I. Abstract

The goal of this experiment was to determine whether nine gene mutations play a role in the eye development of Drosophila melanogaster. The gene mutations to be used were called P elements and are lethal when an organism has two copies of the mutation in all cells of its body. However, they may not necessarily affect the eye cells. Therefore the procedures of this experiment were devised so that in the end, the final fruit flies had two copies of the P elements in only their eyes. To achieve homozygousity of the lethal mutations only in the eye of the flies, and not other parts, mitotic recombination was induced using a protein called FLP placed downstream from the *ey* promoter and two FRT sites.

The eye cells of the final flies with two copies of the lethal mutation either had wildtype eye phenotype with mosaic coloration from the presence or absence of the colored P element, which meant the mutation affected a gene that is not involved in eye development, a mutant eye phenotype (e.g. rough) along with the mosaic coloration if the mutation was involved in eye development, or a small red eye, which meant that the mutation had killed all the eye cells in which it was homozygous. Each of the lines was preserved in at least two balanced stocks.

Of the nine P-element gene mutations studied, four resulted in wildtype eye phenotypes. These were the P-elements that disrupted: CG8036, belle, Dad and CG11722. The P-elements the disrupted CG1965, eIF5, Csk and CG8630 resulted in eye phenotypes that ranged from slightly rough to rough. The P-element that disrupted Hsc70-4 turned out to be cell lethal.

II. Introduction

The organism used in this experiment, Drosophila melanogaster, is an ideal model organism for genetic studies. Its four-chromosome genome has been completely sequenced. This experiment involved lethal mutations inserted into the right arm of the third chromosome.

P elements

The lethal mutation insertions in this experiment are called P elements. There are three main ways to induced mutagenesis: using chemicals, irradiating the fly, and transposons. If the first two ways are used, the exact site of the mutation cannot be identified. P elements utilize transposons. Transposons, first identified by Barbara Mcclintock, are mobile pieces of DNA that can 'jump' from one site to another using an enzyme called transposase (for which it also codes). The transposons insert themselves into select sequences. Fly geneticists used these transposons to insert select P element sequences into known sequences in the flies' genomes. These P elements are recessive lethals, which means that an organism that is homozygous for the P element will die, while organisms with only one copy of the P element will survive. The nine P elements used in this experiment also contain a mini-white gene, which creates a colored eye phenotype when present in an eye cell.

Balancer Chromosomes

These P elements have already been inserted in fly stocks obtained from the Bloomington Stock Center. These stock flies also have a genetic component called a balancer chromosome. The particular balancer chromosome involved in this experiment is called TM6B, which when present will result in a humoral phenotype, which is an increase in the bristle density on the shoulder of the fruit fly. Balancer chromosomes are

used to preserve the heterozygousity of the flies in a particular stock. These chromosomes include three components: a recessive lethal, a dominant marker, and multiple inversions in the genetic region of interest. The recessive lethal leads to any organism with two copies of the balancer to be never be born, the dominant marker makes it easy to identify which organisms have the balancer chromosome, and the multiple inversions cause any gamete in which meiotic recombination has happened to never create an organism because the recombined chromosome will be deformed.

Recombination

In order to make flies with P elements on the same chromosome as FRT sites (whose function will be discussed later) this experiment makes use of meiotic recombination. Meiotic recombination, as its name indicates, happens during meiosis- when a diploid parent cell divides into four haploid daughter cells. In Drosophila, this recombination only happens in the female parent, and so only her gametes will have the products of recombination. When the homologous chromosome pairs line up in the parent cell, they sometimes cross over and exchange sections of DNA. This creates gametes that have different combinations of genes on their chromosomes than the parent. The probability of meiotic recombination happening between two genes on the same chromosome is proportional to the distance between the two genes. The unit for this distance is termed map units, and is the percentage of instances that the recombination will happen.

The other form of genetic recombination, mitotic recombination, also plays a large role in this experiment. Mitotic recombination must be induced; it does not happen naturally, unlike meiotic recombination. One way to induce mitotic recombination is using x rays. However, this method does not allow the researcher to know in which cells the mitotic recombination is occurring. The method to be used in this experiment involves the FLP protein and FRT sites. When there are two FRT sites, one on each homologous chromosome, and FLP in a cell, the FLP will induce mitotic recombination at those FRT sites. The FRT sites used in this experiment are located at cytogenic position 82B. Since homozygousity of the P element is desired only in the eye of the fly, mitotic recombination needs to be induced only in the eye. This is done via limiting expression of the FLP protein by inserting the FLP gene downstream from the *ey* (eyeless) gene promoter region which is only expressed in the eye.

