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JIL-1 and *Su(var)3-7* Interact Genetically and Counteract Each Other's Effect on Position-Effect Variegation in Drosophila

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ABSTRACT

The essential JIL-1 histone H3S10 kinase is a key regulator of chromatin structure that functions to maintain euchromatic domains while counteracting heterochromatization and gene silencing. In the absence of the JIL-1 kinase, two of the major heterochromatin markers H3K9me2 and HP1a spread in tandem to ectopic locations on the chromosome arms. Here we address the role of the third major heterochromatin component, the zinc-finger protein Su(var)3-7. We show that the lethality but not the chromosome morphology defects associated with the null *JIL-1* phenotype to a large degree can be rescued by reducing the dose of the *Su(var)3-7* gene and that *Su(var)3-7* and *JIL-1* loss-of-function mutations have an antagonistic and counterbalancing effect on position-effect variegation (PEV). Furthermore, we show that in the absence of JIL-1 kinase activity, Su(var)3-7 gets redistributed and upregulated on the chromosome arms. Reducing the dose of the *Su(var)3-7* gene dramatically decreases this redistribution; however, the spreading of H3K9me2 to the chromosome arms was unaffected, strongly indicating that ectopic Su(var)3-9 activity is not a direct cause of lethality. These observations suggest a model where Su(var)3-7 functions as an effector downstream of Su(var)3-9 and H3K9 dimethylation in heterochromatic spreading and gene silencing that is normally counteracted by JIL-1 kinase activity.

SU(VAR)3-9, a histone methyltransferase, Su(var)2-5, HP1a, and Su(var)3-7, a 1250-residue zinc-finger protein are all inherent components of pericentric heterochromatin (Rea *et al.* 2000; Eissenberg and Elgin 2000; Schotta *et al.* 2002; Delattre *et al.* 2004; Ebert *et al.* 2004) and are important factors for silencing of reporter genes by heterochromatic spreading in Drosophila (for review see Weiler and Wakimoto 1995; Girton and Johansen 2008). Su(var)3-9 has been shown to catalyze most of the dimethylation of the histone H3K9 residue which in turn can promote HP1a and Su(var)3-7 recruitment (Schotta *et al.* 2002; Jaquet *et al.* 2006). In addition, Su(var)3-9, HP1a, and Su(var)3-7 can directly interact with each other, suggesting a model where interdependent interactions between Su(var)3-9, HP1a, and Su(var)3-7 lead to heterochromatin assembly at pericentric sites (Lachner *et al.* 2001; Schotta *et al.* 2002; Elgin and Grewal 2003; Jaquet *et al.* 2006). Heterochromatin formation in Drosophila is initiated early in development through active removal of H3K4 methylation by the LSD1 demethylase homolog Su(var)3-3 (Rudolph *et al.* 2007). Subsequently, a developmentally regulated balance between Su(var)3-3 H3K4 demethylase, Su(var)3-9 H3K9 methyltransferase, and RPD3 H3K9 deacetylase activity contribute to conserve the distinction between euchromatic and heterochromatic domains (Rudolph *et al.* 2007). Thus, highly complex interactions between multiple heterochromatic and euchromatic factors are likely to contribute to the regulation of a dynamic balance between the distinct chromatin environments promoting gene activity and gene silencing.

It has recently been demonstrated that activity of the essential JIL-1 histone H3S10 kinase (Jin *et al.* 1999; Wang *et al.* 2001) is a major regulator of chromatin structure (Deng *et al.* 2005; 2008) and that it functions to maintain euchromatic domains while counteracting heterochromatization and gene silencing (Ebert *et al.* 2004; Zhang *et al.* 2006; Lerach *et al.* 2006; Bao *et al.* 2007). In the absence of the JIL-1 kinase, the major heterochromatin markers H3K9me2 and HP1a spread in tandem to ectopic locations on the chromosome arms with the most pronounced increase on the X chromosomes (Zhang *et al.* 2006; Deng *et al.* 2007). However, overall levels of the H3K9me2 mark and HP1a were unchanged, suggesting that the spreading was accompanied by a redistribution that reduces the levels in pericentromeric heterochromatin. Genetic interaction assays demonstrated that the lethality as well as

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some of the chromosome morphology defects associated with the null *JIL-1* phenotype to a large degree can be rescued by reducing the dose of the *Su(var)3-9* gene (Zhang *et al.* 2006; Deng *et al.* 2007). This is in contrast to similar experiments performed with alleles of the *Su(var)2-5* gene where no genetic interactions were detectable between *JIL-1* and *Su(var)2-5* (Deng *et al.* 2007) Thus, these findings indicate that while Su(var)3-9 histone methyltransferase activity may be a factor in the lethality and chromatin structure perturbations associated with loss of the JIL-1 histone H3S10 kinase, these effects are likely to be uncoupled from HP1a. However, the potential role of the third major heterochromatin component, Su(var)3-7, was not addressed in these studies. Here we show that *Su(var)3-7*, like *Su(var)3-9*, genetically interacts with *JIL-1*, that reducing the dose of *Su(var)3-7* significantly reduces the lethality of *JIL-1* null mutants, and that *Su(var)3-7* and *JIL-1* loss-of-function mutations have an antagonistic and counterbalacing effect on position-effect variegation (PEV).

MATERIALS AND METHODS

***Drosophila melanogaster* stocks and PEV assays:** Fly stocks were maintained according to standard protocols (Roberts 1998). Canton S was used for wild-type preparations. The $JIL\text{-}1^{z28}$, $JIL\text{-}1^{z60}$, and $JIL\text{-}1^{z2}$ alleles are described in Wang *et al.* (2001) and in Zhang *et al.* (2003). The $Su(var)3\text{-}7^{7.1A}$, $Su(var)3\text{-}7^{14}$, and $Su(var)3\text{-}7^{R2a8}$ alleles are described in Seum *et al.* (2002) and in Spierer *et al.* (2005). The $Su(var)3\text{-}9^{01}$ and $Su(var)3\text{-}9^{02}$ stocks were obtained from the Umeå Stock Center. The *hsp83* promoter-driven *JIL-1-GFP* transgene *GF29.1* is described in Jin *et al.* (1999) and in Wang *et al.* (2001) and the *hsp70* promoter-driven *JIL-1-V5* transgene *JIL-1-FL* is described in Bao *et al.* (2008). The *hsp83* and *hsp70* promoters are leaky and promote expression at or above wild-type levels under non-heat-shock conditions (Wang *et al.* 2001; Bao *et al.* 2008). Recombinant $JIL\text{-}1^{z2}$ $Su(var)3\text{-}7^{7.1A}$, $JIL\text{-}1^{z2}$ $Su(var)3\text{-}7^{14}$, $JIL\text{-}1^{z2}$ $Su(var)3\text{-}7^{R2a8}$, $JIL\text{-}1^{z60}$ $Su(var)3\text{-}7^{R2a8}$, and $JIL\text{-}1^{z2}$ *Hsp70-Gal4* chromosomes were generated as described in Ji *et al.* (2005) except that the *Su(var)3-7* alleles were identified by a *yellow* reporter gene and the presence of $JIL\text{-}1^{z2}$ or $JIL\text{-}1^{z60}$ was confirmed by PCR as in Zhang *et al.* (2003). The $In(1)w^{m4}$ and *DX1* alleles were obtained from the Bloomington Stock Center and the *P*-element insertion line *118E-15* was the generous gift of L. Wallrath. Balancer chromosomes and markers are described in Lindsley and Zimm (1992).

PEV assays were performed as previously described in Lerach *et al.* (2006) and in Bao *et al.* (2007). In short, various combinations of *JIL-1*, *Su(var)3-7*, or *JIL-1 Su(var)3-7* recombinant alleles were introduced into each of the three PEV arrangements by standard crossing. To quantify the variegated phenotype, newly eclosed adults were collected, aged for 5 days at 25°, and were then sorted into different classes on the basis of the percentage of the eye that was red. Eyes from representative individuals from these crosses were photographed using an Olympus stereo microscope and a Spot digital camera (Diagnostic Instruments).

Immunohistochemistry: Polytene chromosome squash preparations were performed as in Kelley *et al.* (1999) using the 1-min or 5-min fixation protocol and labeled with antibody as described in Johansen *et al.* (2009). The preparations were labeled with H3K9me2 pAb (Upstate Biotechnology) or with Su(var)3-7 pAb (Cleard *et al.* 1997) and DNA was visualized by staining with Hoechst 33258 (Molecular Probes) in PBS. The appropriate Texas Red-, TRITC-, or FITC-conjugated secondary antibodies (Cappel/ICN, Southern Biotech) were used (1:200 dilution) to visualize primary antibody labeling. The final preparations were mounted in 90% glycerol containing 0.5% *n*-propyl gallate. The preparations were examined using epifluorescence optics on a Zeiss Axioskop microscope and images were captured and digitized using a high-resolution Spot CCD camera. Images were imported into Photoshop where they were pseudocolored, image processed, and merged. In some images nonlinear adjustments were made to the channel with Hoechst labeling for optimal visualization of chromosomes.

