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Investigating the Influence of CHD1 on Gene Expression in Drosophila Melanogaster Using Position Effect Variegation

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Investigating the influence of CHD1 on gene expression in *Drosophila melanogaster* using Position Effect Variegation

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by

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Abstract

Position Effect Variegation (PEV) is the mosaic expression of a gene that has been moved out of its optimal environment and into a different area on the chromosome. Changing a gene's environment may have profound effects on its eligibility for proper expression, which is a complicated process regulated by many factors. The PEV phenomenon is used as an assay to study gene expression as regulated by chromatin structure. In this study, the *Drosophila melanogaster white* gene was used as a reporter to study the various effects of CHD1, a chromatin regulating factor, on PEV gene expression. Inspired by preliminary data generated by the Armstrong Lab where overexpression of CHD1 resulted in suppression of gene silencing of the *brown* gene and loss of CHD1 resulted in enhancement of gene silencing, this study uses PEV as an assay to examine whether loss of function *chd1* mutant alleles function dominantly to enhance silencing of the *white* gene when it is placed in a repressive chromatin environment. Surprisingly, I found that a *chd1* loss of function mutant allele dominantly suppressed gene silencing (meaning I saw an increase in gene expression), suggesting that the CHD1 protein is normally required for effective silencing. The results demonstrated that CHD1 is a dominant modifier of PEV gene expression. CHD1 significantly modifies gene expression by suppressing silencing of the *white* gene inserted into pericentric heterochromatin on the second and fourth chromosomes and an insertion into the medial region of the fourth chromosome, while it shows no significant modification of the *white* gene inserted into telomeric heterochromatin of the fourth chromosome. Together, these intriguing results regarding varying gene expression at different chromosomal sites show that PEV is a dynamic phenomenon meriting further research and studying the effects of CHD1 as a modifier of PEV may be influential to understanding the mechanism and characteristics of gene expression.

Introduction

Chromatin Structure

The organization of DNA into chromatin in eukaryote is crucial to gene expression. Human cellular DNA measures about two meters long when stretched out but these strands are able to fit inside the cell after tight packaging into chromosomes (Alberts et al 2002). To condense itself, DNA strands wrap around histone protein octamer that forms nucleosome units containing two of each core histones: H2A, H2B, H3, and H4 (Luger 1997). The formation of nucleosomes resembles "beads on a string" and can further be compacted (Figure 1). Nucleosome-rich regions are tightly compacted and remain condensed throughout the cell cycle; they are known as heterochromatin (Heitz 1928). Heterochromatin are observed to be late replicating (Lima de Faria and Jaworka 1968) and are associated with a lack of gene expression (Lyon 1961). Other common characteristics of heterochromatin include repetitive DNA segments and lack of acetylation modifications (Rea et al. 2000). On the other hand, nucleosome-poor regions that are loosely packaged are referred to as euchromatin and are often characterized by presence of many acetylated nucleosomes and active genes. Although there are exceptions, euchromatin usually consists of active chromatin while heterochromatin consists of inactive chromatin. Heterochromatin may be constitutive, permanently silenced chromatin, or facultative, repressed in some cells during specific cycles or developmental stage (Review in Allis et al., 2007).

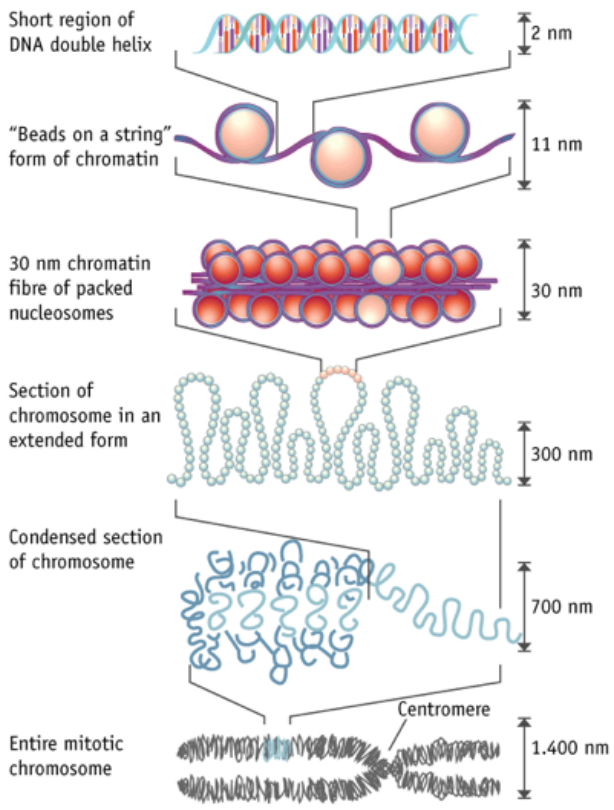


Figure 1. Levels of DNA Compaction
DNA strands wrap around histone proteins forming nucleosomes resembling "beads-on-a-string". The aforementioned structure is considered a loosely packed form of chromatin. The "beads-on-a-string" can further be organized into tightly packed form of chromatin. Eventually through further compaction, the packaged DNA forms chromosome (Illustration adapted from Felsenfeld and Groudine 2003).

Chromatin Modifications

Chromatin is not accessible in the condensed state, therefore covalent or chromatin-remodeling modification must occur to make genetic material available for various processes such as transcription etc. As shown in figure 2, prominent covalent modifications include methylation, ubiquitination, acetylation and phosphorylation; they change chromatin structure by adding different chemical groups to the exposed histone tails at various specific sites (Vaquero et al. 2003).

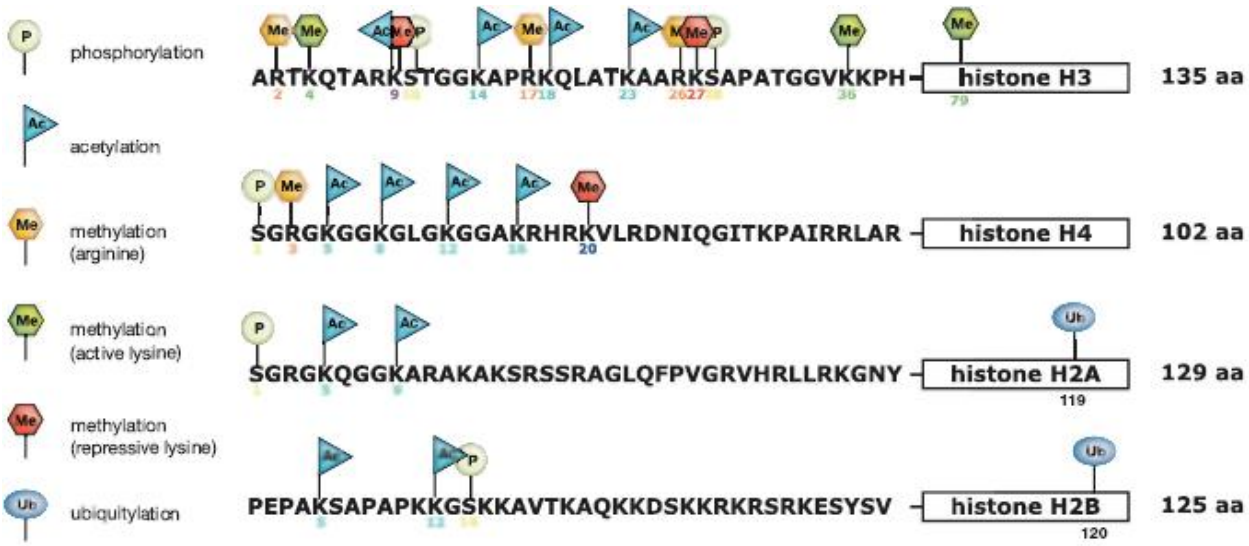


Figure 2. Types of Histone Tail Modifications Modification occurs at the globular domain (boxed) as well but histone tails the host majority of known covalent modifications. (Review in Vaquero et al 2003)

In addition to covalent modifications, chromatin remodeling through remodeling factors can also make DNA available for transcription. These factors are energy-using protein complexes that change chromatin and nucleosome composition in a non-covalent manner. Chromatin remodelers are crucial for a number of reasons. First, after replication, nucleosomes must be properly deposited and spaced so specialized remodelers are used to correctly position the nucleosomes (Review in Vaquero et al 2003). Second, condensed nucleosomes may hide important *cis* elements so remodelers, in this case, can position the *cis* element in a nucleosome-free region or in DNA linkers between nucleosomes and also slide the nucleosome to expose *cis* element (Review in Vaquero et al 2003). Third, nucleosomes must be moved or ejected to provide quick access for DNA repair and recombination. Lastly, after nucleosomes have been moved or ejected, remodelers are needed to move nucleosomes back to its appropriate position

(Reviewed in Vaquero et al 2003). Demonstrated in figure 3, chromatin remodelers physically change chromatin formation and makes DNA accessible for other processes.

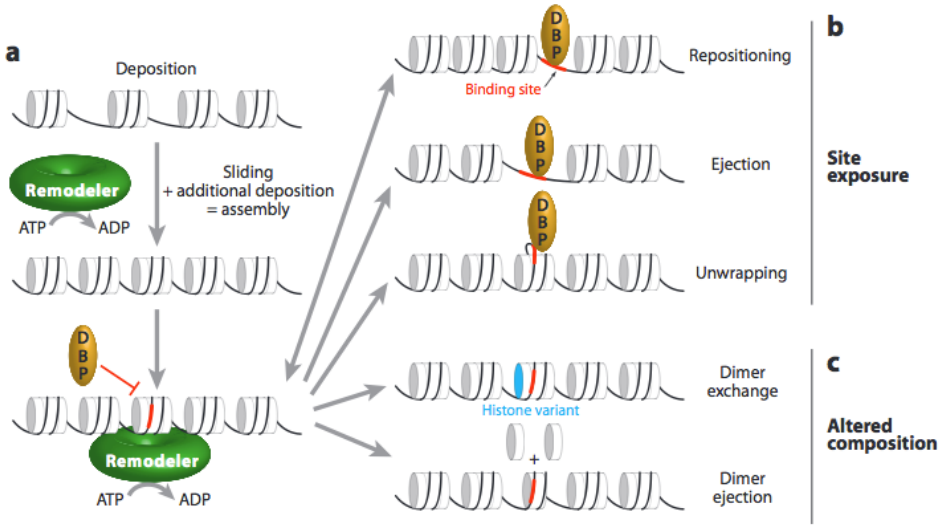


Figure 3. Histone Modifications
The different outcomes of chromatin remodeling. Remodelers (*green*) can assist in chromatin assembly by moving already deposited histone octamers, generating room for additional deposition (*a*). Remodeler action on a nucleosome array results in various products that can be classified in two categories: (*b*) site exposure, in which a site (*red*) for a DNA-binding protein (DBP), initially occluded by the histone octamer, becomes accessible by nucleosomal sliding (repositioning), or nucleosomal eviction (ejection), or localized unwrapping, and (*c*) altered composition, in which the nucleosome content is modified by dimer replacement [exchange of H2A-H2B dimer with an alternative dimer containing a histone variant (*blue*)] or through dimer ejection. (Figure from Clapier and Cairns 2009).

