Abstract

The objective of this research experiment is to determine the function of lethal genes in Drosophila, whether they are cell or organismic lethal and to study the phenotypic expression of these genes. Because lethal genes cannot be studied naturally, several different techniques have been combined to allow the study of lethal genes in the Drosophila eye by making the lethal gene homozygous only in the eye. The main technique used was mitotic recombination, which was induced through the use of flippase (FLP) and FRT on the eyeless gene (ey). Obtaining progeny that phenotypically expresses lethal genes requires several crosses, introducing different genes and mutations to the genome through each cross. By determining the function of certain lethal genes, we can further our knowledge about Drosophila genes in hopes of connecting such information to human genes and disorders and diseases.

Introduction

The basis of this research is on the fundamental biological idea of divergence of all species from a common ancestor. Drosophila and humans share many of the same genes. This research was conducted to study and better understand 9 different lethal genes in Drosophila.

Drosophila has four chromosomes. The focus of this research will be on the left arm of the third chromosome (3L) on which all 9 lethal genes are located.

The eye of the Drosophila was chosen as a site to study the lethal genes because it is not essential for the survival of the flies. The eye was made homozygous for the lethal genes, while the rest of the body was kept heterozygous. All lethal genes are recessive lethal.

The Drosophila eye is made up of approximately 600 to 800 simple eyes called ommatidia. Collectively, the ommatidia create a crystal line structure, an arrangement of all ommatidia in perfect alignment that creates three parallel arrangements for the ommatidia. When the eye is made homozygous for the lethal gene, this crystal line structure may or may not be disrupted. Therefore, the effect of homozygous lethal genes expressed in the eye will be seen as wildtype (normal Drosophila eye), "rough" or "glossy."

One of the methods that make it possible to study the phenotypic expression of lethal genes in the eye is mutagenesis. Mutagenesis was caused by P-elements, which are transposa specific to Drosophila, and are convenient to use because they can be located and forced to land on certain genes, unlike chemical and x-ray mutagenesis. P-elements have two parts: the repeat elements and central area. The repeat elements, called P-end, are on each side of the central part and are required for insertion into DNA. The central area encodes the enzyme transposase, which allows the entire element to move around in the genome. In this research experiment, the central part with transposase was cut out and different genes were inserted. One of the genes inserted was w+ (wildtype red eye color), which serves as a marker. Because of this marker, the flies

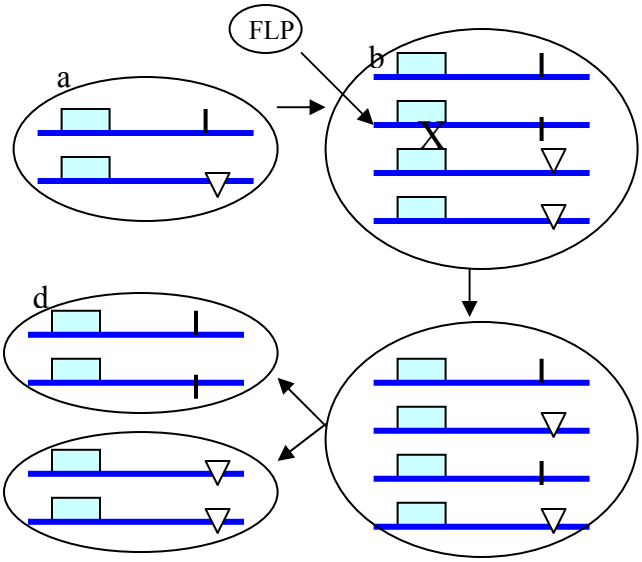


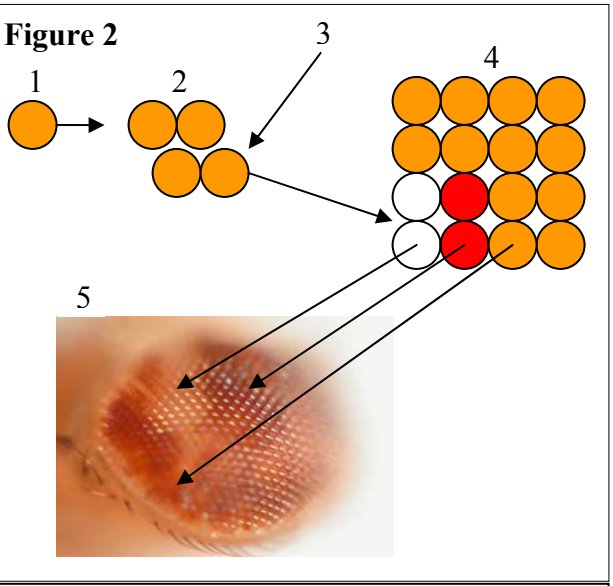
Figure 1 details the mitotic recombination that is induced. The blue rectangles represent FRT, the black lines represent a lethal gene and the triangles represent the wildtype of the lethal genes. A: FRT needs to be present in both chromosomes for FLP to bind to it B: the chromosomes in A have duplicated C: FLP has induced mitotic recombination D: The parental cell has divided into daughter cells

stocks of flies in this research possessed y+. Because the P-element has no transposase, it cannot move around the genome and stays in its specific lethal gene.

Another method that was used to study the phenotypic expression of lethal genes in the eye was mitotic recombination. Mitotic recombination is recombination that occurs during mitosis. Many factors must be present to induce mitotic recombination (**refer to figure 1**). One factor is an enzyme for flippase called FLP, which is taken from yeast and inserted into the Drosophila genome. FLP binds to certain target DNA sequences called FRT, which is also derived from yeast. Recombination only happens at the FRT site because that is where FLP binds. FRT needs to be homozygous for FLP to attach to it. FLP can be expressed in certain areas and not in others, a phenomenon called differential activation. The enhancer used to activate FLP only in the eye is eyeless gene (ey). The ey gene drives FLP to produce flippase only in the eye during early development of the Drosophila, making only the eye homozygous for the lethal gene and keeping the rest of the fly heterozygous for the lethal gene. Therefore, if FRT is homozygous on the 3L chromosome, then FLP binds to FRT. After the chromosomes duplicate, FLP causes mitotic recombination of cells that are heterozygous for the lethal gene and produces a homozygous lethal and wildtype lethal daughter cells. Mitotic recombination does not occur in all of the cells, indicating that only the right conditions result in mitotic recombination.