RESULTS

Viability and chromosome morphology in *JIL-1* and *Su(var)3-7* double mutants: The seven-zinc-finger protein Su(var)3-7 is a major heterochromatic factor that interacts and cooperates with both Su(var)3-9 and HP1a at pericentric heterochromatic regions (Jaquet *et al.* 2006). To determine whether *Su(var)3-7*, like *Su(var)3-9*, genetically interacts with *JIL-1* in the same pathway *in vivo*, we explored interactions between mutant alleles of *Su(var)3-7* and *JIL-1* by generating double mutant individuals. Since *Su(var)3-7* and *JIL-1* both are located on the third chromosome, we first recombined the $Su(var)3\text{-}7^{14}$, $Su(var)3\text{-}7^{7.1A}$, and $Su(var)3\text{-}7^{R2a8}$ alleles onto the $JIL\text{-}1^{z2}$ chromosome. $JIL\text{-}1^{z2}$ is a null allele generated by *P*-element mobilization (Wang *et al.* 2001; Zhang *et al.* 2003), whereas the $Su(var)3\text{-}7^{14}$, $Su(var)3\text{-}7^{7.1A}$, and $Su(var)3\text{-}7^{R2a8}$ alleles were isolated by homologous recombination (Seum *et al.* 2002; Spierer *et al.* 2005). The $Su(var)3\text{-}7^{14}$ and $Su(var)3\text{-}7^{R2a8}$ alleles behave genetically as null mutations, whereas the $Su(var)3\text{-}7^{7.1A}$ allele is a strong hypomorph (Seum *et al.* 2002; Spierer *et al.* 2005). Due to maternal effects, the homozygous *Su(var)3-7* mutant flies from heterozygous parents are viable and fertile. However, in the second generation all the homozygous progeny of homozygous females die during second instar larval stages (Seum *et al.* 2002; H. Deng, unpublished observations). To determine whether a reduction of Su(var)3-7 levels can rescue the lethality normally associated with a null $JIL\text{-}1^{z2}/JIL\text{-}1^{z2}$ mutant background, we crossed $JIL\text{-}1^{z2}$ $Su(var)3\text{-}7^{7.1A}$/*TM6 Sb Tb* males, $JIL\text{-}1^{z2}$ $Su(var)3\text{-}7^{14}$/*TM6 Sb Tb* males, or $JIL\text{-}1^{z2}$ $Su(var)3\text{-}7^{R2a8}$/*TM6 Sb Tb* males with $JIL\text{-}1^{z2}$/*TM6 Sb Tb* virgin females generating $JIL\text{-}1^{z2}$ $Su(var)3\text{-}7^{7.1A}/JIL\text{-}1^{z2}$, $JIL\text{-}1^{z2}$ $Su(var)3\text{-}7^{14}/JIL\text{-}1^{z2}$, or $JIL\text{-}1^{z2}$ $Su(var)3\text{-}7^{R2a8}/JIL\text{-}1^{z2}$ animals identified as non-*Sb* (Table 1). In control experiments in which $JIL\text{-}1^{z2}$/*TM6 Sb Tb* males were crossed with $JIL\text{-}1^{z2}$/*TM6 Sb Tb* virgin females generating $JIL\text{-}1^{z2}/JIL\text{-}1^{z2}$ progeny, no flies of the $JIL\text{-}1^{z2}/JIL\text{-}1^{z2}$ genotype were observed out of a total of 596 eclosed flies, indicating complete lethality (Table 1). However, introduction of one copy of either of the *Su(var)3-7* mutant alleles dramatically increased the number of surviving flies with the $JIL\text{-}1^{z2}/JIL\text{-}1^{z2}$ genotype. In these

TABLE 1

Genetic interaction between *JIL-1* and *Su(var)3-7* alleles

Cross	Genotypes (no. of adult flies)		% of expected ratio[a]
JIL-1^{z2}/TM6 × *JIL-1^{z2}/TM6*	*JIL-1^{z2}/TM6 596*	*JIL-1^{z2}/JIL-1^{z2} 0*	0.0
JIL-1^{z2}/TM6 × *JIL-1^{z2} Su(var)3-7$^{7.1A}$/TM6*	*JIL-1^{z2}/TM6* or *JIL-1^{z2} Su(var)3-7$^{7.1A}$/TM6 531*	*JIL-1^{z2}/JIL-1^{z2} Su(var)3-7$^{7.1A}$ 201*	82.5
JIL-1^{z2}/TM6 × *JIL-1^{z2} Su(var)3-7^{14}/TM6*	*JIL-1^{z2}/TM6* or *JIL-1^{z2} Su(var)3-7^{14}/TM 478*	*JIL-1^{z2}/JIL-1^{z2} Su(var)3-7^{14} 185*	83.7
JIL-1^{z2}/TM6 × *JIL-1^{z2} Su(var)3-7^{R2a8}/TM6*	*JIL-1^{z2}/TM6* or *JIL-1^{z2} Su(var)3-7^{R2a8}/TM6 359*	*JIL-1^{z2}/JIL-1^{z2} Su(var)3-7^{R2a8} 97*	63.8

[a] In these crosses, the *TM6* chromosome was identified by the *Stubble* marker. Consequently, the experimental genotypes could be distinguished from balanced heterozygotic flies by the absence of the *Stubble* marker. The expected Mendelian ratio of non-*Stubble* to *Stubble* flies was 1:2 since *TM6/TM6* is embryonic lethal. The percentage of expected genotypic ratios was calculated as: observed non-*Stubble* flies × 300/total observed flies.

crosses one-third of the eclosed flies would be expected to be of the *JIL-1^{z2}/JIL-1^{z2} Su(var)3-7* genotype assuming full rescue. Therefore, the reduction of Su(var)3-7 levels in these animals resulted in a >60% viability rate compared to a rate of 0% for *JIL-1^{z2}/JIL-1^{z2}* flies without the reduction in Su(var)3-7 levels (Table 1). Similar results were obtained with crosses using the *JIL-1^{z60}* and *Su(var)3-7^{R2a8}* alleles (supporting information, Table S1). Both males and females were rescued but the rate of rescue was higher for females than for males (Table S2). Interestingly, while at least some of the rescued males were fertile, all of the rescued females tested were sterile. In crosses generating double homozygous *JIL-1^{z2} Su(var)3-7$^{7.1A}$/JIL-1^{z2} Su(var)3-7$^{7.1A}$* and *JIL-1^{z2} Su(var)3-7^{14}/JIL-1^{z2} Su(var)3-7^{14}*, rescue of viability was still observed but at a greatly reduced rate of only ~16% (Table S3). To ensure that the *JIL-1^{z2}* chromosome did not have a second site lethal, we performed rescue experiments with the *hsp83* promoter-driven full-length *JIL-1-GFP* transgene, *GF29.1* at 25° (Jin *et al.* 1999; Wang *et al.* 2001). In crosses generating double homozygous *GF29.1/GF29.1; JIL-1^{z2}/JIL-1^{z2}* flies, viability was restored to 65% (Table S4), strongly indicating that the complete lethality of *JIL-1^{z2}/JIL-1^{z2}* flies (Table 1) is not due to a second site lethal. These results were confirmed in crosses with the *hsp70* promoter-driven full-length *JIL-1-V5* transgene, *JIL-1-FL* (Bao *et al.* 2008). In these crosses, viability was restored to 54.8% at 25° (Table S5) and to 94.2% at 21° (Table S6). Thus, these results suggest that the lethality in null *JIL-1* mutant backgrounds to a substantial degree is dependent on the dose of *Su(var)3-7*. Furthermore, since this effect was observed with three different alleles of *Su(var)3-7* it is likely to be specific to *Su(var)3-7* and not to second site modifiers.

It has previously been demonstrated that a reduction in the levels of the heterochromatin factor Su(var)3-9 to a large degree can rescue the severely perturbed polytene chromosome morphology observed in null *JIL-1^{z2}* homozygous larvae (Zhang *et al.* 2006; Deng *et al.* 2007). We therefore investigated whether a reduction in the dose of *Su(var)3-7* would have a similar effect. For this analysis we prepared squashes of polytene chromosomes labeled with Hoechst from *JIL-1^{z2}* homozygous null and wild-type third instar larvae and compared them with squashes from double mutant homozygous *JIL-1^{z2}* larvae with either the *Su(var)3-7^{14}* or the *Su(var)3-7$^{7.1A}$* allele. As illustrated in Figure 1, loss of JIL-1 histone H3S10 kinase activity leads to misalignment of the interband chromatin fibrils, coiling of the chromosomes, and an increase of ectopic contacts between nonhomologous regions. This results in a shortening and folding of the chromosomes with a nonorderly intermixing of euchromatin and the compacted chromatin characteristic of banded regions (Deng *et al.* 2005). The extreme of this phenotype is exhibited by the male X polytene chromosome where no remnants of coherent banded regions can be observed (Figure 1). However, we found that in homozygous *JIL-1^{z2}* double mutant combinations with a reduced dosage of Su(var)3-7 there was little or no improvement in polytene chromosome morphology including that of the male X chromosome (Figure 1).

