Abstract:

The aim of this research is to observe the homozygous lethal mutation, specifically in the eye to see the gene's function in eye development of the *Drosophila melanogaster*. Homozygous lethal mutation is obtained through mitotic recombination caused by FRT19A and ey-Flp. The result of the recombination will be a mosaic of three different eye phenotypes: homozygous P-element mutation, homozygous wild-type, and heterozygous P-element mutation. An additional gene, CL, is then introduced into the genome after mosaic females are obtained. Since CL is homozygous cell lethal, after the mitotic recombination, if a daughter cell is homozygous CL, it will die and its phenotype will not show up in the eye. In addition, one copy of CL will considerably slow down the heterozygous cell's growth compared to homozygous P-element cell growth. Homozygous P-element cells will therefore grow in the majority of the eye. The eye is then observed under the microscope to see if the manipulated gene has a function in eye development based on its phenotype. Based on the mutations in the eye due to the disruption of P-element, we can assume that large clones stocks that are not wild-type have an important role in eye development of *Drosophila*.

Introduction:

Drosophila melanogaster is important to the study of genetic mutations in humans because of its DNA similarity to humans. Seventy-five percents of gene mutations in human diseases show a strong match to those in *Drosophila*. If a new discovery is found in the eyes of *Drosophila*, it is possible to then apply similar developments to higher model organisms, such as mouse, and eventually humans. This research observes specific mutant phenotypes in the eyes to determine whether the gene has a significant role in eye development. This will then lead to a more in-depth research on the specific functions of the genes in eye development.

Drosophila is a good model system to use when working under a time restraint. Due to its short life cycle, it is easy to maintain generations of *Drosophila* during the 12 weeks that this project will be held. In addition, *Drosophila* genome is already sequenced and mapped. When the P-element is inserted into each stock, the sequenced genome allows for fast search of the exact position of the P-element. In order for homozygous mutations to show up in mosaics of the small clones, there must be two copies of the FRT19A present along with the ey-Flp to induce mitotic recombination. *Drosophila* is the ideal organism to use because its chromosomes are physically close to each other, allowing cross over to occur. Human chromosomes, on the other hand, are too far apart for cross over to occur during mitosis.

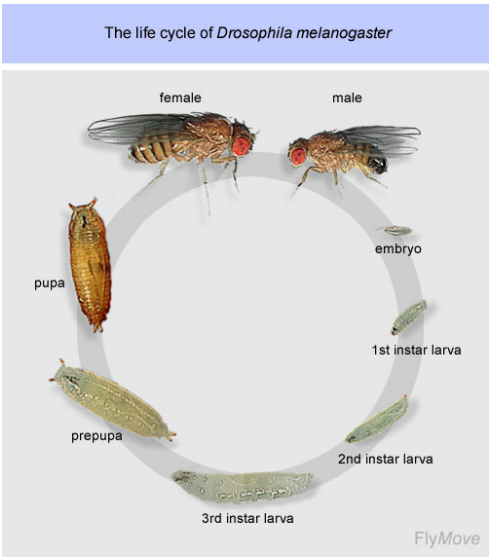

Figure 1: Life cycle of *Drosophila* melanogaster.

P-element disrupts specifics gene in the *Drosophila* genome, causing lethal mutations. Consequently, it is rare for *Drosophila* to survive long enough for researchers to do more detailed studies on the functions of the interrupted genes. Ey-Flp, the master gene regulator of the eye, ensures that any mutation occurs only in the eye. The

Drosophila will then survive for further experimentation, especially ones that concerns with the role the specific gene plays in the development of the eye. Large clones of the eye mutation will then be generated, allowing for a better view under the microscope. The mutant phenotype of the disrupted gene can be seen under the microscope, and further research can be carried out based on the results.

The focus of this research is specifically on the eye of *Drosophila melanogaster*. Each *Drosophila* eye is made up of about 800 ommatidia, each ommatidium containing eight photoreceptors surrounded by 4 cones. In order to examine the functions of the particular gene in eye development, P-element, ey-Flp, and FRT19A are inserted into the *Drosophila*'s genome in order to induce mitotic recombination and mutations in the eye.

Eyeless flipase is an enhancer used to ensure that mutation caused by the interruptions of P-element occurs only in the eye. FRT19A is another component necessary to induce mitotic recombination. The flipase targets the cell at a specific time in the presence of two FRT19A on a homologous chromosome. Mitotic recombination occurs so that some cells in *Drosophila* eye will end up with homozygosity in P-elements, while some end up with as homozygous wild-type. The P-element, a type of transposon, is an efficient way of inserting novel genes into the *Drosophila* genome because the unique thirty base pairs endings make it easy to locate the exact position of the P-element. The inserted P-element disrupts DNA sequences used to produce amino acids. This then will disrupt the proper sequence needed to produce the correct protein needed in eye development, causing a mutation to occur in the eye. The process usually will start in early embryonic stage, during the development of the eye. The mutation will cause parts of the eye to be homozygous P-element, parts to be homozygous wild-type, and the rest to be heterozygous P-elements. In small clones, the mutations will show up as three different phenotypes: cells with homozygous P-element will have a dark red coloration; homozygous wild-type cells will be white; and heterozygous cells will be orange.