The early progenitor cells of the Drosophila eye are heterozygous for the lethal gene that has the p-element { **EQ \f(P[w+],+)** } and has a phenotypic color of orange (In reality, the phenotypic color of the eye can vary from very pale yellow to red because of the position effect of the w+ p-element marker, as described earlier. For explanation purposes, the heterozygous lethal gene will be assumed to have a phenotypic orange eye color.). Mitotic recombination is induced in one of these few cells (Cell A) and when this cell divides, there are two resulting cells, one which is a homozygous lethal cell for the P-element and the other a wildtype cell for the lethal P-element. The homozygous lethal cell is twice the shade of the heterozygous lethal because the homozygous lethal possesses two copies of the P-element and its marker (for explanation purposes, the homozygous lethal cell will be assumed to have a phenotypic dark orange/red eye color). The wildtype lethal cell will be white because it possesses no marker to produce eye color. As all of the progenitor cells further divide and eventually form the eye, the daughters of Cell A will have also divided, forming clusters of white and red spots, called twin spots, in a predominantly orange eye. This pattern is called mosaic and is the small clone phenotype of the mosaic eye (**Refer to Figure 2**). The w+ gene in the P-element serves only as a marker. If the lethal gene is required for eye development, a phenotype will be seen. If the lethal gene is cell lethal, then the cells possessing homozygous lethal genes will be dead. Therefore, there will be no dark orange/red spots. If these

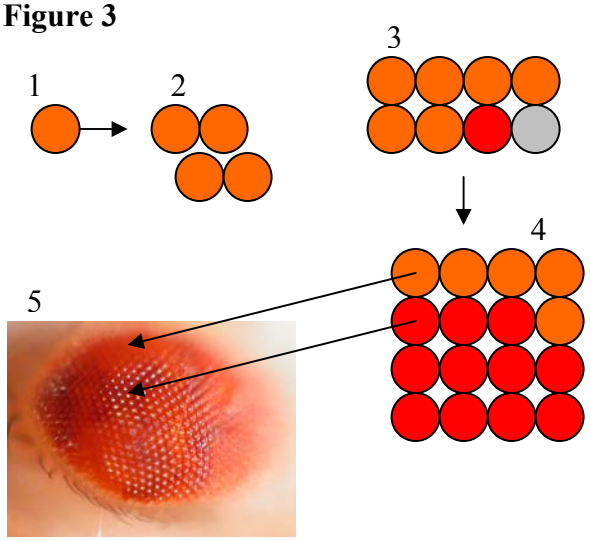
Figure 2



1: progenitor eye cell that is heterozygous for the lethal mutation (orange) **2**: divided cells from progenitor eye cell **3**: mitotic recombination is induced in one cell **4**: mitotic recombination results in a red phenotype that is homozygous lethal and a white phenotype that is wildtype **5**: After many cell divisions, the result of mitotic recombination is twin spots of red/white on a largely orange eye

spots are present, then the lethal gene can be deduced as being an organismic lethal gene.
To further study the phenotype produced by the lethal gene, another mutation is introduced into the chromosome called minute mutation (M), with a w+ marker. Minute mutation causes a mutation in a ribosomal gene which is essential for cell growth. If a cell is heterozygous for M, it will divide more slowly than the wildtype cell because there will only be one functional ribosomal gene. If a cell is homozygous for M, the cell will die because there will be no functional ribosomal genes (M is a cell lethal mutation). The M mutation will be added to the progenitor cells that are heterozygous lethal cells, on the chromosome that does not possess the lethal P-element gene **(refer to figure 3)**. Therefore, when mitotic recombination is induced in one of these cells, its daughter cells will be either homozygous lethal P-element and wildtype M mutation or wildtype lethal P-element and homozygous M mutation. The cell possessing the homozygous minute mutation will die. As the other two cells divide and develop into a cell, the cells possessing the heterozygous M mutation will have a growth disadvantage and will divide at a slower rate. Therefore, when the eye is fully developed, the cells with homozygous lethal P-element and wildtype M mutation will be predominant. This mosaic pattern is called the large clone mosaic because the homozygous lethal P-element cells predominate the eye. The addition of M mutation will not always result in this specific orange spots/red eye mosaic pattern. The exception is when the lethal gene is cell lethal. If this is the case, the eye will be much smaller than the normal Drosophila eye due to the death of the homozygous lethal P-element cells and the inability of the heterozygous M mutation cells to divide quickly enough to create a normal eye.
Using these techniques, the objective of the research will be to introduce different genes ino each of the crosses that will be performed, to obtain the large clone mosaic eye flies from each stock. The phenotype produced by each of the 9 lethal genes will be studied and conclusions will be made whether the genes are cell or organismic lethal.

Figure 3



1: progenitor eye cell that is heterozygous for the lethal and minute mutation (dark orange) **2**: divided cells from progenitor eye cell **3**: mitotic recombination is induced in one cell and results in homozygous lethal/wildtype minute mutation (red) and wildtype lethal/homozygous minute mutation (grey) **4**: continued cell division results in proliferation of homozygous lethal/wildtype M cells 5: the result is large patches of red with smaller patches of dark orange

Although the main goal of this research is to observe the 9 lethal genes, further research can be performed to link Drosophila lethal genes to genes in humans that may cause disorders or diseases. If a common gene is shared by Drosophila and humans, then further study of the lethal gene in Drosophila can lead to a better understanding of the cause of the lethal gene, its effects on cells and the organism, and possible cures for the disorder/disease caused by the gene. For example,

<u>Materials and Methods</u> (refer to figure 4)
There will be five crosses that are conducted. The generation time for Drosophila under optimum conditions is approximately 10 days. These conditions include an environment of 25 degrees Celsius, plenty of food, and no overcrowding in vials. Therefore, between each cross,

approximately 2 weeks is needed for collecting the required progeny, crossing the Drosophila, and transferring the crosses. A total of 10 weeks is required for the five crosses.

The first cross is carried out so that the yw ey-FLP and TM6B, y+ from the female parent and the P[w+] from the male parent can be placed on homologous chromosomes. TM6B and TM6B, y+ was used to prevent recombination during meiosis so the predicted 4 classes of progeny would result. TM6B is a balancer chromosome. D, a dominant marker for dichaete wings, was used to distinguish progeny that did not possess the p[w+] mutation, yw ey-FLP and TM6B, y+ genes from those that did. TM6B, y+ must be inherited by the progeny to prevent meiosis in the next cross; yw ey-FLP must be inherited by the progeny because it is necessary fro mitotic recombination; and p[w+] must be inherited because it disrupts the gene that is under study. When the first cross is carried out, the progeny needed for the next cross is distinguishable because of the y+ marker from the TM6B, y+ gene that only one of the viable male progeny possesses. The male progeny are collected, rather than the females that also have all the genes/mutations necessary for the next cross because if the females were used for the second cross, then the cross would result in two classes of female progeny that do not have differing phenotypic markers to distinguish them. Also, male progeny are used because it does not matter whether they are virgins or not (female flies that are not virgins, store sperm from the males they have mated with so they can continually produce progeny).