Since Zhang *et al.* (2006) and Deng *et al.* (2007) have shown that ectopic Su(var)3-9 histone methyltransferase activity may be a major factor in causing the lethality and chromatin structure perturbations associated with the loss of JIL-1 H3S10 kinase activity, we explored whether a reduction in the dose of *Su(var)3-7* affected the distribution of the H3K9me2 mark in *JIL-1* null mutants. Polytene squashes from third instar larval salivary glands from *JIL-1* and *Su(var)3-7* double mutant combinations were double labeled with Hoechst and an antibody to histone H3K9me2 and compared to wild-type and *JIL-1^{z2}/+* heterozygous preparations (Figure 2). In *JIL-1* null animals histone H3K9 dimethylation is dramatically upregulated on all the chromosome arms; however, the upregulation is most pronounced on the X

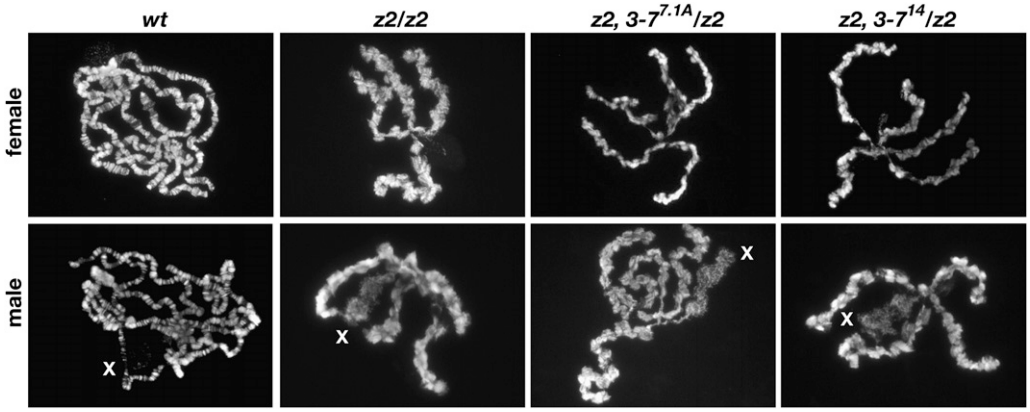

FIGURE 1.—Morphology of polytene chromosomes in *JIL-1* and *Su(var)3-7* double mutant backgrounds. Polytene chromosome preparations from third instar male and female larvae were labeled with Hoechst to visualize the chromatin. Note the misalignment and intermixing of interband and banded regions and the extensive coiling and folding of the chromosome arms in *JIL-1^{z2}/JIL-1^{z2}* (*z2/z2*) mutant chromosomes as compared to wild type (wt). The male X chromosome (X) was particularly affected and no remnants of banded regions were discernible. In *JIL-1* and *Su(var)3-7* double mutant backgrounds from male and female *JIL-1^{z2} Su(var)3-7$^{7.1A}$/JIL-1^{z2}* (*z2, 3-7$^{7.1A}$/z2*) and *JIL-1^{z2} Su(var)3-7^{14}/JIL-1^{z2}* (*z2, 3-7^{14}/z2*) larvae, the polytene chromosome morphology was indistiguishable from that of *JIL-1^{z2}/JIL-1^{z2}* homozygous null mutants.

chromosome (ZHANG *et al.* 2006; DENG *et al.* 2007) (Figure 2). In *JIL-1^{z2}/+* heterozygous preparations both chromosome morphology and H3K9me2 distribution is indistinguishable from wild-type preparations (Figure 2). As further illustrated in Figure 2, a reduction in the dose of Su(var)3-7 affected neither chromosome morphology nor the ectopic spreading of H3K9 dimethylation in *JIL-1^{z2} Su(var)3-7$^{7.1A}$/JIL-1^{z2}*, *JIL-1^{z2} Su(var)3-7^{14}/JIL-1^{z2}*, *JIL-1^{z2} Su(var)3-7^{R2a8}/JIL-1^{z2}*, or *JIL-1^{z2} Su(var)3-7^{14}/JIL-1^{z2} Su(var)3-7^{14}* mutant larvae. Taken together these results suggest that *JIL-1* interacts with *Su(var)3-7* in a genetic pathway and that Su(var)3-7 contributes to the lethality but not the disruption of chromosome morphology observed in *JIL-1* loss-of-function mutants.

To determine whether the distribution of Su(var)3-7 was affected in *JIL-1* null mutants, polytene chromosomes from *JIL-1* and *Su(var)3-7* double mutant combinations were double labeled with Hoechst and an antibody to Su(var)3-7 (CLEARD *et al.* 1997) and compared to wild-type preparations (Figure 3). In wild-type polytene chromosomes, Su(var)3-7 is predominantly located to the chromocenter and the fourth chromosome (CLEARD *et al.* 1997); however, in the absence of JIL-1 the labeling of the chromosome arms is dramatically upregulated in conjunction with a reduced presence at the chromocenter (Figure 3). In contrast to the redistribution of HP1a and H3K9me2 where the upregulation is most pronounced on the X chromosome (ZHANG *et al.* 2006; DENG *et al.* 2007), we did not observe a difference in Su(var)3-7 levels between the X chromosome and the autosomes in the *JIL-1* null background (Figure 3). In *JIL-1^{z2} Su(var)3-7^{14}/JIL-1^{z2}* mutant larvae Su(var)3-7 labeling was substantially reduced at the chromocenter with very little Su(var)3-7 detectable on the chromosome arms (Figure 3). For comparison we also labeled polytene chromosomes heterozygous for the *Su(var)3-9* null alleles *Su(var)3-9^{01}* and *Su(var)3-9^{02}* (REUTER *et al.* 1986; TSCHIERSCH *et al.* 1994; EBERT *et al.* 2004) with Su(var)3-7 antibody. Figure 3 shows that the binding of Su(var)3-7 is greatly reduced at the chromocenter without any spreading to the chromosome arms (Figure 3). This reduced binding pattern in the *Su(var)3-9* null background is similar to that previous reported for the other major heterochromatin component HP1a (SCHOTTA *et al.* 2002). Taken together these results suggest that the distribution pattern of Su(var)3-7 is dependent on both Su(var)3-9 and JIL-1 levels and/or activity.

***JIL-1* and *Su(var)3-7* counteract each other's effect on PEV:** PEV in Drosophila occurs when euchromatic genes are transcriptionally silenced as a result of their placement in or near heterochromatin (reviewed in GIRTON and JOHANSEN 2008). Repression typically occurs in only a subset of cells and can be heritable, leading to mosaic patterns of gene expression. It has been demonstrated that loss-of-function *JIL-1* alleles can act as enhancers of PEV, resulting in increased silencing of gene expression (BAO *et al.* 2007), whereas loci for structural components of heterochromation such as *Su(var)3-9*, *Su(var)2-5*, and *Su(var)3-7* act as strong haplosuppressors (EISSENBERG *et al.* 1990; REUTER *et al.* 1990; TSCHIERSCH *et al.* 1994). This together with the finding that *JIL-1* and *Su(var)3-7* interact genetically suggest that *JIL-1* and *Su(var)3-7* may potentially have a counterbalancing effect on the regulation of PEV. To test this hypothesis we explored the effect of various combinations of loss-of-function alleles of *JIL-1* and *Su(var)3-7* on PEV caused by both *P*-element insertions of reporter genes (*118E-15* and *DX1*) as well as of a chromosome rearrangement (*w^{m4}*).

118E-15: Insertion of the *P* element (*P[hsp26-pt, hsp70-w]*) into euchromatic sites results in a uniform red eye phenotype, whereas insertion into a known heterochromatin region of the fourth chromosome (line *118E-15*) results in a variegating eye phenotype (Figure 4 and Table S7) (WALLRATH and ELGIN 1995; WALLRATH