To better observe the effects of the gene have on the development of the eye, crosses will be carried out to obtain large clones of mutated cells. In the large clones, an additional gene, CL, is inserted to the genotype. Because CL is homozygous cell lethal, cells containing homozygosity in CL will not survive. In addition, CL will cause heterozygous cells to grow more slowly. Therefore, homozygous P-element cells will be able to grow more rapidly than heterozygous cells. Hence, it is easier to observe the homozygous mutation under light microscope to determine whether or not the particular gene has an important function in the eye development of the *Drosophila*.

Material and Methods:

This research requires

In Crossing Scheme:

Drosophila melanogaster
Fresh food in a vial
Yeast

In Fly Observation:

Light microscope
Carbon dioxide and CO_2 pad
Light
Brush
Ethanol

In Data Collecting:

- Light and electron microscope
- Camera/TV
- Zip disks
- Computer
- Tape
- Tweezer
- Slide

Drosophila Crossing Scheme:

♀♀	wP[w+] ; + FM7 +	X	yw FRT19A ; ey-Flp Y ey-Flp	♂♂

The instructors set up the parental cross. Females with the P-element were crossed with FRT19A males to obtain females with FRT19A and P-element. The females containing the P-element were ordered from Bloomington before the start of week 1.

♀♀	wP[w+] ; ey-Flp ywFRT19A +	X	ywFRT19A ; ey-Flp Y ey-Flp	♂♂

The female progenies containing the P-element were crossed with FRT19A males. Meiotic recombination occurred in order to obtain homozygous FRT19A. Two copies of FRT19A along with ey-Flp caused the females to undergo mitotic recombination. The interruption of the P-element in the gene led to the development of three different phenotypes in the eye. The progenies desired can be distinguished through its distinct mosaic eyes.

♀	wP[w+]FRT19A ; ey-Flp ywFRT19A +/ey-Flp	X	FM7c ; + Y +	♂♂

The small clones were crossed with FM7c males in order to preserve the FRT19A chromosome. FM7c, a balancer, will assure that no meiotic recombination occurs. It also visibly distinguishes female progenies containing the P-element, with a deep red coloration of the eyes, from progenies without the P-element. Small clones phenotypes were noted in the notebook and light microscope and SEM pictures of the small clone eye were taken.

♀♀	ywP[w+]FRT19A ; + FM7c +/ey-Flp	X	FM7c ; ey-Flp Y ey-Flp	♂♂

Females with P-element were crossed to FM7c males to preserve the FRT19A chromosomes and reintroduce the ey-Flp. The progeny from this cross is considered as balanced stocks and can be stored for future use.

♀♀	ywP[w+]FRT19A ; ey-Flp FM7c +/ey-Flp	X	w+CLFRT19A ; ey-Flp Dp(1:Y) +/ey-Flp	♂♂

♀♀	ywP[w+]FRT19A FM7c	; ey-Flp +/ey-Flp	X	FM7c Y	; ey-Flp +/ey-Flp	♂♂

Large clone were set up crossing the female progenies with CL males. The males were modified so that there is a duplication of the CL gene on the Y-chromosomes. This is because for CL is hemi-lethal in males. Thus, a duplicated CL is added on to the Y-chromosomes and is marked by curly wings.

	ywP[w+]FRT19A w+CLFRT19A	; ey-Flp ey-Flp

CL genes will cause homozygous CL cells to die early in eye development. It will also slow down growth in heterozygous cells. Therefore, the majority of the eye contained homozygous mutation caused by the disruption of the gene manipulated. The phenotypes of the large clones mosaics were noted and light microscope and SEM pictures of the large clones eye were taken.

During the experiment, only virgin females were used to avoid contaminations. Virgin females were collected soon after the *Drosophila* eclosed. Newly born females with their food bulbs still visible under microscope is considered to be virgins because they are too young to mate. Another method in ensuring the females are virgins is to leave each female in a vial for a minimum of three days. After the flies have mated, females usually hold the males' sperms for an additional period. Females then use the sperms to fertilize their eggs as they lay them. Within three days, the larva will grow large enough to be clearly seen under the microscope. If no larva is seen after the third day, it is safe to assume the female is not holding on to any sperm and is a virgin.