There are four families of chromatin remodeling ATPases: ISWI, SNF2, SWR1, and CHD (Manning and Peterson, 2013). The remodelers share five basic characteristics: 1) an attraction for the nucleosome; 2) domains that recognize covalent histone modifications; 3) a conserved ATPase domain that is DNA-dependent; 4) regulators of the ATPase domain; and 5) domains and or proteins that allow interactions with other transcription factors (Clapier and Cairns, 2009). Even though the four remodeler families share some common properties, they are specialized for different roles when put into biological contexts.

One of the more interesting family of chromatin remodelers to me is Chromodomain Helicase DNA Binding Protein (CHD), which has been associated with human diseases such as prostate cancer (hCHD1), dermatomyositis (hCHD4), Hodgkin's lymphoma (hCHD3), neuroblastoma (hCHD5) and CHARGE syndrome (hCHD7) (Ge Q et al., 1995; Schwab U, 1982; Thompson PM, 2003; Vissers LE, 2004).

Of particular interest to my research and the Armstrong lab is CHD1 as studied in *Drosophila*. *Drosophila* CHD1 has been found to colocalize with RNA Pol II to sites of active transcription on polytene chromosomes (Srivani et al 2005, Marfella et al., 2007). CHD1 also plays a crucial role in the pluripotency of mouse embryonic stem cells where mouse stem cells lacking CHD1 has high inclination for neuronal differentiation, signifying the loss of capability to differentiate into all cell types (Gaspar-Maia et al. 2009). Recent studies have also found that human CHD1 is the second most deleted gene in prostate cancer and the deletion increases cell invasiveness, suggesting that CHD1 may be required for genetic expression of tumor suppressors (Liu et al. 2011, Huang et al. 2011, Burkhardt et al. 2013). Similar to CHD1's suggestive role as a requirement of expression of tumor suppressors, that the loss of CHD1 in clinical specimens has been linked to a significant increase in additional chromosomal deletions, both hemi- and homozygous, perhaps suggesting that CHD1 is required for regulating DNA processes (Liu et al. 2012). With its vast suggestive roles in regulation of DNA and expression of genes, along with its diversity in conservation (Woodage et. al 1997), it is obvious that CHD1 plays important roles biologically. It is then necessary that we continue to conduct research on this specific chromatin remodeler to better understand its role in DNA organization and how that affects gene expression.

Position Effect Variegation

Position Effect Variegation PEV is an exhibition of mosaic gene expression due to a phenomenon where a gene is silenced in some cells yet expressed in others when that gene has been moved into a different environment than its norm. In the classic example of PEV, X-Ray is used as the mutagen that moved the *white* gene, usually in euchromatin, into an area closer to heterochromatin, resulting in the *white* gene being turned off in some cells (Muller, 1930). In 1986, Zhimulev inspected polytene chromosomes of larvae carrying the *white* rearrangement and saw the reporter gene inside a dense block of heterochromatin in all cells where *white* had been silenced (Zhimulev et al., 1986). The fly's eyes have white patches of cells where *white* has been silenced. The number and size of patches as well as level of pigments differ between flies. Because the phenotype is caused by a change in the position of a gene within the chromosome, the condition is referred to as Position Effect Variegation. One idea is that virtually any gene in the appropriate arrangement can variegate in expression (Allis 2007).

One of the most well-known explanations for PEV is the spillover model (Talbert and Henikoff 2006; Wakimoto 1998), suggesting that rearrangement of chromosome has enabled heterochromatin to "spread" and remove a buffer zone that once existed between heterchromatin and euchromatin (Locke et al. 1988). With the buffer zone gone, the resulting effect is that heterochromatin's repressive mechanisms (still unknown) spreads over to euchromatin or nearby zones and silences genes (Locke et al. 1988). One model suggests that transcription factors fight for derepression of heterochromatic silencing (Eissenberg, 2001). The second theory proposed is the turnover model, where silencing can be caused due to a loss of epigenetic marks as nucleosomes are assembled or histones are exchanged and/or replaced (Yankulov 2012). Some suggest PEV is caused by rapid replacement of the H3 histone, which may erase histone marks

(Donze et al. 1999, Ishii et al. 2002, Ishii and Laemmlo 2003). In a study in budding yeast, activation of promoters is often paired with the eviction of nucleosomes (Boeger et al. 2003) and some studies have demonstrated that these activated promoters are sites of H3 histone exchange (Dion et. al. 2007; Jamai et al. 2007; Ruflange et al. 2007), suggesting that perhaps with the ejection of nucleosomes are placement of histone where ejected nucleosomes were so that histones can help arrange for nucleosomes to be in their proper place again. Lastly, an explanation supporting the turnover model is that epigenetic marks may be lost during DNA replications where the entire genome is subjected to unraveling, chromatin's role is to preserve epigenetic memory by recycling histones so that they can properly manage DNA organization (Yankulov 2012).

This study seeks to combine both the heterochromatin spreading and histone turnover theories. I propose that CHD1 antagonizes heterochromatin by preserving epigenetic marks through histone recycling and therefore we predict that the loss of CHD1 will result in enhancement of gene silencing. Using the PEV phenomenon as an assay to investigate the effects of CHD1, a chromatin remodeler, on gene expression at different chromosomal locations, I hope to further provide some new perspectives on gene expression in relation to heterochromatin and also to provide new clues to understanding human diseases that are affected by PEV. Utilizing the *white* gene as a reporter in my PEV assay, I ask two main questions: 1) does CHD1 dominantly modifies PEV 2) what is the effect on PEV when both copies of CHD1 is missing in the fruit flies.

Material and Methods

Drosophila Stocks

Drosophila melanogaster were raised in bottles and vials at 24 °C using standard yeast, agar, cornmeal and molasses. Flies were taken out of their ideal temperature for no more than one hour at a time about every few days for maintenance.

In examining whether CHD1 dominantly modifies PEV, heterozygous, single-generational crosses were made using *yw* as a negative control, *al b c sp* as a negative control and *chd1* ^{5} *b c sp* as the experimental (Appendix).

For our negative control, to test the pattern of expression on our PEV lines, *Su(var)3-9*, a suppressor of variegation, was used in single-generation crosses with our four PEV lines (Appendix).

Table 1: Lines of fruit flies used and sources

Fly Line	**Source**
$w^{1118}; \frac{Dp(3;3)Nap1^{KO1}Nap1^{KO1.5\prime}Nap1^{KO1.3\prime}}{T(2;3)SM6b-TM6B,ap^{RK}Tb^{1}}; +; +$	Bloomington Stock Center
$y^{1}\ w^{*}; +; +; \frac{bip2^{4}}{ln(4)ci^{D},ci^{D}pan^{ciD}}$	Bloomington Stock Center
ln w^{m4}*; Su(var) 3-9*^{1}*/ TM3,* Sb^{1} Ser^{1}	Bloomington Stock Center
$w; \frac{chd1^{5}b\ c\ sp}{CyO,Kr-GFP,w^{+}}; +; +$	Jennifer Armstrong
w; +; +; 39 C-12	Wallwrath LL and Elgin SC, 1995.
w; +;+; 118 E-15	Wallwrath LL and Elgin SC, 1995.
$w;\ +; +; 118\ E-10$	Wallwrath LL and Elgin SC, 1995.
$w; 39\ C-4;\ +;\ +$	Wallwrath LL and Elgin SC, 1995.
$w; al\ b\ c\ sp\ ;+;\ +$	Frances Wang and Bloomington Stock Center

The PEV lines used were gifted by Lori Wallwrath. These fly lines exhibit PEV due to the *white* gene being moved into different chromosomal locations as listed below.

Table 2: Location of *white* insertion in the PEV lines (Wallwrath and Elgin 1995)

Fly Line	**Location**
w; 39 C-4; +; +	Pericentric, second chromosome
w; +; +; 39 C-12	Medial, fourth chromosome
w; +;+; 118 E-15	Telomeric, fourth chromosome
w; +;+; 118 E-10	Pericentric, fourth chromosome

Preparing Flies for Analysis

The progeny of interest were isolated on the thirteenth day after parents have been crossed and then kept alive for another six days in order to ensure adult flies of age six-seven days. After being aged, they were placed in eppendorf tubes in -20°C freezer for visual and quantified analysis of frozen flies within one week. Frances Wang from the Armstrong lab conducted an experiment where she found that freezing resulted in reduced level of pigments detected via the spectrophotometer; however, it was unclear how long the flies in her experiment were frozen for prior to quantification.

Visualizing Phenotypes

Photographs of the eyes of desired progeny were taken using a Nikon Coolpix p6000 camera attached to a Zeiss WPI Stemi 2000C dissecting microscope. The light arms were angled at 45 ° and light intensity at 75% using the Fiber Lite MI 150 High Intensity Illuminator from Dolan-Jenner Industries. The camera setting was set to autofocus, F 6.2 at 1/130 second. The flies were turned to their right side.

Quantifying Phenotypes

Using a razor on the dominant hand and forceps on the non-dominant, fifteen heads were obtained for each replicate. The heads were placed in 0.10ml of 99.9% methanol with 0.1% HCl then homogenized; then, 0.40 ml of the methanol and HCl solution was added before the absorbance was measured. The HCl and methanol were added at different occasions to avoid spilling solution and fly heads when the homogenizer starts. The homogenized mix was centrifuged at 10,000 rpm for five minutes. The supernatant was removed and placed in cuvettes. Then 0.50 ml of methanol and HCl solution was added to the cuvette and mized up and down using a pipette. Absorbance was measured at 480nm using the Thermo Scientific's Genesys 10 Spectrophotometer.

Results

In searching for whether CHD1 significantly modifies PEV, I crossed homozygous *chd1*^{5} *b c sp, al b c sp*, *yw,* and *Su(var) 3-9*^{1} flies to four PEV lines, resulting in 16 different crosses. The *yw* and *al b c sp* crosses were used as negative controls, the *Su(var) 3-9*^{1} was used as a positive control and *chd1* ^{5} *b c sp* as the experimental.

Spectrum analysis

Many studies, when measuring expression level of the *white* gene using the PEV assay, measures the absorbance at 480 nm but it was puzzling to me whether the different shades of red pigments in the eyes among different PEV lines would affect my absorbance results. I expected all four PEV lines with different red-shaded eyes to have different peaks. A spectrum analysis where the absorbance was measured at different wavelengths was done. It was found in this part of the study that 480 nm is the peak of the spectrum in three of four PEV lines (all except 39C-12) and 480 nm is the most consistent wavelength to be used for this experiment (Figure 4).

