Abstract:

This research lab aims to study the functions of the Drosophila genes that affect the eye development through reverse genetics. The whole genome of the Drosophila is sequenced already but the functions of the genes remain obscured. One method of gaining knowledge about the functions of the gene is to observe the problem or presence of mutant phenotypes in the flies associated with the loss or malfunctioning of the gene that is required for proper development. In this case, p-element insertion, a transposon gene, in chromosome 2L will disrupt the proper functioning of the gene, thus inducing mutations in the flies.

For this lab, we are only interested in studying the functions of the genes that contribute to the development of the eye. Two copies of the p-element should be presence in the cells for full expression of the mutant phenotype because now both alleles of the gene will be affected. But the whole fly would die since it is a homozygous lethal mutation. So we induce mitotic recombination in the eye cells by introducing the eyeless enhance. It is an eye specific promoter that will only activate the FLP gene in the eye cells and cause mitotic recombination to occur at the FRT40A site. As a result mosaic eyes will be observed where some clones of cells in the eyes will have two copies of the p-element. Thus we can observe the effects of the p-element insertion into the gene. If indeed the insertion of the p-element into a particular gene blocks the proper function of the gene required for eye development, some kind of mutant phenotypes like roughness, glossiness or some other defects will appear. Then, we will be able to deduce some information about the function since disrupting the gene leads to some sort of abnormalities in the formation of the eye. However not all genes interrupted by the p-element are required for the eye development, and mutant phenotypes will not show up in those cases. By seeing whether problems arise enable us to deduce which genes contribute to the development of the eye.

Introduction:

Formation of the eye happens during the embryonic stage of development, where about 20 cells are set aside to give rise to the eyes. There are two types of cells in the lava stage and they are the lava cells needed for survival and the growing cells, which are the imaginal cells. These imaginal cells will eventually take form as discs, including the eye and antenna discs. Mutant phenotype arises in the eye when there is a mutation or any event that disrupts the normal pathway during the formation and recruitment of the cells that make up the eye. Each facet has eight neurons surrounded by four cone cells, which are supporting cells. When these cells are normally formed in the eyes, the ommatidia and the bristles will be perfectly aligned, giving a very smooth surface and symmetrical pattern. But when there are problems in the formation of the neurons or the supporting cone cells, abnormalities will be observed. The glossy mutant phenotype results from a late defect in the recruitment of cells, causing the malformation of cone cells that affect the proper formation of lens cells. The glossy eye will appear smooth because of the loss of structure of the ommatidia. Glossy eye mutations may affect pigment distributions also, resulting in the presence of white patches. The rough phenotype, on the other hand, is an early defect in the recruitment of photoreceptors cells. When the order of recruitment goes wrong or when too many cells are recruited at the same time, resulting in clusters of nonsymmetrical or unparallel ommatidia, this gives the impression of

roughness in the eyes. When mutations kill off the eye cells, the resulting eye will be very small and has a cell lethal phenotype. Malformations in these cases may be due to problems in cell communication because of disruption of important genes. Cells interact with each other by giving and receiving signals from neighboring cells via junctions. If communication errors happen because the gene is not functioning properly, abnormalities or mutations will occur, giving rise to these mutant phenotypes.

In order to observe these mutant phenotypes and thus study the functions of genes required for eye development, we need to disrupt genes, leading to the abnormal production and expression of proteins encoded by the gene. These disruptions are caused by p-element insertions. P-elements are transposon genes unique to the Drosophila genome. They hop around from chromosomes to chromosomes randomly, but for the purpose of this lab, the p-element is inserted into the genome only once and there it will be stabilized. This is important since our goal is to study the effects of the p-element insertion in a particular gene.

P-element insertions are random, but the Bloomington stocks have already picked out flies with p-elements inserted into known sequences of genes. Our purpose is to study the effects of the p-element insertion into a gene and conclude whether that gene has anything to do with the eye development. If mutant phenotypes arise in the eye like roughness, glossiness or problems in the formation of antennas or head, the disrupted gene is necessary for the eye development.

Two copies of the p-elements cause fatalities since they are disrupting both genes on homologous chromosomes. Flies with one copy will survive but will eventually be replaced by the wild type flies since many unknown side affects and disadvantages affect the growth and productions of the flies with the p-element induced mutation. Thus we will not be able to maintain stocks of flies with the p-element insertion. By introducing the balancer chromosome, we are able to maintain a balanced lethal line because only flies with a copy of p-element and a copy of the balancer are going to survive. The flies in the balanced stock will have the same genotype as the parents. Offsprings with two copies of the balancer chromosomes will die because the balancer chromosome is a homozygous recessive lethal. In addition the balancer chromosome, which is inverted, causes multiple inversions to occur, thus eliminating the recombinant offsprings. If chromosomes line up to pair inorder to recombine and undergo crossing events, inversions and looping must take place to have the correct match of alleles. Inversions will result in deletions of genes required for development or addition of genes, disrupting normal cell activities. The only offsprings viable will be those with a copy of the p-element and a copy of the balancer. The last feature of the balancer chromosome is to allow the experimenter to recognize the presence of the balancer in the flies since it is a dominant marker. Flies with one copy of the balancer will either have curly wings or the scutoid bristles in this lab.

Flies in the balanced stock have only one copy of the p-element induced mutant gene. In order to fully see the extend of the problem or mutations in the flies as a result of the malfunctioning of the gene, two copies of the p-element should be presence to affect both of the alleles on homologous chromosomes. Mentioned earlier, flies will die if two copies of the p-elements are present and expressed in every cell. This is why it is good to study mutations in the eye since lethal mutations induced by two copies of the p-elements or any abnormalities in the eye development will not in most cases affect the

viability of the flies. So we induce mitotic recombination during the developmental stage of the flies in order to form homozygous mutant cells in the eye. Only eye cells will undergo mitotic recombination because the eyeless enhancer is an eye-specific promotor. When activated, it will induce the FLP gene to produce the flpase protein, which will land on the FRT/FRT site and cause mitotic recombination to occur between the chromosomes. Since not all cells in the eyes can sense the protein required for mitotic recombination to take place at the FRT site, only a small amount of cells will undergo mitotic recombination and produce different clones of cells. In addition, only flies with the genotype FRT/FRT can have mitotic recombination in the eye. After mitotic recombination in an eye cell, one daughter cell will have two copies of the p-element and the other daughter cell will be wild type. These cells will individually form clones of cells identical to themselves. The clone of homozygous wild type cells will be white since they don't have the p-element, whereas the homozygous mutant clone of cells with two copies of the p-element, hence two copies of the w+ gene, will be redder or orange darker than the heterozygous cells with one copy of the p-element. When mitotic recombination takes place in the eyes of flies with FRT/FRT sties, they will have mosaic eyes with patches of white, orange and darker orange or red. The white clone along with the reddish or dark orange patches are known as twin spots. However if the p-element is cell lethal, the homozygous mutant cells will die. We can still recognize flies with cells that underwent mitotic recombination because the white clones will be largely expanded with occasional orange spots, which are the heterozygous cells. At this stage, we can also calculate the recombination frequency or map distances between the p-element insertion site and the FRT site. The closer the distance between the p-element insertion and the FRT sites, the lesser the chance of mitotic recombination taking place and thus lower the recombination frequency. The farther the distance between the p-element insertion and the FRT sites, the more chances of mitotic recombination happening. This will give higher recombination frequency.

Observing mosaic color only suggests the occurrence of mitotic recombination since it marks out the homozygous wild type, heterozygous and homozygous mutant, but does not necessary show the mutant phenotype. Further analysis is needed to draw any conclusions, so we set up week 5 crosses. The female in the cross will introduce the minute gene, w+M, into the next generation. The offsprings from this cross do not inherit the two copies of the p-element from the father since only the eye cells, which are somatic cells, underwent mitotic recombination, not gametes. The offsprings showing the mutant or the mosaic phenotype in the eyes is the result of mitotic recombination in their own eye cells, this time with the minute trait in their genome inherited from the mother. The minute gene gives the cells full red color and is homozygous recessive lethal. After mitotic recombination, cells in the eyes with two copies of the minute gene, w+M/w+M, will die while heterozygous cells with one copy bearing the genotype, P[w+]/w+M, will be red and have a slower growth than those cells without the minute trait but carry two copies of the p-elements, p[w+]/p[w+]. These cells will be orange or reddish orange. The minute trait serves as a way to slow the growth of heterozygous cells and kill off homozygous wild type cells. This enables the homozygous mutant cells to form large clones, making it easier for us to spot these mosaic or mutant phenotypes in the eyes. The mosaic eye will have a large clone of orange or reddish orange cells with smaller clones of red cells in the background. If mutant phenotypes do show up in the

eyes, we can conclude that the disrupted gene is required for the proper formation of the eye. However in some cases, we can already observe mutant phenotypes in week 5 from week 3 crosses and thus conclude that the gene is very important for the eye development. Carrying out week 5 crosses serves as a mean to confirm our discoveries.

Research Method:

The whole point of setting up different crosses throughout the weeks is to produce flies with homozygous mutant cells in the eyes. Mutant phenotypes are then easily observed with the formation of large clones of mutant cells. But two copies of the p-elements cause fatalities, so it is impossible to have ready stocks of flies with two copies of the p-element. So flies with one copy of the p-element are put in balanced stocks. In order to study flies with the desired genotype, I need to set up crosses weeks after weeks.

I will be working with 16 P-element lines, some from group A and some from group B. Group A stocks have very small distances between the p-element insertion and the FRT site, so mitotic recombination occurs rarely. Thus group A stocks will have recombination frequencies close to zero. The distance between the p-element insertion and the FRT site in group B stocks is bigger, so mitotic recombination happens more often, hence higher recombination frequency.

I am given 16 Instructor's crosses, done in the previous week. The instructor's cross is between females with ey-FLP gene, curly wing, and Scutoid markers and males with the p-element and the curly wing marker. The mother will pass on either the scutoid marker or the curly marker to their offsprings; this will ensure that viable offsprings have one copy of the balancer chromosome in their genome. The male progenies that I need to pick out from each instructor's cross will be able to inherit the ey-FLP gene and either the curly wing marker with y+ or the scutoid marker from the mother, and the p-element from the father. They will have gray body because of the y+ gene, reddish or dark orange eyes because the p-element has the w+ marker and curly wings. I also need to collect F38 virgins. These flies will carry ey-FLP and FRT40A, two important genes that needed to be passed on to the offsprings. Now, I am ready to set up my week 1 cross between F 38 virgins and the males from the instructor's crosses.

By the end of week 1, I should have 16 crosses set up. Week 2 will be dedicated to transferring these 16 crosses every 2-3 days, group B crosses into vials and group A crosses into big bottles because I need to examine lots of progenies. Its significance will be discussed later. The flies will be laying lots of eggs and transferring them into new vials will reduce crowdedness. Also the presence of fresh food provides them with the optimal environment to mate.

As week 3 rolls around, the progenies are coming out from week 1 crosses. I will be collecting virgin females with ey-FLP and FRT40A inherited from the mother, and the p-element inherited from the father. They will have the characteristics of yellow body, red eyes and straight wings. Other offsprings with different phenotypes like curly wings or white eyes will be discarded. Virgins hatch out the most in the morning, so I will be coming in during that time to catch them. To set up week 3 crosses, I need to collect F38

males, which have yw ey-FLP and FRT40A, with the characteristics of yellow body, straight wings and white eyes. Then I will cross them with the virgin females I collected for each p-element line.

It is important that both FRT and the p-element are present in the virgin females because we want the offsprings from these crosses to have both the p-element and the FRT on the same chromosome. This is achieved through meiotic recombination, which only occurs in Drosophila females. In this case, the female will produce one type of recombinant gametes in which both the p-element and FRT40A are recombined to be on the same chromosome. Meanwhile the father will pass on the FRT gene.

