MEIOTIC recombination mapping is one of the cornerstones of classical genetics. In genetic model organisms, it is commonly used to order genetic loci on chromosomes, and the precise mapping of loci can greatly assist in the identification of the molecular lesion responsible for a particular phenotype of interest. Meiotic mapping provides two distinct types of data that are used in combination to create a genetic map. Recombination distances are used to estimate the lengths of intervals between genetic loci and the behavior of flanking markers is used to infer the order of the loci.

Like all techniques, recombination mapping has its limitations. In particular, it can be used only to map loci that are within 50 cM of one another, because loci that are >50 cM apart behave as if they were on separate chromosomes. To map genetic loci that are >50 cM apart, other loci with intermediate map positions must first be identified and the markers of interest mapped with respect to these loci. A further limitation is that for genetic loci that are very close together, large numbers of progeny must be scored to detect rare recombination events between those markers, making the fine mapping of genetic loci a labor-intensive undertaking.

Recently, Chen et al. (1998) devised a method to circumvent these limitations of meiotic mapping in Drosophila melanogaster, allowing for the rapid ordering of a locus of interest with respect to a series of P-element inserts at known locations along the chromosome. Chen et al. (1998) took advantage of two unusual aspects of Drosophila biology: the general lack of meiotic recombination on all chromosomes in males (Morgan 1912, 1914) and the ability to induce site-specific recombination at the site of P-element insertions by the addition of an external source of transposase (Preston and Engels 1996; Preston et al. 1996). By using site-specific recombination in male Drosophila, it is possible to construct crosses in such a way that all of the observed recombination events are associated with P-element insertion sites. The frequency of these recombination events, like the frequency of P-element transposition events, is due to characteristics of the P-element itself and the nature of the DNA sequence surrounding the insertion site (Berg and Spradling 1991). In addition, because there is no recombination other than that at the site of the P-element insertion, the selection of flanking markers is simplified. The only requirements are that they be easy to score and that a marker be on either side of the locus of interest. Male recombination mapping has been used to fine map several autosomal loci (McKim and Hayashi-Hagihara 1998; Chen et al. 2000; Pai et al. 2000; Chu et al. 2001; Chung et al. 2001; Lee and Treisman 2001).

Male recombination mapping also has limitations. It generally cannot be used to map loci on the sex chromosomes because males are the hemizygous sex in Drosophila (Morgan 1910) and their X and Y chromosomes are without true homologs with which to recombine.

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ABSTRACT

P-element transposons in the Drosophila germline mobilize only in the presence of the appropriate transposase enzyme. Sometimes, instead of mobilizing completely, P elements will undergo site-specific recombination with the homologous chromosome. Site-specific recombination is the basis for male recombination mapping, since the male germline does not normally undergo recombination. Site-specific recombination also takes place in females, but this has been difficult to study because of the obscuring effects of meiotic recombination. Using map functions, I demonstrate that it is possible to employ female site-specific transposase-induced recombination (FaSSTIR) to map loci on the X chromosome and predict that FaSSTIR mapping should be more efficient than meiotic mapping over short genetic intervals. Both FaSSTIR mapping and meiotic mapping were used to fine map the crossveinless locus on the X chromosome. Both techniques identified the same 10-kb interval as the probable location of the crossveinless mutation.

Over short intervals (<~7.6 cM), FaSSTIR produces more informative recombination events than does meiotic recombination. Over longer intervals, FaSSTIR is not always more efficient than meiotic mapping, but it produces the correct gene order. FaSSTIR matches the expectations suggested by the map functions and promises to be a useful technique, particularly for mapping X-linked loci.
(Chen et al. 1998). Researchers wishing to map loci on the X chromosome (which makes up ~25% of the Drosophila genome) have therefore had to use traditional meiotic mapping in females, which becomes increasingly labor intensive as the distance between the locus of interest and the markers being used to map the locus decreases. Presented here is a new technique called female site-specific transposase-induced recombination (FaSSTIR) mapping, which combines some of the benefits of male recombination mapping with the ability to map loci on the X chromosome. This technique relies on the fact that the phenomenon of site-specific recombination associated with P elements is not limited to male Drosophila and occurs in females as well (Broadhead et al. 1977; Kidwell 1977). In addition, because the recombination rate between any two loci in female Drosophila is directly related to the physical distance between them on the chromosome, as increasingly shorter intervals are considered, the recombination distance approaches zero and is therefore analogous to the absence of recombination observed in males. Over sufficiently short intervals, in crosses where both meiotic and site-specific recombination are allowed to take place, site-specific recombination will be more frequent than meiotic recombination and may thus be useful in mapping closely spaced loci on the X chromosome.