The virgin collected were then crossed with several males in a vial. The vial contained several grains of yeast to provide extra nutrition. The flies were also transferred every few days to insure that the larvae were able to grow in an optimum environment with enough food and space. Whenever a cross was created, we noted the eye colors and the body colors and compared them to a calculated punnet squares to make sure there were no contaminations.

Results:

Stock #	Genotype	Insertion Site	Total ♀ Progeny	# Recombinant	Expected Recombination Distance	Observed Recombination Distance
11914	w(67c23) P{lacW}l(1)G0022(G0027)/FM7c	013E07- 013E15	307	14	12	9.11
11886	w(67c23) P{lacW}l(1)G0136(G0136)/FM7c	013E08- 013E09	291	17	13	11.70
11884	w(67c23) P{lacW}l(1)G0168(G0168)/FM7c	013D01- 013D02	155	17	13.5	21.98
11882	w(67c23) P{lacW}l(1)G0186(G0186)/FM7c	012D02	177	20	17	22.56
11877	w(67c23)	010C01-	87	12	29	27.48

	P{lacW}l(1)G0237(G0237)/FM7c	010C02				
11876	w(67c23) P{lacW}dlg1(G0276)/FM7c	010B14- 010B17	305	13	30	8.52
11807	w(67c23) P{lacW}l(1)G0084(G0084)/FM7c	018D05- 018D08	103	17	1	33.12
10138	w(67c23) P{lacW}ras(G0482)/FM7c	009E1- 009E4	135	29	32	42.86

Table 1: Most of the observed recombination distance is similar to the expected value. A significant difference between the observed and the expected values is seen in stock 11876 and 11807.

Stock #	Gene Disrupted	Gene Function	Color of 1 P[w^+]	Color/Phenotype of Small Clone	Color/Phenotype of Large Clone
11914	CG8231	Protein folding	Dark Orange	Dark Red/White, Wild-type	Orange/Red, Wild-type
11886	CG8198	Nitrogen fixation, Iron binding	Dark Orange	White/Red, Wild-type	Orange/Red, Rough/Glossy
11884	CG33206	Thyroid hormone receptor, protein synthesis	Dark Red	Dark Red/White, Rough	Dark Red/Dark Red, Rough
11882	Ben (Bendless)	Protein binding, Protein folding	Medium to Dark Red	White/Red, Wild-type	Orange/Dark Red, Wild-type
11877	CG1558	DNA production, Cell division	Dark Orange	Dark Red/White, Wild-type	Orange/Dark Red, Rough
11876	dlg1	Protein binding	Red Orange	White/Dark Red, Rough/Glossy	Pupal/Larva Lethal
11807	CG12238	Apoptosis, Protein folding	Orange	Dark Red/White/Orange, Wild-type	Orange/Red, Rough
10138	Ras (Rasberry)	Phosphate binding, Amino acid production	----	Dark Red/White, Wild-type	Orange/Dark Red, Wild-type

Table 2: The most common phenotypes found in small clone and large clone are recorded in this table.

11914:

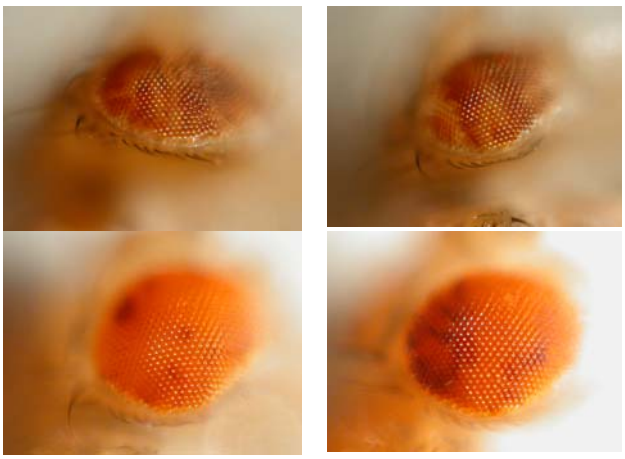

Figure 2: Column- Sub-lines noted from left to right: B, D. Row- Top: Small Clones. Bottom: Large Clones. Both small clones and large clones are wild-type.

Half of the sub-lines in this stock have a phenotype of dark red eye with white spots in the small clones. The other half have white eye with dark red colorings. In this stock, all of my small clone sub-lines have wild-type eye. In the large clone, the females have orange eye with red spots. The eyes are also all wild-type.