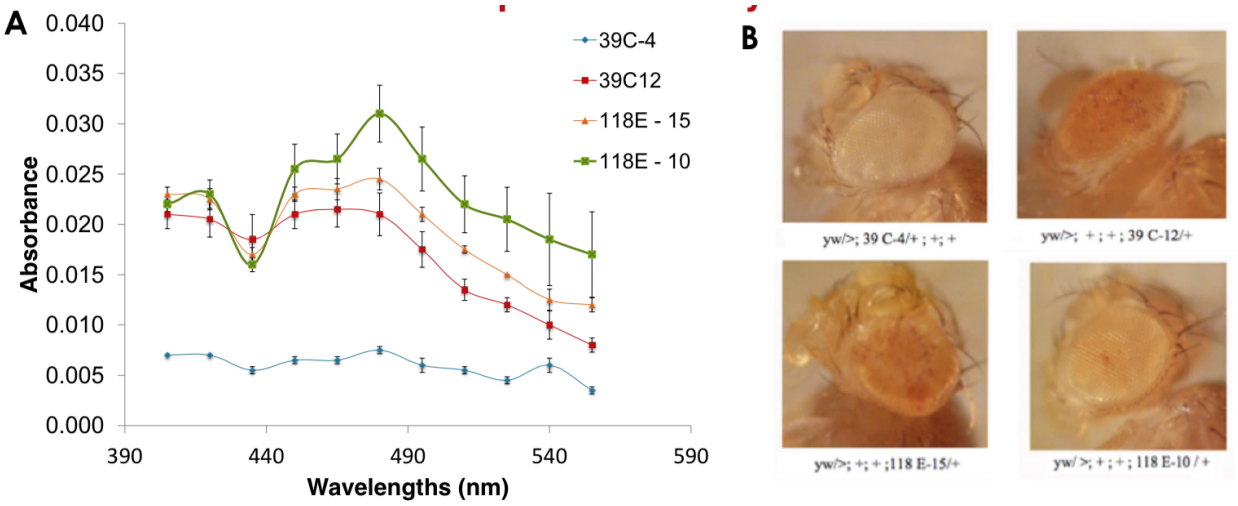


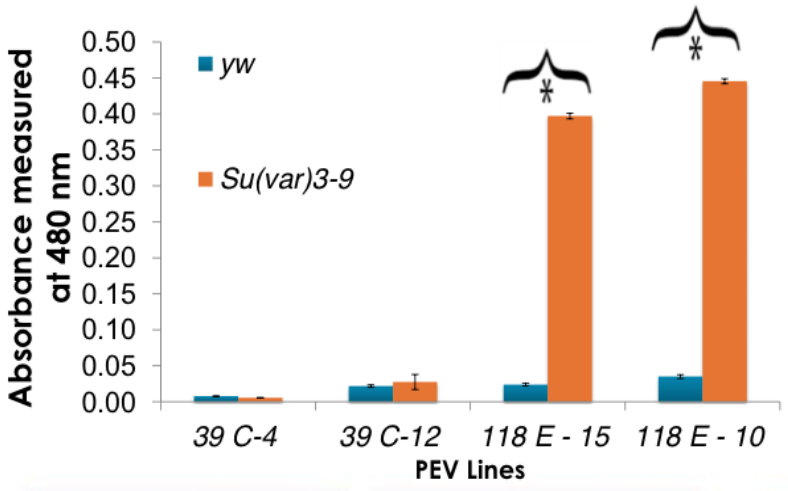
Figure 4. Absorbance at different wavelengths. (A) The absorbance of heterozygous *yw, al b c sp, chd1 b c sp* and PEV mutants at different wavelengths. Measured at each point is the mean of two biological replicates with a total of 30 male fly heads. Error bars represent standard error. (B) Representative visualized phenotype of heterozygous *yw* and PEV mutant male flies.

Positive control with *Su(var)3-9*^{1}

In the positive control where homozygous *Su(var) 3-9*^{1} and the PEV lines were crossed to each other, I tested to see if the four PEV lines being used react to known modifiers of PEV gene expression. I expected the gene expression of the heterozygous progeny of all four PEV lines and *Su(var) 3-9*^{1} to be suppressed (eyes that look more red and higher absorbance levels) since *Su(var) 3-9*^{1} is a known strong suppressor of PEV gene silencing (Ner et al 2002). Surprisingly, *Su(var) 3-9*^{1} suppressed silencing only in lines 118E-15 and 118E-10 but not 39C-4 and 39C-12 (Figure 5). There were negligible absorbance difference between the control and experimental in 39C-4 and 39C-12 lines (Figure 5A). In the 118E-15 line, absorbance in the experimental was 16.77 times higher than the control, meaning *chd1* modifies the gene expression of PEV line 118E-15 by 16.77 fold (Figure 5A). Similarly, *chd1* modifies the gene expression of PEV line 118E-10 by 12.73 fold as absorbance was 12.73 times higher in the experimental than in the control progeny (Figure 5A). The photos taken of the fly eyes of 39C-4

reflect the absorbance data where the eye color did not change between the control and the experimental (Figure 5B). In line 39C-12, the photos taken do not reflect absorbance data where there was a bit higher (but not significant) absorbance in the experimental but what is seen in the experimental photo taken is that the control seems to have eyes that are more red (Figure 5B).

A



B

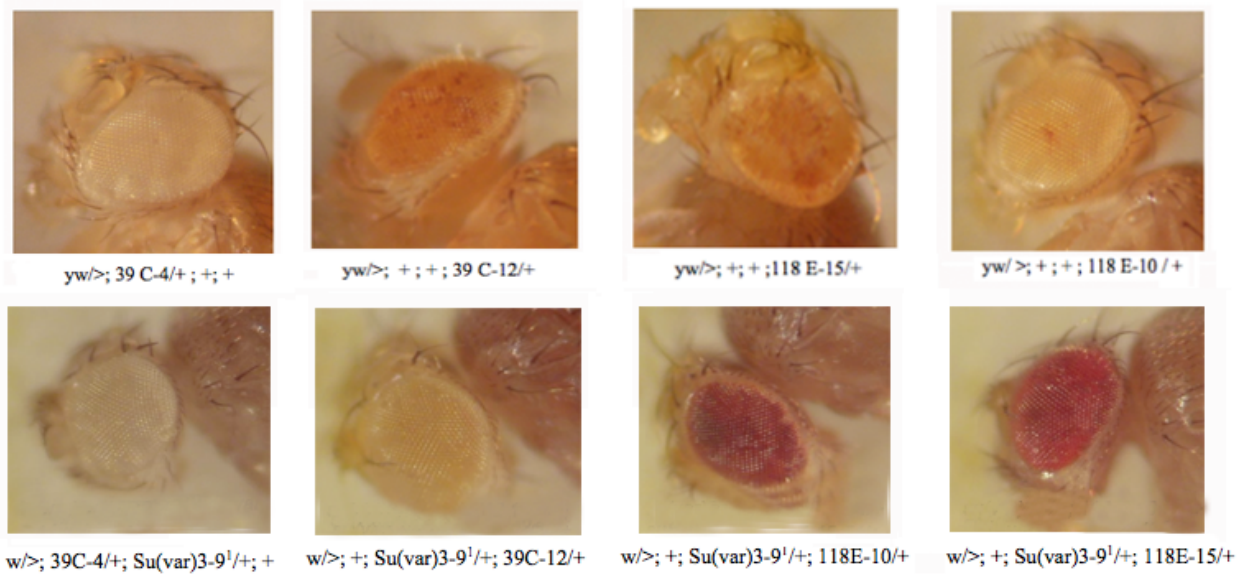


Figure 5. Analysis of suppression of silencing by Su(var)3-9. (A) Absorbance of heterozygous *Su(var)3-9* and PEV mutant flies. Each bar represents the mean absorbance of two biological replicates of 30 total male fly heads. *yw* is control and *Su(var) 3-9* is experimental. * represents significant difference. (B) Representative visualized phenotype of heterozygous *Su(var)3-9* and PEV mutant male flies.

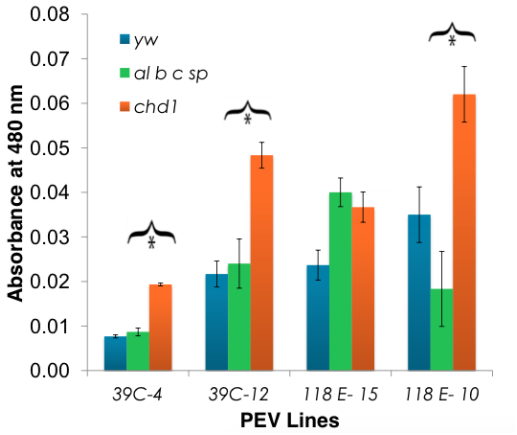
chd1 dominantly modifies PEV gene expression

Moving forward after several control experiments, one of the most crucial experiments in my study seeks to find whether *chd1* dominantly modifies PEV gene expression; that is, when there is one copy of *chd1* missing on *Drosophilas* that exhibit PEV, does the *white* gene get expressed more or less, are the variegated eyes more white or more red. If the eyes look more white, then the *white* gene in PEV expression has been more silenced as opposed to if the eyes are more red, the *white* gene has been less silenced/more expressed. There were two controls done for this experiment where the first control consisted of crossing homozygous *yw* to homozygous PEV lines to eliminate random elements picked up over time as fruit flies breed (Oral fly husbandry process passed down by Professor Armstrong, no literature was found). The second control consisted of crossing homozygous *al b c sp* to the PEV lines to obtain heterozygous progenies was to account for the effects of the *b c sp* markers' effects on PEV gene expression. *chd1* is located on the same chromosome as the markers *b c sp* and this control accounts for the PEV gene expression related to these markers. The absorbance of heterozygous loss of function *chd1* and PEV lines (in orange, Figure 6A) are compared to the second control (in green, Figure 6A). I can use the data from this second control to compare to my experimental of heterozygous *chd1*. Additionally, the body color (yellow and black) seen in the photos of figure 6A does not affect gene expression in the eyes.