As long as appropriate flanking markers are selected so that they are not lost by meiotic recombination, this technique could be used to map loci on the X chromosome and under some circumstances may be more efficient than standard meiotic mapping. Presented here are a mathematical justification for the efficacy of FaSSTIR mapping, a demonstration of the use of the technique for mapping the crossveinless (cv) locus (Bridges 1920), and a comparison of the precision and efficiency of FaSSTIR with traditional meiotic mapping.

MATHEMATICAL JUSTIFICATION FOR FEMALE SITE-SPECIFIC TRANSPOSASE-INDUCED RECOMBINATION MAPPING

In Drosophila females, using a map function first suggested by Haldane (1919), the measured meiotic recombination frequency \( C_{AB} \) between two loci \( A \) and \( B \) can be expressed in terms of the map distance \( x_{AB} \) (in centimorgans) as

\[
C_{AB} = \frac{1}{2} (1 - e^{-2x_{AB}}). \tag{1}
\]

As \( x_{AB} \) increases from 0, \( C_{AB} \) increases from 0 to 0.5. Moreover, as \( x_{AB} \) becomes increasingly small, \( C_{AB} \) and \( x_{AB} \) converge, so that over short intervals (in which multiple crossover events are unlikely), the measured recombination frequency equals the map distance. Similarly, as \( x_{AB} \) approaches 0, the observed recombination frequency also approaches 0, so that

\[
C_{AB} \xrightarrow{\text{lim } x_{AB} \to 0} 0. \tag{2}
\]

In Drosophila males the situation is somewhat different because there is no meiotic recombination in the male germline (Morgan 1912, 1914), so regardless of the distance between \( A \) and \( B \) (\( x_{AB} \)) there is no intrachromosomal recombination in males and

\[
C_{AB} = 0. \tag{3}
\]

Site-specific recombination can be induced in males at \( P \)-element transposon insertion sites by the presence of transposase (Preston and Engels 1996). Male site-specific recombination occurs with a constant frequency, \( k_{M} \), that is independent of genetic distance between a locus \( A \) and a particular transposon insertion \( P \), but instead appears to be due to the nature of the transposon itself and the characteristics of the DNA sequences flanking the transposon (Berg and Spradling 1991). Unlike meiotic recombination, site-specific recombination is essentially a binary process with no theoretical upper limit [although as an empirical matter, site-specific recombination rates >0.5 are unknown (Chen et al. 1998)]. Male site-specific recombination can be written as

\[
C_{AB} = k_{M} x_{AB}. \tag{4}
\]

Recombination frequencies in males are not true map distances and cannot be used to determine distances between loci. Instead, left-right positional information is obtained from the behavior of flanking markers and it is these data that are used to create a map where the order of the loci is specified, but not the distances between them (Chen et al. 1998). However, since many \( P \) elements have known locations on both the physical and the DNA sequence maps, this technique can be used to map genetic loci to small precisely defined intervals between identified \( P \)-element insertions, which can facilitate the molecular characterization of genetic loci.

Site-specific recombination at the site of transposon insertions also occurs in females (Kidwell 1977), but it is much more difficult to study due to obscuring effects of meiotic recombination. Both meiotic and transposase-induced recombination events can influence observed recombination frequencies in females. The effects of both of these processes can be expressed as a function of the meiotic recombination rate \( x_{AB} \) and the site-specific recombination rate \( k_{F} \) as

\[
C_{AB} = k_{F} e^{-2x_{AB}} + \frac{1}{2} (1 - e^{-2x_{AB}}). \tag{5}
\]

