functioning in the eye on the X chromosome are not all part of an eye-specific gene cluster (Figure 4).

The insertions that give a mutant eye phenotype were assigned by instructors to different functional categories on the basis of the molecular and biological gene ontology in their FlyBase gene report (Grumbling and Strelets 2006). The number and percentage of genes within each category are listed for the X chromosome and the autosomes (Table 3). The data indicate that there is no enrichment of genes within a particular functional category on the X chromosome when compared with the autosomes. Our report identified 14 insertions that cause mutant eye phenotypes, but, according to our criteria, do not appear to disrupt any identifiable gene (NG in our database). While these genes could be affecting regulatory elements, such as enhancers for distant genes, they could also be identifying currently unknown and potentially unannotated genes. For instance, one of these NG-characterized genes in our previous study has since been identified as an insertion in *mir-276aS*. These insertions represent a potentially valuable resource for future studies.

To help validate that the phenotypes seen in the mutant stocks are truly from the transposon insertion, excision experiments were performed by the students in the advanced classes to remove the transposon (Chen *et al.* 2005). After making large clone mosaic eyes in the excised stocks from the entire genomic collection, 488 of 674 independent mutant phenotypic stocks reverted to a wild-type phenotype. This indicates that approximately three-quarters of the mutant phenotypes are due solely to the transposon insertion throughout our entire collection of FRT recombinants. This number is most likely even higher, due to some transposons' inability to be excised. One hundred thirty-eight of these excision experiments were performed on X chromosome stocks, with 78% reverting to a wild-type phenotype, indicating

**TABLE 2**

**Number of insertions that lead to mutant eye phenotypes**

| Phenotype | X (%) | 2L (%) | 2R (%) | 3L (%) | 3R (%) | Auto (%) |
|---|---|---|---|---|---|---|
| Wild type | 42 (28) | 199 (54) | 168 (53) | 68 (56) | 61 (62) | 496 (55) |
| Cell lethal | 41 (28) | 59 (16) | 64 (20) | 18 (15) | 12 (12) | 153 (17) |
| Rough | 49 (33) | 81 (22) | 72 (23) | 30 (25) | 18 (18) | 201 (22) |
| Glossy | 17 (11) | 27 (7) | 13 (4) | 5 (4) | 8 (8) | 53 (6) |
| Total mutant | 107 (72) | 167 (46) | 149 (47) | 53 (44) | 38 (38) | 407 (45) |

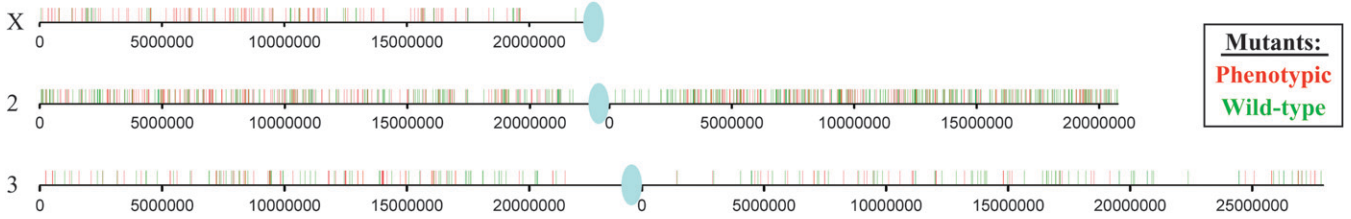


FIGURE 3.—Distribution of unique genes in this study. Genes are mapped by their FlyBase annotated transcriptional start site on the three chromosomes (X, 2, and 3). Green lines indicate transposon insertions that give a wild-type phenotype, while the red lines indicate a mutant phenotype. No centromeric (blue oval) genes were used because they are too close to be recombined with the FRT or are proximal to the FRT and thus inappropriate for mitotic recombination.

that the X chromosome data are consistent with the autosomes.

## DISCUSSION

This study aims to address two different, but interrelated areas: discovery-based research and education. For the research aspect, this study represents the largest functional genomic screen in the Drosophila eye to date. A critically unique aspect of the screen is that the results report mutations that not only give rise to mutant eye phenotypes, but also include stocks with a wild-type eye phenotype. This genomewide catalog of phenotypes will potentially help shape future efforts. The FRT recombinant stocks that were used in this study are now curated in the Kyoto stock center and will allow other Drosophila researchers to perform similar functional genomic screens or to determine the function of an individual gene of interest in the tissue of their choice.