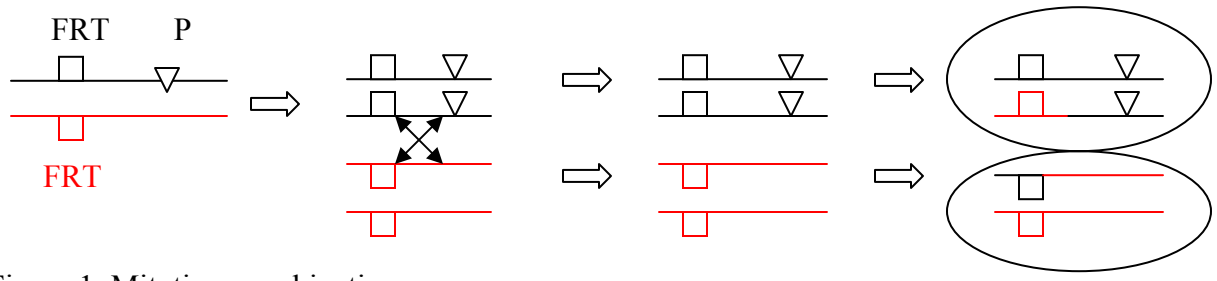


Figure 1. Mitotic recombination

P elements and drosophila eye development

All the P elements are organismic lethals, which means that they lead to the death of an organism if they are homozygous in every cell. However, this does not mean that they necessarily affect the eye cells of a fly. For instance, if the P element disrupts the development of a protein necessary for flies to develop a tracheal system, then a fly with the P element in its tracheal system producing cells will die because it cannot breathe.

However, if the P elements are only homozygous in the eye cells, which aren't needed for tracheal system production, the organism will live and the eye will be unaffected. If the P element is a cell lethal, which means that it disrupts some function that is vital to every type of cell (e.g. mitochondria production), the cells homozygous for the P element won't grow and the eye will be small. If the P element is not involved in eye development, the eye will show no structural phenotype. If the P element does affect eye development, the eye will show a phenotype that is a disruption of its wild-type structure.

The wildtype structure of the drosophila eye is crystalline. It is a compound eye made of 800 simple units, termed ommatidia. Each ommatidium is made of 8 photoreceptor cells and 4 cone cells, which secrete the lens. Each ommatidium has a bristle. In the fly embryo, 10-20 cells become eye cells. During the 3rd instar phase of the drosophila life cycle, the cells differentiate into different types of cells, based on cell-cell communication. The morphogenetic furrow tells the cells when to differentiate. The wildtype eye color is a deep red.

During any step of the eye development process, things may go wrong. If the P-element disrupts a function crucial to cell development early on, the cell may die and give rise to a mutant which has a small eye composed only of the surviving wildtype cells that do not have the P-element in them. If there is a disruption of the normal function of the morphogenetic furrow, the recruitment of cells to become certain types of eye cells will be affected. This can affect the spacing of the ommitidia during development and cause a rough eye phenotype, where the placement of the ommitidia is bumpy. If the P-element disrupts the development and function of the cone cells, the lens will not be formed correctly, and a glossy eye phenotype will be observed.

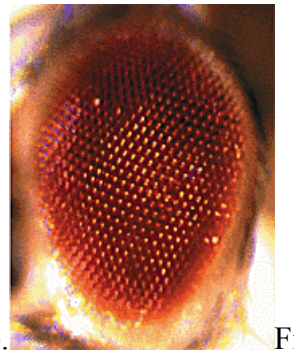
a.  b. 

Figure 2. SEM picture[1](a) and (b)light microscope picture[2] of wildtype eye

III. Materials and Methods

The nine P elements studied came from the Bloomington stocks: 10228, 10219, 10216, 10244, 10245, 10305, 10237, 10222, and 10286.

Stock #	Genotype
10228	`y[1] w[1118]; P{w[+mC]=lacW}l(3)j1B9[j1B9]/TM3, Sb[1]`
10219	`y[1] w[1118]; P{w[+mC]=lacW}l(3)L2100[L2100]/TM3,`

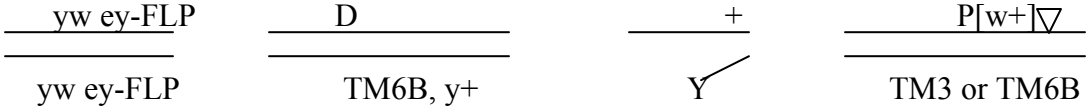
[1] http://www.emory.edu/CELLBIO/moses/caption8.html