Based on a past study by Kelsey Schmidt and Lakshmi Bugga from the Armstrong lab using PEV and the *brown* gene, overexpression of CHD1 resulted in suppression of gene silencing of the brown gene and loss of CHD1 resulted in enhancement of gene silencing, I hypothesize that the loss of chd1 in my study will result in an enhancement of gene silencing, meaning I expected to see flies that have eyes that are more white-looking. Very unexpectedly,

three of four PEV lines actually showed that loss of *chd1* resulted in significant suppression of silencing. The heterozygous progeny of loss of *chd1* and PEV lines *39C-4, 39C-12, 118E-10* resulted in absorbance level that are 2.23, 2.01 and 3.39 times higher than that of the *al b c sp* control, respectively (Figure 6A). The photos presented in figure 6B reflect this change in more expression of the white gene and less expression of silencing (eyes look like they have more red pigments or look more red overall), although it is difficult to tell by looking that the number of red patches have approximately increased by 2.23, 2.01 and 3.39 times. There was no significant difference between the heterozygous *al b c sp* control versus heterozygous *chd1* experimental in line 118E-15; however, there was a 1.67 time increase in absorbance of the heterozygous *al b c sp* in comparison to the heterozygous *yw* (Figure 6A), suggesting the markers *b c sp* may have suppressed PEV gene expression at the telomeric fourth chromosome. The photos from figure 6B reflect the increase in absorbance in the *al b c sp* compared to the *yw* progeny because the eyes in the heterozygous *al b c sp* seems to have more red pigments.

A **B**



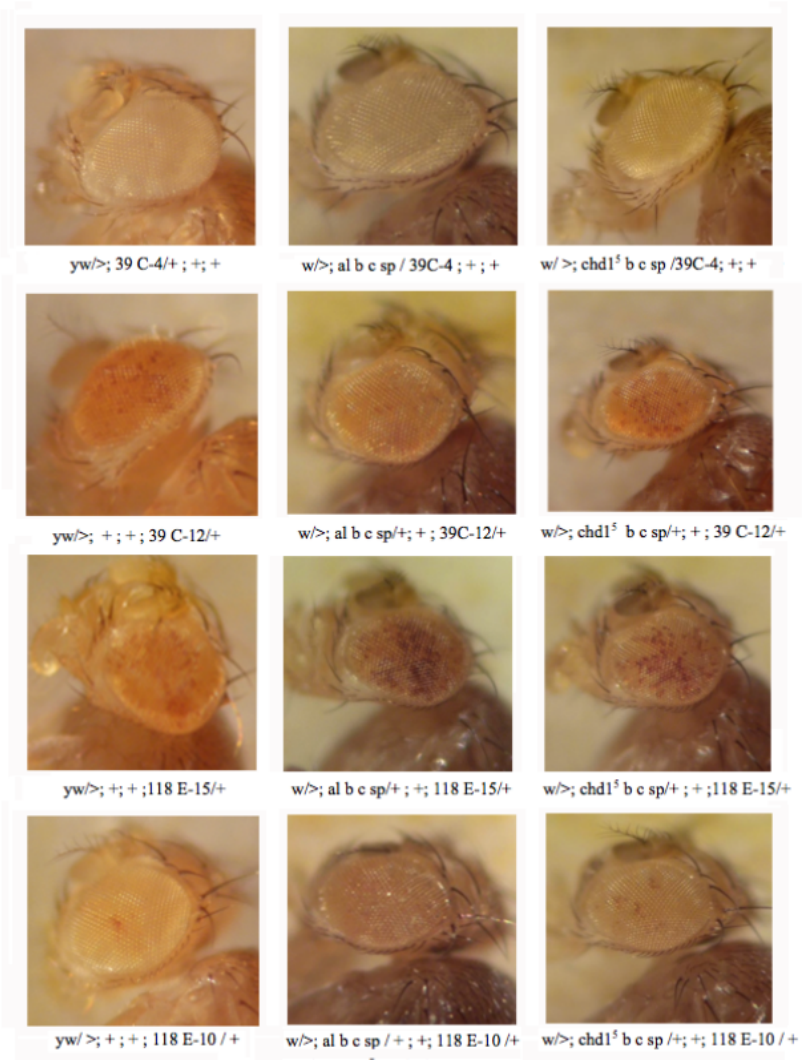


Figure 6. *chd1* dominantly modifiesWord did not find any entries for your table of contents. **PEV gene expression in three of four PEV lines.** Four PEV lines were individually crossed to homozygous *yw,* homozygous *al b c sp*, and heterozygous *chd1 b c sp* to obtain heterozygous progeny for analysis. (A) Each bar contains three biological replicates with a total of 45 male heads. Error bars represent standard error. *yw* is positive control, *al b c sp* is positive control and *chd1* is the experimental. * represents significant difference. Representative visualized phenotype of heterozygous *Su(var)3-9* and PEV mutant male flies. (B) Representative visualized phenotype of heterozygous PEV with *yw, al bc sp, chd1 b c sp* males.

Discussion

Multiple theories regarding repressive heterochromatin's role in PEV gene expression has been studied over the years (Yankulov 2013); however there have been a lack of studies on whether gene expression is different in various areas on the chromosome in relation to heterochromatin. This study seeks to examine whether CHD1, a chromatin remodeling factor,

affects gene expression at various chromosomal locations using the *white* gene as a reporter in the PEV assay. It was proposed that CHD1 represses heterochromatin spreading by preserving epigenetic marks through recycling histones, therefore it was hypothesized that the loss of CHD1 will result in an enhancement of gene silencing.

Absorbance was a quantification method used to measure the degree of gene expression in different progenies. The absorbance (which was measuring for red pigments at 480 nm) corresponded with *white* gene expression level as seen in PEV. Again, the PEV lines used in this study exhibit a variegated eye phenotype because the *white* gene was moved to various spots on different chromosomes and near heterochromatin. The *white* gene, in a different environment than its norm and closer to repressive heterochromatin, is silenced in some cells and expressed in others. When comparing negative, positive control and experimental data, higher absorbance values were associated with higher *white* gene expression/suppression of silencing. My results were opposite from my hypothesis that loss of CHD1 would result in an enhancement of silencing where the *white* gene was more silenced and the fly eyes would look more white. What I found was that CHD1 significantly suppressed gene expression by suppressing silencing of the *white* gene inserted into pericentric heterochromatin on the second and fourth chromosomes and *white* gene insertion into the medial region of the fourth chromosome, while it showed no significant modification of the *white* gene inserted into telomeric heterochromatin of the fourth chromosome (Figure 6).

From the detailed results above, CHD1 is a dominant modifier of PEV gene expression, namely at the pericentric left arms second chromosome, pericentric fourth chromosome, and medial fourth chromosome (Figure 6). CHD1 does not dominantly modifies at the telomeric fourth chromosome (Figure 6) as consistent with a past study where Wallwrath and Elgin found

that pericentric and fourth chromosome transgenes, but not telomeric transgenes, respond to known suppressors of PEV (Wallwrath and Elgin 1995). Overall, the differences in gene expression at different chromosomal sites suggest that various expression mechanisms, not just one, are responsible for gene expression, although no previous study further explored this puzzling phenomenon.

Since the results found were opposite to my hypothesis, several other mechanisms can be proposed to explain why the loss of CHD1 resulted in suppressed silencing (more *white* gene expression). One, CHD1's normal role could be a silencer or a factor helping a silencing factor. Two, in the loss of CHD1, another remodeling factor may be responsible for histone recycling suggesting a perhaps repetitive function in some remodeling factors. Three, with no CHD1 to recycle histones, newly-deposited histone may already have the epigenetic marks necessary to organize DNA that propagate appropriate gene expression.

The results to the second part to my study where I ask whether CHD1 recessively modify PEV are not shown in this report because there were not enough progeny to quantify expression levels. The necessary flies, both females and males, needed to create homozygous *chd1* mutants with one copy of a PEV mutant element were very unhealthy due to multiple mutations, making it difficult to maintain a sizable stock for experimental crosses. The progeny of "F5" was obtained and scored but only 1 male homozygous loss of function *chd1* mutant with PEV hatched; statistically, in my future studies, at least 750 flies in the "F5" progeny will need to hatch for me to have 15 male flies of desired genotype for each biological replicate. The flies will continue to be bred over many generations until the proper number of flies is obtained for quantification. Because only 1 fly of the desired genotype was acquired from the "F5" cross, quantification of whether chd1 recessively modifies the *white* gene in PEV could not be shown.

My study is currently on "F4" where the cross can be repeated many times using fly siblings to grow the stock necessary to make several giant "F5" crosses (See Appendix).

In addition, my study also suggests that different PEV lines respond differently to various modifiers, supporting that there may be multiple mechanism of gene expression at different chromosomal sites. Using a known suppressor of PEV expression, *Su(var)3-9* should have been able to suppress all PEV lines used but it was found that this modifier of PEV suppressed silencing of the *white* gene only at the telomeric site of the fourth chromosome and pericentric site of the fourth chromosome; it did not suppress silencing at the medial site of the fourth chromosome and pericentric site of the second chromosome. These varying results were intriguing and supported my previous theory that gene expression is facilitated by different mechanisms at different chromosomal sites. It was also puzzling that *Su(var)3-9* did not suppress gene silencing in line *39C-12* and 39 C-4 where the *white* gene was moved from euchromatin to the medial site of the fourth chromosome because *Su(var)3-9* is an established strong modifier of PEV (Ner et al 2002). The differences in location of suppression suggest that multiple mechanisms and/or different protein complexes exist in different areas of the chromosomes; these different complexes and mechanisms dictate gene expression that resulted in various PEV phenotypes. In the future, further research using more modifiers are necessary to better the understanding of the PEV phenomenon and gene expression.

Appendix

Below are individual crosses and expected progenies. The highlighted portion displays the progeny desired for optical density quantification.

Su(var)3-9 modifier crosses-Negative control

$$♂\ \frac{w}{>};\ \frac{Su(var)3{-}9^{1}}{TM3,\ Sb^{1}\ Ser^{1}};\ +;\ +\quad\times\quad ♀\quad \frac{w}{w};\ +;\ +;\ \frac{118\ E{-}15\ T4}{118\ E{-}15\ T4}$$

↓

Expected Progeny Genotype	Expected Phenotype
♀ $\frac{w}{w};\ \frac{Su(var)3{-}9^{1}}{+};\ +;\ \frac{118\ E{-}15\ T4}{+}$	Female, no stubble
♀ $\frac{w}{w};\ \frac{TM3,\ Sb^{1}\ Ser^{1}}{+};\ +;\ \frac{118\ E{-}15\ T4}{+}$	Female, stubble
♂ $\frac{w}{>};\ \frac{Su(var)3{-}9^{1}}{+};\ +;\ \frac{118\ E{-}15\ T4}{+}$	Male, no stubble
♂ $\frac{w}{>};\ \frac{TM3,\ Sb^{1}\ Ser^{1}}{+};\ +;\ \frac{118\ E{-}15\ T4}{+}$	Male, stubble

$$♂\ \frac{w}{>};\ \frac{Su(var)3{-}9^{1}}{TM3,\ Sb^{1}\ Ser^{1}};\ +;\ +\quad\times\quad ♀\quad \frac{w}{w};\ +;\ +;\ \frac{118\ E{-}10\ C4}{118\ E{-}10\ C4}$$