The second cross is carried out so that one yw ey-FLP from both parents can be placed on homologous chromosomes and the P[w+] from the male parent and the FRT80B from the female parent can be placed on homologous chromosomes. TM6B, y+ was on the male parent to ensure that there would be no recombination occurring during this cross. TM6B, y+ was also used because it has a y+ (grey body) marker. In the second cross, this marker distinguishes the progeny that have P[w+] from those that do not, which possess TM6B, y+. Progeny with TM6B, y+ must not also be collected because it would prevent recombination in the third cross, a crucial event for mitotic recombination to take place. From each of the 9 stocks of flies that have a P-element in different lethal genes, approximately 10 males were collected and crossed with 10 virgin females (from a different stock) that were homozygous for yw ey-FLP and FRT80B. Every other day, these approximately 20 parent flies were transferred to new vials, with a total of 4 transfers for each of the 9 stocks of flies resulting.

The third cross was carried out by collecting approximately 10 virgin females from the second cross with P[w+] and crossing them with 10 males collected from the same stock as the parent females from the second cross. The main objective of the third cross was to obtain progeny, particularly male, that would have mosaic eyes due to undergoing mitotic recombination. This cross would place P[w+] and FRT80B on one chromosome, and FRT80B on its homologous chromosome (on the third chromosome), with yw ey-FLP on the fourth chromosome to allow mitotic recombination (homozygous FRT and ey-FLP are necessary for mitotic recombination of the lethal gene). From the third cross, four different classes resulted. Two of the classes were expected because they are parental, but the remaining two were recombinant. The relative ratios of parental genotypes to recombinant genotypes vary depending upon the distance (in m.u.) between FRT80B and P[w+]. Meiosis occurred in the female parent, but mitotic recombination occurred in the early stages of development of the male and female progeny that had a certain genotype (from the recombinant class). The genotype necessary for mitotic recombination, as discussed earlier, are FRT on homologous 3L chromosomes with P[w+] on one of the chromosomes, and ey-FLP on the 4h chromosome. The other recombinant and parental classes cannot undergo mitotic recombination because they lack one or more of these factors. The recombinant class that cannot undergo

mitotic recombination is indistinguishable from one of the parental classes. Therefore, when counting the number of progeny for each of the phenotypes, the number of mosaic recombinant phenotypes counted must be doubled for an accurate number of recombinant progeny. For each of the 9 lethal genes under study, 2 different crosses were started, with 10 males and 10 females mated for each cross. These crosses were transferred a total of four items for 10 different vials of progeny for each lethal gene.

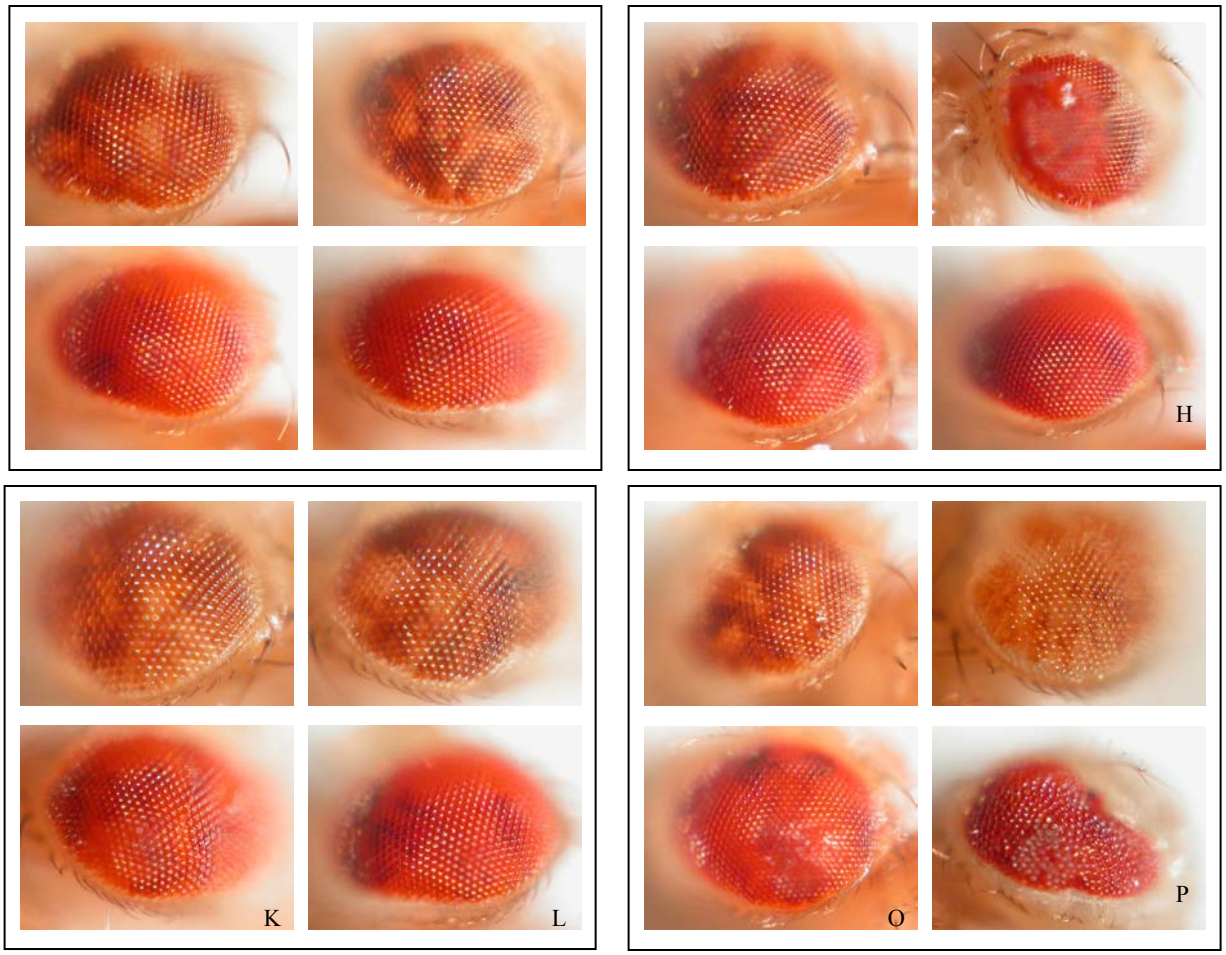
The fourth cross was performed to add the minute mutation (M), which would allow an enlargement of the mosaic (from "small clone" to "large clone") so the phenotype of the lethal gene can be distinguished more clearly. This cross resulted in 4 different classes. These classes were all parental because recombination does not occur in male Drosophila. Also, recombination is prevented because of the TM6B, y+ balancer added to the female parent. TM6B, y+ is also necessary because of the y+ marker which distinguishes the progeny that possess P[w+] from those that do not. Although P[w+] has a w+ marker, the w+ may vary from a pale yellow to red coloration. To ensure that flies can be distinguished, the y+ marker is used. Also, TM6B, y+ distinguishes the progeny without the M mutation from those that do. Because y+ is included in the balancer chromosome, there will be phenotypic difference between these classes. At least four different mosaic male progeny from the third cross (for each of the 9 stocks) will be crossed individually with virgin females that have the minute mutation and TM6B, y+ (which is from a different stock). These mosaic males are mated individually because mitotic recombination can occur anywhere between FRT80B and P[w+], so the small clone mosaic eye will be unique for each resulting mosaic male. There will be four classes of progeny from the fourth cross, with one class being the large clone mosaic eye progeny. At least four light microscope pictures have been taken for the flies with large clone mosaic eyes (from each small clone single male line). SEM pictures have been taken of these flies if they displayed a phenotype other than the wildtype mosaic phenotype.