[2] http://www.hhmi.org/genesweshare/images/sb300_1.gif

	Ser[1]
10216	y[1] w[1118]; P{w[+mC]=lacW}l(3)j9B6[j9B6]/TM3, Sb[1]
10244	y[1] w[1118]; P{w[+mC]=lacW}l(3)j1D8[j1D8]/TM3, Sb[1]
10245	y[1] w[1118]; P{w[+mC]=lacW}l(3)j5A1[j5A1]/TM3, Sb[1]
10305	y[1] w[1118]; P{w[+mC]=lacW}Dad[j1E4]/TM3, Sb[1]
10237	w[1118]; P{w[+mC]=lacW}s2681, l(3)s2681[s2681]/TM3, Sb[1]
10222	y[1] w[1118]; P{w[+mC]=lacW}l(3)L4740[L4740]/TM3, Ser[1]
10286	y[1] w[1118]; P{w[+mC]=lacW}Hsc70-4[L3929]/TM3, Ser[1]

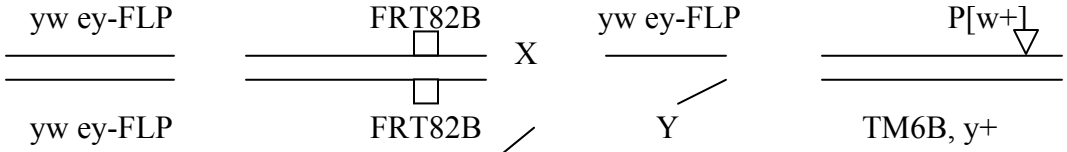
Figure 3. Genotype of Bloomington Stocks used

Three crosses were executed to achieve the final product of a line of flies whose genome leads to mitotic recombination in the eye. The initial parent flies were provided by an instructor cross which was:

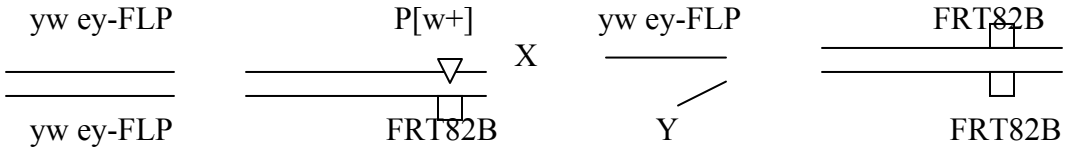


The males chosen to mate in the first cross were gray bodied and humoral with colored eyes.

The first cross was:



This cross was done to create a fly that had the P element and the FRT site on homologous chromosomes. The desired progeny had yellow bodies and nonwhite eyes. The females with this phenotype was selected for the second cross, which was:



In the female parent of this cross, some meiotic recombination occurred. Some of the gametes had a recombined genome in which the FRT site and the P element was on the same chromosome.

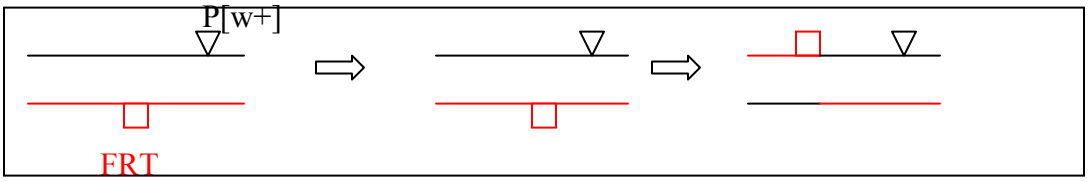


Figure 4. Meiotic recombination

The progeny from those recombined gametes had the necessary genetic conditions for mitotic recombination only in the eye: two FRT sites and the ey-FLP gene. Mitotic recombination occurred in some of these flies' eye cells, giving some of the eye cells two copies of the P element lethal. A fly that had mitotic recombination occurring in its eye showed a mosaic eye phenotype; because the P element contained the mini-white gene, cells with one copy of the P element were orange to yellow, cells with two copies were darker, and cells with no P element were white. The two spots that occurred because of the same mitotic recombination event (in this case, the white and darker spots) are called twin spots. An eye phenotype (e.g. rough, glossy) was seen in one of the stocks.

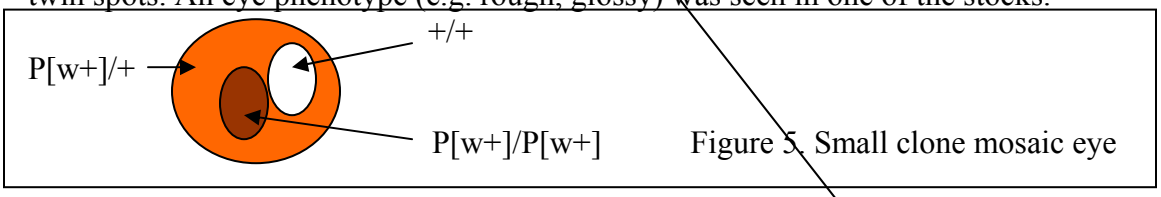
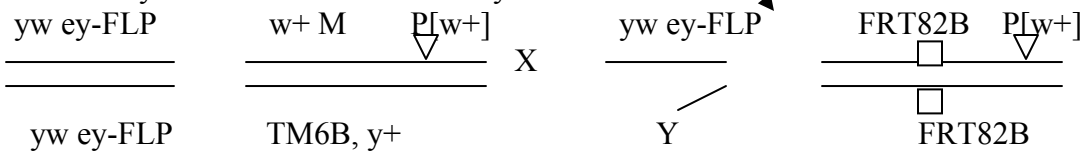