11886:

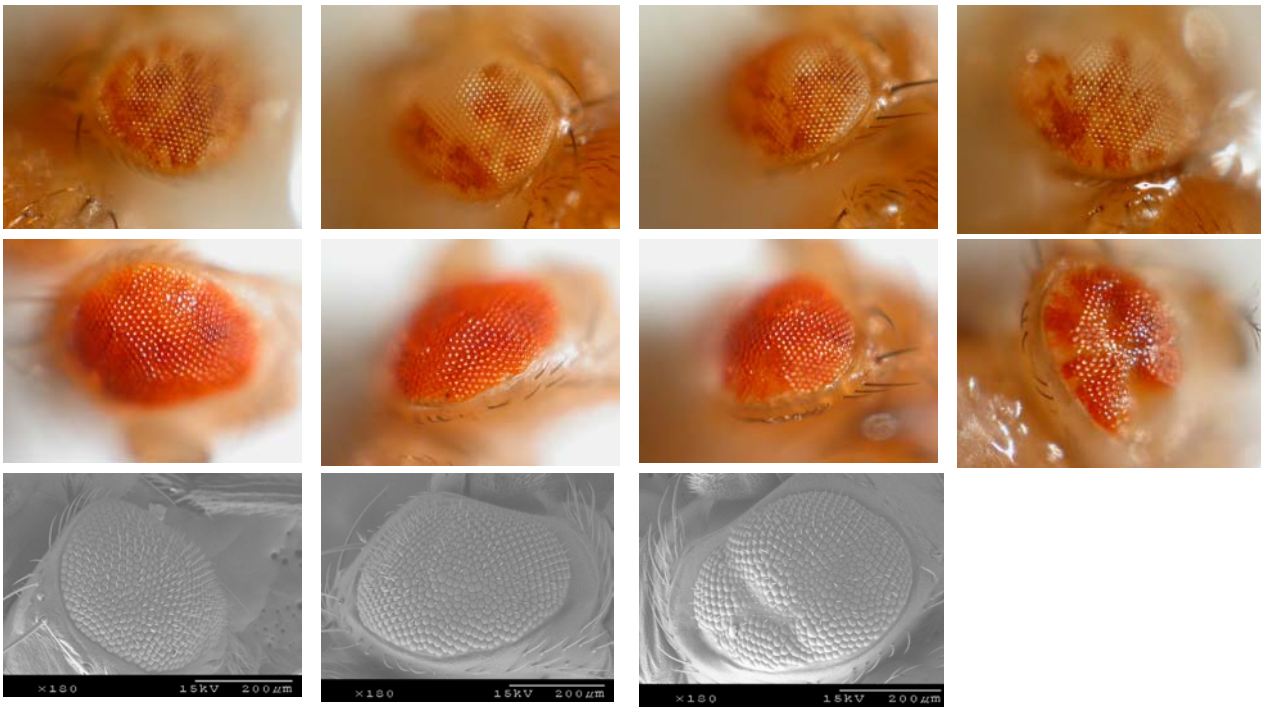

Figure 3: Column- Sub-lines noted from left to right: A, B, G, I. Row- Top: Small Clones. Middle: Large Clones. Bottom: Large Clones SEM. The small clones are wild-type. Large clones all have rough phenotype, but few sub-lines have an additional glossy phenotype.

Most of the sub-lines in the small cones have wild-type white eye with red spots. However, one of the sub-lines is the opposite, with wild-type red eye with white spots. In my large clones, the females have dark orange eyes with a slight tint of red. There are some white cells around the edges in few of the eyes. The large clones for this stock has a very distinct phenotype. It is rough, and we noted that some of the sub-lines have a glossy phenotype. The degree of glossiness varies with each individual flies and sub-lines.

11884:

Figure 4: Column- Sub-lines noted from left to right: A, B, D, H, and N. Row- Top: Small Clones. Middle: Large Clones. Bottom: Large Clones SEM. Both small and large clones have rough phenotype.

All of the small clones sub-lines have rough dark red eye with white patches. In addition, we discovered males containing the P-element in both the small clone and the large clone cross. In the large clones, the roughness is again observed in the eyes. In addition, in the large clones, the homozygous P-element is the same color as heterozygous P-element, so the eye appears to have two red shades.

11882:

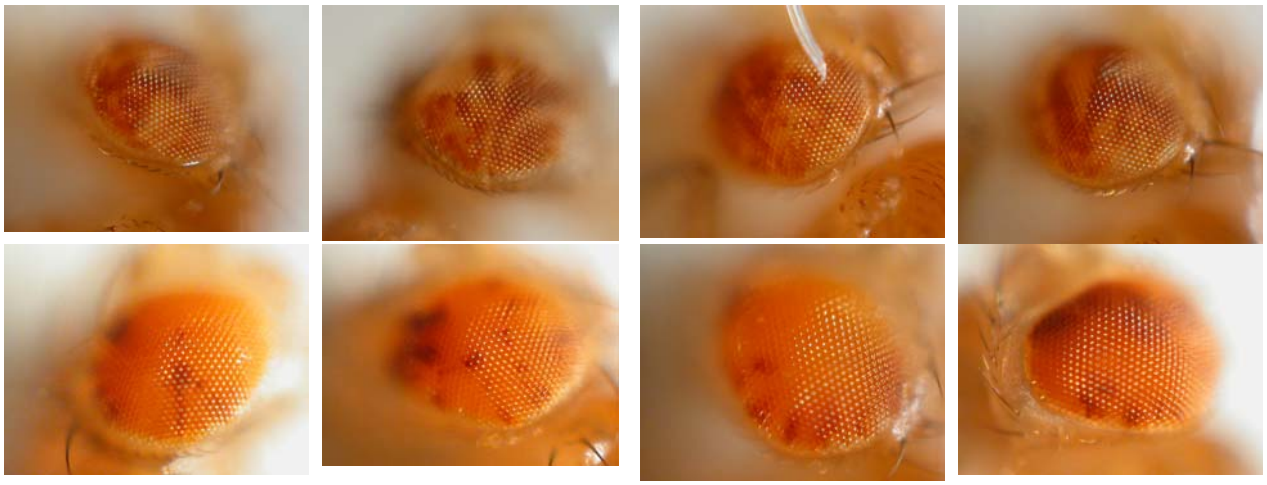

Figure 5: Column- Sub-lines noted from left to right: A, B, D, and F. Row- Top: Small Clones. Bottom: Large Clones. Both small and large clones are wild-type.

In this stock, two of my sub-lines in the small clones have white eye with red patches. There are also two lines with very light orange eye with red patches, and one with red eye and white spots. All of the sub-lines have wild-type eye. In the large clones, the females have orange eye with dark red patches. Most of the progenies in the large clones also have wild-type eye. However, one of the female found in sublime F have a small patch of rough cells near the edge of its eye. The eye collapsed before we could take SEM pictures.

11877:

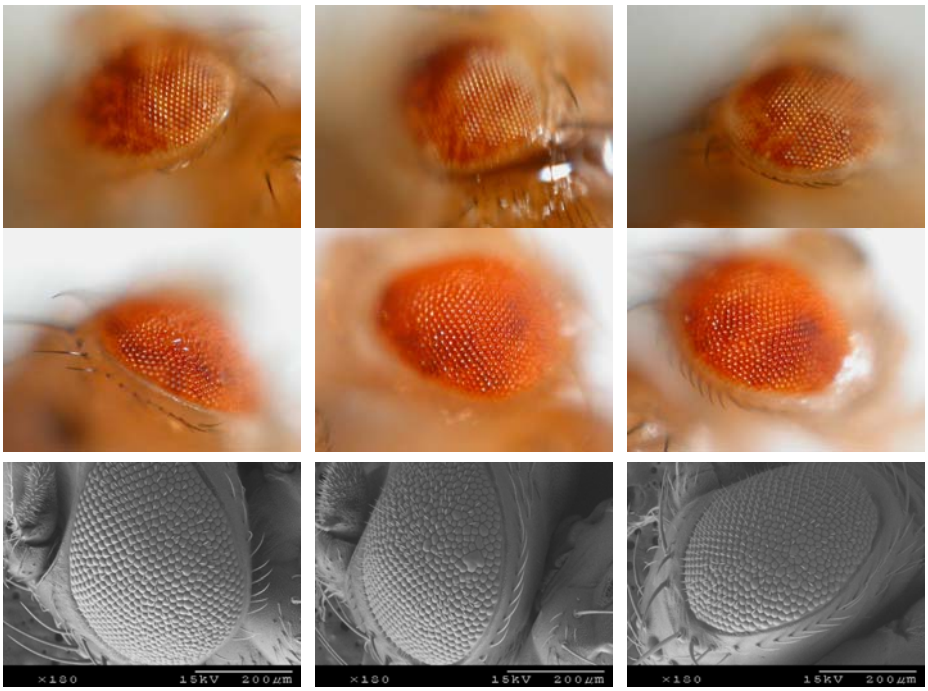

Figure 6: Column- Sub-lines noted from left to right: C, E, and F. Row- Top: Small Clones. Middle: Large Clones. Bottom: Large Clones SEM. The small clones are wild-type. Large clones have rough phenotype.

In the small clones, most of the sub-lines have dark red eye with little white specks. There is one sublime with white eye with red spots. All of the small clones contain wild-type phenotype. In the large clones, all the sub-lines have rough orange eye with similar red spots. In addition, it is noted that males with P-element survived in the large clones.