The fifth cross will be a balanced stock. The balanced stock will be a mating of the virgin females and males of another class from the fourth cross (the progeny with ey-FLP on the fourth chromosome, and TM6B, y+ on one 3L chromosome and FRT80B and P[w+] on the homologous 3L chromosome). The fifth cross is performed because it is the only way to maintain Drosophila because Drosophila cannot be frozen. The TM6B, y+ balancer ensures that recombination does not take place. Also, the heterozygous nature of both parents for P[w+] and TM6B, y+ ensures that only progeny with the desired genotype will result. Any other progeny will not be viable because they will be homozygous for either P[w+] o rTM6B, y+. To obtain large clone mosaic eye flies from this balanced stock, the balanced stock flies can be mated with flies that come from the same stock as the parent female of the fourth cross.

Results

10168: ***(Refer to Figure 4)*** The base P-element is located in scaffold 39900 in AE003559. The small clone phenotype is wildtype. The mosaic is mostly dark red with orange/white patches throughout the eye. The large clone phenotype is also wildtype. The mosaic is slightly distinguishable, as there are dark red spots on a mostly red eye. Because the small clone and large clone were wildtype, this suggests that cdc27 is not a cell lethal gene, but an organismic lethal and that it does not play a significant role in eye development.

10170: ***(Refer to Figure 4)*** The base P-element is located in scaffold 128420 in AE003545; 5828 bases away from CG6175. The small clone phenotype is wildtype. The mosaic is mostly red with small white/clear patches mainly in the peripheral regions of the eye or extending into the middle region of the eye from the peripheral area. The large clone phenotype is also wildtype. The



mosaic is difficult to see because the areas that are homozygous for the lethal gene and those that are heterozygous for the lethal gene are the same shade. The eye is mostly red with slightly darker red patches. The lack of a phenotype suggests that CG6175 is not a cell lethal gene, but an organismic lethal gene. It also indicates that CG6175 does not play a significant role in eye development.

10171: ***(Refer to Figure 4)*** The base P-element is located in scaffold 191967 in AE003555. The small clone is wildtype. The mosaic pattern is mostly dark red with large patches of orange throughout the eye. The large clone is also wildtype. The mosaic pattern is mostly red with small patches of dark red in various regions of the eye. The lack of a phenotype suggests that foi (fear of intimacy) is not a cell lethal, but an organismic lethal. Also, that it does not play a significant role in eye development.

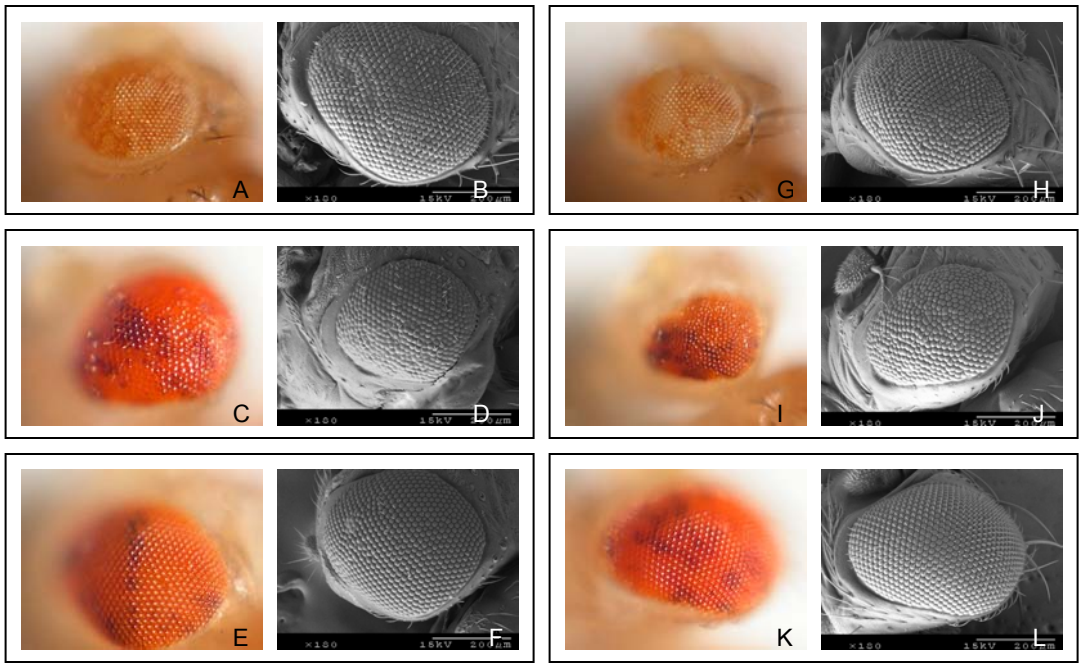
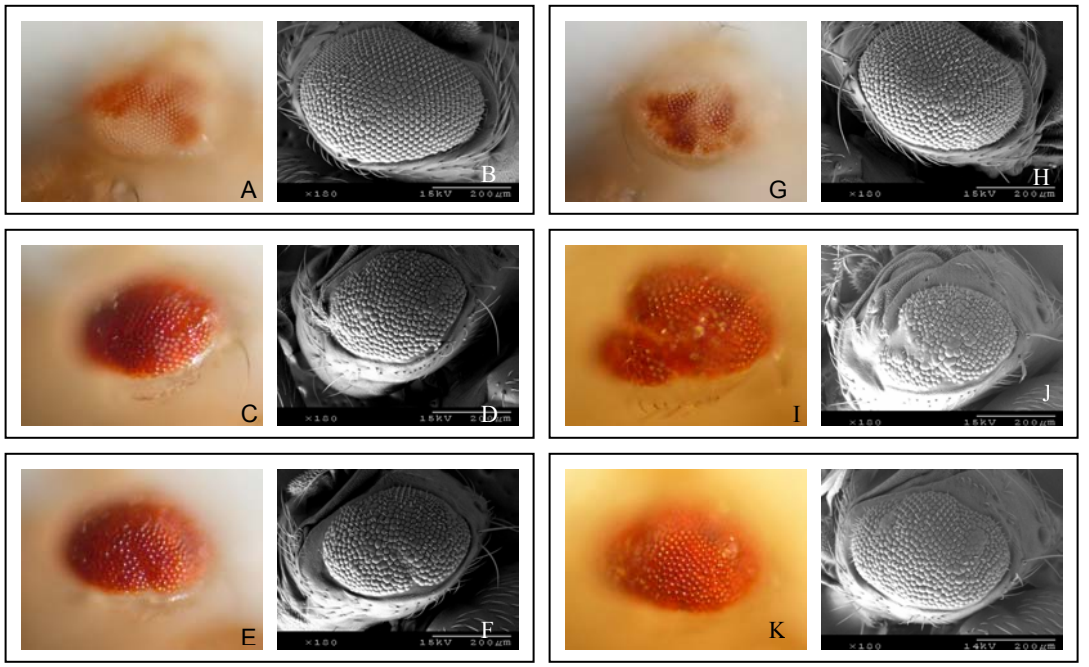