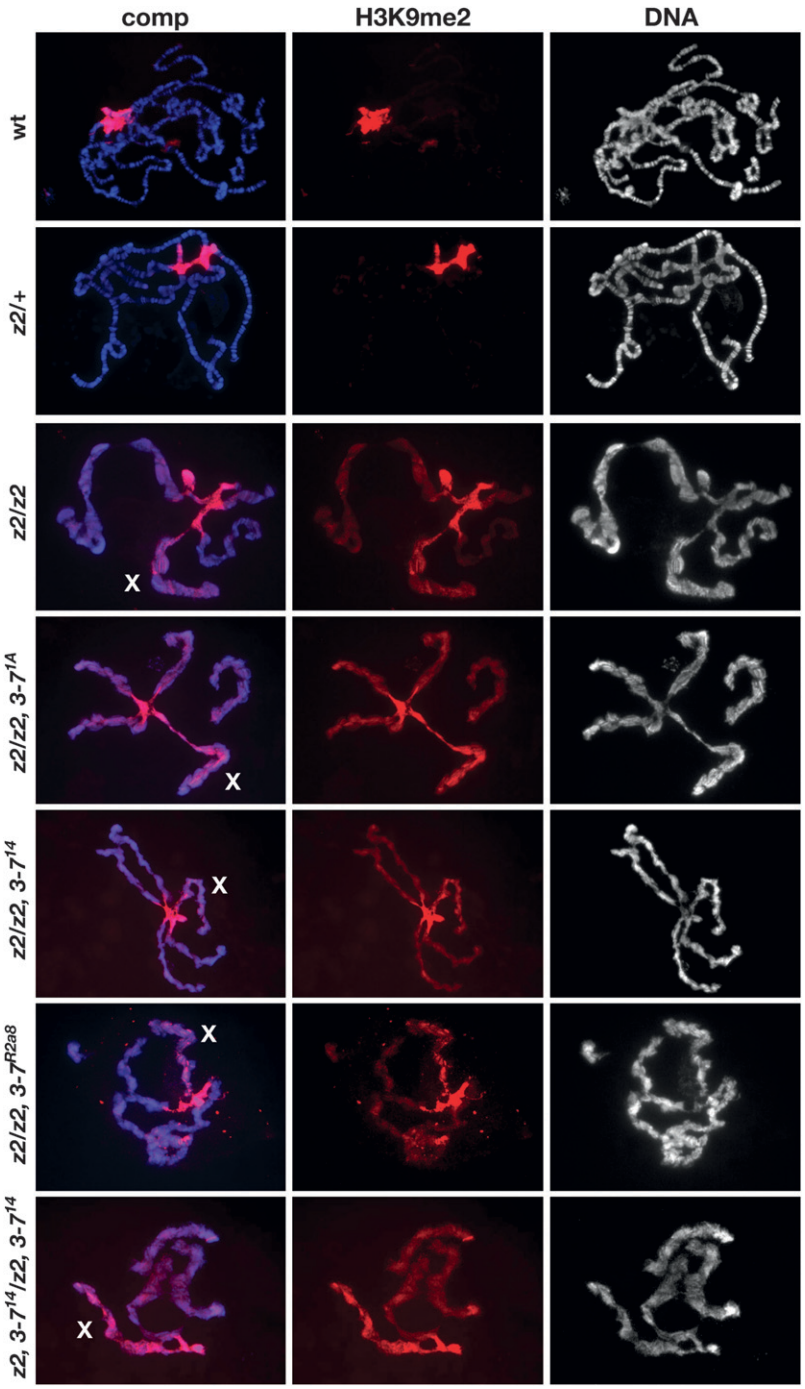

FIGURE 2.—Localization of H3K9me2 in polytene chromosomes from *JIL-1* and *Su(var)3-7* mutant female third instar larvae. The polytene squash preparations were labeled with antibody to H3K9me2 (in red) and with Hoechst (DNA, in blue/gray). The X chromosome is indicated by an X. Preparations from wild-type (wt), heterozygous *JIL-1^{z2}*/+ (*z2*/+), homozygous *JIL-1^{z2}*/*JIL-1^{z2}* (*z2*/*z2*), *JIL-1^{z2} Su(var)3-7$^{7.1A}$*/*JIL-1^{z2}* (*z2, 3-7$^{7.1A}$*/*z2*), *JIL-1^{z2} Su(var)3-7^{14}*/*JIL-1^{z2}* (*z2, 3-7^{14}*/*z2*), *JIL-1^{z2} Su(var)3-7^{R2a8}*/*JIL-1^{z2}* (*z2, 3-7^{R2a8}*/*z2*), and *JIL-1^{z2} Su(var)3-7^{14}*/*JIL-1^{z2} Su(var)3-7^{14}* (*z2, 3-7^{14}*/*z2, 3-7^{14}*) larvae are shown. In wild-type and *JIL-1^{z2}*/+ preparations, H3K9me2 labeling was mainly localized to and abundant at the chromocenter; however, in the absence of the JIL-1 kinase, the H3K9me2 labeling spread to the autosomes and particularly to the X chromosome (see also ZHANG *et al.* 2006; DENG *et al.* 2007). In *JIL-1^{z2} Su(var)3-7$^{7.1A}$*/*JIL-1^{z2}*, *JIL-1^{z2} Su(var)3-7^{14}*/*JIL-1^{z2}*, and *JIL-1^{z2} Su(var)3-7^{14}*/*JIL-1^{z2} Su(var)3-7^{14}* double mutant larvae, the H3K9me2 labeling was indistiguishable from that of *JIL-1^{z2}*/*JIL-1^{z2}* homozygous null mutants.

et al. 1996; CRYDERMAN *et al.* 1998). In the experiments, the transgenic reporter line was crossed into *JIL-1^{z60}*/*JIL-1^{z2}* and *Su(var)3-7$^{7.1A}$*/+ mutant backgrounds as well as into the *JIL-1^{z2} Su(var)3-7$^{7.1A}$*/*JIL-1^{z60}* double mutant background. The *JIL-1^{z60}* allele is a strong hypomorph producing only 0.3% of wild-type JIL-1 protein levels (WANG *et al.* 2001; ZHANG *et al.* 2003). The *JIL-1^{z2}*/*JIL-1^{z60}* heteroallelic combination is semilethal and only a limited number of eclosed animals from large-scale crosses could be analyzed (ZHANG *et al.* 2003). Flies from each of the different genotypes were scored for the percentage of the eye that had red ommatidia and compared to flies containing wild-type levels of the JIL-1 and Su(var)3-7 proteins (Figure 4 and Table S3). Although both male and female flies were scored, due to sex differences, only results from male flies are shown. However, the trend observed in female flies was identical to that in male flies. As illustrated in Figure 4 the hypomorphic allelic combination of the *JIL-1* alleles *JIL-1^{z60}* and *JIL-1^{z2}* leads to a strong enhancement of PEV as indicated by the nearly completely white eye phenotype, whereas in contrast, the heterozygous *Su(var)3-7$^{7.1A}$*/+ allele leads to strong suppression of PEV as indicated by the nearly completely red eyes. However, in the *JIL-1^{z2}*

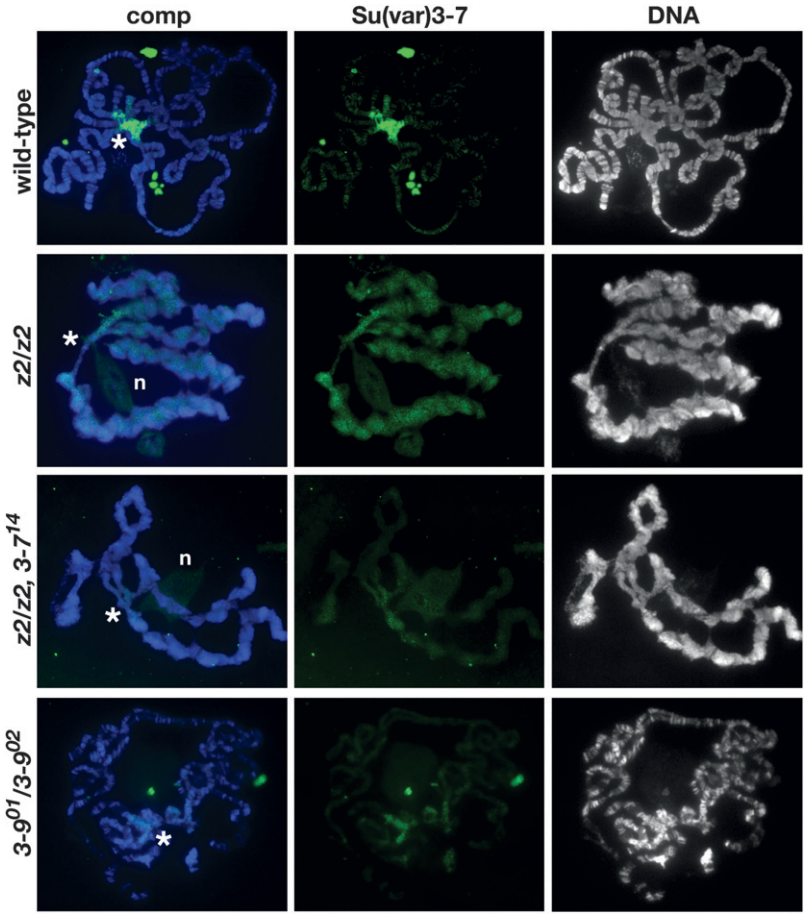

FIGURE 3.—Localization of Su(var)3-7 in polytene chromosomes from *JIL-1*, *Su(var)3-7*, and *Su(var)3-9* mutant female third instar larvae. The polytene squashes were labeled with antibody to Su(var)3-7 (in green) and with Hoechst (DNA, in blue/gray). The chromocenter is indicated with an asterisk and n indicates weak background labeling of the nucleolus in some of the preparations. Preparations from wild-type, *JIL-1^{z2}* homozygous (*z2/z2*), *JIL-1^{z2} Su(var)3-7^{14}/JIL-1^{z2}* (*z2, 3-7^{14}/z2*), and *Su(var)3-9^{01}/Su(var)3-9^{02}* (*3-9^{01}/3-9^{02}*) larvae are shown. In wild-type preparations, Su(var)3-7 labeling was mainly localized to and abundant at the chromocenter; however, in the absence of the JIL-1 kinase, the Su(var)3-7 labeling spread to the chromosome arms with a concomitant decrease at the chromocenter. In *JIL-1^{z2} Su(var)3-7^{14}/JIL-1^{z2}* and *Su(var)3-9^{01}/Su(var)3-9^{02}* mutant larvae Su(var)3-7 labeling was greatly reduced and mainly confined to the chromocenter.

Su(var)3-7$^{7.1A}$/JIL-1^{z60} double mutant background variegation of the proportion of red ommatitidia was substantially restored and closer to the distribution when wild-type levels of the JIL-1 and Su(var)3-7 proteins were present (Figure 4 and Table S7).

w^{m4}: The *In(1)w^{m4}* X chromosome contains an inversion that juxtaposes the euchromatic *white* gene and heterochromatic sequences adjacent to the centromere (MULLER 1930; SCHULTZ 1936). The resulting somatic variegation of *w^{m4}* expression occurs in clonal patches in the eye, reflecting heterochromatic spreading from the inversion breakpoint that silences *w^{m4}* expression in the white patches and euchromatic packaging of the *w* gene in those patches that appear red (reviewed in GREWAL and ELGIN 2002). Studies of this effect suggest that the degree of spreading may depend on the amount of heterochromatic factors at the breakpoint (reviewed in WEILER and WAKIMOTO 1995; GIRTON and JOHANSEN 2008). Interestingly, strong hypomorphic combinations of *JIL-1* alleles, in which heterochromatic factors spread to ectopic locations (ZHANG *et al.* 2006; DENG *et al.* 2007), act as suppressors not enhancers of PEV of the