Week 4 is another week dedicated to transferring group A and B crosses and also making sure that the flies are producing lots of eggs. The distance between FRT and the p-element is very small in group A flies, thus it is less probable to find recombinant offsprings. This is the reason I need to transfer group A crosses into big bottles so I could examine lots of progenies.

As week 5 comes along, the offsprings from week 3 will hatch out. This time I need to collect males and females with white/orange mosaic eyes, and yellow body. Mosaic eyes result from the onset of mitotic recombination, which is targeted only in the eye cells. Mitotic recombination will take place only in flies with two copies of the FRT in the same location on homologous chromosomes. The mosaic patches mark the homozygous mutant cells, heterozygous and the homozygous wild types. The males will be used to set up week 5 crosses while the females will be taken light microscope pictures and SEM pictures if mosaic eyes show mutant phenotypes.

For each p-element line I need to set up three single week 5 crosses. Each is a cross between the mosaic eye male with 5 F39 virgin females. Setting up three single crosses reduces the risk. If something went wrong in the cross, I don't need to waste all of them. Also three separate crosses serve as a comparison of the results obtained.

I could approximate the distance between the p-element insertion site and the FRT site by calculating the recombinant frequencies and converting the data into map units. Doubling the amount of recombinant offsprings and then dividing that by the total number of offsprings and multiplying by 100 will give the number of map units between the p-element and the FRT sites. I double the amount of recombinant offsprings because one type of recombinant flies gives white eyes, thus not being able to distinguish them from the wildtype flies, which also have white eyes. By doubling the amount of the flies with the observable recombinant trait, which is the presence of the mosaic eye, I can account for the recombinant offsprings that are not distinguishable. The data on recombination frequencies and distances of the stocks are given on chart 1 in the result section.

Mosaic eyes indicate the presence of mitotic recombination, not necessary the mutant phenotype. The purpose of week 5 crosses is to make these mutant or mosaic phenotypes easier to spot. The F39 virgins will carry the minute gene, w+M, which is then passed on. The eye cells of the offsprings will undergo mitotic recombination at the FRT/FRT site. Wild type cells with two copies of the minute gene will die, and the homozygous mutant cells will have no minute gene. Heterozygous cells with one copy of

the minute will slow down in growth. So homozygous mutant cells can form a large clone of cells, making it easier to spot the mosaic color and the presence of mutant phenotypes like roughness, glossiness, etc, if present.

During week 7 and 8, I will be setting up balanced stocks of flies with one copy of the p-element, FRT and the balancer chromosome. The offsprings will have the same genotype as the parents, thus maintaining the stock. I will also pick out offsprings with mosaic eyes having large clones of orange cells with smaller clones of red cells and possibly the mutant phenotype. If mutant phenotype does arise, we can conclude that the function of the disrupted gene is associated with the eye development. I will take both the light microscope and SEM pictures to document the mutant phenotypes in the eye. The light microscope picture gives a general representation of the phenotype and the SEM picture gives a clearer view of what and where the malformations take place.

Results:

Chart 1: Recombination Data

Group B

Stock #	# of recombinant offsprings	Total # of offsprings	Experimental recombination frequency	Experimental recombinantion map distance between p[w+] and FRT40A (m.u.)	Theoretical recombination map distance between P[w+] and FRT40A (m.u.)
10583	48 x 2 = 96	237	.405	40	38
10520	6 x 2 = 12	174	.068	6.8	10
10646	42 x 2 = 84	201	.42	42	54
10523	15 x 2 = 30	294	.10	10	10
10617	15 x 2 = 30	219	.14	14	15
10457	36 x 2 = 72	208	.346	35	47
10692	Head/eye phenotype 24 x 2 = 48 wild type 12 x 2 = 24 Total=72	232	.31	31	51
10644	12 x 2 = 24	240	.1	10	13

12384	21 x 2 = 42	242	.17	17	11
10687	8 x 2 = 16	316	.05	5	6
10476	16 x 2 = 32	226	.14	14	12
11070	21 x 2 = 42	245	.17	17	22

Group A

Stock #	# of recombinant offsprings	Total # of offsprings	Experimental recombination frequency	Experimental recombinantion map distance between p[w+] and FRT40A	Theoretical recombination map distance between P[w+] and FRT40A (at 54)
15108	30 x 2 = 60	1039	.057	5.7	4 m.u.
10995	5 x 2 = 10	353	.028	2.8	1
13071	18 x 2 = 36	742	.048	4.8	3
10562	0	1958	0	0	0

The summary of each stock, including the genotype, gene and molecular information, can be found in chart 2. The full description of the phenotypes that are observed from each stock is found in the following paragraphs.

Group B

Stock 10583

Orange/white mosaic eyes are observed with at least half of the eye white in color for the small clone analysis as seen in fig.1a. The male eyes tend to be darker and redder than the female eyes, expressing the red color more (fig.1b). The small clone phenotype is wildtype. Homozygous mutant twin spot clones are difficult to visualize because there is little difference in color between dark orange and orange. Dark reddish orange / red mosaic eyes are observed for large clone analysis and appear to be wildtype according to fig.1c.

Stock 10520

Orange/white mosaic eyes are observed and appear to be wildtype for the small clone analysis (fig.2a). About half of the eye is white and the homozygous mutant twin spot clones are hard to visualize. Orange/red mosaic eyes are not observable for large clone analysis. Instead the whole eye appears dull red and about half the size of the normal eyes according to fig.2b. This gives the cell lethal phenotype. Rough edges are visible along the outer rims of the eyes as seen in both the light microscope and the SEM pictures (fig.2b-c). There are different sizes of the eyes but all of the cell lethal eyes are at least half the size of the wildtype ones, some being even less than $1/4^{th}$ the size.

Stock 10646

Orange/red/white mosaic eyes are visible in small clone analysis and appear to be wildtype as seen in fig.3a. Twin spots, which are regions made up by the homozygous mutant cells (red) and the homozygous wildtype cells (white), are discernable because of

the obvious differences between these colors. Dark orange/dull red mosaic eyes are visible in the large clone analysis and appear to be wildtype also as indicated by the light microscope picture, fig.3b. Male eyes tend to give a redder color than females'.

Stock 10523

Orange/white wildtype mosaic eyes are observable, but the white clones are largely expanded with occasional tiny orange spots for the small clone analysis as seen in fig.4a. The phenotype is wildtype. Twin spots are not visible. The large clone phenotype is cell lethal thus the orange/red mosaic eyes are not observable as seen in fig.4b. The eye has a dull red color, indicated by the light microscope picture. Rough edges along the outer rims of the eyes as well as roughness appearing in the eye due to the unparallel alignment of the ommatidia are visible in the SEM picture, fig.4c. Eyes affected by the p-element insertions are relatively small, less than half the size of the normal eyes.

Stock 10617

Orange/red/white mosaic eyes are clearly observable with the presence of twin spots in the small clone analysis, fig.3c. The eye appears wildtype. Dark reddish orange/red mosaic eyes are visible in large clone analysis and appear to be wildtype also, fig.3d. Because males have eyes that are brighter in color, they tend to express the red color more than the females'.

Stock 10457

Orange/white mosaic eyes are visible, but the white clones are largely expanded with occasional orange spots in small clone analysis, fig.5a. The phenotype is wildtype. The homozygous mutant twin spots are not observable. Two mutant phenotypes are observed in the three single male crosses for the large clone analysis. Majority of the mutant eyes in large clone analysis are cell lethal. Orange/red mosaic eyes are barely observable as indicated by the light microscope picture, fig.5d. The cells of the eyes are not lined up correctly and rough edges along the outer rims are visible as shown clearly in the SEM picture, fig.5e. They are somewhat smaller than normal eyes. The other mutant phenotype is rough eye. Orange/red mosaic eyes are visible (fig. 5b). SEM picture shows roughness occurring in areas where the ommatidia are not lined in a parallel fashion, fig. 5c.

Stock 10692

Two phenotypes are observed in small clone analysis. One phenotype is wildtype. Orange/red/white mosaic eyes are observable in wildtype flies for the small clone analysis as seen in fig.6a. Twin spots are clearly visible. The other phenotype is rough eye with an enlarged head (fig.6d). Orange/white mosaic patches are also observable with the presence of twin spots. Roughness is confirmed with the SEM picture of the small clone (fig.6e). Two phenotypes are also observed in the large clone analysis. For the wildtype phenotype, orange/red mosaic eyes are hard to visualize in males because they have very bright red eyes as seen in the light microscope picture fig.6c. Orange/red wildtype mosaic eyes are easier to be observed in females since they have a lighter color, fig.6b. The mutant phenotype is rough eye with an enlarged head just like in the small clone analysis except this time the mosaic eye is reddish orange/dull red as in fig. 7a. The

mosaic character is very hard to visualize. SEM picture shows roughness occurring in areas where the ommatidia are not lined up in a parallel way and where the bristles are not pointing in the right direction (fig. 7b). For example, some are pointing toward each other. Compared to the wildtype head, fig.7d, the mutant head looks bigger and malformed as shown in fig. 7c.

Stock 10644

Orange/red/white mosaic eyes are visible and appear to be wildtype in small clone analysis, with the presence of twin spots, fig.8a. Two phenotypes arise in the large clone analysis. A and B single male crosses yield flies with wildtype eyes. Dark reddish orange/red mosaic eyes are clearly discernable, fig.8b. Male eyes tend to be darker and redder than the female eyes. However in single cross C, the large clone phenotype appears to be slight glossy and rough as seen in the light microscope picture, fig.8c. Orange/red mosaic eyes are still observable. Where the mutant clones are glossy, white patches are apparent as seen in the light microscope picture, fig.8c. SEM picture shows slight roughness and partial loss of ommatidia structure in glossy areas, fig.8d. Bristles not pointing in unison are also observable in the SEM picture.

Stock 12384

Orange/red/white mosaic eyes are visible in small clone analysis and appear to be wildtype, fig.3e. The homozygous twin spot clones are clearly observable. Dark orange/red mosaic eyes are visible in large clone analysis and appear to be wildtype as indicated by the light microscope picture, fig.3f. Male eyes tend to have a brighter red color than the females'.

Stock 10687

Orange/white mosaic eyes are visible and appear to be wildtype for the small clone analysis. At least half of the eye is white as indicated by light microscope picture, fig.9a. Twin spots are not too apparent since there is not much difference between the colors, dark orange and orange. Dark reddish orange/red mosaic eyes are observable in large clone analysis and appear to be wildtype shown in fig.9b-c. The male eyes tend to be redder and harder to observe the mosaic character as seen in fig.9b than the female eyes, fig.9c.

Stock 10476

Orange/red/white mosaic eyes are discernable in small clone analysis and appear to be wildtype fig.9d. The homozygous mutant twin spot clones are clearly observable. Orange/red mosaic eyes are quite difficult to be observed because the eyes are very red in large clone analysis as shown by the light microscope picture, fig.9e. The phenotype appears to be wildtype with the ommatidia lining up in a symmetrical pattern.

Stock 11070

Orange/white mosaic eyes are visible and appear to be wildtype in small clone analysis as seen in fig.10a. The white clones are greatly expanded with occasional orange spots. Twin spots are not apparent. The large clone phenotype is cell lethal and the orange/red mosaic eyes are not observable. Rough edges are discernable in the light

microscope picture, fig.10b. The mutant eyes appear smaller than normal eyes and have a dull red color. SEM shows roughness in areas where the ommatidia are not parallel and where the bristles are not pointing in the right direction, fig.10c. Also there are blisters in some of the ommatidia.

Group A

Stock 15108

Dark reddish orange/white mosaic eyes are observed for small clone analysis and appear to be wildtype as seen in fig.11a. The homozygous mutant twin spot clones are clearly visible along the edges of the eye. Orange/red mosaic eyes are quite hard to visualize in the large clone analysis due to the redness of the eyes. The mutant clones appear rough as indicated by the light microscope picture, fig.11b. SEM confirms the roughness of the eyes in areas where the ommatidia are not lined up in a parallel fashion and where the bristles are not pointing in a unison pattern, fig.11c. There is also an abnormal antenna formation. Enlarged black nodes of the antennas are visible in both the light microscope and the SEM pictures, fig.11e-f compared with the wildtype antenna in fig.11d.