11876:

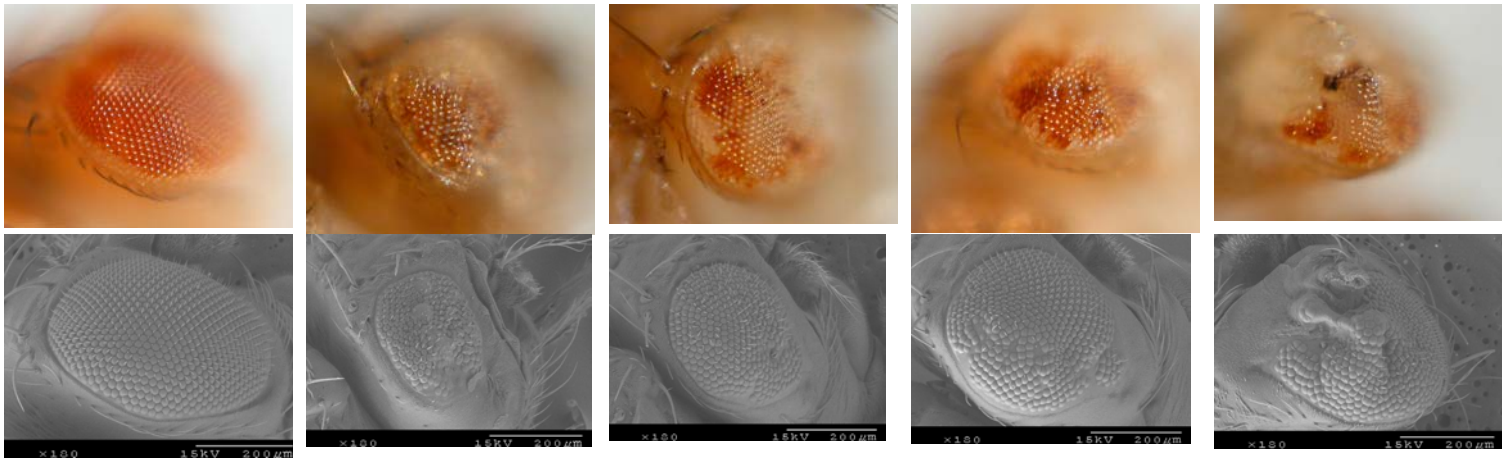

Figure 7: Column- Sub-lines noted from left to right: A, B, F, H, I. Row- Top: Small Clones. Bottom: Small Clones SEM. Small clones have rough and glossy phenotypes. Large Clones are pupal lethal.

In the small clones, most of the sub-lines have a very distinct rough, and glossy phenotype. Most of them have white eyes with dark red spots in the remaining ommatidia that survived. One of the sub-lines, on the other hand, has a normal red eye, with tiny specks of white toward the side of the eye. It appears to be wild-type, and only slight mutation can be seen around the edges on SEM. In the large clones, the sub-lines show pupal lethality. When viewing the closed pupa under the microscope, we observed that some of the pupas are deformed and the head appears to have shrunken into its body.

11807:

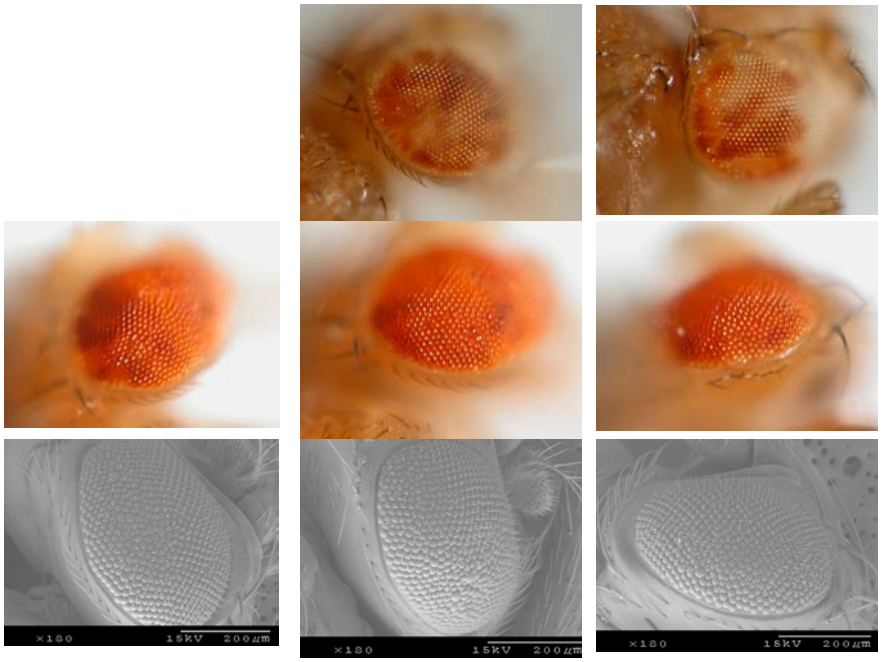

Figure 8: Column- Sub-lines noted from left to right: A, B, D. Row- Top: Small Clones. Middle: Large Clones. Bottom: Large Clones SEM. Small clones are wild-type. Large clones have rough phenotype.