For the educational aspect, this study was performed with numerous undergraduate students, who constitute the majority of the authors of this article (264 students) and who were predominantly in their first or second year in college. (The student authors are presented alphabetically in three groups: advanced students who have participated for more than two quarters over the past 2 years, beginning students from the past 2 years, and the beginning and advanced students from the first 2 years.) They performed this work through their involvement in the UCLA Undergraduate Research Consortium in Functional Genomics (URCFG), the main goal of which is to involve undergraduate students in real scientific research early in their undergraduate career, while also educating them about scientific

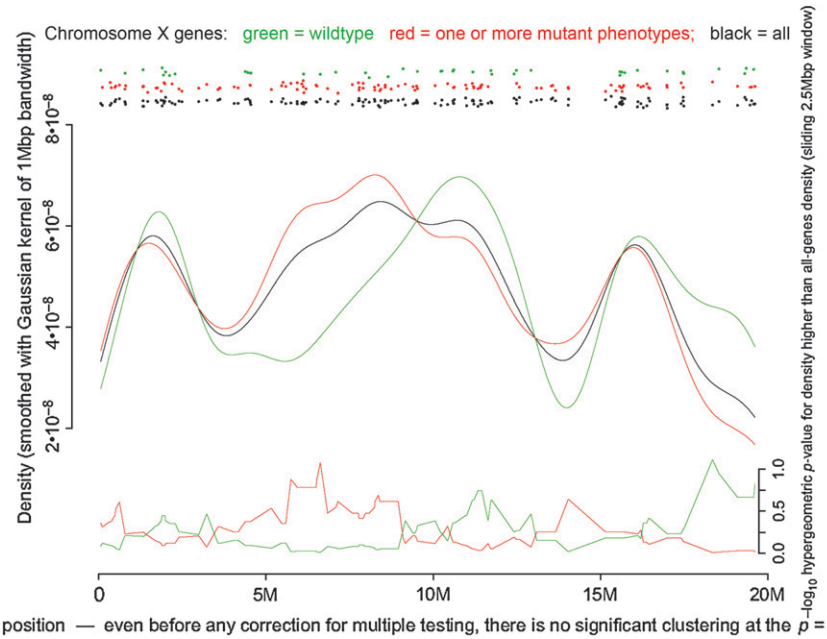


FIGURE 4.—Clustering analysis of genes on the X chromosome. The scattered dots near the top of the plot show the gene positions. A small amount of random *y*-axis jitter was added so that dots close to each other can be seen distinctly. Black represents all genes, green represents genes that give a wild-type phenotype, while red represents mutant phenotype. A 2.5-Mbp window (1.25 Mbp inclusive on each side) was centered on each gene. A typical window encompassed ~10–25 genes; the average number of genes per window was ~20.1. The negative base 10 log of the hypergeometric *P*-value for enrichment in a subset (no. of genes in a window that have a phenotype)/(no. of genes in a window) > (no. of genes on X chromosome having a phenotype)/(no. of total genes on X chromosome examined) was computed for each window; higher values indicate more significant enrichment and a $P = 0.05$ level corresponds to $-\log_{10}(0.05) = \sim 1.301$. No corrections were made for multiple testing as without them there was no significantly enriched window (represented by the lines at the bottom). The curves in the middle of the plot show estimated density as a visual aid; they are 1-Mbp-bandwidth Gaussian kernel-smoothed versions of the gene positions.

**TABLE 3**

**Functional categorization of genes that give a mutant eye phenotype**

| Gene function category | X (%) | Auto (%) | All (%) |
|---|---|---|---|
| Apoptosis | 0 (0) | 3 (1) | 3 (1) |
| Cell adhesion/ECM | 4 (4) | 7 (2) | 11 (2) |
| Cell cycle | 1 (1) | 10 (2) | 11 (2) |
| Channel and transporter | 5 (5) | 12 (3) | 17 (3) |
| Chaperone and protein folding/binding | 1 (1) | 13 (3) | 14 (3) |
| Chromatin remodeling/binding | 1 (1) | 10 (2) | 11 (2) |
| Cytoskeleton | 6 (6) | 23 (6) | 29 (6) |
| DNA replication, repair, and recombination | 2 (2) | 4 (1) | 6 (1) |
| Intracellular transport | 6 (6) | 23 (6) | 29 (6) |
| Metabolic enzyme | 2 (2) | 22 (5) | 24 (5) |
| Mitochondrial | 5 (5) | 16 (4) | 21 (4) |
| Novel | 19 (17) | 74 (18) | 93 (18) |
| Other cellular processes | 3 (3) | 6 (1) | 9 (2) |
| Protein modification/metabolism | 7 (6) | 22 (5) | 29 (6) |
| RNA binding/processing | 5 (5) | 39 (9) | 44 (8) |
| Signal transduction | 22 (20) | 48 (12) | 70 (13) |
| Transcription and gene regulation | 12 (11) | 46 (11) | 58 (11) |
| Translation and protein synthesis | 8 (7) | 25 (6) | 33 (6) |
| Ubiquitination/degradation | 0 (0) | 8 (2) | 8 (2) |