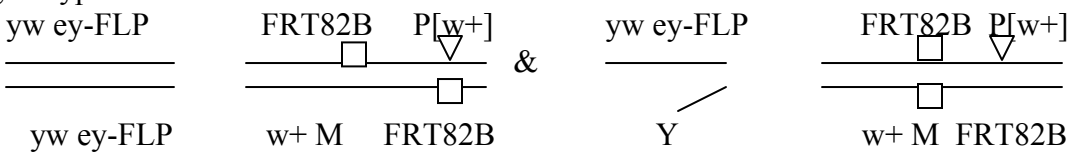
Figure 5. Small clone mosaic eye

The males with yellow bodies and mosaic eyes were chosen for the next cross:

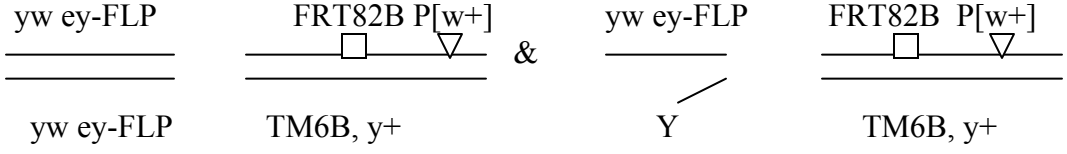


This cross introduced the minute gene into the fly stock. This exaggerated the phenotype of the P element homozygous cells. The minute gene was introduced in a way so that eye cells that are wildtype had two copies of the minute gene, cells with one copy of the P element had one copy of the minute gene and cells with two copies of the P element did not have the minute gene at all.

Cells with two copies of the minute gene died, cells with one copy grew slowly, and cells with no copies of the gene grew at a normal pace. The cells with two copies of the P element were at an advantage and grew faster and so became a bigger part of the eye and easier to examine for eye phenotypes. The progeny that were chosen for eye phenotype examination and pictures were the yellow bodied ones with mosaic/small eyes and genotype:



This cross also reintroduced the TM6B balancer chromosome into the experiment to create a balanced stock of flies. The progeny crossed to set up the balanced stock were gray bodied with nonwhite eyes and genotype:



IV. Results

The small clone mosaic progeny of the second cross were counted to determine the meiotic recombination distance between the FRT sites and the P-element. This data was compared with the theoretical recombination distances calculated from Flybase data. Some of the distances were different from each other, but this can be attributed to low numbers of flies counted. Also, the larger the recombination distance, the less accurate it is.

Stock #	P element insertion site	#mosaic progeny	#total progeny counted	Theoretical recombination distance(map units)	Experimental Recombination distance (map units)
10228	85A9	13	970	1	2.68
10219	84B2	2	863	1	0.46
10237	85F7	11	444	2	4.95
10305	89E10	10	261	12	7.66
10286	88E4	9	429	9	4.19
10222	85A5	26	305	1	17.04
10216	83B4	2	975	0	0.41
10244	86E16	14	706	4	3.96
10245	87E5	6	375	6	3.2

Figure 6. Recombination distances of P-elements

Stock 10228

This P element disrupts a gene called CG8036. The experimental recombination distance of 2.68 m.u. is close to the theoretical of 1 m.u. The mosaic small clone progeny of this line were wildtype, with visible color contrast between the patches of different color(fig. 7a). The large clone progeny of the single male crosses (the third cross) had a wildtype phenotype with orange and red patches(fig. 7b). In one of the single male crosses, a small percentage of the mosaic progeny did not exhibit the orange/red patches, but instead had a cell lethal small eye phenotype(fig. 7c).

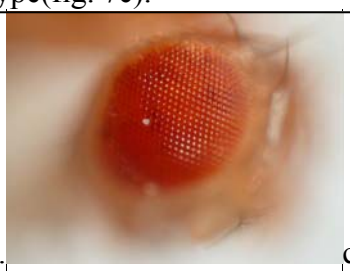
a.  b.  c. 