It is necessary to distinguish between male site-specific recombination rates \( k_{M} \) and female site-specific recombination rates \( k_{F} \) because there are currently no data on the relationship between these two rates for individual transposon insertions. The generalized behavior of Equations 1, 3, and 5 is shown graphically in Figure 1.
Figure 1.—(Left axis) Theoretical predictions of the behavior of meiotic mapping (Equation 1), male recombination mapping (Equation 3), and FaSSTIR mapping (Equation 5). (Right axis) Empirical determination of the relative efficiency of meiotic mapping and FaSSTIR mapping as meiotic map distance from the locus of interest increases. Meiotic recombination rates approach 0 as distance approaches 0. Both male recombination rates and FaSSTIR rates approach nonzero constants ($k_p^M$ and $k_p^F$, respectively) as distance approaches 0. As distances become large, meiotic recombination rates and FaSSTIR rates approach 50, while male recombination rates remain constant. For the comparisons of relative efficiency, meiotic recombination distance on the x-axis has been centered here on the map position of the cv locus (1-13.7). Efficiencies of 1 indicate that FaSSTIR and meiotic recombination rates are equal. Efficiencies $>1$ indicate FaSSTIR is more efficient, and those $<1$ indicate that meiotic recombination is more efficient. The greatest increase in efficiency is over the shortest intervals and generally declines as the distance from the locus of interest increases. The shaded box shows the region over which FaSSTIR was consistently more efficient than meiotic recombination, a region that encompasses 7.65 cM to the left of cv and 7.55 cM to the right of the cv locus. However, the relative increase in efficiency achieved by FaSSTIR beyond the 5-cM meiotic recombination distance to either side of the locus of interest is marginal.

In Drosophila females, as $x_{AB}$ becomes increasingly small, the effects of meiotic recombination diminish, and distance-independent site-specific recombination predominates. Therefore, over sufficiently short intervals, the measured recombination rate will be due entirely to the rate of site-specific recombination and thus will be a constant $k_p$ so that

$$C_{AB} = \lim_{x \to 0} k_p e^{-2x} + \frac{1}{2}(1 - e^{-2x}) = k_p.$$  \hspace{1cm} (6)

This observation suggests that FaSSTIR can be used to map loci over short intervals in females just as it is used to map loci over longer intervals in males (cf. Equations 4 and 6). Just as in male site-specific recombination, only left-right information determined from flanking markers can be used to produce maps because FaSSTIR recombination rates are not true map distances. The choice of flanking markers is somewhat more difficult in females than in males in that they must be close enough to the loci being examined so that they are not lost by recombination. This flanking marker recombination is analogous to that encountered in typical meiotic mapping in females, which is not an issue in Drosophila male recombination mapping.

Equation 6 suggests that over short intervals and for female site-specific recombination rates greater than zero ($k_p > 0$), FaSSTIR will take place more often than meiotic recombination alone. Due to this increase in potentially informative recombination events, FaSSTIR may be efficient in providing high-resolution fine-mapping data for regions of the Drosophila genome that are not amenable to male site-specific recombination mapping, particularly the X chromosome. These predictions are not predicated by the choice of map function. A similar set of equations can be derived from the alternative generalized map function of Kosambi (1944) and give substantially similar results to those shown here for the map function proposed by Haldane (1919; derivation not shown).

 MATERIALS AND METHODS

Drosophila stocks: Flies were raised on standard cornmeal-molasses Drosophila medium and grown at 25°C unless otherwise noted. The $y^{1} w^{1} v^{1} sn^{1}$ mapping chromosome was created by recombination. The transposase stock $Sp^{1}/CyO$; Dr1 Delta2-3/TM6 Ubx and all P elements were obtained from the Bloomington Fly Stock Center except for EP(X)1368, EP(X)1405, EP(X)0371, and EP(X)1604, which were obtained from Exelixis. The information about cytological locations and flanking sequences of P elements is from the BERKELEY DROSOPHILA GENOME PROJECT (2002). Only homozygous viable P-element inserts with sequenced flanking regions were used. All genetic symbols not described in the text are in the
Drosophila reference works (Lindsley and Zimm 1992). P-element flanking sequences were compared to the Drosophila genomic scaffold sequence (Adams et al. 2000) by BLAST (Altschul et al. 1997) to determine the exact locations of the insertion sites.

**Drosophila crosses:** For FaSSTIR-mapping experiments, female flies carrying the mapping chromosome $y^+ w^{118} \text{cv} \text{ sn}^1$; +; + were mated to male flies from the transposase stock that were +; $Sp^e/CyO; \text{Dr}^2 \Delta 3/\text{TM6} \text{ Ultra}$. Male progeny from this stock that were $y^+ w^{118} \text{cv} \text{ sn}^1$; $Sp^e/+; \text{Dr}^2 \Delta 3/++; \text{Dr}^2 \Delta 3/+$ or $y^+ w^{118} \text{cv} \text{ sn}^1$; $CyO/+; \text{Dr}^2 \Delta 3/++$ were then crossed to flies carrying the $\text{P}$ element chromosome $[y^+ w^{118} E\text{P}(X) \text{cv} \text{ sn}^1] +; +$. Virgin female progeny from this cross that were $y^+ w^{118} \text{cv} \text{ sn}^1/y^+ w^{118} E\text{P}(X) \text{cv} \text{ sn}^1$; +; +; + were mated to males carrying the mapping chromosome $y^+ w^{118} \text{cv} \text{ sn}^1$; +; + and the progeny from this cross were scored.