Numbers of genes on the X chromosome, autosomes (auto), or all chromosomes were identified on the basis of publicly available information from FlyBase, using *Drosophila melanogaster* genome annotation 5.1. Gene ontology for each gene that gives a phenotype was used to assign a single functional category. Parentheses indicate percentage of the genes on the X chromosome or autosomes belonging to that category.

research as a way of thinking, analyzing, and interrelating concepts learned in didactic lectures. Each student worked on 10 individual mutant stocks, which is a small part of the project, but when taken as a whole, the sum of their results is impressive. While working with these stocks, the students gained a sense of ownership of them. The student was the only person responsible for the maintenance, successful recombination, data collection, and website creation for each stock. We found the URCFG stimulated camaraderie among the students, increasing their enjoyment of working together in a large laboratory setting. It was not uncommon to see students compare their own results with their fellow classmates, and feel a sense of pride when they accomplished a particularly difficult recombination or identified a mutant eye phenotype. The ratio of mutant phenotypes was high enough that each student generally had at least one stock in his or her collection that gave a mutant phenotype. This high success rate helped improve the student's attitude and desire to work hard, often encouraging other members of the class to do the same. Additionally, in retrospect, many of the introductory course students who later completed a core curriculum genetics class found it to be easier than their non-URCFG peers did, as a result of having done such experiments as mapping FRT sites with respect to a gene or phenotyping firsthand.

The contribution of the many undergraduates in the program is what made this genomewide analysis possible, overcoming the considerable effort inherent in this project by splitting up the work into small parts that were manageable by the individual student. For instance, we have estimated that for this project to be completed, our 264 undergraduate students have performed >3000 separate recombination experiments, >2000 phenotype verification experiments, and >670 excision experiments, for a conservative estimate of >150,000 independent Drosophila crosses over a 3.5-year period. This level of productivity was accomplished by the individual students while continuing a full load of didactic education. During the program, the students were introduced to the "bigger picture," but care was taken not to overburden them with only the long-term implications. Summer students and instructors of the program repeated and confirmed each data point and compiled the data for analysis. All of the students knew that their work would eventually be published, and this was a motivational force for them.

In conjunction with their laboratory effort, the students also received specialized didactic instruction to help them understand the basis for their work. This included providing them with the ability to appreciate scientific research as an endeavor, and educating them in substantive aspects of research such as proper record keeping, ethics, scientific writing, and career options. This novel curricular approach appeared to amplify the student's overall education, including a better grasp of abstract concepts in genetics.

At the end of each quarter, students filled out the standard UCLA course evaluations. Among the questions

asked was if there was a change in interest in the course subject matter following the course. Since our course was directed at research, our interpretation of this question was: How did the student's interest in research change because of our course? Figure 5A shows the results from all 223 students who responded to these questions. Figure 5A demonstrates that our course significantly increased the student's interest in research. To further quantitate the educational impact of our program, we had our students participate in the Survey of Undergraduate Research Experiences II (SURE II), an extension of the original SURE survey, which intends to collect quantitative data on the benefits of undergraduate research (Lopatto 2004). While the majority of students that take this survey across the nation are involved in a full-time summer research experience, we felt that it would be an accurate assessment for our program, given our main goal, despite it being only 10 weeks long with the students busy with a full academic load. This survey quantifies the benefit gained from the student's research experience in multiple areas. Eighty-eight introductory URCFG students took the survey, and overall the scores were typically above the students who had a more intensive summer research experience (Figure 5B). This was especially true in the aspects that we focus on in our class. For instance, the midterm of the course is an NIH-style grant proposal for the work the students are performing in the class, and the final is a research article-formatted paper detailing their results. These assignments are covered in the survey under scientific writing where our surveyed students indicated that their experience in the class helped them have a large gain. We have also found that our high teacher: student ratio (1:10) was very important so that the students never floundered in their work. The instructors were able to benefit from the program as well. Instructors were given the opportunity to develop their teaching styles while learning how to maintain a large research laboratory. Overall, we have found this unique educational opportunity to be extremely rewarding for all involved, for both education and research.