Figure 7. 10228 wildtype small clone(a) and wildtype large clone (b) eyes, both wildtype; 10228C small eye progeny(c)

Stock 10286

The P element of this stock disrupts the Heat shock protein cognate 4(Hsc70-4) gene. The experimental recombination distance of 4.19 m.u. was considerably less than the theoretical of 9m.u. The mosaic small clone of this progeny were wildtype with expanded white clones and pale orange patches(Fig. 8a). The color contrast was difficult to spot at

first, but got darker as the flies got older. One of the males used in the single male crosses turned out to be sterile. The large clone progeny of the second cross had a cell lethal phenotype, with small eyes composed of only misshapen red ommatidia (fig 8b). SEM pictures (fig 8c) show that some of the ommatidia have fused together.

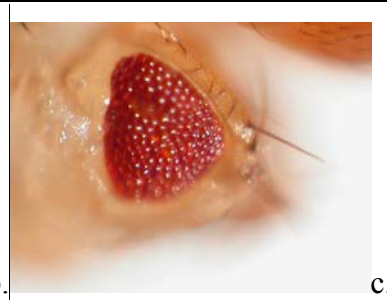
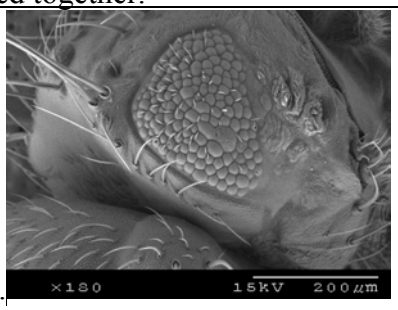
a.  b.  c. 

Figure 8. 10286 wildtype small clone (a) and small eye large clone(b) light microscope and large clone SEM (c) pictures

Stock 10222

The gene this P element disrupted was belle. The experimental recombination distance of 17.04 m.u. was much different than the theoretical recombination distance of 1 m.u. The mosaic small clone progeny were wildtype, with visible color contrast between patches(fig. 9a). The large clone orange/red mosaic progeny also had wildtype eye phenotype(fig. 9b). The progeny of one of the single male crosses had a slightly rough phenotype, but since this phenotype was also observed in the progeny without the P element in their eyes, it was determined that this phenotype was caused by a background mutation and not the P element.

a.  b. 

Figure 9. 10222 small clone (a) and large clone (b) light microscope pictures

Stock 10219

The gene this P element disrupted was CG1965. The experimental recombination distance of 0.46 was less than the theoretical distance of 1 m.u. The two small clone mosaic progeny found were both nonvirgin females. The small clone mosaic male progeny of one of these females were used in the third cross. The mosaic small clone progeny were wildtype with expanded white clones and light orange patches(fig 10a). The large clone orange/red mosaic progeny had rough eye phenotype(fig 10b). SEM pictures of the large clone show uneven ommatidia and misshapen eye shape with a slight cleavage in some flies (fig 10c). It was noted that the 10219 large clone eyes collapsed much faster than those of other lines.

Figure 10. 10219 wildtype small clone (a) and rough large clone(b) light microscope pictures and SEM picture of 10219 large clone(c) with arrow pointing to cleavage in eye

Stock 10216

The gene this P element disrupted was elongation initiation factor 5C (eIF5C). The experimental recombination distance was 0.41 m.u. which was close to the theoretical distance of 0 m.u. Because of the small recombination distance, only two single male crosses could be set up. The mosaic small clone progeny had rough eyes with much color contrast(fig 11a). SEM shows uneven ommatidia placement (fig 11b) along with missing bristles. The large clone progeny had orange/red mosaic eyes that ranged from slightly rough to rough (fig 11c-e).