Stock 10995

Orange/white mosaic eyes are observed and appear to be wildtype for the small clone analysis, fig.12a. The homozygous mutant twin spot clones are difficult to visualize due to little difference in color between dark orange and orange. Orange/red mosaic eyes are observed for large clone analysis and appear to be wildtype as shown in the light microscope picture, fig.12b.

Stock 13071

Dark orange/white mosaic eyes are apparent and appear to be wildtype in small clone analysis, but the white clones are greatly expanded, fig.13a. Twin spots are not visible. The large clone phenotype is cell lethal, so the orange/red mosaic eyes are not observable. The eyes are very dull red and small as indicated by the light microscope picture, fig.13b. SEM shows abnormal eye shape and roughness in the eye where the ommatidia are not lined up correctly due to cell deaths, fig.13c. In some cases, the mutant eyes are extremely small and have protruding characteristic as seen in fig.13d-e.

Discussion:

We want to study the functions of the genes in the eye development by disrupting genes with p-element insertions. These mutations are lethal and the flies would die before they could produce offsprings if two copies of the p-element are present in all cells. We must have a means to localize the mutation and the eye seems to be the perfect choice. First it is easy to see the mutations in the eye areas under a microscope. Flies with mosaic eyes definitely are recombinant with clones of cells containing two copies of the p-element. Also the eye is a dispensable organ. Lethal mutations in the eye or even the absence of the eye will not affect the viability of the flies in most cases. As can be seen, localizing the mutation in the eye cells not only make it easier to observe the mutation but also allow us to study which genes are required for proper eye development. By gaining knowledge about the Drosophila's genome may be beneficial to the understanding of diseases as well as other manifestations that occur in our biological systems. Studying human subjects is impossible and since many similarities arise between sequences of genes and development in Drosophila and Humans, we can infer information that could apply to understanding our systems from the studies already done on flies. More understanding help scientists find possible treatments and even cures to certain diseases resulted from mutations or disruptions of genes. In addition, Drosophila is a good candidate to do research on because of certain characteristics in their genome like the presence of p-elements, balancer chromosomes and the existence of only 4 chromosomes.

Group B

Stock 10583

The disrupted gene is vrille. The gene encodes a product with transcriptional regulatory activity, which is involved in locomotor rhythm. Its amino acid contains a basic region leucin zipper transcription factor, which is involved in protein kinase signaling in the smooth muscle cell development during metamorphosis. The gene may have some functions in regulating gene expression, apoptosis, motility, proliferation and more. Hypomorphic mutations affect the maternal wing and are embryonic recessive.

Since both small and large clones are wildtype with no signs of mutant phenotypes, this suggests the insignificance of the gene in the development of the eye.

Stock 10520

The disrupted gene is abrupt. The gene encodes a product with specific RNA polymerase II transcriptional factor activity involved in axon choice point recognition. The regulatory protein controls the specificity of neuromuscular connections. Two conserved domains are found and they are the BTB/POZ domain and the Zn finger proteins. Amorphic mutations affect the abdominal posterior fascicle, abdominal transver nerve, and other tissues.

Mutant phenotype shows up in the large clone. Very small eyes suggests that the p-element affects cell growth at the late stage of eye development because if it is affected in an earlier stage, the heterozygous cells could have replaced the mutant, dead cells. Since it is at a late stage, there is no time to replace those malformed cells. Also, heterozygous cells grow at a slow rate because of the presence of one copy of the minute

gene. Cell lethal results in small eyes with significant amount of roughness and rough edges along the outer rims of the eye. Small clone analysis doesn't show any mutant phenotype because the number of homozygous mutant cells is small and unable to show the phenotype at that early stage. Since mutant phenotype arises due to the p-element insertion into the gene, this implies the importance of the abrupt gene in the eye development. The gene may be involved in regulating RNA polymerases and transcriptional activities during eye development.

Stock 10646

The disrupted gene is split ends. The gene encodes a product involved in glia cell migration. It also regulates the development of midline glial cells. Its amino acid sequence contains a RNA-binding region. The gene may be important in regulating the expression of transcriptional effectors in signaling pathways.

Since both small and large clone phenotypes are wildtype, the gene is not necessary for eye development.

Stock 10523

The disrupted gene is scar. The gene encodes a product with cytoskeletal regulatory activity. Its amino acid sequence contains the Wiskott Aldrich syndrome proteins, which are involved in cytoskeletal activities by regulating actin nucleation. It may also be involved in the development of the embryonic central nervous system.

In the small clone analysis, the white clones are largely expanded, while the homozygous mutant red clones died off because two copies of the p-elements causes cell lethality. Twin spot clones are not visible since the red clones are not formed. One copy of the p-element already affects the growth of the cells, so heterozygous cells grow slower than the wildtype cells. Thus the majority of the eye is white. In the large clone analysis, it is clearly visible that the phenotype is cell lethal. This causes very small rough eyes to form as seen in the SEM. Eyes affected by the p-element insertions are relatively small, less than half the size of the wildtype eyes, suggesting that the p-element affects the eye at a late stage. At a later stage, more cells die, and it is harder to replace these many dead cells with heterozygous ones. Also, the heterozygous cells grow slower because of the presence of a copy of the minute gene. The wildtype cells which give white color are already killed off because they carry two copies of the minute gene after mitotic recombination. The presence of mutant phenotypes implies that the scar gene is essential to the proper formation of the eye. The presence of the WAS protein domains suggests that the gene may take part in regulating cytoskeletal activities during the development of the eye.

Stock 10617

The disrupted gene is pendulin. It encodes a product with protein carrier activity. It is involved in the intercellular transport of NLS-containing proteins from the nurse cells to the oocytes. Its amino acid sequence contains an importin B binding domain, which is involved in intracellular trafficking, secretion, and vesicular transport. Mutations affect spermatid, dorsal appendage, and the egg.

Because small and large clone phenotypes are wildtype, the pendulin gene is not required for the proper formation of the eye.

Stock 10457

The disrupted gene is overgrown hematopoietic organs at 23B. It encodes a product with structural constituent of ribosome, which is involved in protein biosynthesis. Its amino acid sequence contains a ribosomal protein s21e, which is involved in translational activities and protein biosynthesis. Mutations show defective developmental rate.

The cells with two copies of the p-element, normally red in color, have died and replaced by wildtype cells so twin spot clones are not observable in the small clone analysis. White clones are thus largely expanded with occasional orange spots. These heterozygous cells are already affected by the copy of the p-element insertion, causing them to grow slowly. Two mutant phenotypes arise in the three single male crosses. Majority of the mutant eyes in large clone analysis are cell lethal. The eyes have rough edges and are somewhat smaller than wildtype eyes, which suggests the p-element insertion affects the eye development at an early stage. Thus, the heterozygous cells have more time to replace these dead mutant cells before development is finished even if they carry one copy of the minute gene, which affects their growth rate. The other mutant phenotype is rough eye with smoother edges along the sides. Slight roughness occurs when the ommatidia are not parallel and the bristles are not all pointing in the same direction. Since rough eye is the minority mutant phenotype, their presence may be the result of second site mutation during recombination events and development. Both mutant phenotypes do suggest the important of the gene in the formation of the eye. The presence of the ribosomal protein s21 e domain implies that the gene may be involved in translational activities and protein biosynthesis in cells, which is quite important.

Stock 10692

There are two disrupted genes and they are glycogen phosphorylase and fat (ft). Glycogen phosphorylase encodes a product with glycogen phosphorylase activity, which is involved in carbohydrate metabolism. Malfunctioning or absence of the protein could lead to abnormal development. Its amino acid sequence contains the carbohydrate phosphorylase domain. Fat (ft) encodes a product with calcium-dependent cell adhesion activity, which is involved in cell proliferation. According to Flybase, its amino acid seuqnece contains a REV protein, which is an anti-repression trans activator protein, a cadherin domain and thrombospondin N-terminal domains. The gene may be involved in conveying dorsal ventral positional information to developing ommatidia to create the dorsal ventral midline. Amorphic mutations affect the imaginal disc and the wings. This results in the overgrowth of the brain and imaginal discs

Two phenotypes are observable in the small clone analysis and they are wildtype and rough eye with an enlarged head. Mutant phenotype is already observable in small clone analysis, suggesting the importance of the gene in eye development. The typical orange/red/white mosaic is observable for the wildtype eye. Orange/white mosaic eyes are still observable for the rough eye. In this case, there is also an enlarged head that may be due to the formation of an oversized brain as a result of the malfunctioning of the fat gene after the insertion of the p-element. So a total of six single male crosses have been done, three for each phenotype. Large clone analysis confirms the result from the small clone analysis. Wildtype flies give rise to wildtype flies with the presence of orange/red

mosaic eyes. Flies with the rough phenotype also give rise to flies with the rough phenotype. Roughness in the eye may be caused by the abnormal growth of the cells due to abnormal glycogen phosphorylase activity that is going on and/or the malfunctioning of the fat gene that causes abnormal formation of the imaginal discs. Thus the ommatidia do not have a parallel alignment. Also along with the rough eye, there is an enlarged head, confirming the mutant phenotype that is present in the small clone analysis. Because of the presence of a head/eye mutant phenotype, this suggests the necessity of the genes, GlyP and Ft, which may function in regulating glycogen phosphorylase activities and cell proliferation activities respectively, during development of the ommatidia and imaginal discs.

Stock 10644

The disrupted gene is ribonucleoside diphosphate reductase large subunit. It encodes a product with ribonucleoside-diphosphate reductase activity, which is involved in DNA replication. Three conserved domains are found and they are the ribonucleotide reductase barrel domain, the ribonucleotide reductase- alpha subunit, which is involved in nucleotide transport and metabolism, and the ATP cone domain, which is involved in the ATP binding in nucleotidereductases.

Two single male crosses, A and B yield wildtype flies for the large clone analysis and the C single male cross yields flies with a slight glossy and rough mutant phenotype. Glossy eye mutations may affect pigment distributions, so in areas where glossiness shows up, there are white patches. Also the ommatidia appear to have a lost of structure because of the malformation of the cone cells or structural cells. Where the ommatidia are not lined up in a parallel way, roughness occurs. Because the mutant phenotype shows up in only one single male cross, it could be inferred that second site mutations may have occurred during recombination events in the single male of that cross. But it can also be implied that the gene may be important in the eye development by regulating DNA replication events. Since majority of the flies are wildtype, it can be speculated that the ribonucleoside diphosphate reductase large subunit gene may be relatively unimportant in the eye development.

Stock 12384

Two p-elements are inserted into two different genes. The first gene is l(2)k09015a. Its function is unknown. Mutations are lethal. The second gene is unknown. No mutation is available on Flybase and no functional analysis has been done for this gene. No conserved domains are founded in the protein blast search.

Since small and large clone phenotypes are wildtype, this implies that the genes are not required for eye development.

Stock 10687

The disrupted gene is Nnp-1. Its amino acid sequence contains the nucleolar protein domain, which is involved in rRNA processing and metabolism. The gene may be involved in rRNA metabolism.

Wildtype phenotypes in small and large clones suggest that the gene is not required for the eye formation.

Stock 10476

The disrupted gene is KDEL Receptor. It encodes a product with KDEL sequence binding, which is involved in retrograde transport (from golgi to ER). One conserved domain is found and it is ER lumen protein retaining receptor, which is involved in intracellular trafficking, secretion, and vesicular transport. The gene may be involved in regulating membrane traffic of proteins in and out of the cells.

Small and large clone phenotypes are wildtype. This implies that the gene is not needed for eye development.

Stock 11070

The disrupted gene is CG13393. It encodes a product with apoptosis inhibitor activity, which is involved in anti-apoptosis. Its amino acid sequence contains KOG1746, the defender against cell death protein/ oligosaccharyltransferase, epsilon subunit domain. No mutation is available in Flybase. The p-element insertion is also close to two other genes and they are CG17295 and CG13384.