The small clones for most of the sub-lines in this stock show a wild-type dark red eye with light orange or white spots. There is one subline that showed wild-type eye with white eye and red spots; the rest has orange eye with dark red spots. In the large clones, most of the stocks have rough orange eye with varying degrees of red spots. From the SEM, we noted that only half of the eye contains the rough phenotype. We also noted that there are fused ommatidia in the rough area of the eye. The other half of the eye appears to be wild-type.

10138:

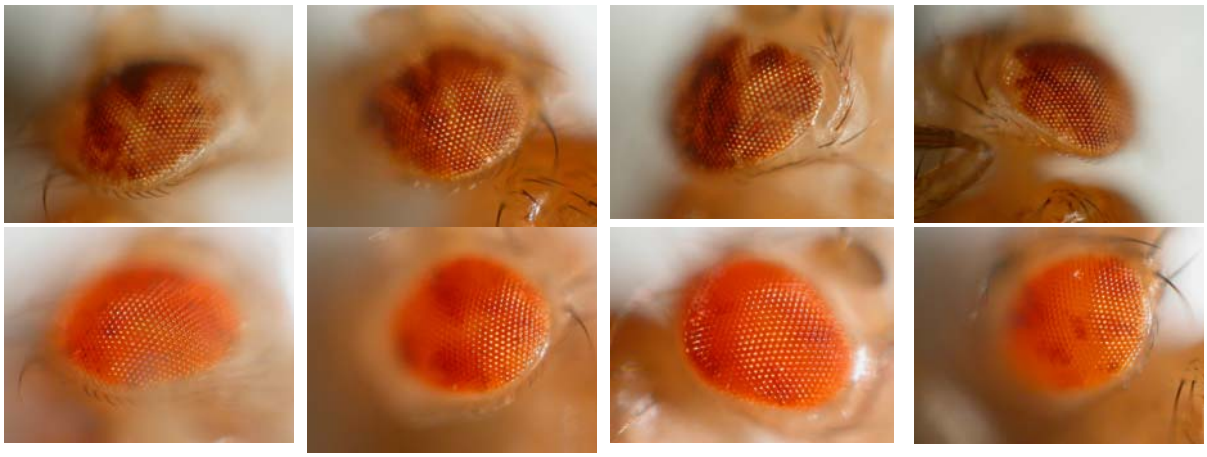

Figure 9: Column- Sub-lines noted from left to right: A, B, C, and D. Row- Top: Small Clones. Bottom: Large Clones. Both small and large clones are wild-type.

All of the small clones of this stock has wild-type dark red eye with white spots. In the large clones, wild-type phenotype is observed along with orange eye and dark red spots. However, in one of the sub-lines, some flies with small, slight rough patches are found.

Discussions:

The phenotypes of the homozygous lethal mutation in the eye of *Drosophila* are used to determine the functions of the specific genes disrupted in eye development. We will discuss here the different large clones phenotypes obtained and interpret whether the gene plays an important role in eye development.

Wild-Type:

Three out of my eight stocks show a wild-type phenotype in the large clones. These results signal several different possibilities. The first possibility is that the gene is not express in the development of the eye. If the gene has no function in eye development, then however severely the gene may be disrupted, there will still be no phenotype in the eye. The second possibility is that the gene regulates an important function in eye development. The fly genome has various genes to control one important function in case a mutation occurs in one of the regulatory gene. If there is no backup gene to take over the production of that protein, then it will lack of the protein and a whole chain of process necessary for *Drosophila* to develop will stop. If there is another gene controlling the same function as the disrupted gene, then it is possible that that the other gene will be able to produce enough protein necessary for development continue without any problem. The last possibility is that the P-element in inserted into intron and was spliced out during development process. With the P-element gone, it will have no effect on the interrupted gene.

In stock 11914, gene CG8231 is disrupted. CG8231 controls protein folding, an important function in development. It is possible that because of its importance, the *Drosophila* genome has another gene that also regulates the same function as CG8231 to continue the development process in case of a mutation.

In stock 11882, the gene Ben is disrupted by the P-element. Like CG8231, Ben controls protein folding and DNA binding. It produces ubiquitin-conjugated enzymes

that are critical for cell's viability. With such an important function, it is also most likely that there is another gene to take over for Ben in case it is unable to code for the enzyme. In one of the sub-lines for this stock, however, I discovered a large clone mosaics that has a slightly rough phenotype. This is the only one that I found in its sub-line and the entire stock. This phenotype may be due to a contamination. The second possibility is that another gene is disrupted during mitotic recombination that is vital to eye development. This prevents the production of protein coded by this hypothetical gene, and therefore, will produce a slightly rough phenotype in the eye.