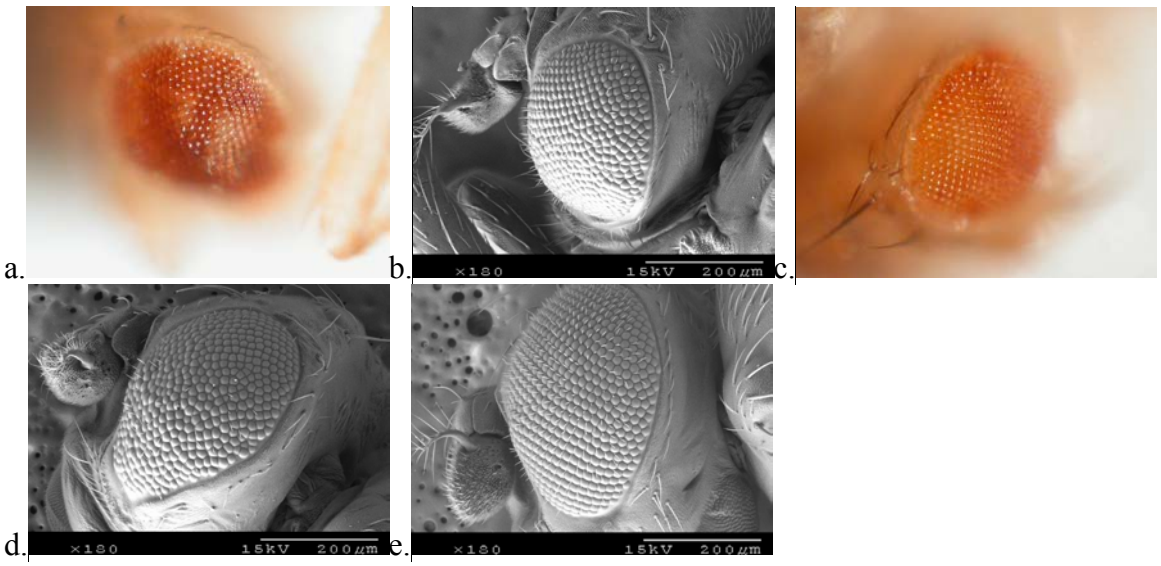
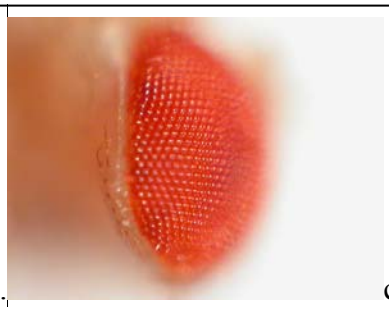
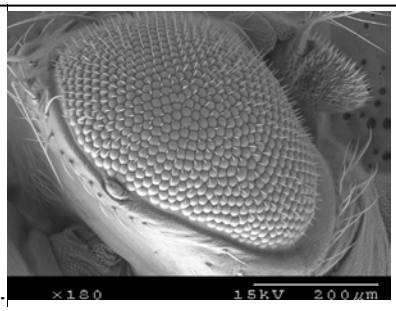


Figure 11. 10216 rough small clone light microscope(a) and SEM(b)pictures; large clone rough light microscope(c) and SEM (d) pictures and SEM of slightly rough progeny(e)

Stock 10244

The gene disrupted by this P element is C-terminal Src kinase(Csk). The experimental recombination distance of 3.96 m.u. is very close to the theoretical distance of 4 m.u. The mosaic small clone progeny had wildtype eyes with strong color contrast(fig 12a). The large clone orange/red mosaic progeny had phenotypes ranging from slightly rough to rough(fig 12-c). SEM shows uneven and bumpy ommatidia placement(fig 12b).

a.  b.  c. 

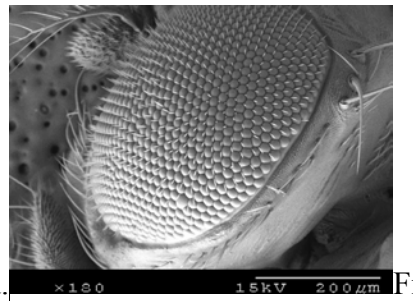
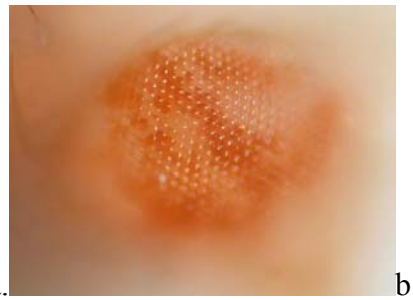
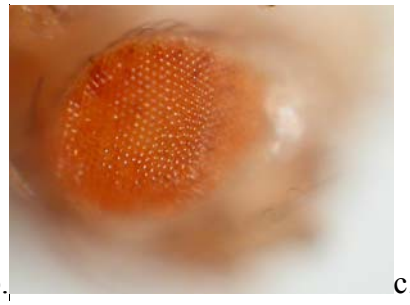
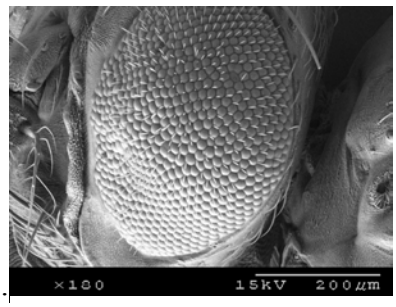
d. 

Figure 12. 10244 wildtype small clone(a) and rough large clone(b) light microscope pictures and SEM pictures of rough large clone(c) and slightly rough large clone(e)

Stock 10245

The gene this P element disrupted was CG8630. The experimental recombination distance of 3.2 m.u. is less than the theoretical distance of 6 m.u. The small clone mosaic progeny had wildtype eyes with strong color contrast between the color patches(fig 13a). The orange/red mosaic large clone progeny had phenotypes ranging from slightly rough to rough(fig. 13b-d). SEM shows uneven ommatidia placement.

a.  b.  c. 