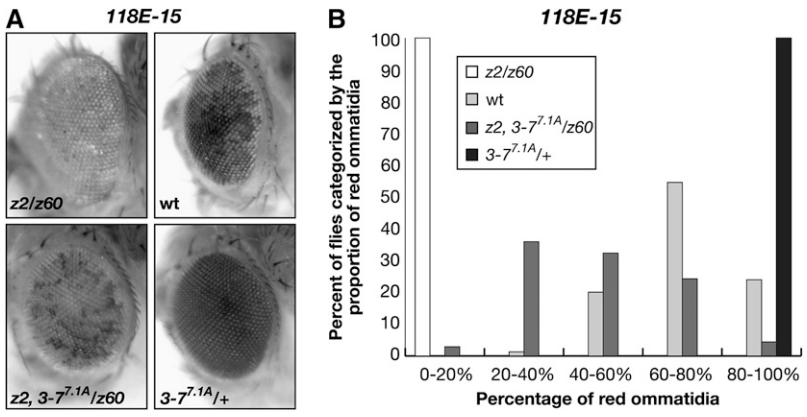

FIGURE 4.—Counterbalancing effect of *JIL-1* and *Su(var)3-7* loss-of-function alleles on PEV of the *P*-element insertion line *118E-15*. (A) Examples of the degree of PEV in the eyes of wild-type *JIL-1* and *Su(var)3-7* (wt), *JIL-1^{z60}/JIL-1^{z2}* (*z2/z60*), *Su(var)3-7$^{7.1A}$/+*, and *JIL-1^{z2} Su(var)3-7$^{7.1A}$/JIL-1^{z60}* (*z2, 3-7$^{7.1A}$/z60*) flies in a *118E-15* background. All images are from male flies. (B) Histograms of the distribution of the percentage of red ommatidia in wt, *JIL-1^{z60}/JIL-1^{z2}* (*z2/z60*), *Su(var)3-7$^{7.1A}$/+*, and *JIL-1^{z2} Su(var)3-7$^{7.1A}$/JIL-1^{z60}* (*z2, 3-7$^{7.1A}$/z60*) male flies homozygous for *118E-15*.

TABLE 2

***JIL-1* alleles act as haploenhancers of PEV of w^{m4}**

Genotype[a]	*n*	Percentage of flies categorized by the proportion of red ommatidia				
		0% red	0–25% red	25–75% red	75–99% red	100% red
+/+	274	0.0	23.0	59.0	19.0	0.0
zz28/+	182	8.8	64.3	26.9	0.0	0.0
z60/+	126	2.4	83.3	14.3	0.0	0.0
z2/+	162	21.0	65.4	13.6	0.0	0.0
z60/z2	19	0.0	0.0	0.0	0.0	100.0

[a] Genotype of the third chromosome. Only male flies hemizygous for w^{m4} on the X chromosome were tabulated.

w^{m4} allele (Lerach *et al.* 2006). On the basis of these findings, Lerach *et al.* (2006) proposed a model where the suppression of PEV of w^{m4} in strong *JIL-1* hypomorphic backgrounds is due to a reduction in the level of heterochromatic factors at the pericentromeric heterochromatin near the inversion breakpoint site that reduces its potential for heterochromatic spreading and silencing. However, as illustrated in Figure 2, in heterozygous preparations of the null $JIL\text{-}1^{z2}$ allele both chromosome morphology and H3K9me2 distribution are indistinguishable from wild-type preparations. Therefore, a prediction of the model of Lerach *et al.* (2006) is that *JIL-1* should have no effect or act as a haploenhancer of PEV at the w^{m4} allele. To test this hypothesis, the $In(1)w^{m4}$ chromosome was crossed into different heterozygous *JIL-1* mutant backgrounds of hypomorphic and null *JIL-1* alleles ($JIL\text{-}1^{z28}$, $JIL\text{-}1^{z60}$, and $JIL\text{-}1^{z2}$). The $JIL\text{-}1^{z28}$ allele is a weak hypomorph producing 45% the normal level of wild-type JIL-1 protein (Zhang *et al.* 2003). The strong hypomorphic $JIL\text{-}1^{z2}/JIL\text{-}1^{z60}$ heteroallelic combination was included for comparison. Male flies with the different genotypes were scored for the percentage of the eye that was red and variegated w^{m4}; +/+ flies containing wild-type levels of JIL-1 protein were used as controls (Table 2). As shown in Table 2, all three heterozygous *JIL-1* alleles reduced the proportion of red ommatidia as compared to +/+ flies, whereas the strong hypomorphic $JIL\text{-}1^{z2}/JIL\text{-}1^{z60}$ heteroallelic combination resulted in completely red eyes. Thus, these results strongly indicate that *JIL-1* acts as a haploenhancer of PEV of w^{m4} in male flies.

To test whether a heterozygous *JIL-1* allele could counterbalance the suppression of a *Su(var)3-7* hypomorphic allele of PEV of w^{m4}, we performed experiments similar to those described above for *118E-15*. In the experiments, the $In(1)w^{m4}$ chromosome was crossed into $JIL\text{-}1^{z2}$/+ and $Su(var)3\text{-}7^{7.1A}$/+ mutant backgrounds as well as into the $JIL\text{-}1^{z2}$ $Su(var)3\text{-}7^{7.1A}$/+ double mutant background. As illustrated in Figure 5, heterozygous $JIL\text{-}1^{z2}$/+ led to enhancement of PEV as indicated by the increased proportion of white ommatidia, whereas in contrast, the heterozygous $Su(var)3\text{-}7^{7.1A}$/+ allele led to suppression of PEV as indicated by an increase of the proportion of red ommatidia. However, in the $JIL\text{-}1^{z2}$ $Su(var)3\text{-}7^{7.1A}$/+ double mutant background, variegation of the proportion of red ommatitidia was intermediate and closer to the distribution when wild-type levels of the JIL-1 and Su(var)3-7 proteins were present (Figure 5 and Table S7). These results suggest that the haploenhancer effect of JIL-1 can counterbalance the haplosupressor effect of Su(var)3-7 on PEV of w^{m4}.

DX1: We also tested the counterbalancing effect of *JIL-1* and *Su(var)3-7* alleles on PEV of the *DX1* transgenic line. In the *DX1* line, seven tandem copies of the *p[lacW]* transgene, which contains a *mini-white* and a *lacZ* gene, were inserted into the 50C euchromatic region of the

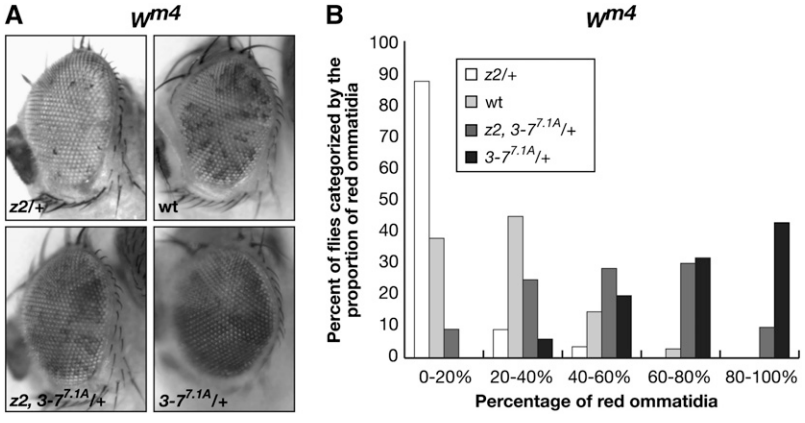

Figure 5.—Counterbalancing effect of *JIL-1* and *Su(var)3-7* loss-of-function alleles on PEV in the eyes of w^{m4} flies. (A) Examples of the degree of PEV in the eyes of wild-type *JIL-1* and *Su(var)3-7* (wt), $JIL\text{-}1^{z2}$/+ (*z2*/+), $Su(var)3\text{-}7^{7.1A}$/+, and $JIL\text{-}1^{z2}$ $Su(var)3\text{-}7^{7.1A}$/+ (*z2, 3-7*$^{7.1A}$/+) flies in a w^{m4} background. All images are from male flies. (B) Histograms of the distribution of the percentage of red ommatidia in wt, $JIL\text{-}1^{z2}$/+ (*z2*/+), $Su(var)3\text{-}7^{7.1A}$/+, and $JIL\text{-}1^{z2}$ $Su(var)3\text{-}7^{7.1A}$/+ (*z2, 3-7*$^{7.1A}$/+) male flies in a w^{m4} background.

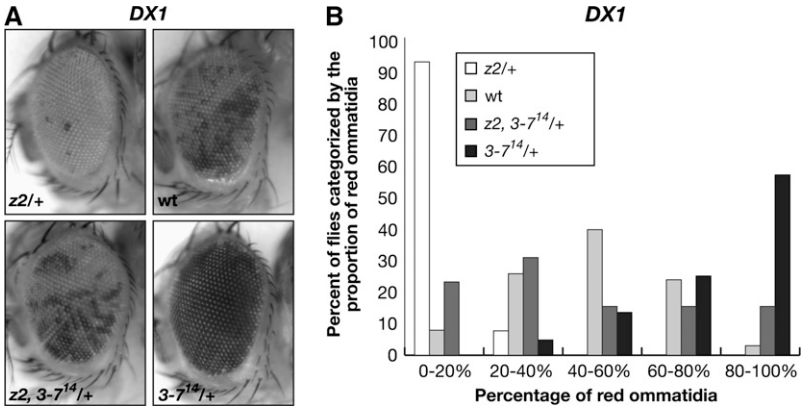

FIGURE 6.—Counterbalancing effect of *JIL-1* and *Su(var)3-7* loss-of-function alleles on PEV in the eyes of *DX1* flies. (A) Examples of the degree of PEV in the eyes of wild-type *JIL-1* and *Su(var)3-7* (wt), $JIL\text{-}1^{z2}/+$ (*z2/+*), $Su(var)3\text{-}7^{14}/+$, and $JIL\text{-}1^{z2}\ Su(var)3\text{-}7^{14}/+$ (*z2, 3-7*^{14}*/+*) flies in a *DX1* background. All images are from male flies. (B) Histograms of the distribution of the percentage of red ommatidia in wt, $JIL\text{-}1^{z2}/+$ (*z2/+*), $Su(var)3\text{-}7^{14}/+$, and $JIL\text{-}1^{z2}\ Su(var)3\text{-}7^{14}/+$ (*z2, 3-7*^{14}*/+*) male flies in a *DX1* background.