For the small clone analysis, twin spots are not visible because the homozygous mutant cells, those that give red color, are killed since two copies of the p-element cause cell lethality. One copy of the p-element already has an effect in cell growth as seen by the smaller number of heterozygous cell, orange, compared to the wildtype cells. Thus the white clones are largely expanded. The large clone phenotype is cell lethal. This results in a smaller eye formed with rough edges along the sides. Also the ommatidia are not lined up in a parallel fashion and the bristles are not pointing in unison, giving the rough impression in the eye. The eyes are generally small, suggesting that the p-element affects the cells at a late stage in development where the homozygous mutant cells die off. The gene CG13393 may be involved in anti-apoptosis activities in cells, and disrupting the gene will interfere with its function. The fact that blisters form, showing the presence of abnormal apoptosis events happening in the cells of the eyes during development, strongly implies that the likely function of the gene is indeed involved in anti-apoptosis events. The presence of the cell lethal mutant phenotype in the large clone suggests the importance of the gene in eye development.

Group A

Stock 15108

The disrupted gene is outspread. The product it encodes is unknown. However its amino acid sequence contains the PH domain, which is usually found in eukaryotic signaling proteins, and the F-actin binding protein, which regulates actin cytoskeletal organization. Mutations are visible and affect the wing.

Large clone analysis shows roughness and antenna defects. The antenna looks like an enlarged black node. Roughness occurs because the ommatidia are not lined up correctly. Antenna defects may be due to the fact that the eye cells and the antennas are formed from closely linked discs. If the eye has been affected and the rough mutant phenotype occurs, this could also affect the proper formation of the antennas since they are so close to each other. The presence of the eye/antenna defect suggests the important of the gene, which may be involved in regulating or participating in the signaling pathway during the developmental stages of the eye and the antennas.

Stock 10995

The disrupted gene is myosin heavy chain. It encodes a product with muscle motor activity, involved in strained muscle contraction. Its amino acid sequence contains a myosin head. The gene may be involved in the regulation of myosin induced skeletal muscle development and movement. Amorphic mutations affect the indirect flight, muscle, other tissues and are dominant flightless.

Small and large clone phenotypes are wildtype, implying that the gene is not required for the eye development.

Stock 13071

The disrupted gene is cyclin E. The gene encodes a product with cyclin-dependent protein kinase, intrinsic regulatory activity, involved in the G1/S transition of the mitotic cell cycle. CycE also has a role in controlling cell proliferation during the eye imaginal disc development. Amorphic mutations are recessive and affect the eye and hindgut.

Twin spot clones are not visible because mutant cells with two copies of the p-element are not formed in the small clone analysis. Two copies of the p-element is cell lethal, so one copy of the p-element affects the growth of the heterozygous cells. Thus the white clones, which are wildtype, are greatly expanded. The large clone phenotype is cell lethal. This results in very small rough eyes. Some eyes are even less than $1/4^{th}$ the size of regular eyes. This implies that the p-element affects the eye development at a late stage, so the slowly growing heterozygous cells cannot replace most of the dying mutant cells before development is over. The gene is definitely important for the eye development. The functions of the gene during the eye development may include regulating cell cycle, cell division and chromosome partitioning.

Stock 10562

There are two disrupted genes and they are the Mlx interactor and the gustatory receptor 39a genes. The mlx interactor encodes a product with transcriptional factor activity, which is involved in regulating transcription. Its amino acid sequence contains a helix-loop-helix dimerization domain. The gustatory receptor 39a gene encodes a product with taste receptor activity. Flies with mosaic eyes were not found in week 3 because mitotic recombination happens so rarely in this stock as a result of a very small distance between the p-element insertion site and the FRT40A site. The recombination frequency as calculated is 0, suggesting that the distance between those sites is zero. Thus no further crosses can be set up for this stock and no analyses of small and large clones are done.

References:

1. Flybase http://flybase.bio.indiana.edu
2. PubMed: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi
3. Berkeley Drosophila Genome Project: http://www.fruitfly.org
4. Baylor P-screen Database: http://flypush.imgen.bcm.tmc.edu/pscreen/

Acknowledgment:

At this moment, I would like to thank all those who have contributed and made this research successful. First of all I would like to thank NIH for giving out the grant, thus allowing us to do research on Drosophila that may vastly add knowledge to the understanding of our own biological system and the onset of certain diseases. This leads us a step closer to treating and hopefully curing some of the deadly diseases like cancer that bugs us for many years. I also want to thank Bloomington for providing the stocks and all the information relating to the p-element insertion sites and the affected genes. In addition I would like to thank Dr. Banerjee for giving us lectures on related topics that help in our understanding of the concepts pertaining to genetics and also open doors and introduce us to the various opportunities in the research field. Also, Dr. Chen has helped us enormously in the lab and answer many questions that clarified the procedures being carried out in the experiments. He also double checked our results and confirmed the presence of mutant phenotypes. Besides the instructors, I would also like to thank the TAs, Eric and Sherlene, for their time and commitment in helping us take SEM pictures. I also want to thank the lab assistant, Helena for her help. Lastly I would like to acknowledge the help of my sister, Jenny Chan. I truly thank her for her time and effort in helping me organize my stocks and take some light microscope pictures for me.

Chart 2: Stock Summary

Name	Stock #	Genotype	Cytological position	Gene and Molecular Info (both full and abbreviated gene names)	Small-clone phenotype (eg. Wildtype, rough, glossy, or wildtype but white clones greatly expanded)	Large-clone phenotype (eg. Wildtype, rough, glossy, cell-lethal, or other)
Amy	10583	y[d2]w[1118] P{ey-FLP}; P{w[+mc]=lacW}vri[k05901]P{neoFRT}40A/Cyo, y[+]	025D04-05	The disrupted gene is vrille (vri). The p-element is inserted within the 1st intron of the vrille gene. Vrille encodes a product with transcriptional regulatory activity involved in locomotor rhythm. Its amino acid contains a bZIP transcription factor family. It genetically interacts with Mad, dpp, and ea. 13 classical mutants are record. Hypomorphic mutations affect the maternal effect wing vein L5 and L2 which are embryonic recessive non-rescuable maternal effect lethal and recessive female sterile.	Orange/white wildtype mosaic	Dark orange/red wildtype mosaic
Amy	10520	y[d2]w[1118] P{ey-FLP}; P{w[+mc]=lacW}ab[k02807]P{neoFRT}40A/Cyo, y[+]	032E01-02	The disrupted gene is abrupt (ab). The p-element is inserted within the 2nd intron of the abrupt gene. Abrupt encodes a product with specific RNA polymerase II transcriptional factor activity involved in axon choice point recognition. Its amino acid sequence contains a BTB/POZ domain and a zinc finger, C2H2 type. The regulatory protein controls the specificity of neuromuscular connections. 16 classical mutants are recorded. Amorphic mutations are recessive male sterile and affect the abdominal posterior fascicle, abdominal transverse nerve, and other tissues.	Orange/white wildtype mosaic	Cell-lethal, very small eyes, rough, dull red color
Amy	10646	y[d2]w[1118] P{ey-FLP}; P{w[+mc]=lacW}spen[k06805] P{neoFRT}40A/ Cyo, y[+]	021B04-06	The disrupted gene is split ends (spen). The p-element is inserted within the 1st intron. It encodes a product involved in glia cell migration, which is localized to the nucleus. It also	Orange/red/white wildtype mosaic	Reddish orange/red wildtype mosaic

				regulates development of midline glial cells. Its amino acid sequence contains a RNA- binding region. 43 classical mutants are recorded. Mutations are recessive lethal and affect the ommatidium and photoreceptor cell formation.		
Amy	10523	y[d2]w[1118] P{ey-FLP}; P{w[+mc]=lacW}l(2)k03107[k03107] P{neoFRT}40A/Cyo, y[+]	032C01-02	The disrupted gene is scar. The p-element is inserted within the 1st exon. It encodes a product with cytoskeletal regulatory activity. It may be involved in the development of the embryonic central nervous system. Its amino acid sequence contains the Wiskott Aldrich syndrome proteins conserved domains. 4 classical mutants are recorded. No mutation is available in Flybase.	Wildtype but white clones largely expanded with little orange spots	Cell-lethal Rough Dull red Very small eyes
Amy	10617	y[d2]w[1118] P{ey-FLP}; P{w[+mc]=lacW}l(2)k06324[k06324] P{neoFRT}40A/Cyo, y[+]	031A01-02	The disrupted gene is pendulin (pen). The p-element is inserted within the 1st exon. It encodes a product with protein carrier activity. It is involved in the intercellular transport of NLS-containing proteins from the nurse cells to the oocytes. Its amino acid sequence contains an importin B binding domain. 6 classical mutants are recorded. Mutations affect spermatid, dorsal appendage, and the egg and are recessive male and female sterile.	Orange/red/white wildtype mosaic	Reddish orange/red wildtype mosaic
Amy	10457	y[d2]w[1118] P{ey-FLP}; P{w[+mc]=lacW}oho23B[k16804a] P{neoFRT}40A/Cyo, y[+]	023B05-06	The disrupted gene is overgrown hematopoietic organs at 23B (oho23B). It encodes a product with structural constituent of ribosome, which is involved in protein biosynthesis, a component of cytosolic small ribosomal subunit. Its amino acid sequence contains a ribosomal protein s21e. There are 7 classical mutants. The lymph gland, larval brain, and imaginal disc are affected by the mutations. The mutations are recessive lethal, recessive hyperplastic, dominant visible and show defective developmental rate.	White clones largely expanded with tiny orange spots, wildtype	Each of the three single male cross gives 2 phenotypes: a. Cell-lethal Orange/red Mosaic visible b. rough eye Dark orange/red mosaic
Amy	10692	y[d2]w[1118] P{ey-FLP};	022B06-07	There are two disrupted genes. One of them is	2 phenotypes:	

		P{w[+mc]=lacW}Gly[pk07918]ft[k07918] P{neoFRT}40A/Cyo, y[+]		glycogen phosphorylase (GlyP). The p-element is inserted within the 1st exon. It encodes a product with glycogen phosphorylase activity which is involved in carbohydrate metabolism. Its amino acid sequence contains a glycogen phosphorylase. There are 2 classical mutants recorded. Mutations reduce viability. The other gene is fat (ft). It encodes a product with calcium-dependent cell adhesion activity, which is involved in cell proliferation. According to Flybase, its amino acid sequence contains a REV protein, which is an anti-repression trans activator protein, a cadherin domain and thrombospondin N-terminal domains. There are 24 classical mutants recorded. The fat gene may be involved in conveying dorsal ventral positional information to the developing ommatidia to create the dorsal ventral midline. Amorphic mutations affect the imaginal disc and the wings. This results in overgrowth of the brain and imaginal discs.	a. wildtype reddish orange/ white mosaic b.big head/rough eye orange/white mosaic	a. wild type Red/dull red mosaic b. big head/rough eye orange/red mosaic 2 balanced stocks
Amy	10644	y[d2]w[1118] P{ey-FLP}; P{w[+mc]=lacW}Rnrl[k06709] P{neoFRT}40A/ Cyo, y[+]	031D08-09	The disrupted gene is ribonucleoside diphosphate reductase large subunit (RnrL). The p-element is inserted within the 1st exon. It encodes a product with ribonucleoside-diphosphate reductase activity which is involved in DNA replication. Its amino acid sequence contains a ribonucleotide reductase large subunit. There are 2 recorded classical mutants. Mutations are recessive lethal.	Orange/red/white wildtype mosaic	2 single male crosses (A+B) gave Orange/red wildtype mosaic The other single male cross (C) gave glossy & slight rough eyes
Amy	12384	y[d2]w[1118] P{ey-FLP}; P{w[+mc]=lacW}l(2)k09015a[k09015a] P{lacW}k09015b P{neoFRT}40A/Cyo, y[+]	032A04-05; 047A11-14	No flanking sequence. There are two p-element insertions, thus two disrupted genes. The first gene is l(2)k09015a. One classical mutant is recorded; mutations are lethal. No functional analysis has been done for this gene. The other	Orange/red/white wildtype mosaic	Dark orange/dull red wildtype mosaic