↓

Expected Progeny Genotype	Expected Phenotype
♀ $\frac{w}{w};\ \frac{Su(var)3{-}9^{1}}{+};\ +;\ \frac{118\ E{-}10\ C4}{+}$	Female, no stubble
♀ $\frac{w}{w};\ \frac{TM3,\ Sb^{1}\ Ser^{1}}{+};\ +;\ \frac{118\ E{-}10\ C4}{+}$	Female, stubble
♂ $\frac{w}{>};\ \frac{Su(var)3{-}9^{1}}{+};\ +;\ \frac{118\ E{-}10\ C4}{+}$	Male, no stubble
♂ $\frac{w}{>};\ \frac{TM3,\ Sb^{1}\ Ser^{1}}{+};\ +;\ \frac{118\ E{-}10\ C4}{+}$	Male, stubble

$$♂\ \frac{w}{>};\ \frac{Su(var)3{-}9^{1}}{TM3,\ Sb^{1}\ Ser^{1}};\ +;\ +\quad\times\quad ♀\quad \frac{w}{w};\ +;\ +;\ \frac{39C{-}12\ 1020}{39C{-}12\ 1020}$$

↓

Expected Progeny Genotype	Expected Phenotype
♀ $\frac{w}{w};\ \frac{Su(var)3{-}9^{1}}{+};\ +;\ \frac{39C{-}12\ 1020}{+}$	Female, no stubble
♀ $\frac{w}{w};\ \frac{TM3,\ Sb^{1}\ Ser^{1}}{+};\ +;\ \frac{39C{-}12\ 1020}{+}$	Female, stubble
♂ $\frac{w}{>};\ \frac{Su(var)3{-}9^{1}}{+};\ +;\ \frac{39C{-}12\ 1020}{+}$	Male, no stubble
♂ $\frac{w}{>};\ \frac{TM3,\ Sb^{1}\ Ser^{1}}{+};\ +;\ \frac{39C{-}12\ 1020}{+}$	Male, stubble

$$♂\ \frac{w}{>};\ \frac{Su(var)3{-}9^{1}}{TM3,\ Sb^{1}\ Ser^{1}};\ +;\ +\quad\times\quad ♀\quad \frac{w}{w};\ \frac{39C{-}4\ oxy\ c22}{39C{-}4\ oxy\ c22};\ +;\ +$$

↓

Expected Progeny Genotype	Expected Phenotype
♀ $\frac{w}{w};\ \frac{Su(var)3{-}9^{1}}{+};\ +;\ \frac{39C{-}4\ oxy\ c22}{+}$	Female, no stubble
♀ $\frac{w}{w};\ \frac{TM3,\ Sb^{1}\ Ser^{1}}{+};\ +;\ \frac{39C{-}4\ oxy\ c22}{+}$	Female, stubble
♂ $\frac{w}{>};\ \frac{Su(var)3{-}9^{1}}{+};\ +;\ \frac{39C{-}4\ oxy\ c22}{+}$	Male, no stubble
♂ $\frac{w}{>};\ \frac{TM3,\ Sb^{1}\ Ser^{1}}{+};\ +;\ \frac{39C{-}4\ oxy\ c22}{+}$	Male, stubble

<u>Heterozygous mutant crosses</u>

$$♀\ \frac{w}{w};\ \frac{chd1^{5}\ \text{b c sp}}{CyO,Kr{-}GFP,w^{+}};\ +;\ +\ \times\ ♂\ \frac{w}{>};\ +;\ +;\ \frac{118\ E{-}15\ T4}{118\ E{-}15\ T4}$$

↓

Expected Progeny Genotype	Expected Phenotype
♀ $\frac{w}{w};\ \frac{\text{chd1}^{5}\ \text{b c sp}}{+};\ +;\ \frac{118\ E{-}15\ T4}{+}$	Female, straight wings
♀ $\frac{w}{w};\ \frac{CyO,Kr{-}GFP,w^{+}}{+};\ +;\ \frac{118\ E{-}15\ T4}{+}$	Female, curly wings
♂ $\frac{w}{>};\ \frac{\text{chd1}^{5}\ \text{b c sp}}{+};\ +;\ \frac{118\ E{-}15\ T4}{+}$	Male, straight wings
♂ $\frac{w}{>};\ \frac{CyO,Kr{-}GFP,w^{+}}{+};\ +;\ \frac{118\ E{-}15\ T4}{+}$	Male, curly wings

$$♀\ \frac{w}{w};\ \frac{chd1^5\ \text{b c sp}}{CyO,Kr-GFP,w^+};+;+\quad\times\quad♂\ \frac{w}{>};+;+;\frac{118\,E-10\,C4}{118\,E-10\,T4}$$

↓

Expected Progeny Genotype	Expected Phenotype
$♀\ \frac{w}{w};\frac{\text{chd1}^5\ \text{b c sp}}{+};+;\frac{118\,E-10\,C4}{+}$	Female, straight wings
$♀\ \frac{w}{w};\frac{CyO,Kr-GFP,w^+}{+};+;\frac{118\,E-10\,C4}{+}$	Female, curly wings
$♂\ \frac{w}{>};\frac{\text{chd1}^5\ \text{b c sp}}{+};+;\frac{118\,E-10\,C4}{+}$	Male, straight wings
$♂\ \frac{w}{>};\frac{CyO,Kr-GFP,w^+}{+};+;\frac{118\,E-10\,C4}{+}$	Male, curly wings

$$♀\ \frac{w}{w};\ \frac{chd1^5\ \text{b c sp}}{CyO,Kr-GFP,w^+};+;+\quad\times\quad♂\ \frac{w}{>};\frac{39\,C-4\,oxy\,c22}{39\,C-4\,oxy\,c22};+;+$$

↓

Expected Progeny Genotype	Expected Phenotype
$♀\ \frac{w}{w};\frac{\text{chd1}^5\ \text{b c sp}}{39\,C-4\,oxy\,c22};+;+$	Female, straight wings
$♀\ \frac{w}{w};\frac{CyO,Kr-GFP,w^+}{39\,C-4\,oxy\,c22};+;+$	Female, curly wings
$♂\ \frac{w}{>};\frac{\text{chd1}^5\ \text{b c sp}}{39\,C-4\,oxy\,c22};+;+$	Male, straight wings
$♂\ \frac{w}{>};\frac{CyO,Kr-GFP,w^+}{+39\,C-4\,oxy\,c22};+;+$	Male, curly wings

$$♀\ \frac{w}{w};\ \frac{chd1^5\ \text{b c sp}}{CyO,Kr-GFP,w^+};+;+\quad\times\quad♂\ \frac{w}{>};+;+;\frac{39\,C-12\ \ 1020}{39\,C-12\ 1020}$$

↓

Expected Progeny Genotype	Expected Phenotype
$♀\ \frac{w}{w};\frac{\text{chd1}^5\ \text{b c sp}}{+};+;\frac{39\,C-12\ \ 1020}{+}$	Female, straight wings
$♀\ \frac{w}{w};\frac{CyO,Kr-GFP,w^+}{+};+;\frac{39\,C-12\ \ 1020}{+}$	Female, curly wings
$♂\ \frac{w}{>};\frac{\text{chd1}^5\ \text{b c sp}}{+};+;\frac{39\,C-12\ \ 1020}{+}$	Male, straight wings
$♂\ \frac{w}{>};\frac{CyO,Kr-GFP,w^+}{+};+;\frac{39\,C-12\ \ 1020}{+}$	Male, curly wings

Negative control crosses

$$♀\ \frac{w}{w};\ \frac{al\ b\ c\ sp}{al\ b\ c\ sp};+;+\quad\times\quad♂\ \frac{w}{>};+;+;\frac{118\,E-15\,T4}{118\,E-15\,T4}$$

↓

Expected Progeny Genotype	Expected Phenotype
$♀\ \frac{w}{w};\frac{al\ b\ c\ sp}{+};+;\frac{118\,E-15\,T4}{+}$	Female
$♂\ \frac{w}{>};\frac{al\ b\ c\ sp}{+};+;\frac{118\,E-15\,T4}{+}$	Male

$$♀\ \frac{w}{w};\ \frac{al\ b\ c\ sp}{al\ b\ c\ sp};+;+\quad\times\quad♂\ \frac{w}{>};+;+;\frac{118\,E-10\,C4}{118\,E-10\,C4}$$

↓

Expected Progeny Genotype	Expected Phenotype
$♀\ \frac{w}{w};\frac{al\ b\ c\ sp}{+};+;\frac{118\,E-10\,C4}{+}$	Female
$♂\ \frac{w}{>};\frac{al\ b\ c\ sp}{+};+;\frac{118\,E-10\,C4}{+}$	Male

$$♀\ \frac{w}{w};\ \frac{al\ b\ c\ sp}{al\ b\ c\ sp};+;+\quad\times\quad♂\ \frac{w}{>};+;+;\frac{39\ C-12\ 1020}{39\ C-12\ 1020}$$

↓

Expected Progeny Genotype	Expected Phenotype
$♀\ \frac{w}{w};\frac{al\ b\ c\ sp}{+};+;\frac{39\ C-12\ 1020}{+}$	Female
$♂\ \frac{w}{>};\frac{al\ b\ c\ sp}{+};+;\frac{39\ C-12\ 1020}{+}$	Male

$$♀\ \frac{w}{w};\ \frac{al\ b\ c\ sp}{al\ b\ c\ sp};+;+\quad\times\quad♂\ \frac{w}{>};\frac{39\ C-4\ oxy\ c\ 22}{39\ C-4\ oxy\ c22};+;+$$

↓

Expected Progeny Genotype	Expected Phenotype
$♀\ \frac{w}{w};\frac{al\ b\ c\ sp}{39\ C-4\ oxy\ c\ 22};+;+$	Female
$♂\ \frac{w}{>};\frac{al\ b\ c\ sp}{39\ C-4\ oxy\ c\ 22};+;+$	Male

Negative control crosses

$$♀\ \frac{yw}{yw};+;+;+\quad\times\quad♂\ \frac{w}{>};+;+;\frac{118\ E-15\ T4}{118\ E-15\ T4}$$

↓

Expected Progeny Genotype	Expected Phenotype
$♀\ \frac{yw}{w};+;+;\frac{118\ E-15\ T4}{+}$	Female
$♂\ \frac{yw}{>};+;+;\frac{118\ E-15\ T4}{+}$	Male

$$♀ \frac{yw}{yw};\ +;\ +;\ +\quad \times \quad ♂ \frac{w}{>}\ ;\ +;\ +;\ \frac{39\,C{-}12\ 1020}{39\,C{-}12\ 1020}$$

↓

Expected Progeny Genotype	Expected Phenotype
♀ $\frac{yw}{w}$; +; + ; $\frac{39\,C{-}12\ 1020}{+}$	Female
♂ $\frac{yw}{>}$;+; + ; $\frac{39\,C{-}12\ 1020}{+}$	Male

$$♀ \frac{yw}{yw};\ +;\ +;\ +\quad \times \quad ♂ \frac{w}{>}\ ;\frac{39\,C{-}4\ oxy\ c22}{39\,C{-}4\ oxy\ c22};\ +;\ +$$

↓

Expected Progeny Genotype	Expected Phenotype
♀ $\frac{yw}{w}$; +; + ; $\frac{39\,C{-}4\ oxy\ c22}{+}$	Female
♂ $\frac{yw}{>}$;+; + ; $\frac{39\,C{-}4\ oxy\ c22}{+}$	Male

"Four" generation of crosses to obtain progenies with homozygous *chd1* mutants and a PEV affected gene. Labeled as four generations to keep track, but in reality "F3" has to be repeated multiple times to have enough virgin progeny to start F4.