second chromosome (DORER and HENIKOFF 1994). The tandem sequence repetition induces heterochromatin formation resulting in partial silencing of the *mini-white* reporter and a variegated eye phenotype (DORER and HENIKOFF 1994). In the experiments, the *DX1* reporter line was crossed into $JIL\text{-}1^{z2}/+$ and $Su(var)3\text{-}7^{14}/+$ mutant backgrounds as well as into the $JIL\text{-}1^{z2}\ Su(var)3\text{-}7^{14}/+$ double mutant background. As illustrated in Figure 6, heterozygous $JIL\text{-}1^{z2}/+$ led to strong enhancement of PEV as indicated by the increased proportion of white ommatidia, whereas in contrast, the heterozygous $Su(var)3\text{-}7^{14}/+$ allele led to suppression of PEV as indicated by an increase of the proportion of red ommatidia. However, in the $JIL\text{-}1^{z2}\ Su(var)3\text{-}7^{14}/+$ double mutant background, variegation of the proportion of red ommatitidia was intermediate and closer to the distribution when wild-type levels of the JIL-1 and Su(var)3-7 proteins were present (Figure 6 and Table S7). These results suggest that the haploenhancer effect of *JIL-1* also can counterbalance the haplosupressor effect of *Su(var)3-7* on PEV of the *DX1* transgenic insertion line.

DISCUSSION

While Su(var)3-9, Su(var)3-7, and HP1a reciprocal interactions are well documented at pericentric regions (SCHOTTA *et al.* 2002; GREIL *et al.* 2003; DANZER and WALLRATH 2004) they are not universal. For example, HP1 binding on the fourth chromosome has been shown to be independent of Su(var)3-9 (SCHOTTA *et al.* 2002), and DANZER and WALLRATH (2004) using a tethering system to recruit HP1a to euchromatic sites have shown that HP1a-mediated silencing can operate in a Su(var)3-9-independent manner. Moreover, DENG *et al.* (2007) have provided evidence that at least two different molecular mechanisms regulate Su(var)3-9 localization, one dependent on HP1 and one dependent on the JIL-1 kinase. These findings indicate that although Su(var)3-9, Su(var)3-7, and HP1a cooperate in heterochromatin formation and gene silencing at pericentric chromosome sites, they may function independently at other regions such as the chromosome arms. In this study we show that the lethality but not the chromosome morphology defects associated with the null *JIL-1* phenotype to a large degree can be rescued by reducing the dose of the *Su(var)3-7* gene. This effect was observed with three different alleles of *Su(var)3-7*, strongly suggesting it is likely to be specific to *Su(var)3-7* and not to second site modifiers. Furthermore, we provide evidence that JIL-1 levels and/or activity regulate the chromosome localization of Su(var)3-7 and that Su(var)3-7 levels are dramatically redistributed to the chromosome arms in conjunction with a reduced presence at the chromocenter in the absence of JIL-1.

Previously, it has been demonstrated that *JIL-1* genetically interacts with *Su(var)3-9* but not with *Su(var)2-5*, suggesting that the lethality and disruption of chromosome morphology observed when JIL-1 levels are decreased are associated with ectopic Su(var)3-9 activity on the chromosomal arms and unrelated to HP1a recruitment (DENG *et al.* 2007). In this scenario, the spreading of the H3K9me2 mark to ectopic locations on the chromosomes is likely to lead to heterochromatization and repression of gene expression at these sites, leading to increased lethality (ZHANG *et al.* 2006; DENG *et al.* 2007). This hypothesis has been supported by genetic interaction assays that demonstrated that the lethality of *JIL-1* null mutants could be almost completely rescued by a reduction in *Su(var)3-9* dosage that prevented ectopic dimethylation of histone H3K9 (DENG *et al.* 2007). However, in this study we show that while reducing the dose of *Su(var)3-7* also rescues viability of *JIL-1* null mutant larvae, H3K9me2 in polytene squashes still spreads to the chromosome arms, strongly indicating that ectopic Su(var)3-9 activity is not a direct cause of lethality, but rather that Su(var)3-9-mediated recruitment of Su(var)3-7 is a necessary factor. Futhermore, since viability was rescued despite no obvious improvement in chromosome morphology, the lethality caused by loss of JIL-1 function is not likely to be a consequence of perturbed chromosome morphology. Taken together these observations give rise to a model where Su(var)3-7 functions as an effector downstream of Su(var)3-9 and

H3K9 dimethylation in heterochromatic spreading and gene silencing that is normally counteracted by JIL-1 kinase activity. How Su(var)3-7 may mediate these effects is unknown and will require additional studies.

The inherent components of heterochromatin Su(var)3-9, HP1a, and Su(var)3-7 display a haplosuppressor/triploenhancer dosage-dependent effect on PEV (Schotta *et al.* 2002). Additional copies of all three genes cause strong enhancement of *white* variegation in w^{m4}, and in genetic interaction tests, the suppressor effect of *Su(var)3-9* null mutations dominates the triplo-dependent enhancer effect of *Su(var)2-5)* and *Su(var)3-7* (Schotta *et al.* 2002). Furthermore, it has been recently demonstrated that the gain-of-function $JIL\text{-}1^{Su(var)3\text{-}1}$ allele is one of the strongest suppressors of PEV so far described at all the PEV arrangements that have been tested (Ebert *et al.* 2004; Lerach *et al.* 2006; Bao *et al.* 2007). This allele even counteracts gene repression that is caused by overexpression of the major determinants of heterochromatin formation, *e.g.*, *Su(var)3-9*, *Su(var)2-5*, and *Su(var)3-7* (Ebert *et al.* 2004). The $JIL\text{-}1^{Su(var)3\text{-}1}$ allele generates truncated proteins with COOH-terminal deletions that mislocalize to ectopic chromatin sites, leading to expanded histone H3S10 phosphorylation (Ebert *et al.* 2004; Zhang *et al.* 2006; Bao *et al.* 2008). On the basis of these findings, Ebert *et al.* (2004) proposed a model for a dynamic balance between euchromatin and heterochromatin, where as can be monitored in PEV arrangements, the boundary between these two states is determined by antagonistic functions of euchromatic regulators (JIL-1) and the determinants of heterochromatin assembly. In this study we have further tested this hypothesis using *JIL-1* loss-of-function alleles which we show can act as haploenhancers of PEV. This included PEV of the w^{m4} allele where, interestingly, combinations of strong hypomorphic *JIL-1* alleles act as suppressors, not enhancers. Lerach *et al.* (2006) have proposed that this is due to a reduction in the levels of heterochromatic factors near the inversion breakpoint that reduces its potential for heterochromatic spreading and silencing (reviewed in Girton and Johansen 2008). As predicted by this hypothesis we show that in chromosome squash preparations from $JIL^{z2}/+$ larvae there was no discernible redistribution of the H3K9me2 heterochromatic mark. We further demonstrate that *JIL-1* and *Su(var)3-7* alleles can counteract each other's effect on PEV. In all three PEV arrangements tested, *Su(var)3-7* loss-of-function alleles acted as strong haplosuppressors as indicated by a high proportion of nearly completely red eyes, whereas *JIL-1* loss-of-function alleles acted as strong haploenhancers as indicated by a high proportion of flies with nearly completely white ommatidia. However, in double mutant backgrounds, variegation of the proportion of red ommatidia was substantively restored and closer to the distribution when wild-type levels of JIL-1 and Su(var)3-7 proteins were present. These results strongly support a genetic interaction between *JIL-1* and *Su(var)3-7* and provide evidence that a finely tuned balance between the levels of JIL-1 and Su(var)3-7 contributes to the regulation of PEV.

While several potential mechanisms for heterochromatin spreading and gene silencing have been identified (reviewed in Girton and Johansen 2008), the concept of a dynamic balance between euchromatin and heterochromatin implies that euchromatic factors may have similar spreading potential. However, the mechanisms that actively may lead to the expansion of euchromatic domains have received comparatively less attention. In Drosophila, the studies of the JIL-1 kinase have demonstrated that histone H3S10 phosphorylation is an important epigenetic modification potentially regulating both the establishment and maintenance of euchromatin (reviewed in Johansen and Johansen 2006). For example, Deng *et al.* (2008) have shown using a LacI tethering system, that JIL-1-mediated ectopic H3S10 phosphorylation can cause a change in higher-order chromatin structure from a condensed heterochromatin-like state to a more open euchromatic state. Thus, spreading of JIL-1 activity has the potential to expand euchromatic domains and counteract gene silencing in heterochromatic regions. However, while it has been shown that the COOH-terminal region of JIL-1 can directly interact with the histone H3 tail region (Bao *et al.* 2008), it remains to be established how JIL-1 targeting to specific chromatin regions is regulated and how dynamic this regulation is.

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LITERATURE CITED

Bao, X., H. Deng, J. Johansen, J. Girton and K. M. Johansen, 2007 Loss-of-function alleles of the JIL-1 histone H3S10 kinase enhance position-effect-variegation at pericentric sites in Drosophila heterochromatin. Genetics **176:** 1355–1358.