A project of this magnitude is not without its challenges. In a large public university, generating enough funds to keep an acceptable instructor-to-student ratio is a difficult goal to achieve. In this respect, the support from the Howard Hughes Medical Institute (HHMI) was critical and allowed us to demonstrate to the university that student instruction can improve by investigative teaching techniques. This allowed presentations to higher administration and fundraising to cause expansion of the project through internal funding. It was also difficult to maintain the students who participated in our first program into more detailed research programs led by us since running such laboratories proved too expensive even with an HHMI budget. The program was therefore modified to place successful students from the initial program into individual research laboratories of the large body of faculty at UCLA. This process has now given rise to a new minor in biomedical research that is run on an interdepartmental

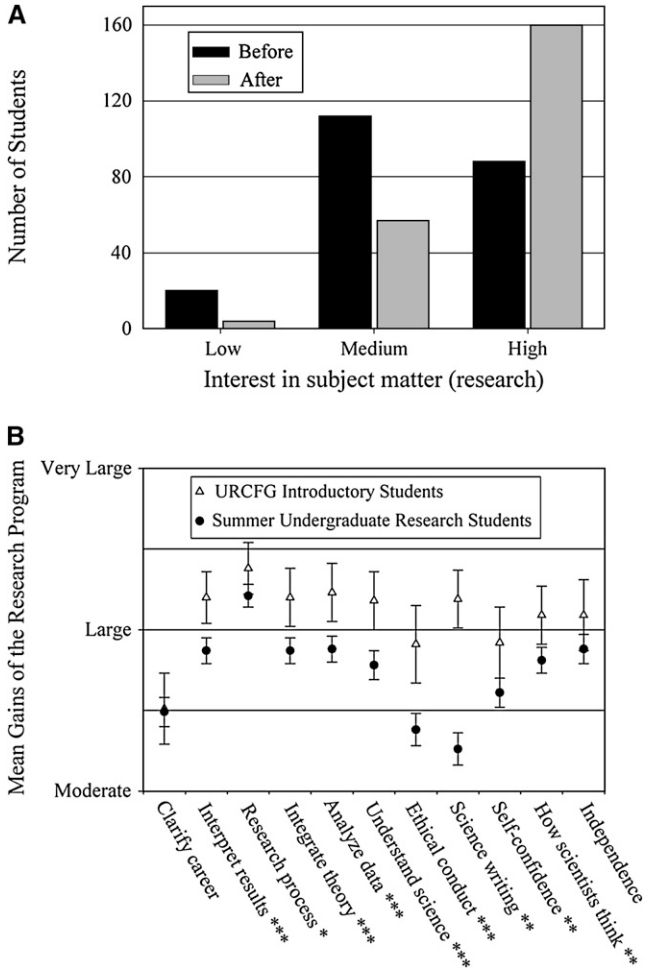


Figure 5.—Educational results of introductory URCFG students. (A) Results from standard course evaluations of 223 introductory students when asked about their interest in the subject (research) before and after the course. (B) Eighty-eight students, consisting of students from all four years of our program, voluntarily participated in the SURE II survey (Lopatto 2004) in 2006. The survey has a number of questions measuring gains of knowledge in areas related to scientific research. A five-point scale was used to measure the level of gain: no gain or very small gain, small gain, moderate gain, large gain, and very large gain. All students reported moderate gains or greater from the URCFG program and their average benefit for each subject is plotted on the graph *vs.* the results from 532 students from across the nation at multiple universities and colleges that had participated in a summer research program in 2006. The full-subject descriptions for the *x*-axis are as follows: clarification of a career path, skill in the interpretation of results, understanding of the research process in your field, ability to integrate theory and practice, ability to analyze data and other information, understanding science, learning ethical conduct in your field, skill in science writing, self-confidence, understanding of how scientists think, and learning to work independently. The mean scores are graphed ± 95% confidence intervals. *T*-tests were performed for each question to determine significant differences; *P*-values are as follows: *<0.05, **<0.01, and ***<0.001.

basis and uses our class as a means to place successful students into other laboratories in a way that matches the student's interest with that of the individual faculty. Another difficulty with a program such as this involves the logistics of keeping large amounts of data in one single place. This was solved by running summer programs purely dedicated to rechecking and tabulating the data acquired during the academic quarters. Finally, the success of the program itself poses a difficulty. The instructors hired are very employable and all find lucrative teaching offers elsewhere, making it difficult to maintain continuity. This can only be overcome by having multiple instructors for the same purpose at one time.

The educational goals of this program would be incomplete without being able to complete a proper scientific inquiry-based project. This report demonstrates that there is a genomic bias of essential genes involved in eye development on the X chromosome, a conclusion that required a genomewide functional analysis. While the reason for this bias or the genetic significance of this result is unknown, this phenomenon is not entirely new. It is known that in humans there are more mutations that lead to mental retardation on the X chromosome (Inlow and Restifo 2004), although the basis for this is still unknown. Further functional genomic studies similar to ours in other tissues might reveal whether such genomic biases are seen in other developmental contexts. To obtain more detailed information on our data, program, educational goals, and methods, see our website at http://www.BruinFly.ucla.edu.

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