Figure 5 A: 10199B small clone light microscope (LM), rough **B**: 10199B small clone, rough SEM **C**: 10199B large clone LM, rough **D**: 10199B large clone SEM, rough, most of bristles missing **E**: 10199B large clone LM, slightly rough **F**: 10199B large clone SEM, slightly rough, most of bristles missing **G**: 10199C small clone LM, slightly rough **H**: 10199C small clone SEM, few bristles missing **I**: 10199C large clone LM, rough **J**: 10199C large clone SEM, most bristles missing **K**: 10199C large clone LM, wildtype **L**: 10199C large clone SEM, wildtype

12061: ***(Refer to Figure 4)*** The base P-element is located in scaffold 97387 in AE003471. The small clone phenotype is wildtype. The mosaic pattern varies and is either mostly red with orange patches, or is mostly red-orange with white patches. The large clone phenotype is wildtype. The mosaic is mostly red with a few patches of more dark red which are present in various places of the eye. Four single male lines were created. All of the small clone progeny (which were large clone) were wildtype, except
the fourth single male cross progeny. This suggests that the fourth single male cross mostly likely had a background mutation that was expressed as the rough phenotype that was seen. Since the other three single male crosses were wildtype, it was concluded that mtacp1 (mitochondrial acyl carrier protein 1) does not play a significant role in drosophila eye development. Also, because the phenotype did not suggest cell lethality, mtacp1 is an organismic lethal gene.

10880: No flanking sequence was available. The small clone is wildtype. The mosaic pattern is mostly red-dark red with orange-white patches. The mosaic pattern appears only in the dorsal half of the eye. The ventral half is all white. The large clone is wildtype. The mosaic pattern in the dorsal half of the eye has more dark red patches on a dark red background. The ventral half has a few red patches on a white background. These patches are large and appear throughout the ventral area, but more towards the dorsal half of the eye. Because all small clone and large clone are wildtype, this suggests that mirr (mirror) does not have a significant impact on drosophila eye development. This also shows that mirr is not a cell lethal gene, but rather an organismic lethal gene.



10199: The base P-element is located in scaffold 82001 in AE003593. The small clone phenotype is slightly rough. One of the small clone parents had some of its bristles missing. The mosaic pattern is mostly orange with many white patches. The large clone phenotype varies within each single female small clone line. Some progeny are wildtype, but others have varying degrees of rough phenotype. The ones with rough phenotype have bristles missing. The mosaic is mostly orange with very dark red/almost black patches.

The presence of wildtype large clone progeny suggests that the rough phenotype and the lack of bristles may be a result of a background mutation.

The lack of cell lethality suggests that loner is not a cell lethal gene, but an organismic lethal gene.

10198: The base P-element is located in scaffold 59387 in AE003593. Small clone and large clone mosaic phenotypic expressions were unable to be studied because no small clone mosaic progeny were found.

10189: The base P-element is located in scaffold 208192 in AE003519. Small clone and large clone mosaic phenotypic expressions were unable to be studied because more than four classes of progeny resulted from the week one cross. There were a total of 4 classes of progeny. These included yellow body, white eye progeny and yellow body, orange eye progeny. The orange eye was unexpected because the color of the eyes of this stock is dark red. Also, there were grey body, red eye progeny; grey body, white eye progeny. These last two were concluded to be the expected progeny because the grey body, red eye progeny had the correct eye color. It was assumed that the grey body color was the result of a y+ marker in the P-element. After the week three cross had been performed and no small clone progeny appeared for this stock, it was discovered that this stock did not have the y+ marker on the P-element. The crosses must have been contaminated during one of the crosses.

10180: There was no flanking sequence available. The small clone is slightly rough with a very few bristles missing. The mosaic pattern is mostly white with large red patches. The red patches are present in a large portion of the eye. The large clone is rough and slightly cell lethal. The large clone also has missing bristles. The mosaic pattern is indistinguishable because the eye is dark red and rough.
The presence of a minimal amount of cell lethality suggests that the gene of this stock may be a cell lethal gene. Also, the presence of rough phenotype with some bristles missing suggests that it plays an important role in drosophila eye development.

Discussion

10168: The disrupted gene is cdc27. The P-element is inserted within the 1st exon of the cdc27 gene. Cdc27 encodes tetratricopeptide repeat domain. It is involved in a variety of functions including protein-protein interactions, but common features in the interaction partners have not been defined. Also, it is involved in chaperone, cell-cycle, transcription, and protein transport complexes. Mutations have been isolated which affect the mitotic cycle and the mitotic chromosome and are mitotic and recessive pharate adult lethal. Cdc27 plays an essential role in DNA replication by recruiting PCNA to the pol delta holoenzyme.
Because cdc27 has important functions that are necessary for eye development, such as DNA replication, which suggests that cdc27 is most likely a redundant gene. Therefore, even if this gene were mutated, there would other genes to take over the function of the gene and the organism would be unaffected.

10170: The disrupted gene is CG6175. The P-element is 5.8 kilobases 3' of CG6175 gene. CG6175 gene encodes smart00595, MADF, subfamily of SANT domain and Adh distal factor-1 (Adf-1), a sequence-specific DNA-binding activity. Adf-1 binds to upstream recognition elements in each of the two promoters of the Drosophila alcohol dehydrogenase gene (Adh), and binding of Adf-1 to the Adh distal promoter site activates transcription. Adf-1 may play an important role not only in the regulation of Adh expression but also in the transcription of other Drosophila genes as well. CG6175 is a product putatively involved in cell communication.
Because only wildtype mosaic eye progeny are seen, it is clear that a lethal gene, which is most likely CG6175 or one close to the P-element insertion sight, does not play a significant role in drosophila eye development. Or, it could be that

any gene is too far away from the P-insertion sight to be greatly affected by the mutation.