				gene is unknown.		
Amy	10687	y[d2]w[1118] P{ey-FLP}; P{w[+mc]=lacW}Nnp-1[k07826] P{neoFRT} 40A/Cyo, y[+]	034B06-07	The disrupted gene is Nnp-1. The p-element is inserted within the 1st exon. Its amino acid sequence contains the nucleolar protein domain, which is involved in rRNA processing and metabolism. Three classical mutants are recorded; mutations are recessive lethal.	Orange/white wildtype mosaic	Orange/red wildtype mosaic
Amy	10476	y[d2]w[1118] P{ey-FLP}; P{w[+mc]=lacW}KdelR[k00311] P{neoFRT}40A/Cyo, y[+]	031E01-02	The disrupted gene is KDEL Receptor (KdelR). The p-element is inserted within the 1st exon. It encodes a product with KDEL sequence binding which is involved in retrograde transport (from golgi to ER). One classical mutant is recorded; mutations are recessive lethal.	Orange/red/white wildtype mosaic	Red/dull red wildtype mosaic
Amy	11070	y[d2]w[1118] P{ey-FLP}; P{w[+mc]=lacW} l(2)k12914[k12914] P{neoFRT}40A/Cyo, y[+]	029C01-02	The disrupted gene is CG13393. It encodes a product with apoptosis inhibitor activity, which is involved in anti-apoptosis. Its amino acid sequence contains KOG1746, the defender against cell death protein/ oligosaccharyltransferase, epsilon subunit domain. The p-element is inserted within the 1st exon. No classical mutant is recorded. No mutation is available in Flybase. Not much functional information is given for this gene. The p-element insertion is also close to two other genes. They are CG17295 and CG13384.	Wildtype but white clones greatly expanded (white majority)	Cell-lethal Small eyes Dull red Blisters
Amy	15108	y[d2]w[1118] P{ey-FLP}; P{y[+mDint^2]w[BR.E.BR]=SUPor-P}KG06771 P{neoFRT} 40A/Cyo, y[+]	035B04	The disrupted gene is outspread (osp). The p-element is inserted within the 1st intron. Its amino acid contains the PH domain, usually found in eukaryotic signaling proteins, and the F-actin binding protein, which regulates actin cytoskeletal organization. There are 35 recorded classical mutants. Mutations are viable, fertile, and visible and affect the wing. Not much functional information is given on Flybase.	Orange/red/white wildtype mosaic	Rough eye; antenna defect (enlarged black node) Very red eye, cannot see mosaic

Amy	10995	y[d2]w[1118] P{ey-FLP}; P{w[+mc]=lacW}Mhc[k10423] P{neoFRT}40A/ Cyo, y[+]	036B01-02	The disrupted gene is myosin heavy chain (Mhc). The p-element is inserted about 2kb from the 5' end of the disrupted gene. Mhc encodes a product with muscle motor activity. It is involved in strained muscle contraction, a component of striated muscle thick filament. Its amino acid sequence contains a myosin head. Thirty classical mutants are recorded. Amorphic mutations affect the indirect flight, muscle, mesothoracic extracoxal depressor muscle 66, skeletal muscle of the leg and other tissues. These mutations are viable and dominant flightless.	Orange/white wildtype mosaic	Orange/red wildtype mosaic
Amy	13071	y[d2]w[1118] P{ey-FLP}; P{y[+mDint2]w[BR.E.BR]=SUPor-P}CycE [KG00239] P{neoFRT} 40A/Cyo, y[+]	035D05	The disrupted gene is cyclin E (CycE). The p-element is inserted within the 3rd intron. The gene encodes a product with cyclin-dependent protein kinase, intrinsic regulatory activity, which is involved in the G1/S transition of the mitotic cell cycle. CycE also has a role in controlling cell proliferation during the eye imaginal disc development. It is expressed in embryo, larva and the ovary. Thirty classical mutants are recorded. Amorphic mutations are recessive visible and lethal and affect the eye and embryonic hindgut.	Wildtype but white clones greatly expanded (white majority)	Cell-lethal Dull red Rough Very small eyes
Amy	10562	y[d2]w[1118] P{ey-FLP}; P{w[+mc]=lacW}l(2)k05106[k05106] P{neoFRT} 40A/Cyo, y[+]	039C01-02	There are two disrupted gene. The first disrupted gene is Mlx interactor (Mio). The p-element is inserted within the 3rd intron. It encodes a product with transcriptional factor activity which is involved in regulating transcription. Its amino acid sequence contains a helix-loop-helix dimerization domain. One classical mutant is recorded. The other gene is gustatory receptor 39a (gr39a). The p-element is inserted within the 1st intron. It encodes a product with taste receptor activity, which is involved in taste,	----------N/A----	-------N/A--------

				component of integral to membrane.		

Figure 1: Group B wildtype stock 10583

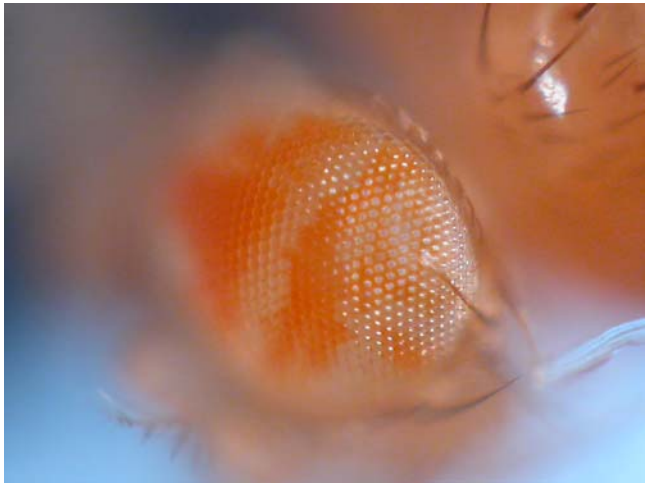


Fig. 1a. Small clone of wildtype orange/white mosaic female eye

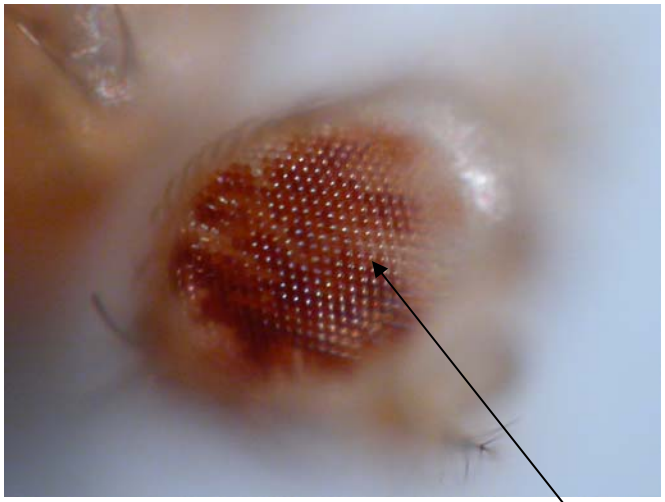


Fig. 1b. Small clone of wildtype red/white mosaic male eye

Twin spot

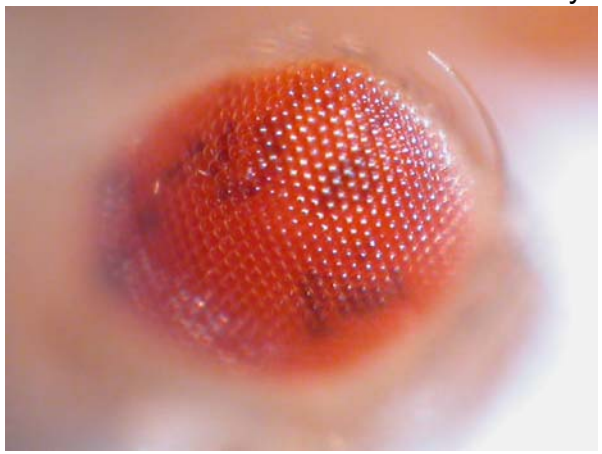


Fig. 1c. Large clone of wildtype

Reddish orange/red mosaic eye

Figure 2: Group B Mutant Stock 10520

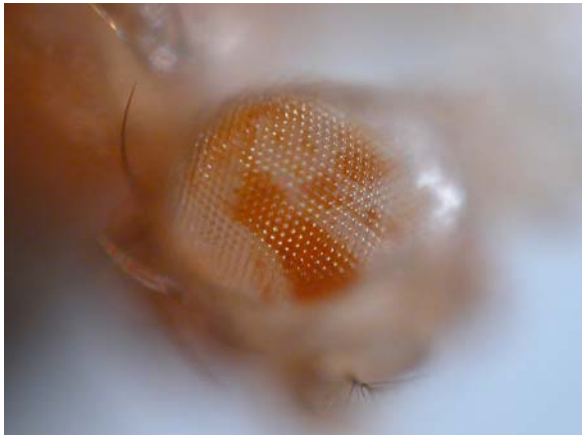


Fig. 2a. Small Clone of wildtype orange/white mosaic eye

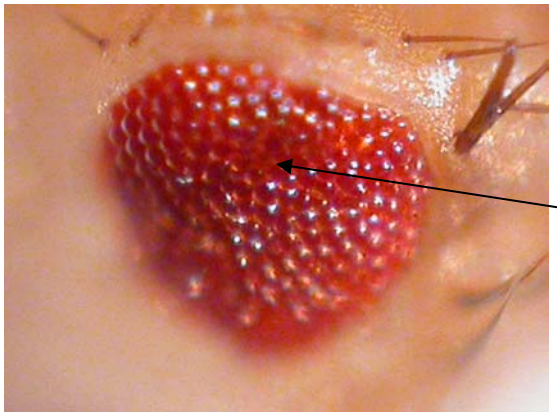


Fig. 2b. Large clone

Cell lethal phenotype. Eye is small and has a very dull red color.

Cell lethal causes roughness

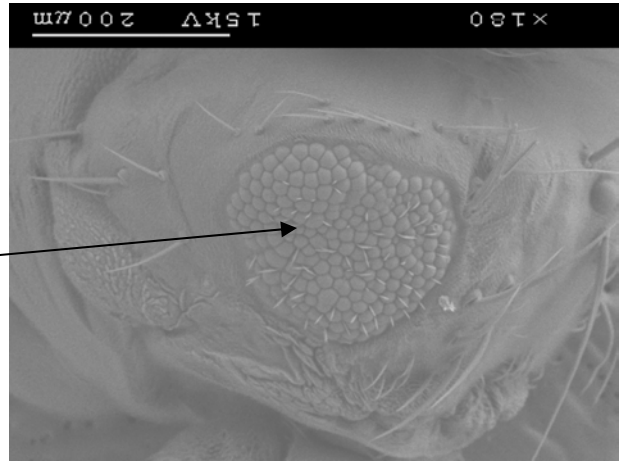


Fig. 2c. SEM of large clone showing rough and cell lethal characteristics

Figure 3: Group B Wildtype Stocks

10646 (fig.a-b), 10617(fig. c-d), 12384 (fig. e-f)

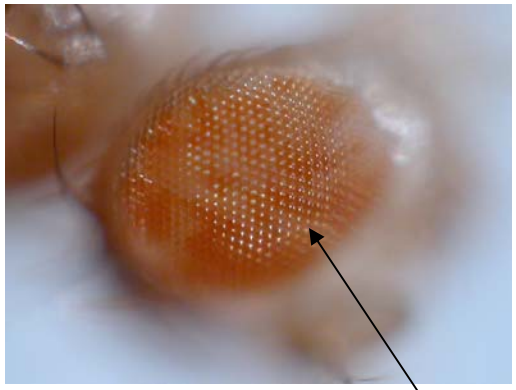


Fig. 3a. stock 10646

Small clone of orange/red/ white mosaic eye

Twin spot (dark orange/ white)