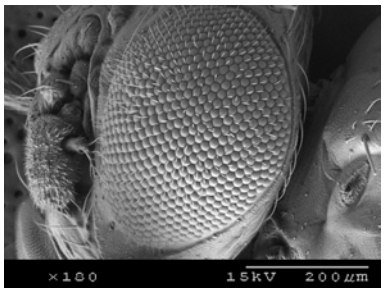
d. 

Figure 13. Light microscope pictures of 10245 wildtype small clone(a) and rough large clone(b); SEM pictures of rough large clone(c) and slightly rough large clone(d)

Stock 10305

The gene disrupted by this P element is Daughters against dpp(Dad). The experimental recombination distance is 7.66 m.u. which is less than the theoretical distance of 12 m.u. The small clone progeny were wildtype with visible color contrasts(fig. 14a). The large clone orange/red mosaic progeny were wildtype as well(fig. 14b).

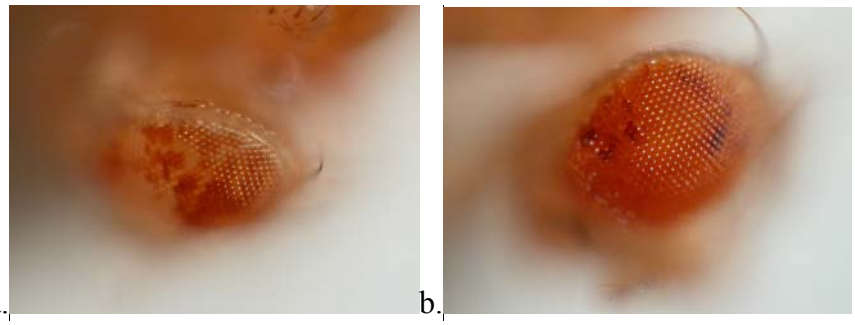


Fig 14. Light microscope pictures of 10305 wildtype small clone(a) and wildtype large clone(b)

Stock 10237

The genes possibly affected by this P element are CG11722 and CG3910. The experimental recombination distance is 4.95 m.u. which is more than the theoretical distance of 3 m.u. The small clone progeny had wildtype phenotype with visible color contrasts(fig. 15a). The large clone mosaic progeny also had wildtype phenotype with visible color contrasts(fig. 15b).

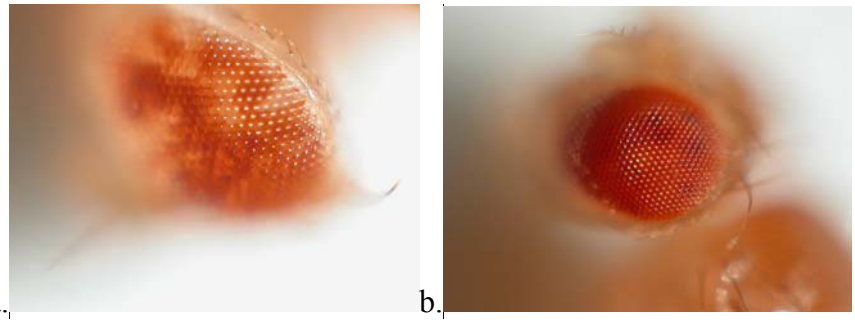


Figure 15. Light microscope pictures of wildtype small clone (a) and wildtype large clone(b)

V. Discussion

There is information about the functions of some of the genes disrupted by the P elements in used in this experiment, but many have functions that have not been clearly determined yet. The effects these particular P elements (and the genes they disrupt) have on eye development have not been studied before for the most part. Their possible effects on eye development, based on the results obtained, are discussed.

Stock 10228

The gene disrupted, CG8036, encodes a product with transkeletolase activity- it is involved in carbohydrate transport and metabolism. The domain of the product is linked to energy production/conservation and lipid metabolism. The wildtype phenotype of the large clone suggests that the lethal P element mutation does not disrupt eye development, so this gene is not involved in eye development. Its function, however, is very important to cell survival, so perhaps the function of carbohydrate transport and metabolism is controlled by more than one gene, so when CG8036 was disrupted, another gene kept on making the necessary product so the cell could still function. An interesting observation is that a small percentage of the large clone progeny of 10228C had a cell lethal mutation. A possible explanation for this occurrence is that one of the females had a background mutation in the other gene responsible for carbohydrate transport and metabolism, and so

her large clone progeny had both transkeletolase genes disrupted and so had cells that could not survive. This is supported by the fact that all of the other progeny (the ones without two copies of the P element) were wildtype, and so the background mutation in that female only affected the flies with a disrupted CG8036 gene. A possible future experiment can be to examine other P elements that disrupt transkeletolase genes, and if they also give rise to wildtype phenotypes, to try create flies with both transkeletolase genes disrupted and so identify which genes are redundant.