10171: The gene disrupted is foi (fear of intimacy). The P-element is inserted within the 1st exon of the 1st transcript. Foi encodes pfam02535, Zip, ZIP Zinc transporter. The ZIP family consists of zinc transport proteins and many putative metal transporters. Foi is required for tracheal branch fusion during tracheal development. Mutations have been isolated which affect the gonadal mesoderm and the precursor gonad and are embryonic lethal. Foi affects gonad formation without affecting gonad cell identity, and is therefore specifically required for the morphogenesis of this organ.
Mutation of foi most likely did not produce a phenotype because it is not an essential gene in eye development. As discussed in the previous paragraph, foi is involved with other organs of the body. Therefore, when the lethal gene was expressed in the eye, it had no affect on the eye and produced wildtype.

12061: The disrupted gene is mtacp1 (mitochondrial acyl carrier protein 1). The P-element is inserted within the 1st exon of the 2 transcripts of the mtacp1 gene. Mtacp1 encodes for acyl carrier protein (ACP) and is essential for viability and is required for both male and female gametogenesis.
Mtacp1 also encodes PP-binding: Phosphopantetheine attachment site. A 4'-phosphopantetheine prosthetic group is attached through a serine. This prosthetic group acts as a 'swinging arm' for the attachment of activated fatty acid and amino-acid groups. This domain forms a four helix bundle. The attachment serine is replaced by an alanine in some members. A P-element-induced loss-of-function mutation in the mtacp1 gene causes lethality.
Mtacp1 gene is highly expressed in the tracheal system and is involved with the biosynthesis of octanoate, a precursor to lipoic acid.
Because it has been shown that a P-element-induced loss-of-function mutation in the mtacp1 causes lethality, this indicates that mtacp1 does not have a redundant gene. Therefore, the lack of a phenotype must be the result of mtacp1 not playing a significant role in drosophila eye development.

10880: The gene disrupted is mirr (mirror). Mirr encodes HOX; cd00086, homeodomain. They are DNA binding domains involved in the transcriptional regulation of key eukaryotic developmental processes. Phenotype of mirr mutations implicates it in wing and peripheral nervous system development. Mirr is involved in prepatterning sensory precursor cells in the lateral notum; required for the formation of the alula and a subset of sensory bristles in the lateral domain of the notum. Studies have indicated a link between the epidermal growth factor receptor (EGFR) signaling pathway to the Fringe signaling pathway via Mirror. Also, mirror controls the Toll signaling pathway, leading to dorsal nuclear transport. Mirror homeodomain protein provides a link that coordinates the Gurken/EGFR signaling pathway (initiated in the oocyte) with the Fringe/Notch/Delta pathway (in follicle cells). This coordination is required for epithelial morphogenesis, and for producing the signal in ventral follicle cells that determines the dorsal/ventral axis of the embryo.
The clear distinction between the dorsal and ventral parts of the eye in this stock is most likely due to the function of this gene in determining the dorsal/ventral axis of the embryo. Mutation of mirr may have disrupted the signal to the ventral follicle cells so that the ventral half of the drosophila eye would not be able to express the correct phenotype. It is possible that the ventral half of the eye is also homozygous for the lethal gene in the small clone. Yet, there is no phenotypic expression because the w+ marker in the P-element is not expressed in this area. The red patches from the large clone mosaic can be seen in the ventral half because the cells that make up these patches are heterozygous for the minute mutation, which has a w+ mutation.

Therefore, a phenotype can be seen for the large clone mosaic.

10199: The disrupted gene is loner. The P-element is inserted within the first exon of the 1st transcript. Loner encodes for Sec7, PH domain-like. The Sec7 domain is the central domain of the guanine-nucleotide-exchange factors (GEFs) of the ADP-ribosylation factor family of small GTPases (ARFs). It carries the exchange factor activity. Loner also encodes pleckstrin homology domain, a domain commonly found in eukaryotic signaling proteins. The domain family possesses multiple functions including the abilities to bind inositol phosphates, and various proteins. PH domains have been found to possess inserted domains (such as in PLC gamma, syntrophins) and to be inserted within other domains. This gene is also required for myoblast fusion which is essential for the formation and regeneration of skeletal muscle.
Because of the varying phenotypes of the large clone progeny, it is difficult to determine the function of the gene. Because there are wildtype large clone mosaic progeny, it is most likely that loner is organismic lethal. Because it has many important functions, it is possible that loner has redundant genes, which prevents all of the large clone progeny from showing a phenotype. The varying degrees of rough phenotype are hypothesized to be due to a background mutation.

10198: The disrupted gene is pap. The P-element is inserted within the 10th intron of the 2nd transcript of the pap gene. Pap (also known as skd) encodes Homeodomain, DNA binding domains involved in the transcriptional regulation of key eukaryotic developmental processes. Skd is required for the normal progression of photoreceptor differentiation in the eye disc and for the normal patterning of other imaginal discs. Loss-of-function mutations have been isolated which affect the larval mouth and are embryonic recessive lethal.
Although there were no small clone mosaic progeny that were found, disruption of pap most likely would result in a phenotype, providing that there is no redundant gene for pap, because skd is involved in photoreceptor differentiation.

10189: The disrupted gene is Indy (I'm not dead yet). The P-element is inserted within the 8th intron of all three transcripts. Indy encodes for pfam00939 and Na_sulph_symp (sodium: sulfate symporter family). INDY functions as a novel sodium-independent mechanism for transporting Krebs and citric acid cycle intermediates through the epithelium of the gut and across the plasma membranes of organs involved in intermediary metabolism and storage. Heterozygous Indy mutants show a near doubling of the average adult life-span without a decline in fertility or physical activity. The life-extending effect of mutations in Indy is likely caused by an alteration in energy balance caused by a decrease in INDY transport function.
Although there were no small clone or large clone mosaic progeny obtained, disruption of indy would most likely not have a great impact on the development of drosophila eye because a heterozygous mutant of indy extends the life span of drosophila. Therefore, the heterozygous lethal large clone progeny would most likely not show a phenotype. It is difficult to determine whether indy is cell lethal or organismic lethal.

10180: There was no flanking sequence found for this stock so information about the stock could not be obtained.
Based on the data obtained, this mutation has a role in eye development, yet its significance in eye development is difficult to determine because the phenotype was slightly rough.