Bao, X., W. Cai, H. Deng, W. Zhang, R. Krencik *et al.*, 2008 The COOH-terminal domain of the JIL-1 histone H3S10 kinase interacts with histone H3 and is required for correct targeting to chromatin. J. Biol. Chem. **283:** 32741–32750.

Cleard, F., M. Delattre and P. Spierer, 1997 Su(var)3–7, a *Drosophila* heterochromatin-associated protein and companion of HP1 in the genomic silencing of position-effect variegation. EMBO J. **16:** 5280–5288.

Cryderman, D. E., M. H. Cuaycong, S. C. R. Elgin and L. L. Wallrath, 1998 Characterization of sequences associated with position-effect-variegation at pericentric sites in *Drosophila* heterochromatin. Chromosoma **107:** 277–285.

Danzer, J. R., and L. L. Wallrath, 2004 Mechanisms of HP1-mediated gene silencing in *Drosophila*. Development **131:** 3571–3580.

Delattre, M., A. Spierer, Y. Jaquet and P. Spierer, 2004 Increased expression of *Drosophila* Su(var)3–7 triggers Su(var)3–9-dependent heterochromatin formation. J. Cell. Sci. **117:** 6239–6247.

Deng, H., W. Zhang, X. Bao, J. N. Martin, J. Girton *et al.*, 2005 The JIL-1 kinase regulates the structure of *Drosophila* polytene chromosomes. Chromosoma **114:** 173–182.

Deng, H., X. Bao, W. Zhang, J. Girton, J. Johansen *et al.*, 2007 Reduced levels of Su(var)3–9 but not Su(var)2–5 (HP1) counteract the effects on chromatin structure and viability in loss-of-function mutants of the JIL-1 histone H3S10 kinase. Genetics **177:** 79–87.

Deng, H., X. Bao, W. Cai, M. J. Blacketer, A. S. Belmont *et al.*, 2008 Ectopic histone H3S10 phosphorylation causes chromatin structure remodeling in *Drosophila*. Development **135:** 699–705.

Dorer, D. R., and S. Henikoff, 1994 Expansions of transgene repeats cause heterochromatin formation and gene silencing in *Drosophila*. Cell **77:** 993–1002.

Ebert, A., G. Schotta, S. Lein, S. Kubicek, V. Krauss *et al.*, 2004 Su(var) genes regulate the balance between euchromatin and heterochromatin in *Drosophila*. Genes Dev. **18:** 2973–2983.

Eissenberg, J. C., and S. C. Elgin, 2000 The HP1 protein family: getting a grip on chromatin. Curr. Opin. Genet. Dev. **10:** 204–210.

Eissenberg, J. C., T. James, D. Foster-Hartnett, D. Hartnett, V. Ngan *et al.*, 1990 Mutation in a heterochromatin-specific chromosomal protein is associated with suppression of position-effect variegation in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **87:** 9923–9927.

Elgin, S. C., and S. I. Grewal, 2003 Heterochromatin: silence is golden. Curr. Biol. **13:** R895–R898.

Girton, J., and K. M. Johansen, 2008 Chromatin structure and regulation of gene expression: the lessons of PEV in *Drosophila*. Adv. Genet. **61:** 1–43.

Greil, F., I. Van Der Kraan, J. Delrow, J. F. Smothers, E. De Wit *et al.*, 2003 Distinct HP1 and Su(var)3–9 complexes bind to sets of developmentally coexpressed genes depending on chromosomal location. Genes Dev. **17:** 2825–2838.

Grewal, S. I., and S. C. Elgin, 2002 Heterochromatin: new possibilities for the inheritance of structure. Curr. Opin. Genet. Dev. **12:** 178–187.

Jaquet, Y., M. Delattre, J. Montoya-Burgos, A. Spirer and P. Spierer, 2006 Conserved domains control heterochromatin localization and silencing properties of Su(var)3–7. Chromosoma **115:** 139–150.

Ji, Y., U. Rath, J. Girton, K. M. Johansen and J. Johansen, 2005 D-Hillarin, a novel W180-domain protein, affects cytokinesis through interaction with the septin family member Pnut. J. Neurobiol. **64:** 157–169.

Jin, Y., Y. Wang, D. L. Walker, H. Dong, C. Conley *et al.*, 1999 JIL-1: a novel chromosomal tandem kinase implicated in transcriptional regulation in *Drosophila*. Mol. Cell **4:** 129–135.

Johansen, K. M., and J. Johansen, 2006 Regulation of chromatin structure by histone H3S10 phosphorylation. Chromosome Res. **14:** 393–404.

Johansen, K. M., W. Cai, H. Deng, X. Bao, W. Zhang *et al.*, 2009 Methods for studying transcription and epigenetic chromatin modification in *Drosophila* polytene chromosome squash preparations using antibodies. Methods **48:** 387–397.

Kelley, R. L., V. H. Meller, P. R. Gordadze, G. Roman, R. L. Davis *et al.*, 1999 Epigenetic spreading of the *Drosophila* dosage compensation complex from *roX* RNA genes into flanking chromatin. Cell **98:** 513–522.

Lachner, M., D. O'Carroll, S. Rea, K. Mechtler and T. Jenuwein, 2001 Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. Nature **410:** 116–120.

Lerach, S., W. Zhang, X. Bao, H. Deng, J. Girton *et al.*, 2006 Loss-of-function alleles of the JIL-1 kinase are strong suppressors of position effect variegation of the w^{m4} allele in Drosophila. Genetics **173:** 2403–2406.

Lindsley, D. L., and G. G. Zimm, 1992 The Genome of *Drosophila melanogaster*. Academic Press, New York.

Muller, H. J., 1930 Types of visible variegations induced by X-rays in *Drosophila*. J. Genet. **22:** 299–335.

Rea, S., F. Eisenhaber, D. O'Carroll, B. D. Strahl, Z. W. Sun *et al.*, 2000 Regulation of chromatin structure by site-specific histone H3 methyltransferases. Nature **406:** 481–487.

Reuter, G., R. Dorn, G. Wustmann, B. Friede and G. Rauh, 1986 Third chromosome suppressor of position-effect variegation loci in *Drosophila melanogaster*. Mol. Gen. Genet. **202:** 481–487.

Reuter, G., M. Giarre, J. Farah, J. Gausz, A. Spierer *et al.*, 1990 Dependence of position-effect varigation in Drosophila on dose of a gene encoding an unusual zinc-finger protein. Nature **344:** 219–223.

Roberts, D. B., 1998 *Drosophila*: *A Practical Approach*. IRL Press, Oxford.

Rudolph, T., M. Yonezawa, S. Lein, K. Heidrich, S. Kubicek *et al.*, 2007 Heterochromatin formation in *Drosophila* is initiated through active removal of H3K4 methylation by the LSD1 homolog Su(var)3–3. Mol. Cell **26:** 103–115.

Schotta, G., A. Ebert, V. Krauss, A. Fischer, J. Hoffmann *et al.*, 2002 Central role of *Drosophila* SU(VAR)3–9 in histone H3–K9 methylation and heterochromatic gene silencing. EMBO J. **21:** 1121–1131.

Schultz, J., 1936 Variegation in *Drosophila* and the inert chromosome regions. Proc. Natl. Acad. Sci. USA **22:** 27–33.

Seum, C., D. Pauli, M. Delattre, Y. Jaquet, A. Spierer *et al.*, 2002 Isolation of *Su(var)3–7* mutations by homologous recombination in *Drosophila melanogaster*. Genetics **161:** 1125–1136.

Spierer, A., C. Seum, M. Delattre and P. Spierer, 2005 Loss of the modifiers of variegation Su(var)3–7 or HP1 impacts male X polytene chromosome morphology and dosage compensation. J. Cell Sci. **118:** 5047–5057.

Tschiersch, B., A. Hofmann, V. Krauss, R. Dorn, G. Korge *et al.*, 1994 The protein encoded by the *Drosophila* position-effect variegation suppressor gene *Su(var)3–9* combines domains of antagonistic regulators of homeotic gene complexes. EMBO J. **13:** 3822–3831.

Wallrath, L. L., and S. C. R. Elgin, 1995 Position effect variegation in *Drosophila* is associated with altered chromatin structure. Genes Dev. **9:** 1263–1277.

Wallrath, L. L., V. P. Guntur, L. E. Rosman and S. C. R. Elgin, 1996 DNA representation of variegating heterochromatic P-element inserts in diploid and polytene tissues of *Drosophila melanogaster*. Chromosoma **104:** 519–527.

Wang, Y., W. Zhang, Y. Jin, J. Johansen and K. M. Johansen, 2001 The JIL-1 tandem kinase mediates histone H3 phosphorylation and is required for maintenance of chromatin structure in *Drosophila*. Cell **105:** 433–443.

Weiler, K. S., and B. T. Wakimoto, 1995 Heterochromatin and gene expression in *Drosophila*. Annu. Rev. Genet. **29:** 577–605.

Zhang, W., Y. Jin, Y. Ji, J. Girton, J. Johansen *et al.*, 2003 Genetic and phenotypic analysis of alleles of the Drosophila chromosomal JIL-1 kinase reveals a functional requirement at multiple developmental stages. Genetics **165:** 1341–1354.

Zhang, W., H. Deng, X. Bao, S. Lerach, J. Girton *et al.*, 2006 The JIL-1 histone H3S10 kinase regulates dimethyl H3K9 modifications and heterochromatic spreading in *Drosophila*. Development **133:** 229–235.