F1 ♀ $\frac{w^{1118}}{w^{1118}}$; $\frac{Dp(3;3)Nap1^{KO1}Nap1^{KO1.5\prime}Nap1^{KO1.3\prime}}{T(2;3)SM6b-TM6B,ap^{RK}Tb^{1}}$; +; + × ♂ $\frac{y^{1}w^{*}}{>}$; +; +; $\frac{bip2^{4}}{\ln(4)ci^{D},ci^{D}pan^{ciD}}$

↓

Expected Progeny Genotype	Expected Phenotype
♂ $\frac{w^{1118}}{>}$; $\frac{+}{T(2;3)SM6b-TM6B,ap^{RK}Tb^{1}}$; +; $\frac{\ln(4)ci^{D},ci^{D}pan^{ciD}}{+}$	Male, tubby body, unorganized eye pattern, missing end part of third vein
♂ $\frac{w^{1118}}{>}$; $\frac{+}{T(2;3)SM6b-TM6B,ap^{RK}Tb^{1}}$; +; $\frac{bip2^{4}}{+}$	Male, tubby body, unorganized eyes
♂ $\frac{w^{1118}}{>}$; $\frac{+}{\mathrm{Dp}(3;3)Nap1^{KO1}Nap1^{KO1.5\prime}Nap1^{KO1.3\prime}}$; +; $\frac{\ln(4)ci^{D},ci^{D}pan^{ciD}}{+}$	Male, normal size body, missing end part of third vein
♂ $\frac{w^{1118}}{>}$; ; $\frac{+}{\mathrm{Dp}(3;3)Nap1^{KO1}Nap1^{KO1.5\prime}Nap1^{KO1.3\prime}}$; +; $\frac{bip2^{4}}{+}$	Male, normal size body
♀ $\frac{w^{1118}}{y^{1}w^{*}}$; $\frac{+}{T(2;3)SM6b-TM6B,ap^{RK}Tb^{1}}$; +; $\frac{\ln(4)ci^{D},ci^{D}pan^{ciD}}{+}$	Female, tubby body, unorganized eyes, missing end part of third vein
♀ $\frac{w^{1118}}{y^{1}w^{*}}$; $\frac{+}{T(2;3)SM6b-TM6B,ap^{RK}Tb^{1}}$; +; $\frac{bip2^{4}}{+}$	Female, tubby body, unorganized eyes
♀ $\frac{w^{1118}}{y^{1}w^{*}}$; $\frac{+}{\mathrm{Dp}(3;3)Nap1^{KO1}Nap1^{KO1.5\prime}Nap1^{KO1.3\prime}}$; +; $\frac{\ln(4)ci^{D},ci^{D}pan^{ciD}}{+}$	Female, normal size body, unorganized eyes, missing end part of third vein
♀ $\frac{w^{1118}}{y^{1}w^{*}}$; $\frac{+}{\mathrm{Dp}(3;3)Nap1^{KO1}Nap1^{KO1.5\prime}Nap1^{KO1.3\prime}}$; +; $\frac{bip2^{4}}{+}$	Female, normal size body

F2 ♀ $\frac{w}{w}$; $\frac{chd1^5 b\ c\ sp}{CyO,Kr-GFP,w^+}$; +; + × ♂ $\frac{w}{>}$; $\frac{+}{T(2;3)SM6b-TM6B,ap^{RK}Tb^1}$; +; $\frac{\ln(4)ci^D,ci^D pan^{ciD}}{+}$

↓

Expected Progeny Genotype	Expected Phenotype
♀ $\frac{w}{w}$; $\frac{chd1^5\ b\ c\ sp,+}{T(2;3)SM6b-TM6B,ap^{RK}Tb^1}$; +; $\frac{+}{\ln(4)ci^D,ci^D pan^{ciD}}$	Female, tubby body, missing end of third vein, white eye, disorganized eye pattern
♀ $\frac{w}{w}$; $\frac{chd1^5\ b\ c\ sp,+}{+}$; +; $\frac{+}{\ln(4)ci^D,ci^D pan^{ciD}}$	Female, white eye, straight winged, missing end part of third vein
♀ $\frac{w}{w}$; $\frac{chd1^5\ b\ c\ sp,+}{(2;3)SM6b-TM6B,ap^{RK}Tb^1}$; +;+	Female, white eye, tubby body, curly wings, unorganized eye pattern
♀ $\frac{w}{w}$; $\frac{chd1^5\ b\ c\ sp,+}{+}$; +; +	Female, white eye, straight wings
♀ $\frac{w}{w}$; $\frac{C□O,Kr-GFP,w^+}{T(2;3)SM6b-TM6B,ap^{RK}Tb^1}$; +; $\frac{+}{\ln(4)ci^D,ci^D pan^{ciD}}$	Female, curly wings, red eyes, tubby body, unorganized eye pattern, missing end of third vein, disorganized pattern of eye
♀ $\frac{□}{w}$; $\frac{CyO,Kr-GFP,w^+}{+}$; +; $\frac{+}{\ln(4)ci^D,ci^D pan^{ciD}}$	Female, red eye, curly wings, missing end part of third vein
♀ $\frac{w}{w}$; $\frac{CyO,Kr-GFP,w^+}{(2;3)SM6b-TM6B,ap^{RK}Tb^1}$; +;+	Female, red eye, tubby body, curly wings, unorganized eye pattern
♀ $\frac{w}{w}$; $\frac{CyO,Kr-GFP,w^+}{+}$; +; +	Female, red eye, curly wings
♂ $\frac{w}{>}$; $\frac{chd1^5\ b\ c\ sp,+}{T(2;3)SM6b-TM6B,ap^{RK}Tb^1}$; +; $\frac{+}{\ln(4)ci^D,ci^D pan^{ciD}}$	Male, tubby body, missing end of third vein, white eye, disorganized eye pattern
♂ $\frac{w}{>}$; $\frac{chd1^5\ b\ c\ sp,+}{+}$; +; $\frac{+}{\ln(4)ci^D,ci^D pan^{ciD}}$	Male, white eye, straight winged, missing end part of third vein

$\male \frac{w}{>}; \frac{chd1^5\ b\ c\ sp,+}{(2;3)SM6b-TM6B,ap^{RK}Tb^1}; +; +$	Male, white eye, tubby body, curly wings, unorganized eye pattern
$\male \frac{w}{>}; \frac{chd1^5\ b\ c\ sp,+}{+}; +; +$	Male, white eye, straight wings
$\male \frac{w}{>}; \frac{CyO,Kr-GFP,w^+}{T(2;3)SM6b-TM6B,ap^{RK}Tb^1}; +; \frac{+}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Male, curly wings, red eyes, tubby body, unorganized eye pattern, missing end of third vein, disorganized pattern of eye
$\male \frac{w}{>}; \frac{CyO,Kr-GFP,w^+}{+}; +; \frac{+}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Male, red eye, curly wings, missing end part of third vein
$\male \frac{w}{>}; \frac{CyO,Kr-GFP,w^+}{(2;3)SM6b-TM6B,ap^{RK}Tb^1}; +; +$	Male, red eye, tubby body, curly wings, unorganized eye pattern
$\male \frac{w}{>}; \frac{CyO,Kr-GFP,w^+}{+}; +; +$	Male, red eye, curly wings

This first part of the third generation of crosses seeks to breed a semi-stable stock. Each generation, the progeny with all expected phenotype characteristics must be selected to breed the next generation. Half of the progeny loses the dominant *ci* marker every generation so selective breeding occurs to produce more desired flies for more "F3" stock and F4 crosses.

F3♀ $\frac{w}{w}; \frac{chd1^5\ b\ c\ sp,+}{T(2;3)SM6b-TM6B,ap^{RK}Tb^1}; +; \frac{+}{\ln(4)ci^{\square},ci^D pan^{ciD}}$ × ♂ $\frac{w}{>}; \frac{chd1^5\ b\ c\ sp,+}{T(2;3)SM6b-TM6B,ap^{RK}Tb^1}; +; \frac{+}{\ln(4)ci^D,ci^D pan^{ciD}}$

↓

Expected Progeny Genotype	Expected Phenotype
♀ $\frac{w}{w}; \frac{chd1^5\ b\ c\ sp,+}{T(2;3)SM6b-TM6B,ap^{RK}Tb^1}; +; \frac{+}{\ln(4)ci^D,ci^D pan^{ciD}}$	Female, white eye, tubby body, unorganized eye pattern, missing end part of third vein
♀ $\frac{w}{w}; \frac{chd1^5\ b\ c\ sp,+}{T(2;3)SM6b-TM6B,ap^{RK}Tb^1}; +; +$	Female, full veins
♂ $\frac{w}{>}; \frac{chd1^5\ b\ c\ sp,+}{T(2;3)SM6b-TM6B,ap^{RK}Tb^1}; +; \frac{+}{\ln(4)ci^D,ci^D pan^{ciD}}$	Male, white eye, tubby body, unorganized eye pattern, missing end part of third vein
♂ $\frac{w}{>}; \frac{chd1^5\ b\ c\ sp,+}{T(2;3)SM6b-TM6B,ap^{RK}Tb^1}; +; +$	Male, full veins

F3♀ $\frac{w}{w}; \frac{chd1^5\ b\ c\ sp,+}{T(2;3)SM6b-TM6B,ap^{RK}Tb^1}; +; \frac{+}{\ln(4)ci^D,ci^D pan^{ciD}}$ × ♂ $\frac{w}{>}; +; +; \frac{39\ C-12\ 1020}{39\ C-12\ 1020}$

↓

Expected Progeny Genotype	Expected Phenotype
♀ $\frac{w}{w}; \frac{chd1^5\ b\ c\ sp,+}{+}; +; \frac{39\ C-12\ 1020}{\ln(4)ci^D,ci^D pan^{ciD}}$	Female, white eyes, missing last part of third vein
♀ $\frac{w}{w}; \frac{chd1^5\ b\ c\ sp,+}{+}; +; \frac{39\ C-12\ 1020}{+}$	Female, white eyes
♀ $\frac{w}{w}; \frac{T(2;3)SM6b-TM6B,ap^{RK}Tb^1}{+}; +; \frac{39\ C-12\ 1020}{+}$	Female, white eyes, tubby body, unorganized eye pattern
♀ $\frac{w}{w}; \frac{T(2;3)SM6b-TM6B,ap^{RK}Tb^1}{+}; +; \frac{39\ C-12\ 1020}{\ln(4)ci^D,ci^D pan^{ciD}}$	Female, white eyes, missing last part of third vein, tubby body, unorganized eye pattern
♂ $\frac{w}{>}; \frac{chd1^5\ b\ c\ sp,+}{+}; +; \frac{+39\ C-12\ 1020}{\ln(4)ci^D,ci^D pan^{ciD}}$	Male, white eyes, missing last part of third vein

♂ $\frac{w}{>}; \frac{chd1^5\ b\ c\ sp,+}{+}; +; \frac{+}{39\ C{-}2\ 1020}$	Male, white eyes, tubby body, unorganized eye pattern
♂ $\frac{w}{>}; \frac{+}{T(2;3)SM6b{-}TM6B,ap^{RK}Tb^1}; +; \frac{39\ C{-}12\ 1020}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Male, white eyes, missing last part of third vein, tubby body, unorganized eye pattern
♂ $\frac{w}{>}; \frac{+}{T(2;3)SM6b{-}TM6B,ap^{RK}Tb^1}; +; \frac{+}{39\ C{-}12\ 1020}$	Male, white eyes, tubby body, unorganized eye pattern

The fourth generation of crosses consists of crossing desired *chd1* mutant flies from F3 with the three PEV lines.