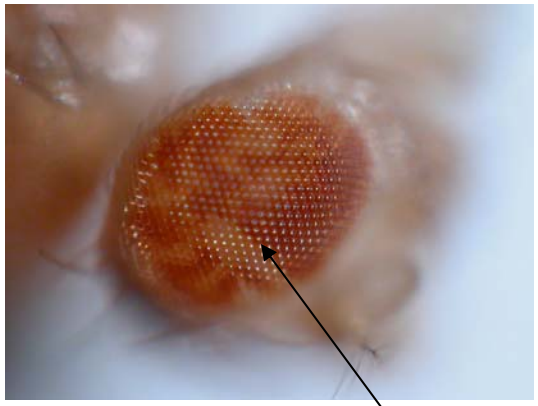


Fig. 3c. stock 10617

Small clone of orange/red/white mosaic eye

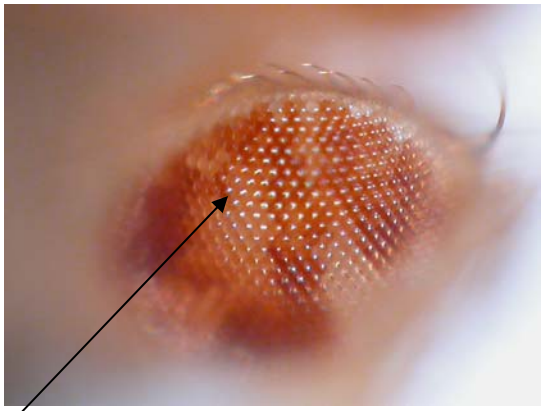


Fig. 3e. stock 12384

Small clone of orange/red/white mosaic eye

Twin spot (red/white)

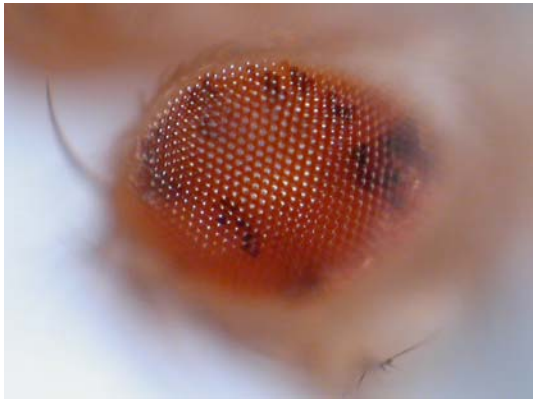


Fig. 3b. stock 10646

Large clone of dark orange/ dull red mosaic eye

Fig. 3d. stock 10617

Large clone of reddish orange/red mosaic eye

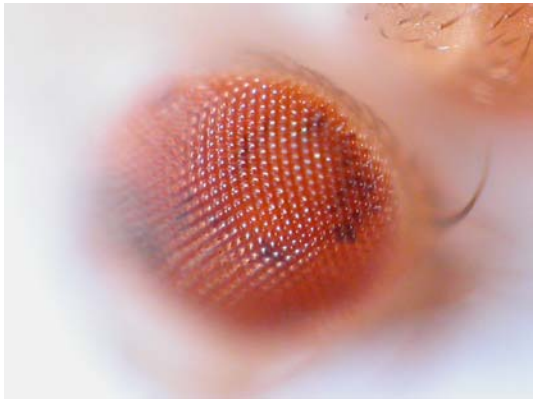


Fig. 3f. stock 12384

Large clone of dark orange/ red mosaic eye

Figure 4: Group B Mutant stock 10523

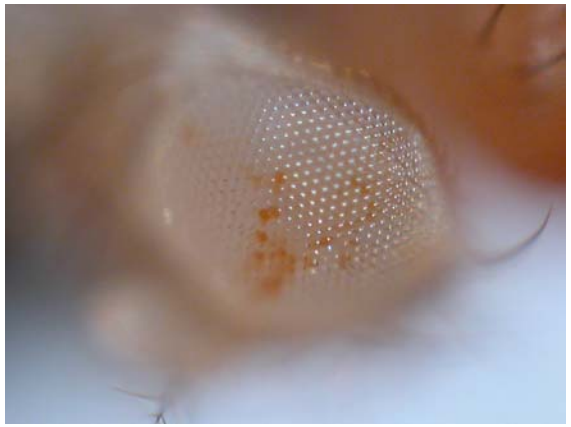


Fig. 4a. Small clone, wildtype phenotype,

White majority with some orange spots

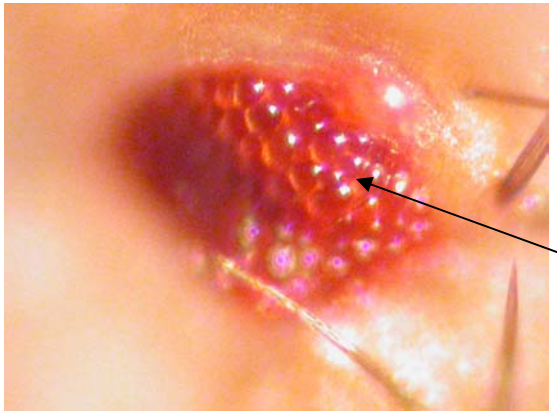


Fig. 4b. Large clone.
Cell lethal causes
roughness

Unparallel
ommatidia
alignment

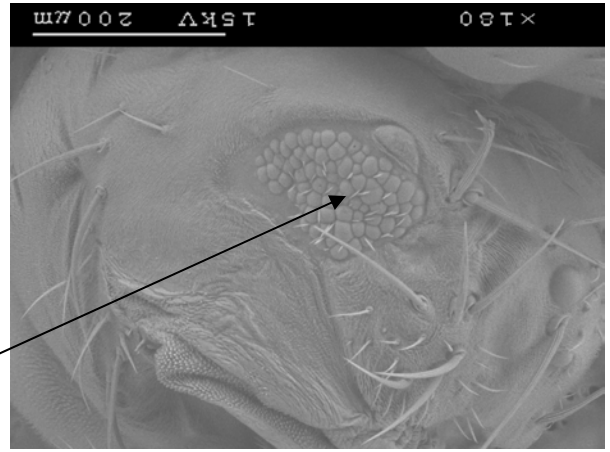


Fig. 4c. SEM of cell
lethal phenotype showing
roughness and small eye

Figure 5: Group B Mutant Stock 10457

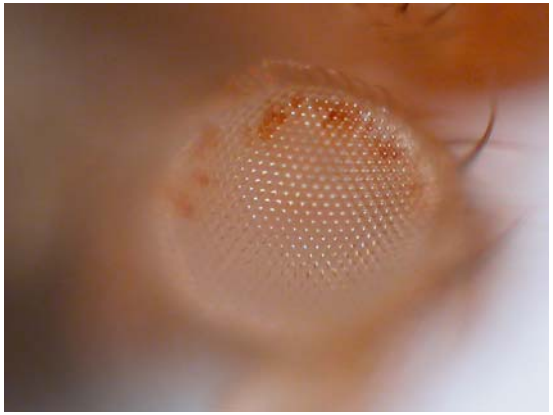


Fig 5a. Small Clone
White majority with occasional orange spots, wildtype

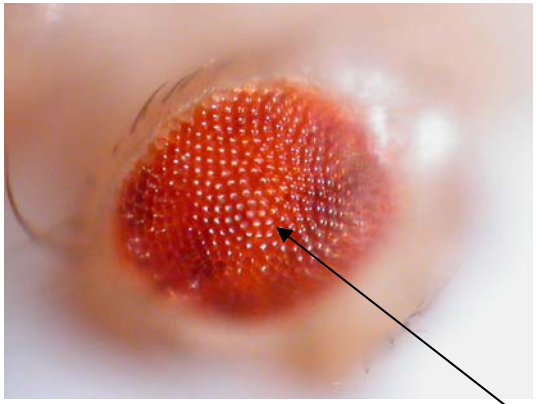


Fig. 5b. Large clone of reddish orange/ red rough mosaic eye

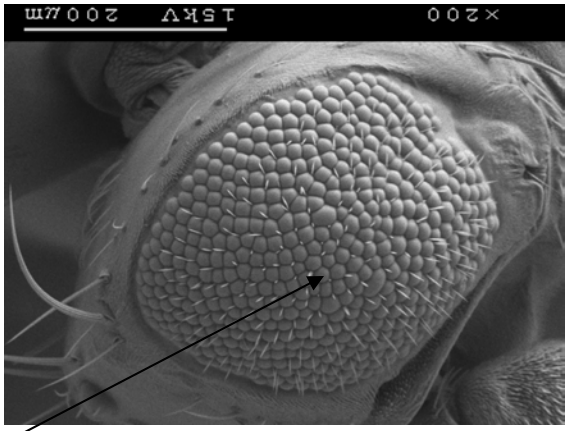


Fig. 5c. SEM of rough eye

Rough characteristics

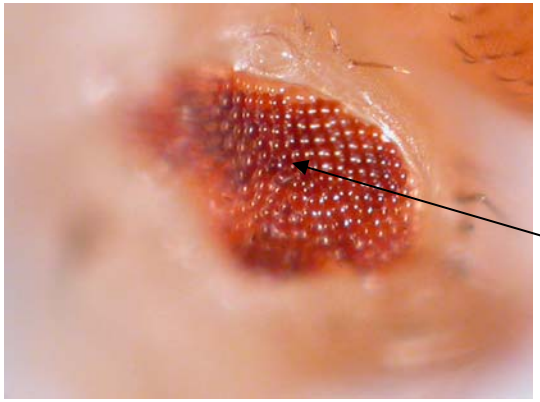


Fig. 5d. Large clone of orange/red mosaic eye, cell lethal

Cell lethal causes roughness and small eye formation

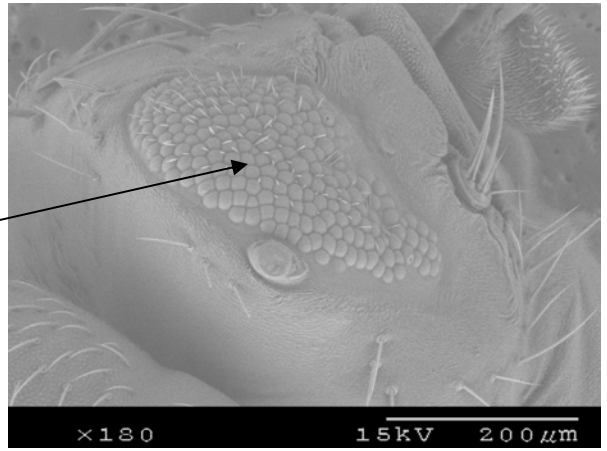
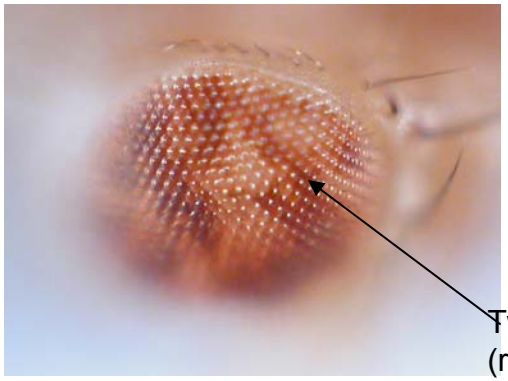


Fig. 5e. SEM of cell lethal large clone

Figure 6: Group B Mutant Stock 10692



Twin spots (red/white patches)