Lastly, stock 10138 also shows wild-type phenotype in the large clones mosaics. In 10138, the P-element disrupts the gene Ras. This produces KDPG and KHG aldolase, cystathionine beta-synthase, glutamate synthase protein, and is a phosphate binding site. A likely function of this gene is the production of amino acid. Amino acids are critical to cellular development. It is also very likely that there is another gene that helps regulate the same functions as Ras. However, there was slight roughness found in one of my sub-lines. This may due to contamination on my part, or as in 11882, another gene is disrupted during mitotic recombination.

Rough:

Four of my stocks were found with rough phenotypes. Because of the results, we can conclude that the interrupted gene plays an important role in eye development. By disrupting the gene with P-element, specific amino acids production cannot occur. This has a cascade effect, inhibiting future process that requires the usage of these proteins.

In stock 11886, the large clones mosaics show a rough phenotype. The gene disrupted, CG8198, controls nitrogen fixation and iron binding. By disrupting CG8198, it also inhibits the production of the protein necessary for protein and transcript to occur. If transcription is unable to occur, this will stop a majority of the protein synthesis. Since in the large clones, majority of the eye contains the homozygous P-element mutation, many of the ommatidia will be incomplete due to this obstruction during the beginning of eye development. No roughness is observed in the small clones. This may be because the area disrupted is too small to observed under microscope. Another reason for this may be because other normal cells surrounding the mutated ones are able to produce the proteins necessary for transcription to occur and share these proteins with the mutated cells. Thus, these mutated cells have enough proteins necessary to continue with their normal functions. However, in many of my sub-lines, I also found flies with both rough and glossy phenotype. This may because there are not enough normal cells around the mutated cells to help produce the missing proteins, causing the cells to die in the glossy eye. It may also be because another gene was disrupted during mitotic recombination, and the disruption of its functions causes the glossiness seen in the eye. From the results seen in the large clone mutations, we can conclude that CG8198 has an important function in eye development.

In stock 11884, the rough phenotype is seen in both the small clones and the large clones. The gene disrupted, CG33206, is involved in the production of DNA, regulation of nucleic acid binding and organization of the cytoskeleton. Many of the primary development will not occur when the gene is interrupted. This interruption may be enough to cause a slightly rough phenotype to be seen in small clones. The small clone phenotype is not as severe as the large clones and it may be because of the sharing of the missing protein by neighboring cells. The gene is also responsible for cell metabolism and protein synthesis. With many of the cells lacking this gene, no proteins are produced

to promote cells' uptake of amino acids. If the metabolism does not occur properly, more severe roughness can be seen in the eyes. In addition, males are found containing the P-element in both the small and large clones. This suggests that the interruptions in the gene may not be as lethal to males as previously expected.

The large clones of stock 11877 also have rough phenotype. The P-element disrupts CG1558, which controls DNA production and cell division. When the gene is disrupted, cell division will not occur properly. This is shown with fused ommatidia in the fly eye. From the pictures of SEM, we can see that only about half of the eye is rough. This suggests that neighboring cells are able to share the protein produced by CG1558 with the mutated cells, thereby allowing for DNA production and cell division to occur normally. The rough area lacks normal cells to produce the proteins and therefore cell divisions cannot occur properly, resulting in fused ommatidia. Few males are also found containing the P-element. As in stock 11884, this suggests that the mutation does not cause lethality in males.

In stock 11807, the P-element interrupts CG12238. This gene is needed to produce PHD zinc finger. Zinc finger is used in DNA binding, so a disruption in CG12238 means that the DNA replication process will be affected in some way. Similar to stock 11877, only about half of the eye is rough in most of the sub-lines. This implies that normal cells are able to share zinc fingers with neighboring mutated cells. Moreover, CG12238 have a regulatory role in cell growth and cell division. This explains why fused ommatidia are observed in large clones SEM pictures.

Pupal Lethal:

In stock 11876, the P-element interrupts dlg1. Dlg1 controls protein receptors and bindings. It is also responsible for regulations of enzymes, metabolism, and transportation of nucleotide around the cells. In the small clones, the mutation produces an eye that is rough and glossy. This suggests that Dlg1 is vital to *Drosophila* eye development. Without the proteins produced by this gene, many of the primary functions, such as polypeptides and protein productions cannot occur. Lack of this gene also impedes nucleotide transport and the metabolism of the cells. Since the gene may be expressed as early as oogenesis, the mutations in the eye of the flies may vary according to the time this gene is first expressed in development. In large clones, the mutation is found to be pupal lethal. When observed under the light microscope, the pupal are found to be headless inside its cocoon. This suggests that not only is this gene vital in eye development, it may also contributes to the development of other parts of the body. If the cell cannot produce the necessary enzymes, it may lead to incorrect development of the head.

Reference:
FlyBase. http://www.flybase.org/.
National Center for Biotechnology Information. http://www.ncbi.nlm.nih.gov/.

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