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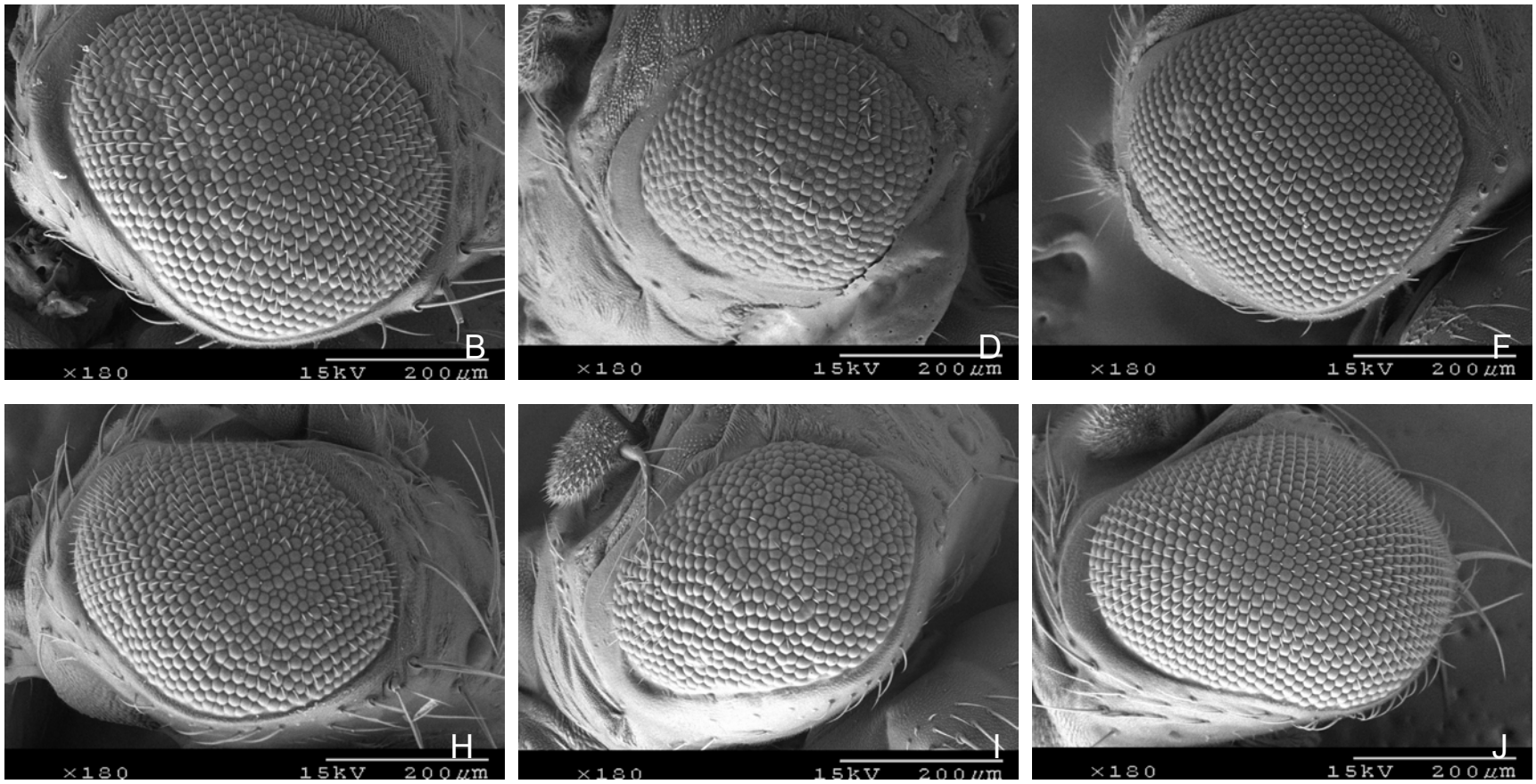
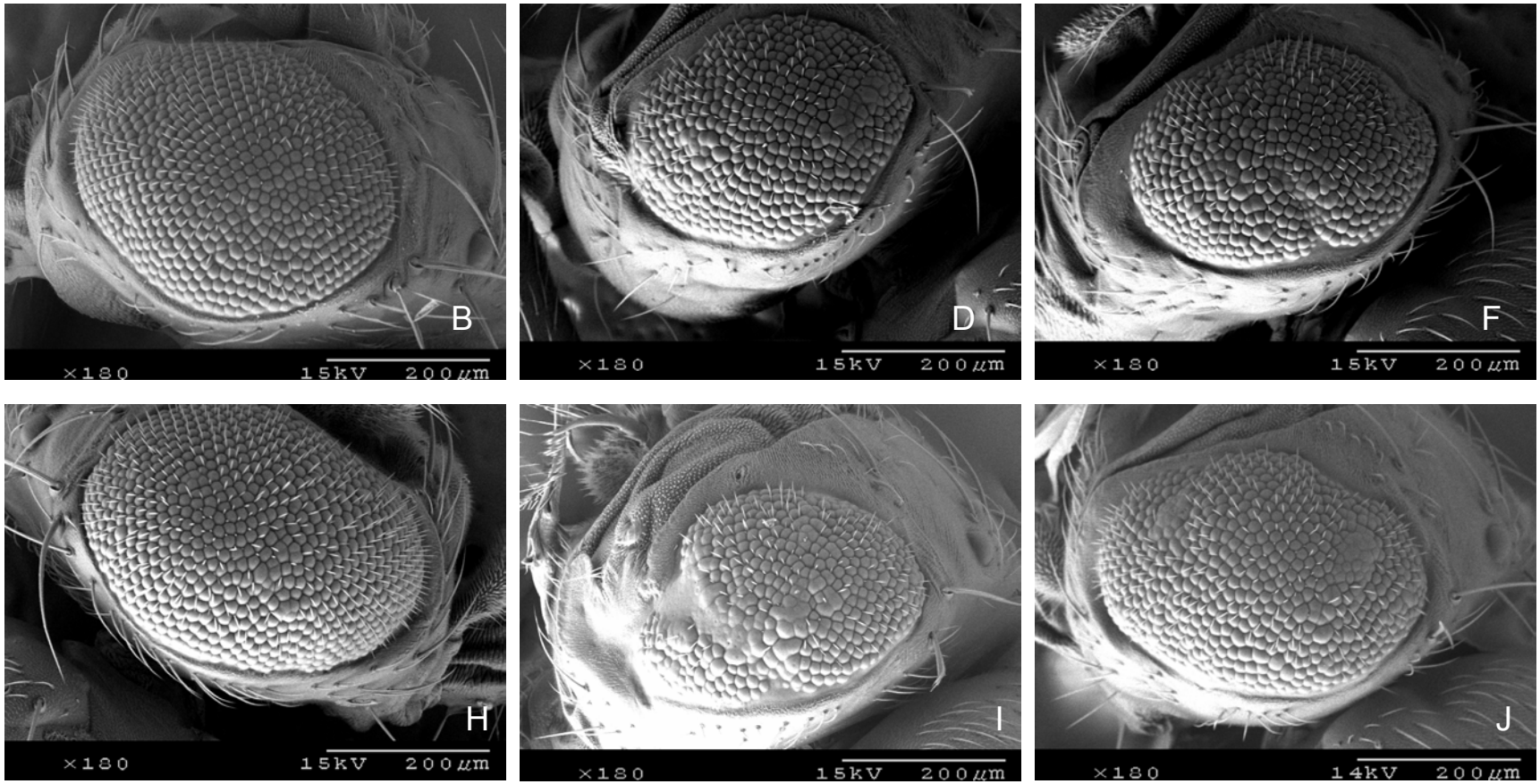
References:

http://flybase.bio.indiana.edu
http://www.ncbi.nlm.nih.gov/blast/
http://www.fruitfly.org/blast
http://flypush.imgen.bcm.tmc.edu/pscreen/
http://flybase.net/blast/