For meiotic-mapping experiments, female flies carrying the mapping chromosome $y^+ w^{118} \text{cv} \text{ sn}^1$; +; + were mated to male flies carrying the $\text{P}$ element chromosome $y^+ w^{118} E\text{P}(X) \text{cv} \text{ sn}^1$; +; +. Virgin female progeny from that were $y^+ w^{118} \text{cv} \text{ sn}^1/y^+ w^{118} E\text{P}(X) \text{cv} \text{ sn}^1$; +; +; + were crossed back to males carrying the mapping chromosome $y^+ w^{118} \text{cv} \text{ sn}^1$; +; + and the progeny from this cross were scored.

About five males and five females were placed in each vial. For each chosen $\text{P}$ element line, from 10 to 70 crosses were set up for both FaSSTIR and meiotic mapping, and progeny were scored for recombinants. Meiotic recombination events between $\text{cv}$ and $P$ elements close to $\text{cv}$ are very rare, requiring greater numbers of progeny to calculate recombination frequencies and the larger number of crosses. Informatively single recombinants were identified by the phenotypes produced by the flanking markers yellow and singed and by eye color produced by the mini-white construct carried by the $P$ elements. Recombination distances and standard errors were calculated by standard methods (Weir 1996). Comparisons of FaSSTIR and meiotic mapping were made by calculating the relative efficiency of the two methods by dividing the FaSSTIR recombination rate by the meiotic recombination rate for each $\text{P}$ element insertion. When this ratio was greater than one, FaSSTIR was more efficient than meiotic recombination. The limits of the interval in which FaSSTIR was more efficient were determined by linear interpolation.

**RESULTS**

**Scheme for mapping:** Figure 2 illustrates the schemes used for mapping a mutation using FaSSTIR and meiotic recombination. Hemizygous lethal and homozygous lethal mutations can also be mapped by FaSSTIR. Such a scheme requires the creation of double-balanced stocks, and only some recombinant progeny may be easily scored in the F1 backcross, but is otherwise essentially similar to what is described here.

In the case of a hemizygous viable mutation for both FaSSTIR and meiotic recombination, two visible markers ($m_1$ and $m_2$) that flank a mutation of interest ($g$) are selected. The chromosome containing the $P$ element is put in trans to a chromosome carrying mutation $g$ that also carries mutant alleles of the visible markers. The transposase source ($\Delta 2-3$) for FaSSTIR is provided by another chromosome carrying a dominant visible marker ($D$). The cross must be conducted in a background (usually eye color $w^+$ or $ry^+$) in which a visible marker associated with the $P$ element ($w^+$ or $ry^+$) can also be scored to distinguish between single- and multiple-recombination events. For both types of mapping, a single-recombination event between the $P$ element and $g$ locus results in the $g$ mutation cosegregating with a wild-type eye color marker and with either $m_1$ or $m_2$. The other single-recombination product can also be identified, because it carries only the other flanking marker on a $g^+$ and $w^+$ or $ry^+$ chromosome. By identifying probable single recombinants, it is then possible to test whether $g$ cosegregates with either $m_1$ or $m_2$.

**Mapping the crossveinless gene:** The $cv$ gene has been mapped to X chromosome region 5A8–5C2 in complementation tests with $Df(1)C149$ (fails to complement, breakpoints at 5A8–9 and 5C5–6) and $Df(1)N73$ (complements, breakpoints 5C2 and 5D5–6; Slizynska 1964) and so $P$ elements that mapped to this region were initially chosen for analysis. The $P$ element insertion sites were mapped by in situ hybridization by the Berkeley Drosophila Genome Project (2002). Additional $P$ elements that mapped to X chromosome divisions 1–10 were also tested to determine the genetic distances over which FaSSTIR is effective. A total of 23 $P$ element insertion lines, which span approximately half of the X chromosome, were tested. The body color locus yellow

**Figure 2.—Mapping schemes. (A) Meiotic recombination-mapping scheme. (B) FaSSTIR-mapping scheme.**
but still indicated the correct order of loci along the markers. In addition, FaSSTIR may also be affected by frequency of FaSSTIR than of meiotic recombination, allows for greater flexibility in the selection of flanking markers, the efficiency of FaSSTIR and meiotic recombination. Over longer intervals, it is probably advantageous to use meiotic mapping because it requires fewer generations to set up the appropriate mapping cross.