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JIL-1 and *Su(var)3-7* Interact Genetically and Counteract Each Other's Effect on Position-Effect Variegation in Drosophila

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TABLE S1

Genetic interaction between the *JIL-1^{z60}* and *Su(var)3-7^{R2a8}* alleles

Cross	Genotypes (no. of adult flies)		% of expected ratio[a]
JIL-1^{z60}/TM6 X *JIL-1^{z60}/TM6*	*JIL-1^{z60}/TM6*	*JIL-1^{z60}/ JIL-1^{z60}*	
	570	*1*	*0.5%*
JIL-1^{60}/TM6 X *JIL-1^{z60} Su(var)3-7^{R2a8}/TM3*	*JIL-1^{z60}/TM3 or JIL-1^{z60} Su(var)3-7^{R2a8}/TM6*	*JIL-1^{z60}/ JIL-1^{z60} Su(var)3-7^{R2a8}*	
	415	*228*	*106.4%*

[a] In these crosses the *TM6 and TM3* chromosome was identified by the *Stubble* marker. Consequently, the experimental genotypes could be distinguished from balanced heterozygotic flies by absence of the *Stubble* marker. The expected Mendelian ratio of non-*Stubble* to *Stubble* flies was 1:2 since the *TM6/TM6* and *TM6/TM3* genotypes are embryonic lethal. The percentage of expected genotypic ratios were calculated as: observed non-*Stubble* flies X 300/total observed flies.

TABLE S2

Genetic interaction between *JIL-1* and *Su(var)3-7* alleles of male and female flies

Cross	Genotypes (no. of adult flies)		% of expected ratio[a]
JIL-1^{z2}/TM6 X *JIL-1^{z2} Su(var)3-7$^{7.1A}$/TM6*	*JIL-1^{z2}/TM6* or *JIL-1^{z2} Su(var)3-7$^{7.1A}$/TM6*	*JIL-1^{z2}/ JIL-1^{z2} Su(var)3-7$^{7.1A}$*	
males	212	52	59.0%
females	319	149	95.5%
JIL-1^{z2}/TM6 X *JIL-1^{z2} Su(var)3-7^{14}/TM6*	*JIL-1^{z2}/TM6* or *JIL-1^{z2} Su(var)3-7^{14}/TM6*	*JIL-1^{z2}/ JIL-1^{z2} Su(var)3-7^{14}*	
males	230	78	76.0%
females	248	107	90.4%
JIL-1^{z2}/TM6 X *JIL-1^{z2} Su(var)3-7^{R2a8}/TM6*	*JIL-1^{z2}/TM6* or *JIL-1^{z2} Su(var)3-7^{R2a8}/TM6*	*JIL-1^{z2}/ JIL-1^{z2} Su(var)3-7^{R2a8}*	
males	132	26	49.4%
females	227	71	71.5%

[a] In these crosses the *TM6* chromosome was identified by the *Stubble* marker. Consequently, the experimental genotypes could be distinguished from balanced heterozygotic flies by absence of the *Stubble* marker. The expected Mendelian ratio of non-*Stubble* to *Stubble* flies was 1:2 since *TM6/TM6* is embryonic lethal. The percentage of expected genotypic ratios were calculated as: observed non-*Stubble* flies X 300/total observed flies.

TABLE S3

Genetic interaction between homozygous *JIL-1* and *Su(var)3-7* alleles

Cross	Genotypes (no. of adult flies)		% of expected ratio[a]
JIL-1^{z2} Su(var)3-7$^{7.1A}$/TM6 X *JIL-1^{z2} Su(var)3-7$^{7.1A}$/TM6*	*JIL-1^{z2} Su(var)3-7$^{7.1A}$/TM6* 531	*JIL-1^{z2} Su(var)3-7$^{7.1A}$/ JIL-1^{z2} Su(var)3-7$^{7.1A}$* 30	16.0%
JIL-1^{z2} Su(var)3-7^{14}/TM6 X *JIL-1^{z2} Su(var)3-7^{14}/TM6*	*JIL-1^{z2} Su(var)3-7^{14}/TM6* 426	*JIL-1^{z2} Su(var)3-7^{14}/ JIL-1^{z2} Su(var)3-7^{14}* 24	16.0%

[a] In these crosses the *TM6* chromosome was identified by the *Stubble* marker. Consequently, the experimental genotypes could be distinguished from balanced heterozygotic flies by absence of the *Stubble* marker. The expected Mendelian ratio of non-*Stubble* to *Stubble* flies was 1:2 since *TM6/TM6* is embryonic lethal. The percentage of expected genotypic ratios were calculated as: observed non-*Stubble* flies X 300/total observed flies.

TABLE S4

Rescue of homozygous *JIL-1^{z2}* lethality by the *JIL-1-GFP* transgene, *GF29.1 at 25°C*

Cross		Genotypes (no. of adult flies)		% of expected ratio[a]
GF29.1/GF29.1 ; JIL-1^{z2}/TM6	X	*GF29.1/GF29.1 ; JIL-1^{z2}/TM6*	*GF29.1/GF29.1 ; JIL-1^{z2}/ JIL-1^{z2}*	
GF29.1/GF29.1 ; JIL-1^{z2}/TM6		1344	373	65.2%

[a] In these crosses the *TM6* chromosome was identified by the *Stubble* marker. Consequently, the experimental genotypes could be distinguished from balanced heterozygotic flies by absence of the *Stubble* marker. The expected Mendelian ratio of non-*Stubble* to *Stubble* flies was 1:2 since *TM6/TM6* is embryonic lethal. The percentage of the expected genotypic ratio was calculated as: observed non-*Stubble* flies X 300/total observed flies.

TABLE S5

Rescue of homozygous *JIL-1*^{z2} lethality by the *JIL-1-V5* transgene, *JIL-1-FL*, at 25°C

Cross	Genotypes (no. of adult flies)		% of expected ratio[a]
JIL-1-FL/JIL-1-FL ; JIL-1^{z2}*/TM6* X *+/+ ; JIL-1*^{z2} *Hsp70-Gal4/TM6*	*JIL-1-FL/+ ; JIL-1*^{z2}*/TM6 or JIL-1-FL/+; JIL-1*^{z2} *Hsp70-Gal4/TM6*	*JIL-1-FL/+ ; JIL-1*^{z2}*/ JIL-1*^{z2} *Hsp70-Gal4*	
	376	*84*	*54.8%*

[a] In these crosses the *TM6* chromosome was identified by the *Stubble* marker. Consequently, the experimental genotypes could be distinguished from balanced heterozygotic flies by absence of the *Stubble* marker. The expected Mendelian ratio of non-*Stubble* to *Stubble* flies was 1:2 since *TM6/TM6* is embryonic lethal. The percentage of expected genotypic ratios were calculated as: observed non-*Stubble* flies X 300/total observed flies. The *JIL-1-FL* rescue construct is described in BAO *et al.* (2008) and the *JIL-1*^{z2} allele was recombined with the *Hsp70-Gal4* driver line.

TABLE S6

Rescue of homozygous *JIL-1*^{z2} lethality by the *JIL-1-V5* transgene, *JIL-1-FL*, at 21°C

Cross	Genotypes (no. of adult flies)		% of expected ratio[a]
JIL-1-FL/JIL-1-FL ; JIL-1^{z2}*/TM6* X *+/+ ; JIL-1*^{z2} *Hsp70-Gal4/TM6*	*JIL-1-FL/+ ; JIL-1*^{z2}*/TM6 or JIL-1-FL/+; JIL-1*^{z2} *Hsp70-Gal4/TM6*	*JIL-1-FL/+ ; JIL-1*^{z2}*/ JIL-1*^{z2} *Hsp70-Gal4*	
	751	*344*	*94.2%*

[a] In these crosses the *TM6* chromosome was identified by the *Stubble* marker. Consequently, the experimental genotypes could be distinguished from balanced heterozygotic flies by absence of the *Stubble* marker. The expected Mendelian ratio of non-*Stubble* to *Stubble* flies was 1:2 since *TM6/TM6* is embryonic lethal. The percentage of expected genotypic ratios were calculated as: observed non-*Stubble* flies X 300/total observed flies. The *JIL-1-FL* rescue construct is described in BAO *et al.* (2008) and the *JIL-1*^{z2} allele was recombined with the *Hsp70-Gal4* driver line.

TABLE S7

The counterbalancing effect of *JIL-1* and *Su(var)3-7* alleles on PEV

Genotype[a]	*n*	% of flies categorized by the proportion of red ommatidia				
		0-20% red	20-40% red	40-60% red	60-80% red	80-100% red
118E-15/+						
JIL-1^{z2}/JIL-1^{z60}	5	100.0	0.0	0.0	0.0	0.0
+/+	154	0.0	1.3	20.1	54.5	24.0
JIL-1^{z2}, 3-7$^{7.1A}$/JIL-1^{z60}	136	2.9	36.0	32.4	24.3	4.4
3-7$^{7.1A}$/+	70	0.0	0.0	0.0	0.0	100.0
w^{m4}						
JIL-1^{z2}/+	111	87.4	9.0	3.6	0.0	0.0
+/+	103	37.9	44.7	14.6	2.9	0.0
JIL-1^{z2}, 3-7$^{7.1A}$/+	154	9.1	24.7	26.6	29.9	9.7
3-7$^{7.1A}$/+	117	0.0	6.0	19.7	31.6	42.7
DX1/+						
JIL-1^{z2}/+	91	92.3	7.7	0.0	0.0	0.0
+/+	101	7.9	25.7	39.6	23.8	3.0
JIL-1^{z2}, 3-7^{14}/+	100	34.0	34.0	12.0	11.0	9.0
3-7^{14}/+	104	0.0	4.8	13.5	25.0	56.7

[a] Only results from male flies were tabulated.