Fig. 6a. Small Clone of orange/red/white wildtype mosaic eye

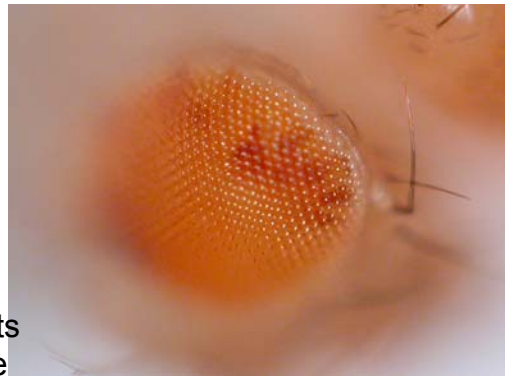


Fig. 6b. Large Clone of female's wildtype orange/red mosaic eye

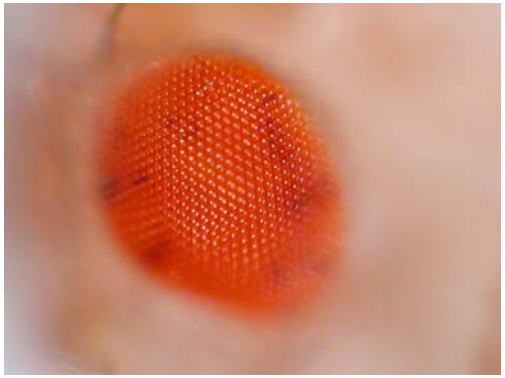


Fig. 6c. Large Clone of male's wildtype reddish orange/red mosaic eye

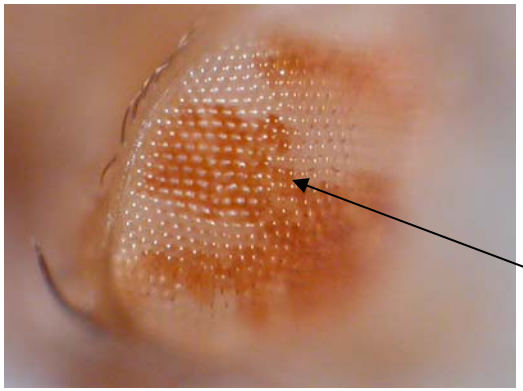


Fig .6d. Small Clone of orange/white rough mosaic eye

Rough patch

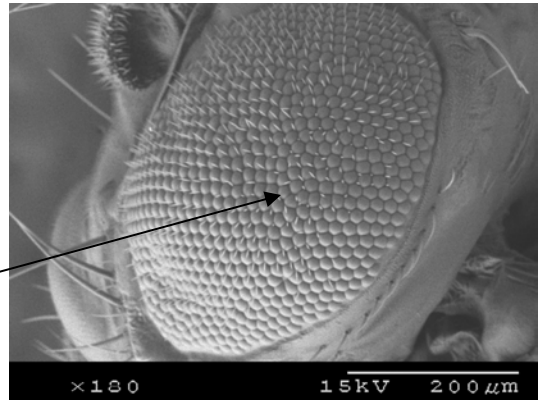


Fig. 6e. SEM of small clone showing roughness

Figure 7: Group B Mutant Stock 10692

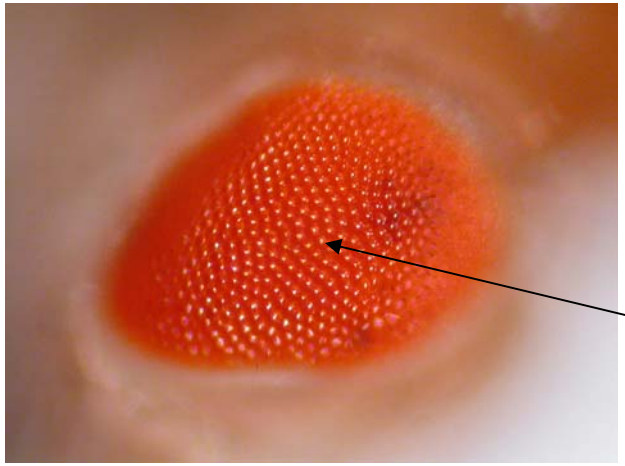


Fig.7a. Large clone of rough eye; reddish orange/red mosaic eye

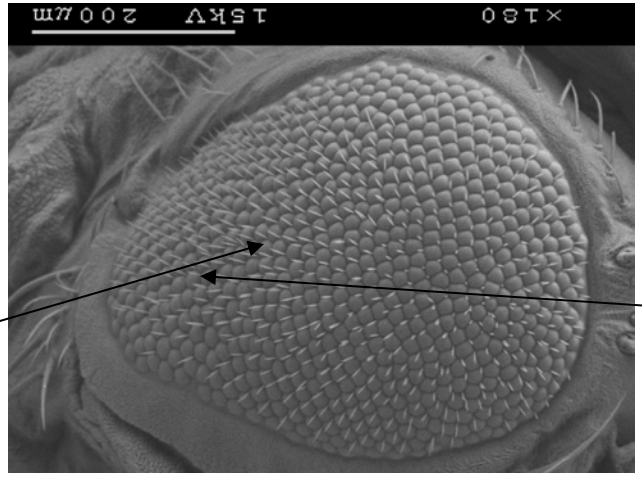


Fig.7b. SEM of large clone rough eye

Fig.7c. Enlarged head from large clone

Fig.7d. Wildtype head

Figure 8: Group B Mutant stock 10644

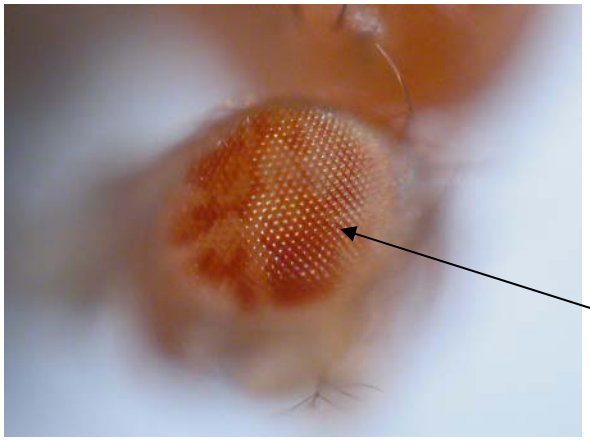


Fig. 8a Small Clone of wildtype orange/red/white mosaic eye

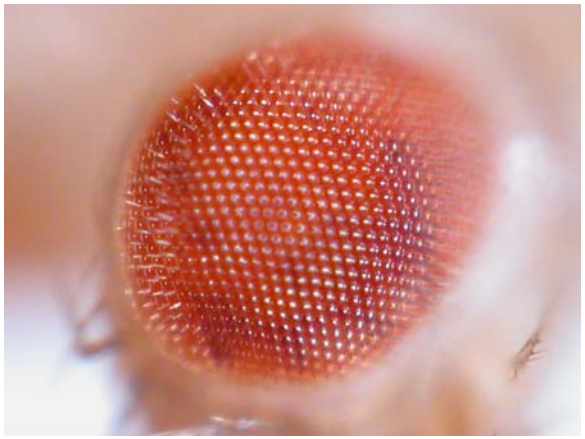


Fig. 8b. Large clone of wildtype reddish orange/red mosaic eye

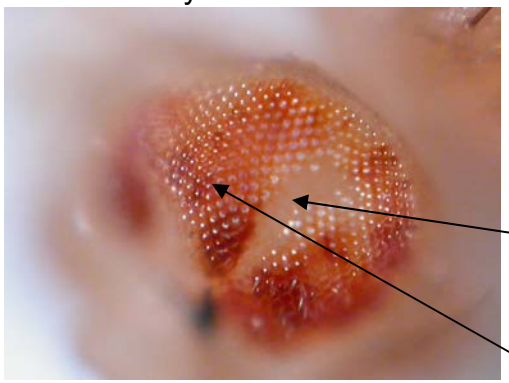


Fig. 8c. Large clone of slight glossy, slight rough eye from single cross C

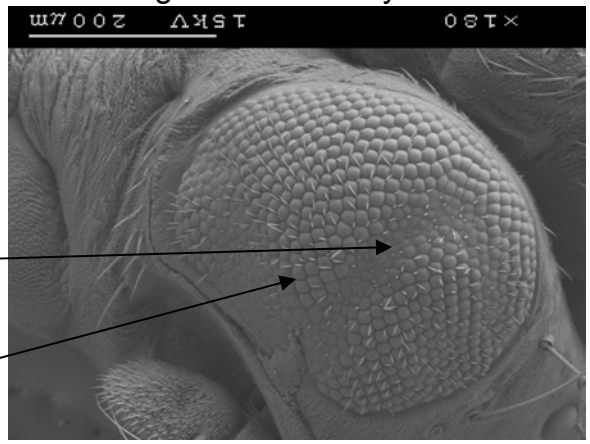


Fig. 8d. SEM shows slight rough and glossiness

Figure 9: Group B Wildtype Stocks

10687 (fig. a-c), 10476 (fig. d-e)

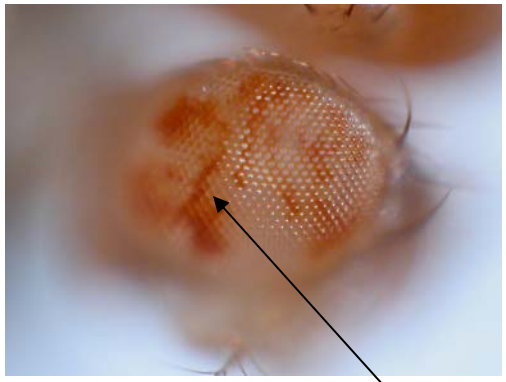


Fig. 9a. stock 10687

Small clone of wildtype orange/white mosaic

Twin spot (dark orange/ white)