The data produced by different methods of mapping crossovers are entirely congruent with each other. Deficiency mapping defined the interval 5A8–C2. Meiotic recombination mapping and FaSSTIR mapping both define a smaller interval, 5A13–B1, between $P$-element inserts $EP0496$ and $EP1349$ within the deficiency interval. Meiotic mapping further defines this interval as measuring 0.07 cM. By examining the insertion sites of the $P$ elements on the Drosophila genome scaffold sequence, this interval can also be defined as being 10 kb in length. This interval contains three complete open reading frames and probably contains regulatory sequences for at least two other loci (Figure 4).

**DISCUSSION**

I have shown that FaSSTIR can be used to map a locus to the same interval as meiotic recombination mapping and that, over short intervals, FaSSTIR is more efficient than meiotic recombination. Over longer intervals, the efficiency of FaSSTIR and meiotic recombination rates becomes similar. This conforms to the mathematical predictions that over short intervals FaSSTIR rates will be higher and over longer intervals FaSSTIR and meiotic recombination rates are expected to converge at 50%. Transposase-induced recombination in females was generated in 23 $P$-element insertion lines, and in each case the order of the insertion relative to the mutant of interest and to two flanking markers was obtained unambiguously. FaSSTIR allows some of the increases in the efficiency of mapping that have been derived from male recombination mapping on the autosome to be applied to genes on the X chromosome. The increasingly large collection of $P$-element insertions on the X chromosome will make this a highly useful technique for characterizing such loci.

Although FaSSTIR can be used on any chromosome with a homolog (in Drosophila, all except the Y chromosome), male recombination is likely to be an easier mapping technique for most autosomal loci because of the absence of meiotic recombination in males, which allows for greater flexibility in the selection of flanking markers. In addition, FaSSTIR may also be affected by phenomena similar to the misleading recombination phenomenon observed in this study.

![Figure 3](image-url)  
*Figure 3.—Recombination frequencies for meiotic recombination and FaSSTIR mapping. Sequence position refers to the locations of the $P$-element insertion sites on the Drosophila genome scaffold sequence. The sequence position is numbered such that the distal end is 0 and increases proximally. $\bullet$, meiotic recombination frequencies; $\triangle$, FaSSTIR recombination frequencies. Standard errors were generally small and error bars for most points would have been inside the symbols, so they are not shown.*

(y) and the bristle morphology locus *singed* (*sn*) were used as visible flanking markers. The $cv^o$ allele has a visible recessive phenotype and is both homozygous and hemizygous viable, so determining whether it cosegregated with $y$ or $sn$ was unambiguous.

For all of the $P$ elements tested, it was possible to unambiguously map *crossveinless* with respect to the $P$ element by both the meiotic-mapping and the FaSSTIR-mapping techniques. Eight $P$ elements were found to be distal to the *crossveinless* locus and recombinants between the $P$ and $cv$ tended to produce $y^+ w P cv sn$ and $y w cv^+ sn^+$ recombination products. Fifteen $P$ elements were found to be proximal to the *crossveinless* locus and recombinants between the $P$ and $cv$ tended to produce $y w cv P sn^+$ and $y^+ w cv^+ sn$ recombination products. Additional meiotic recombination events that removed or added flanking markers also took place on these recombinant chromosomes at low frequency. These events did not obscure the mapping signal, but do necessitate examining multiple recombinant progeny.