Enlargement of Figure 5 (SEM) - 10199



Enlargement of Figure 6 (SEM) – 10180



Stock #	Genotype	Cytological position	Gene P element is inserted in	eye color	Small-clone phenotype	Large-clone phenotype	# SC mosaic	Total #	Actual m.u.	Theoretical m.u.
10170	y[1] w[1118]; P{w[+mC]=lacW}l(3)L4111[L4111]/TM3, Ser[1]	068C01-04	5.8 kilobases 3' of CG6175	dark red-orange	wildtype, mostly red with small white/clear patches	wildtype, mosaic is difficult to see, mostly red with slightly darker red patches	152	605	25.1	11
10168	y[1] w[1118]; P{w[+mC]=lacW}Cdc27[L7123]/TM3, Ser[1]	065F01-02	cdc27	orange	wildtype, mostly dark red with orange/white patches	wildtype, dark red spots on a mostly red eye.	100	387	25.8	27
12061	y[1] w[*]; P{w[+mC]=lacW}mtacp1[j4A6]/TM6B, Tb[+]	061F06-07	mtacp1 (mitochondrial acyl carrier)	orange	wildtype, mosaic pattern varies and is red/orange or orange/white.	wildtype, mostly red with a few patches of more dark red	82	199	41.2	46.5
10198	y[1] w[1118]; P{w[+mC]=lacW}skd[L7062]/TM3, Ser[1]	078A02-03	pap	pale orange	No small clone mosaic progeny were found.	N/A	0	1299	0	0
10199	y[1] w[1118]; P{w[+mC]=lacW}l(3)L5541[L5541]/TM3, Ser[1]	078A05-06	loner	light orange	slightly rough, one of the small clone parents had some of its bristles missing, mostly orange with many white patches.	phenotype varies within each single female small clone line; some wildtype, others have varying degrees of rough phenotype with bristles missing; mosaic is mostly orange with very dark red/almost black patches	6	861	0.01	0
10189	y[1] w[1118]; P{w[+mC]=lacW}l(3)j14E7[j14E7]/TM3, Sb[1]	075E01-02	indy (I'm not dead yet)	dark red	No small clone mosaic progeny were found.	N/A	0	821	0	2
10171	y[1] w[1118]; P{w[+mC]=lacW}foi[j8E8]/TM3, Sb[1]	066D05-06	foi (fear of intimacy)	yellow-orange	wildtype, mostly dark red with large patches of orange	wildtype, mostly red with small patches of dark red	100	419	23.9	21
10880	y[1] w[*]; P{w[+mC]=lacW}mirr[cre2]/TM3, Sb[1]	069D	mirr (mirror)	dorsal is red, ventral is white	wildtype, mostly red-dark red with orange-white patches; mosaic pattern appears only in the dorsal half of the eye, ventral half is all white	wildtype, in dorsal half of the eye, more dark red patches on a dark red background; in ventral half, a few red patches on a white background	58	505	11.5	9
10180	y[1] w[1118]; P{w[+mC]=lacW}l(3)L0499[L0499]/TM3, Ser[1]	070D01-02	none found	dark orange-red	slightly rough with a few bristles missing, mostly white with large red patches	rough and slightly cell lethal, mosaic is indistinguishable because the eye is dark red	4	831	0.01	5

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Stock # (eg 10179)	Cytological location of P element insertion site (eg 052F05-07)	Base P element is inserted within flanking sequence (eg 228 in 498)	Base P element is located in scaffold (eg 236118)	Gene P element is inserted (eg *Lis1)*	Exon or Intron P element is inserted in (52 base of 1st exon of first 4 transcripts)	Type & function of protein made (eg microtubule associated complex)	# of mutant alleles (eg 19)	Where & what stage is the gene expressed Eg adult, oogenesis (germarium, nurse cell, oocyte), adult
10170	068C01-04	base 048 in the 55 bases	128420 in AE003545; 5828 bases away from CG6175	near CG6175 (68C1--2) base 80 in the 87 bases; 122592 in AE003545; 1152 base of 2nd intron of 1 transcript	N/A	It encodes a product putatively involved in cell communication.	1	N/A
10168	065F01-02	base 158 in the 537 bases	39900 in AE003559	Cdc27	555 base of 3rd exon of 1 transcript	Tetratricopeptide repeat (TPR) encodes a product involved in mitosis Mutations have been isolated which affect the mitotic cycle and the mitotic chromosome and are mitotic and recessive pharate adult lethal	2	N/A

12061	061F06-07□	base 041 in the 48 bases□	97387 in AE003471	mtacp1	208 base of 1st exon of 2 transcript	Acyl carrier protein (ACP) *mtacp1* is essential for viability and is required for both male and female gametogenesis. □	2	expressed in the embryo (embryonic/larval dorsal trunk , embryonic/larval lateral trunk and embryonic/larval tracheal system) and ovary (nurse cell). □
10198	078A02-03□	base 256 in the 592 bases□	59387 in AE003593 □	pap	5136 base of 10th intron of 2 transcripts	Loss-of-function mutations have been isolated which affect the larval mouth and are embryonic recessive lethal *skd* is required for the normal progression of photoreceptor differentiation in the eye disc and for the normal patterning of other imaginal discs. □	14	affect antennal disc, eye disc, larval mouth, maxillary palpus, photoreceptor cell, wing margin□
10199	078A05-06□	base 141 in the 176 bases□	82001 in AE003593□	loner	base 12 of first exon of 1 transcript	Sec7 domain, , PH domain-like	0	N?A
10171	066D05-06□	base 177 in the 441 bases□	191967 in AE003555□	foi	base 119 of 1st exon of 1 transcript	It encodes a product involved in tracheal system development (sensu Insecta) . Mutations have been isolated which affect the gonadal mesoderm and the precursor gonad and are embryonic lethal.	10	N/A

10880	069D □	N/A	N/A	N/A	N/A	homeobox domain and an iroquois-class homeodomain protein motif phenotype of *mirr* mutations implicates it in wing and peripheral nervous system development. □	25	expressed in the embryo (dorsal fold , embryo , embryo , embryonic/larval proventriculus and 3 other listed tissues), larva (dorsal mesothoracic disc , eye-antennal disc , eye-antennal disc , imaginal disc and 3 other listed tissues) and ovary (follicle cell □
10180	070D01-02 □	N/A	N/A	N/A	N/A	N/A	N/A	N/A
10189	075E01-02□	base 108 in the 594 bases□	208192 in AE003519 □	indy	base 2068 of 8th intron of three transcripts	Sodium:sulfate symporter family heterozygous *Indy* mutants show a near doubling of the average adult life-span without a decline in fertility or physical activity□	10	N/A