Fig. 9b. stock 10687

Large clone of male's wildtype red/dull red mosaic eye

Fig. 9c. stock 10687

Large clone of female's wildtype orange/red mosaic eye

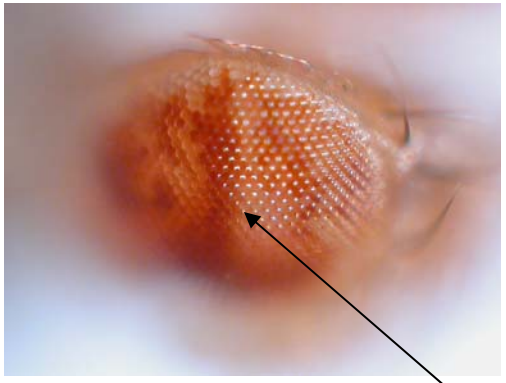


Fig. 9d. stock 10476

Small clone of wildtype orange/red/white mosaic eye

Twin spot

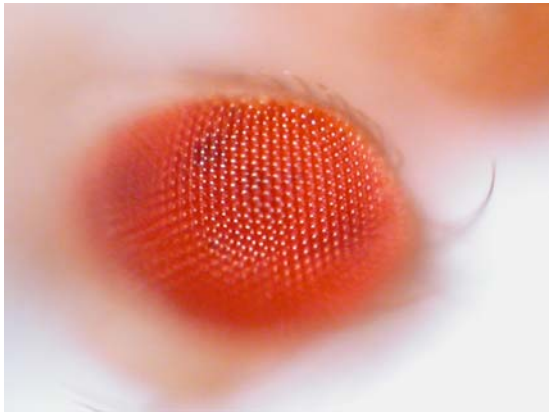


Fig. 9e. stock 10476
Large clone of wildtype reddish orange/red mosaic eye

Figure 10: Group B Mutant Stock 11070

Fig. 10a. Small Clone of wildtype eye; White Majority with some orange spots

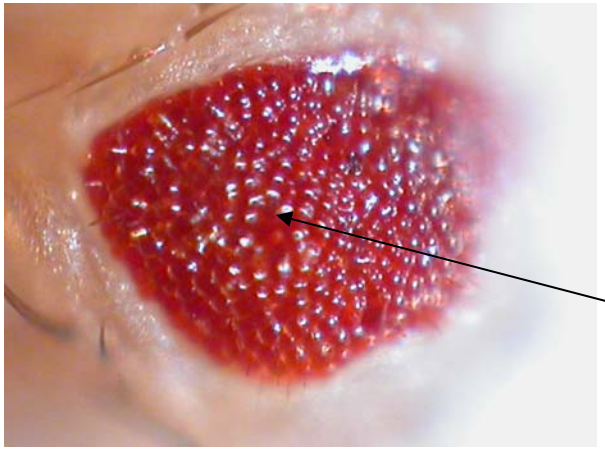


Fig. 10b. Large clone showing cell lethal phenotype

Cell lethal causes roughness

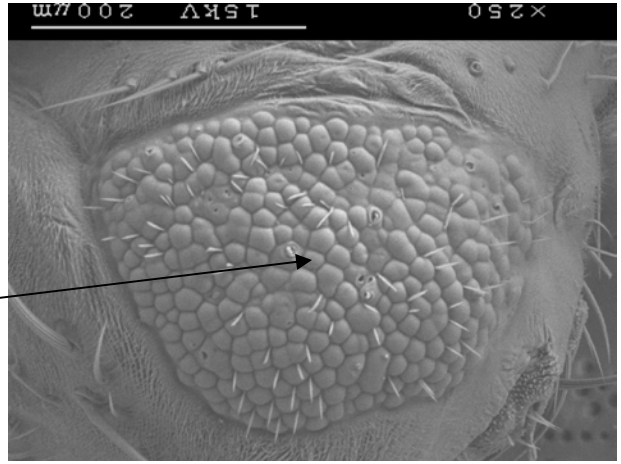
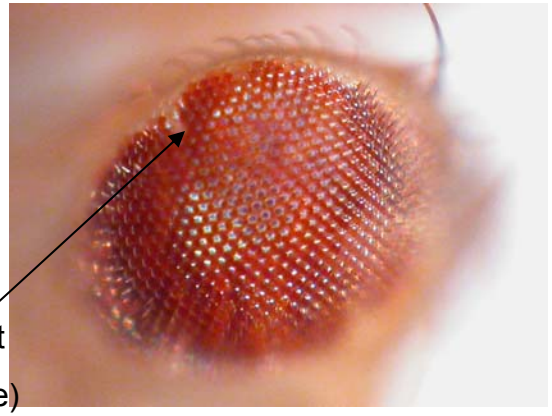


Fig 10c. SEM of large clone shows cell lethality

Figure 11: Group A Mutant Stock 15108



Twin spot

(red/white)

Fig. 11a. Small Clone of wildtype orange/red/white mosaic eye

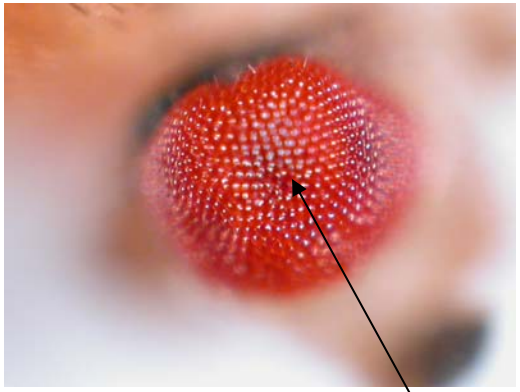
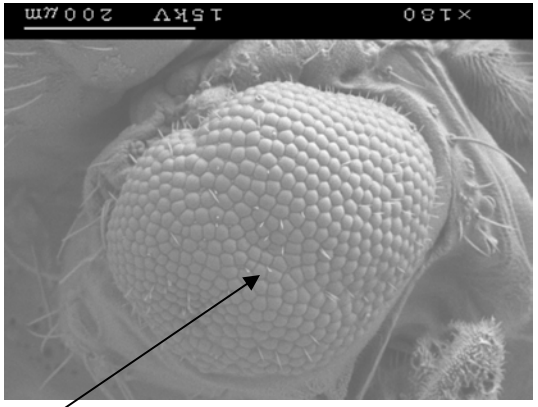


Fig. 11b. Large clone of rough eye



Rough patch

Fig. 11c. SEM of large clone shows roughness

Fig. 11d. wildtype antenna

Fig. 11e. antenna defect

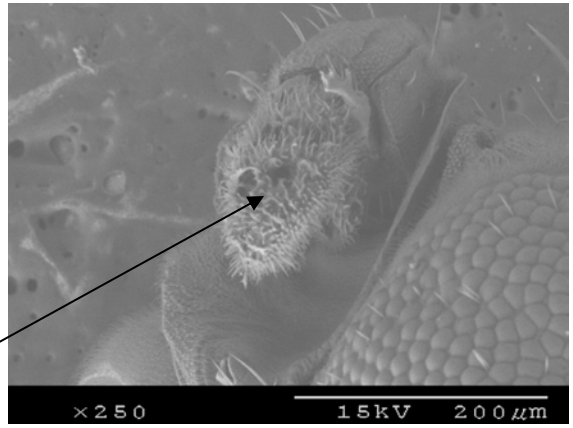
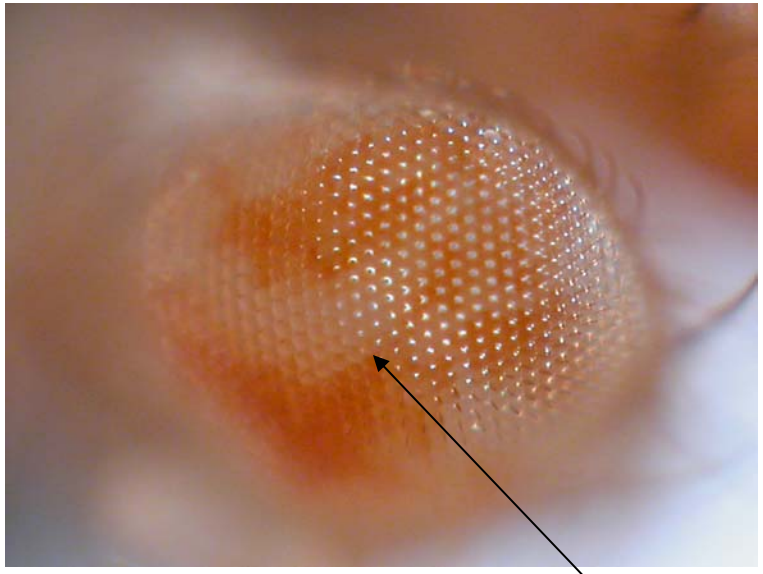


Fig. 11f. SEM shows antenna defect

Figure 12: Group A Wildtype Stock 10995



Twin spot (dark orange/white)

Fig. 12a. Small clone of wildtype orange/white mosaic eye

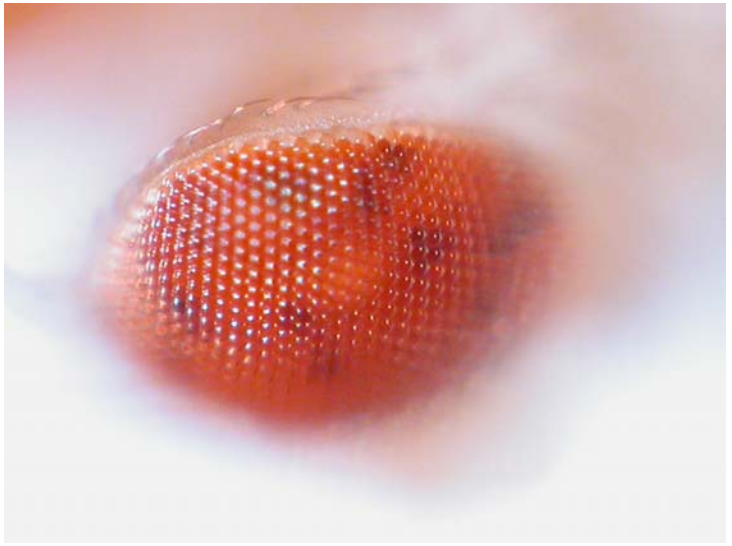


Fig. 12b. Large Clone of wildtype dark orange/red mosaic eye

Figure 13: Group A Mutant Stock 13071

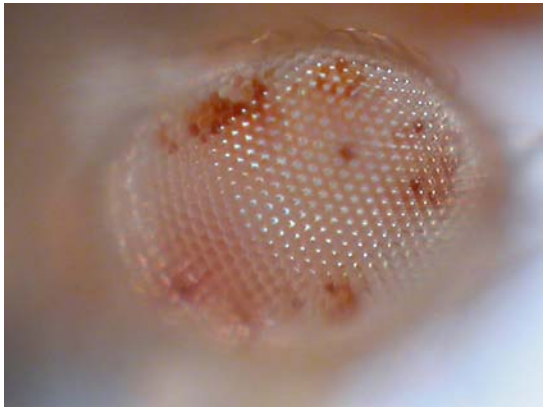


Fig. 13a. Small Clone of wildtype eye,

White clones greatly expanded with some orange spots

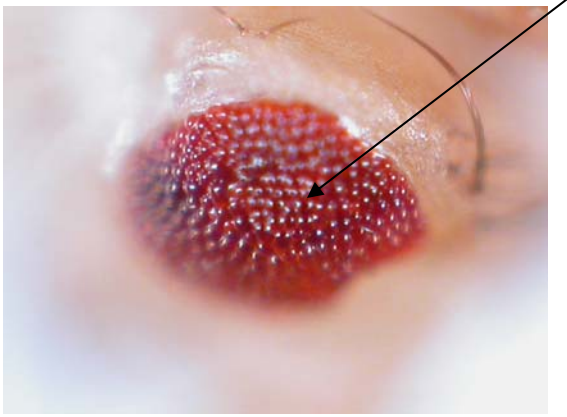


Fig. 13b. Large clone

Cell lethal

Rough patch

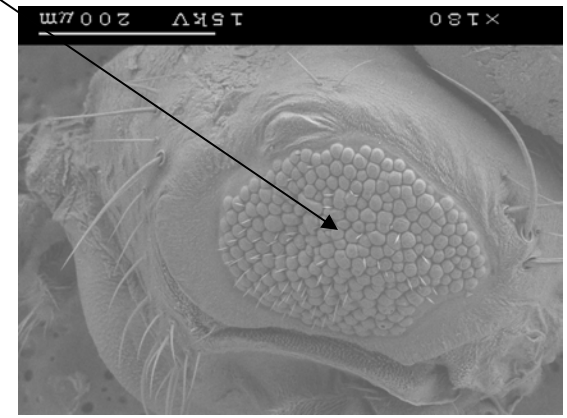


Fig. 13c. SEM of large clone

Cell lethal

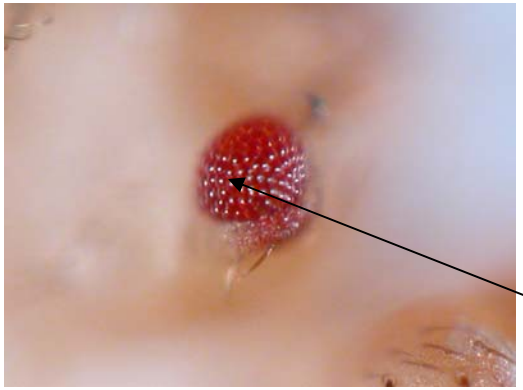


Fig. 13d. Large clone

Cell lethal and very small eye

Severe cell lethality causes very small bulging eye

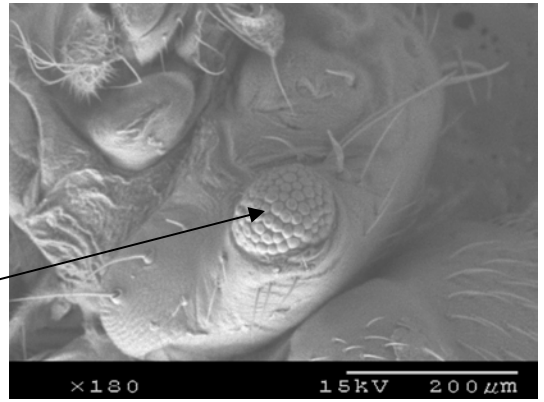


Fig. 13e. SEM of large clone

Cell lethal and small eye