In almost all cases, the frequency of FaSSTIR exceeded the frequency of meiotic recombination between the $P$ element and the *crossveinless* locus (Figure 3). Two exceptions ($EP1395$ and $EP0912$) had a lower frequency of FaSSTIR than of meiotic recombination, but still indicated the correct order of loci along the chromosome. It appears that FaSSTIR gives the greatest increase in mapping efficiency relative to meiotic mapping when the $P$ element is relatively close to the locus of interest with a maximum relative efficiency of >200-fold. This increase in efficiency declines for insertion sites that are farther away, and for $P$ elements that are separated by $>\sim5$ cM from locus of interest, FaSSTIR produces minimal increases in efficiency (Figure 1). This suggests that, as a mapping tool, FaSSTIR is most appropriate for mapping intervals within 5 cM of a locus of interest on the meiotic recombination map. For intervals >5 cM, it is probably advantageous to use meiotic mapping because it requires fewer generations to set up the appropriate mapping cross.
events that are produced when some \( P \)-element inserts are used for male recombination mapping (Citu et al. 2001). The mechanism by which these misleading results are produced is unknown, but may also affect transposase-induced recombination in females. To minimize the effects of these misleading events, researchers using FaSSTIR should try to score as many recombinant flies as is practical. Finally, as shown above, FaSSTIR efficiency is similar to meiotic mapping for intervals \( > 7.6 \) cM and is only marginally more efficient over intervals \( > 5 \) cM.

FaSSTIR may find application in two other areas. First, FaSSTIR, like male recombination, can be used to generate recombination events in regions of the genome where meiotic recombination is infrequent, such as near centromeres. This has the potential to greatly facilitate the recombination-dependent interval mapping of single-nucleotide polymorphism (SNP) markers relative to genes of interest (Hoskins et al. 2001).

Second, FaSSTIR can be used to compare the transposase-induced site-specific recombination rates in male and female Drosophila. The germline in the two sexes is organized very differently and may present significantly different environments in which \( P \)-element transposition and recombination take place. However, if a set of \( P \)-elements is mapped via male recombination, FaSSTIR, and female recombination, it will be possible to measure \( k_p \) (Equation 4) and \( k_f \) (Equation 6) for the same individual inserts. Comparing these rates in the context of male and female germ cell formation may provide insights into the biology of these transposon insertions that have contributed so significantly to the field of genetics.

Recently, it has been suggested that CG12410, the predicted open reading frame immediately proximal to the interval defined by these mapping experiments, encodes a homolog of the twisted gastrulation \( (tsg) \) locus and has been called \( \text{twisted gastrulation-2} (tsg_2; \text{Ross et al. 2001}). Twisted gastrulation protein regulates signaling by bone morphogenetic protein (BMP) ligands, such as decapentaplegic and glass-bottom boat [which play roles in crossvein formation (Conley et al. 2000; Marcus 2001)], by facilitating the binding of the BMP antagonist chordin and also by facilitating the degrada-

Figure 4.—Fine-scale map around the crossveinless locus. Deficiency mapping, meiotic mapping, and FaSSTIR mapping all produce congruent results for the location of the crossveinless locus. The hatched box shows the region defined by deficiency mapping and the solid box shows the region defined by meiotic and FaSSTIR mapping. The order of the \( P \) elements was determined by reported cytology and by comparing flanking sequences to the genomic scaffold sequence. These two methods produced entirely congruent orders of the \( P \) elements. The asterisk indicates cytological map positions inferred from the mapping data presented here; the star indicates map positions obtained from FlyBase (2002); and the black cross indicates \( P \) elements with map positions, as determined by meiotic recombination frequencies, which would change the order of the insertions from that suggested by cytology and by comparisons of flanking sequence with the genomic scaffold (BERKELEY DROSOPHYLA GENOME PROJECT 2002). For these insertions, linear interpolation with respect to the two nearest neighboring insertions was used to assign an adjusted map position for these elements. Also shown is the 10-kb genomic region to which the crossveinless lesion was mapped. It contains three complete open reading frames and may also contain regulatory sequences from neighboring genes.
tion of cleaved chordin molecules (Larrain et al. 2001). In this manner, tsg can both antagonize and promote BMP signaling and it has been hypothesized that tsg2 may function in a similar manner (Larrain et al. 2001). It has also been reported that mutations in CG12410 fail to complement cv0 (L. March, personal communication to FlyBase: Fbgn0000394), suggesting that this predicted open reading frame corresponds to the crossveinless locus. If these findings and the genetic mapping data from this study are both correct, this suggests further that the lesion that produced the cv0 mutation is probably a regulatory mutation in the 5′ sequence upstream of CG12410 and may be located in between the EP1349 insertion site and the 5′ transcription start site of CG3160 (Figure 4). Confirmation of this interpretation will require additional experiments.

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