F4 to obtain homozygous *chd1* mutant with one mutant copy of *39 C-12 1020*

♀ $\frac{w}{w}; \frac{chd1^5\ b\ c\ sp,+}{T(2;3)SM6b{-}TM6B,ap^{RK}Tb^1}; +; \frac{+}{\ln(4)ci^D,ci^Dpan^{ciD}}$ × ♂ $\frac{w}{>}; \frac{chd1^5 b\ c\ sp}{+}; +; \frac{39\ C{-}12\ 1020}{\ln(4)ci^D,ci^Dpan^{ciD}}$

↓

Expected Progeny Genotype	Expected Phenotype
♀ $\frac{w}{w}; \frac{chd1^5\ b\ c\ sp,+}{chd1^5\ b\ c\ sp,++}; +; \frac{39\ C{-}12\ 1020}{+}$	Female, black body, curved wings, full veins
♀ $\frac{w}{w}; \frac{chd1^5\ b\ c\ sp,+}{chd1^5\ b\ c\ sp,++}; +; \frac{+}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Female, missing end part of third vein, curved wings, black body, straight wings
♀ $\frac{w}{w}; \frac{chd1^5\ b\ c\ sp,+}{chd1^5\ b\ c\ sp,++}; +; \frac{\ln(4)ci^D,ci^Dpan^{ciD}}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Female, lethal
♀ $\frac{w}{w}; \frac{chd1^5\ b\ c\ sp,+}{+}; +; \frac{39\ C{-}12\ 1020}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Female, straight wings, red eyes, missing end part of third vein
♀ $\frac{w}{w}; \frac{chd1^5\ b\ c\ sp,+}{+}; +; \frac{39\ C{-}12\ 1020}{+}$	Female, straight wings, full veins
♀ $\frac{w}{w}; \frac{chd1^5\ b\ c\ sp,+}{+}; +; \frac{\ln(4)ci^D,ci^Dpan^{ciD}}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Female, lethal
♀ $\frac{w}{w}; \frac{chd1^5\ b\ c\ sp,+}{T(2;3)SM6b{-}TM6B,ap^{RK}Tb^1}; +; \frac{39\ C{-}12\ 1020}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Female, tubby body, unorganized eye pattern, missing end part of third vein, curly wings

♀ $\frac{w}{w}$; $\frac{chd1^5\ b\ c\ sp,+}{T(2;3)SM6b-TM6B,ap^{RK}Tb^1}$; +; $\frac{+}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Female, tubby body, missing end part of third vein, tubby body, unorganized eye patter
♀ $\frac{w}{w}$; $\frac{chd1^5\ b\ c\ sp,+}{T(2;3)SM6b-TM6B,ap^{RK}Tb^1}$; +; $\frac{\ln(4)ci^D,ci^Dpan^{ciD}}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Female, lethal
♀ $\frac{w}{w}$; $\frac{T(2;3)SM6b-TM6B,ap^{RK}Tb^1}{+}$; +; $\frac{39\ C-12\ 1020}{+}$	Female, tubby body, unorganized eye pattern, full veins
♀ $\frac{w}{w}$; $\frac{T(2;3)SM6b-TM6B,ap^{RK}Tb^1}{+}$; +; $\frac{39\ C-12\ 1020}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Female, tubby body, unorganized eye pattern, curly wings, missing end part of third vein
♀ $\frac{w}{w}$; $\frac{T(2;3)SM6b-TM6B,ap^{RK}Tb^1}{+}$; +; $\frac{\ln(4)ci^D,ci^Dpan^{ciD}}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Female, lethal
♂ $\frac{w}{>}$; $\frac{chd1^5\ b\ c\ sp,+}{chd1^5\ b\ c\ sp,++}$; +; $\frac{39\ C-12\ 1020}{+}$	Male, black body, curved wings, full veins
♂ $\frac{w}{>}$; $\frac{chd1^5\ b\ c\ sp,+}{chd1^5\ b\ c\ sp,++}$; +; $\frac{+}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Male, missing last part of third vein, black body, curved wings
♂ $\frac{w}{>}$; $\frac{chd1^5\ b\ c\ sp,+}{chd1^5\ b\ c\ sp,++}$; +; $\frac{\ln(4)ci^D,ci^Dpan^{ciD}}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Male, lethal
♂ $\frac{w}{>}$; $\frac{chd1^5\ b\ c\ sp,+}{+}$; +; $\frac{39\ C-12\ 1020}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Male, missing end part of third vein, straight wings
♂ $\frac{w}{>}$; $\frac{chd1^5\ b\ c\ sp,+}{+}$; +; $\frac{39\ C-12\ 1020}{+}$	Male, no black body, no curved wings, full veins
♂ $\frac{w}{>}$; $\frac{chd1^5\ b\ c\ sp,+}{+}$; +; $\frac{\ln(4)ci^D,ci^Dpan^{ciD}}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Male, lethal
♂ $\frac{w}{>}$; $\frac{T(2;3)SM6b-TM6B,ap^{RK}Tb^1}{+}$; +; $\frac{39\ C-12\ 1020}{+}$	Male, tubby body, unorganized eye pattern, straight wings
♂ $\frac{w}{>}$; $\frac{T(2;3)SM6b-TM6B,ap^{RK}Tb^1}{+}$; +; $\frac{39\ C-12\ 1020}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Male, red eyes, tubby body, unorganized eye pattern curly wings, missing end part of third vein
♂ $\frac{w}{>}$; $\frac{T(2;3)SM6b-TM6B,ap^{RK}Tb^1}{+}$; +; $\frac{\ln(4)ci^D,ci^Dpan^{ciD}}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Male, lethal
♂ $\frac{w}{>}$; $\frac{chd1^5\ b\ c\ sp,+}{T(2;3)SM6b-TM6B,ap^{RK}Tb^1}$; +; $\frac{39\ C-12\ 1020}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Male, tubby body, unorganized eye pattern, curly wings, missing last part of third vein

♂ $\frac{w}{>}; \frac{chd1^5\ b\ c\ sp,+}{T(2;3)SM6b-TM6B,ap^{RK}Tb^1}; +; \frac{+}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Male, missing end part of third vein, tubby body, unorganized eye pattern, curly wings
♂ $\frac{w}{>}; \frac{chd1^5\ b\ c\ sp,+}{T(2;3)SM6b-TM6B,ap^{RK}Tb^1}; +; \frac{\ln(4)ci^D,ci^Dpan^{ciD}}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Male, lethal

F4 to obtain homozygous *chd1* mutant with one copy of mutant *118 E-15 T4*

♀ $\frac{w}{w}; \frac{chd1^5\ b\ c\ sp,+}{T(2;3)SM6b-TM6B,ap^{RK}Tb^1}; +; \frac{+}{\ln(4)ci^D,ci^Dpan^{ciD}}$ × ♂ $\frac{w}{>}; \frac{chd1^5 b\ c\ sp}{CyO,Kr-GFP,w^+}; +; \frac{118\ E-15\ T4}{\ln(4)ci^D,ci^Dpan^{ciD}}$

↓

Expected Progeny Genotype	Expected Phenotype
♀ $\frac{w}{w}; \frac{chd1^5\ b\ c\ sp,+}{chd1^5\ b\ c\ sp,++}; +; \frac{3118\ E-15\ T4}{+}$	Female, black body, curved wings, full veins
♀ $\frac{w}{w}; \frac{chd1^5\ b\ c\ sp,+}{chd1^5\ b\ c\ sp,++}; +; \frac{+}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Female, missing end part of third vein, curved wings, black body, straight wings
♀ $\frac{w}{w}; \frac{chd1^5\ b\ c\ sp,+}{chd1^5\ b\ c\ sp,++}; +; \frac{\ln(4)ci^D,ci^Dpan^{ciD}}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Female, lethal
♀ $\frac{w}{w}; \frac{chd1^5\ b\ c\ sp,+}{+}; +; \frac{118\ E-15\ T4}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Female, straight wings, red eyes, missing end part of third vein
♀ $\frac{w}{w}; \frac{chd1^5\ b\ c\ sp,+}{+}; +; \frac{118\ E-15\ T4}{+}$	Female, straight wings, full veins
♀ $\frac{w}{w}; \frac{chd1^5\ b\ c\ sp,+}{+}; +; \frac{\ln(4)ci^D,ci^Dpan^{ciD}}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Female, lethal
♀ $\frac{w}{w}; \frac{chd1^5\ b\ c\ sp,+}{T(2;3)SM6b-TM6B,ap^{RK}Tb^1}; +; \frac{118\ E-15\ T4}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Female, tubby body, unorganized eye pattern, missing end part of third vein, curly wings