Stock 10286

The gene disrupted, Hsc70-4, encodes a product with ATPase activity involved in neurotransmitter secretion localized to the mitochondrion inner membrane. ATPases are protein complexes that synthesize ATP, used as fuel. The product is also involved in chaperone activity- helping other proteins to fold. Surprisingly, its domain is linked to actin, a protein involved in the cell cytoskeleton. The large clone phenotype of small eye means that this product is vital to cell survival. Hsc70-4's product's function of fueling neurotransmitter secretion is vital to cell survival, which means it must be involved with important neurotransmitters or the proteins that Hsc70-4 helps fold must be important to cell survival.

Stock 10222

The gene disrupted, belle, encodes a product that is a RNA helicase involved in spermatid development. RNA helicases unwind strands of RNA and are vital to RNA metabolism. The mosaic large clones with this mutation had wildtype eyes, which means that this gene is not involved in eye development. Because spermatid development does not happen in the eye and is not crucial for eye development, the drosophila eye is unaffected by a mutation affected the belle gene.

Stock 10219

The gene disrupted by this P element is CG1965 which encodes a product involved in DNA binding generally regarded to help regulate the pol II promoter. The pol II promoter regulates the transcription for RNA polymerase. The rough phenotype seen in the large clone with this mutation show that the RNA polymerase this gene controls is involved in eye development. The RNA polymerase may be involved in transcription of proteins that are vital to normal eye development. Because it has a rough phenotype, that means there is something wrong with the cell arrangement process that happens early in fly eye development. Some of the large clone eyes exhibit a slight cleavage that can be seen in the SEM pictures; perhaps this cleavage further indicates that CG1965 is involved in eye cell arrangement.

Stock 10216

The gene disrupted by this P element is eIF5C, which encodes a product involved in translation initiation of a component of the cytosol. The rough to slightly rough phenotype seen in the large clones with this P element indicate that the component of the cytosol disrupted by the mutation is vital to the correct placement of ommatidia.

Stock 10244

The gene disrupted by this P element is Csk, which encodes a product with protein-tyrosine kinase activity that regulates protein activity by amino acid phosphorylation. When the gene is mutated, some proteins are either left on all the time or turned off all the time. The slightly rough to rough phenotype observed in the large clones with this mutation indicate that one or more the proteins regulated by the product of this gene are involved in eye development.

Stock 10245

The gene disrupted by this P element is CG8630, which encodes a product with stearoyl-CoA 9-desaturase activity involved in fatty acid metabolism. The rough phenotype observed in the large clones with this mutation show that this product is somehow involved in eye development. A possible situation could be that when the gene is mutated, its product is not available to break down fatty acids, so the fatty acids build up in the cell, causing problems.

Stock 10305

The gene disrupted by this P element is Dad, which encodes a product with inhibitory cytoplasmic mediator activity involved in the negative regulation of transforming growth factor beta receptor signaling pathway expressed in the larval dorsal mesothoracic disc. In other words, the product of this gene helps turn off a growth factor. The wildtype phenotype of the large clones with this mutation shows that this product is not involved in eye development. Since the growth factor pathway is expressed in the dorsal mesothoracic disc of larvae and not in their eyes, mutation of the Dad gene does not affect eye development.

Stock 10237

The genes located near this P element are CG11722 and CG3910. No molecular information is available for either gene, but genes with similar sequences to CG11722 in different organisms encode products that regulate hormones, and the conserved domain of the product of CG3910 is linked to methyltransferases. The P element most likely affects CG11722 because it is upstream from that gene; it is downstream from CG3910 and likely does not affect it. The wildtype phenotype of the large clone progeny with this mutation shows that the hormones that CG11722 regulate (if it is indeed a hormone regulator) do not affect eye development, or perhaps are so crucial to eye cell development that two genes code for the same function.

The results of this experiment only touch the surface of the functions of these nine P elements and the genes they disrupt. Of the five lines that gave rise to phenotypes, further study is needed to pinpoint exactly at what stage in drosophila eye development the mutations start affecting the eye. Further study is also needed to identify the exact functions in the eye of the products of the disrupted genes. Only with much more information and many more experiments can the effects of these P elements on drosophila eye development be fully known. Once determined, this information may prove to be very valuable to Drosophila researchers. Results from the Human Genome Project reveal that 50% of proteins are conserved between humans and fruit flies. Many developmental and regulatory pathways are similar in humans and flies. Perhaps one of the developmental pathways involving one of the genes disrupted by these P elements will prove to be important to biomedicine.

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