♀ $\frac{w}{w}$; $\frac{chd1^5\ b\ c\ sp,+}{T(2;3)SM6b-TM6B,ap^{RK}Tb^1}$; + ; $\frac{+}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Female, tubby body, missing end part of third vein, tubby body, unorganized eye patter
♀ $\frac{w}{w}$; $\frac{chd1^5\ b\ c\ sp,+}{T(2;3)SM6b-TM6B,ap^{RK}Tb^1}$; + ; $\frac{\ln(4)ci^D,ci^Dpan^{ciD}}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Female, lethal
♀ $\frac{w}{w}$; $\frac{T(2;3)SM6b-TM6B,ap^{RK}Tb^1}{+}$; + ; $\frac{118\ E-15\ T4}{+}$	Female, tubby body, unorganized eye pattern, full veins
♀ $\frac{w}{w}$; $\frac{T(2;3)SM6b-TM6B,ap^{RK}Tb^1}{+}$; + ; $\frac{118\ E-15\ T4}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Female, tubby body, unorganized eye pattern, curly wings, missing end part of third vein
♀ $\frac{w}{w}$; $\frac{T(2;3)SM6b-TM6B,ap^{RK}Tb^1}{+}$; + ; $\frac{\ln(4)ci^D,ci^Dpan^{ciD}}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Female, lethal
♂ $\frac{w}{>}$; $\frac{chd1^5\ b\ c\ sp,+}{chd1^5\ b\ c\ sp,++}$; + ; $\frac{118\ E-15\ T4}{+}$	Male, black body, curved wings, full veins
♂ $\frac{w}{>}$; $\frac{chd1^5\ b\ c\ sp,+}{chd1^5\ b\ c\ sp,++}$; + ; $\frac{+}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Male, missing last part of third vein, black body, curved wings
♂ $\frac{w}{>}$; $\frac{chd1^5\ b\ c\ sp,+}{chd1^5\ b\ c\ sp,++}$; + ; $\frac{\ln(4)ci^D,ci^Dpan^{ciD}}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Male, lethal
♂ $\frac{w}{>}$; $\frac{chd1^5\ b\ c\ sp,+}{+}$; + ; $\frac{118\ E-15\ T4}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Male, missing end part of third vein, straight wings
♂ $\frac{w}{>}$; $\frac{chd1^5\ b\ c\ sp,+}{+}$; + ; $\frac{118\ E-15\ T4}{+}$	Male, no black body, no curved wings, full veins
♂ $\frac{w}{>}$; $\frac{chd1^5\ b\ c\ sp,+}{+}$; + ; $\frac{\ln(4)ci^D,ci^Dpan^{ciD}}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Male, lethal
♂ $\frac{w}{>}$; $\frac{T(2;3)SM6b-TM6B,ap^{RK}Tb^1}{+}$; + ; $\frac{118\ E-15\ T4}{+}$	Male, tubby body, unorganized eye pattern, straight wings
♂ $\frac{w}{>}$; $\frac{T(2;3)SM6b-TM6B,ap^{RK}Tb^1}{+}$; + ; $\frac{118\ E-15\ T4}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Male, red eyes, tubby body, unorganized eye pattern curly wings, missing end part of third vein
♂ $\frac{w}{>}$; $\frac{T(2;3)SM6b-TM6B,ap^{RK}Tb^1}{+}$; + ; $\frac{\ln(4)ci^D,ci^Dpan^{ciD}}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Male, lethal
♂ $\frac{w}{>}$; $\frac{chd1^5\ b\ c\ sp,+}{T(2;3)SM6b-TM6B,ap^{RK}Tb^1}$; + ; $\frac{118\ E-15\ T4}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Male, tubby body, unorganized eye pattern, curly wings, missing last part of third vein

♂ $\frac{w}{>}; \frac{chd1^5\ b\ c\ sp,+}{T(2;3)SM6b-TM6B,ap^{RK}Tb^1}; +; \frac{+}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Male, missing end part of third vein, tubby body, unorganized eye pattern, curly wings
♂ $\frac{w}{>}; \frac{chd1^5\ b\ c\ sp,+}{T(2;3)SM6b-TM6B,ap^{RK}Tb^1}; +; \frac{\ln(4)ci^D,ci^Dpan^{ciD}}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Male, lethal

F4 to obtain homozygous mutant *chd1* with one copy of mutant *118 E-10 C4*

♀ $\frac{w}{w}; \frac{chd1^5\ b\ c\ sp,+}{T(2;3)SM6b-TM6B,ap^{RK}Tb^1}; +; \frac{+}{\ln(4)ci^D,ci^Dpan^{ciD}}$ × ♂ $\frac{w}{>}; \frac{chd1^5 b\ c\ sp}{CyO,Kr-GFP,w^+}; +; \frac{118\ E-10\ C4}{\ln(4)ci^D,ci^Dpan^{ciD}}$

↓

Expected Progeny Genotype	Expected Phenotype
♀ $\frac{w}{w}; \frac{chd1^5\ b\ c\ sp,+}{chd1^5\ b\ c\ sp,++}; +; \frac{118\ E-10\ C4}{+}$	Female, black body, curved wings, full veins
♀ $\frac{w}{w}; \frac{chd1^5\ b\ c\ sp,+}{chd1^5\ b\ c\ sp,++}; +; \frac{+}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Female, missing end part of third vein, curved wings, black body, straight wings
♀ $\frac{w}{w}; \frac{chd1^5\ b\ c\ sp,+}{chd1^5\ b\ c\ sp,++}; +; \frac{\ln(4)ci^D,ci^Dpan^{ciD}}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Female, lethal
♀ $\frac{w}{w}; \frac{chd1^5\ b\ c\ sp,+}{+}; +; \frac{118\ E-10\ C4}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Female, straight wings, red eyes, missing end part of third vein
♀ $\frac{w}{w}; \frac{chd1^5\ b\ c\ sp,+}{+}; +; \frac{118\ E-10\ C4}{+}$	Female, straight wings, full veins
♀ $\frac{w}{w}; \frac{chd1^5\ b\ c\ sp,+}{+}; +; \frac{\ln(4)ci^D,ci^Dpan^{ciD}}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Female, lethal
♀ $\frac{w}{w}; \frac{chd1^5\ b\ c\ sp,+}{T(2;3)SM6b-TM6B,ap^{RK}Tb^1}; +; \frac{118\ E-10\ C4}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Female, tubby body, unorganized eye pattern, missing end part of third vein, curly wings
♀ $\frac{w}{w}; \frac{chd1^5\ b\ c\ sp,+}{T(2;3)SM6b-TM6B,ap^{RK}Tb^1}; +; \frac{+}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Female, tubby body,missing end part of third vein, tubby body, unorganized eye patter
♀ $\frac{w}{w}; \frac{chd1^5\ b\ c\ sp,+}{T(2;3)SM6b-TM6B,ap^{RK}Tb^1}; +; \frac{\ln(4)ci^D,ci^Dpan^{ciD}}{\ln(4)ci^D,ci^Dpan^{ciD}}$	Female, lethal

♀ $\frac{w}{w}; \frac{T(2;3)SM6b-TM6B,ap^{RK}Tb^{1}}{+}; +; \frac{118\ E-10\ C4}{+}$	Female, tubby body, unorganized eye pattern, full veins
♀ $\frac{w}{w}; \frac{T(2;3)SM6b-TM6B,ap^{RK}Tb^{1}}{+}; +; \frac{118\ E-10\ C4}{\ln(4)ci^{D},ci^{D}pan^{ciD}}$	Female, tubby body, unorganized eye pattern, curly wings, missing end part of third vein
♀ $\frac{w}{w}; \frac{T(2;3)SM6b-TM6B,ap^{RK}Tb^{1}}{+}; +; \frac{\ln(4)ci^{D},ci^{D}pan^{ciD}}{\ln(4)ci^{D},ci^{D}pan^{ciD}}$	Female, lethal
♂ $\frac{w}{>}; \frac{chd1^{5}\ b\ c\ sp,+}{chd1^{5}\ b\ c\ sp,++}; +; \frac{118\ E-10\ C4}{+}$	Male, black body, curved wings, full veins
♂ $\frac{w}{>}; \frac{chd1^{5}\ b\ c\ sp,+}{chd1^{5}\ b\ c\ sp,++}; +; \frac{+}{\ln(4)ci^{D},ci^{D}pan^{ciD}}$	Male, missing last part of third vein, black body, curved wings
♂ $\frac{w}{>}; \frac{chd1^{5}\ b\ c\ sp,+}{chd1^{5}\ b\ c\ sp,++}; +; \frac{\ln(4)ci^{D},ci^{D}pan^{ciD}}{\ln(4)ci^{D},ci^{D}pan^{ciD}}$	Male, lethal
♂ $\frac{w}{>}; \frac{chd1^{5}\ b\ c\ sp,+}{+}; +; \frac{118\ E-10\ C4}{\ln(4)ci^{D},ci^{D}pan^{ciD}}$	Male, missing end part of third vein, straight wings
♂ $\frac{w}{>}; \frac{chd1^{5}\ b\ c\ sp,+}{+}; +; \frac{118\ E-10\ C4}{+}$	Male, no black body, no curved wings, full veins
♂ $\frac{w}{>}; \frac{chd1^{5}\ b\ c\ sp,+}{+}; +; \frac{\ln(4)ci^{D},ci^{D}pan^{ciD}}{\ln(4)ci^{D},ci^{D}pan^{ciD}}$	Male, lethal
♂ $\frac{w}{>}; \frac{T(2;3)SM6b-TM6B,ap^{RK}Tb^{1}}{+}; +; \frac{118\ E-10\ C4}{+}$	Male, tubby body, unorganized eye pattern, straight wings
♂ $\frac{w}{>}; \frac{T(2;3)SM6b-TM6B,ap^{RK}Tb^{1}}{+}; +; \frac{118\ E-10\ C4}{\ln(4)ci^{D},ci^{D}pan^{ciD}}$	Male, red eyes, tubby body, unorganized eye pattern curly wings, missing end part of third vein
♂ $\frac{w}{>}; \frac{T(2;3)SM6b-TM6B,ap^{RK}Tb^{1}}{+}; +; \frac{\ln(4)ci^{D},ci^{D}pan^{ciD}}{\ln(4)ci^{D},ci^{D}pan^{ciD}}$	Male, lethal
♂ $\frac{w}{>}; \frac{chd1^{5}\ b\ c\ sp,+}{T(2;3)SM6b-TM6B,ap^{RK}Tb^{1}}; +; \frac{118\ E-10\ C4}{\ln(4)ci^{D},ci^{D}pan^{ciD}}$	Male, tubby body, unorganized eye pattern, curly wings, missing last part of third vein
♂ $\frac{w}{>}; \frac{chd1^{5}\ b\ c\ sp,+}{T(2;3)SM6b-TM6B,ap^{RK}Tb^{1}}; +; \frac{+}{\ln(4)ci^{D},ci^{D}pan^{ciD}}$	Male, missing end part of third vein, tubby body, unorganized eye pattern, curly wings
♂ $\frac{w}{>}; \frac{chd1^{5}\ b\ c\ sp,+}{T(2;3)SM6b-TM6B,ap^{RK}Tb^{1}}; +; \frac{\ln(4)ci^{D},ci^{D}pan^{ciD}}{\ln(4)ci^{D},ci^{D}pan^{ciD}}$	Male, lethal

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