Transvection at the *eyes absent* Gene of Drosophila

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ABSTRACT

The Drosophila *eyes absent* (*eya*) gene is required for survival and differentiation of eye progenitor cells. Loss of gene function in the eye results in reduction or absence of the adult compound eye. Certain combinations of *eya* alleles undergo partial complementation, with dramatic restoration of eye size. This interaction is sensitive to the relative positions of the two alleles in the genome; rearrangements predicted to disrupt pairing of chromosomal homologs in the *eya* region disrupt complementation. Ten X-ray-induced rearrangements that suppress the interaction obey the same general rules as those that disrupt transvection at the bithorax complex and the *decapentaplegic* gene. Moreover, like transvection in those cases, the interaction at *eya* depends on the presence of normal *zeste* function. The discovery of transvection at *eya* suggests that transvection interactions of this type may be more prevalent than generally thought.

CHANGING the location of a gene in the eukaryotic genome can have a dramatic influence on its expression. This observation, first made by STURTEVANT in Drosophila, is known as the position effect (STURTEVANT 1925; reviewed in LEWIS 1950). The study of position effects has led to advances in understanding the molecular basis of gene regulation, chromosome structure, and the relationship between the two (WU 1993; REUTER and SPIERER 1992; PARO 1990; SHAFFER *et al.* 1993). While most of these studies have been carried out in Drosophila, they have been shown to be relevant to vertebrates and mammals, as well (WU 1993; EISSENBERG and ELGIN 1991).

Known position effects fall into several categories (for reviews, see LEWIS 1950; WILSON *et al.* 1990). A stable type occurs in rearrangements or transposon insertions that allow a gene to come under the influence of foreign *cis*-regulatory regions. This kind of position effect is exemplified in transgenic mice, where *cis*-regulatory regions near the insertion point can affect the expression of a transgene (reviewed in WILSON *et al.* 1990). In Drosophila, such position effects have been used to advantage for detecting *cis*-regulatory regions by the "enhancer-trap" method (O'KANE and GEHRING 1987).

Another category is known as position effect variegation (PEV) (reviewed in SPOFFORD 1976; REUTER and SPIERER 1992; SPRADLING and KARPEN 1990). It occurs with rearrangements that bring a wild-type allele into the vicinity of heterochromatin, a compacted form of chromatin near the centromere. This results in a mutant phenotype which, rather than being equally expressed in all cells, is differentially expressed in various patches of tissue. While best characterized in Drosophila, PEV is known also in mice. Mice exhibiting a mottled phenotype have been shown to possess a translocation of the X-chromosome to an autosome, which is thought to result in heterochromatin formation in the neighborhood of the affected gene (RUSSELL and BANGHAM 1961; reviewed in EICHER 1970).

A third category of position effect, the subject of the work presented here, is transvection: a genetic interaction sensitive to the relative positions of two alleles in the genome (reviewed in JUDD 1988; ASHBURNER 1989; WU and GOLDBERG 1989; WU 1993). Such interactions are thought to be mediated by chromosomal pairing. The phenotypic severity depends on how close the two alleles are to each other. Perhaps the most widely studied of these phenomena is the interaction between certain alleles of the bithorax complex (BX-C), first described by LEWIS, for which he coined the term "transvection" (LEWIS 1954). Complementation between a number of allele pairs, such as *bithorax*^{34e} and *Ultrabithorax* (*Ubx*) or *Contrabithorax* (*Cbx*) and *Ubx* is disrupted by rearrangements that disrupt pairing of chromosomal homologs in the salivary gland nuclei (LEWIS 1955, 1964). Such rearrangements have been referred to as transvection-disrupting rearrangements (GELBART 1982). Other terms used for similar phenomena have been "synapsis-dependent allelic complementation" (GELBART 1982), or "*trans*-sensing effects" (TARTOF and HENIKOFF 1991).

Most transvection-disrupting rearrangements isolated for BX-C have two attributes: (1) one breakpoint is in a "critical region" of the chromosome between the gene and the centromere; and (2) the rearrangement is such that the gene has become attached to a different chromosome arm (LEWIS 1954). We use the term transvection-disrupting rearrangements of the type

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reported by LEWIS to describe a chromosomal rearrangement with these key properties. Certain allelic combinations of *decapentaplegic* (*dpp*) also interact by transvection; their complementation is sensitive to rearrangements similar to those described for BX-C (GELBART 1982). For both BX-C and *dpp*, transvection-disrupting rearrangements are thought to reduce pairing between the alleles, thus causing the loss of complementation. Transvection for both BX-C and *dpp* requires the normal function of the gene *zeste* (KAUFMAN *et al.* 1973; BABU and BHAT 1981; GELBART and WU 1982), which produces a protein that binds to multiple sites of chromosomal DNA (PIRROTTA *et al.* 1988).

Transvection effects have been described for nearly a dozen other loci, including the *white* gene in the *zeste*^{1} mutant background (the *zeste-white* interaction; GANS 1953; reviewed in WU and GOLDBERG 1989; PIRROTTA 1991), puffing at 64C (ASHBURNER 1967), the *Sgs-4* gene (KORGE 1977), and the *yellow* gene (GEYER *et al.* 1990). While these interactions have features similar to transvection at BX-C and *dpp*, there are some notable differences. For example, allelic interactions at BX-C and *dpp* are positive, that is, the phenotype is closer to wild type when the alleles are able to pair. The opposite is true for the *white* alleles in the *zeste-white* interaction (JACK and JUDD 1979). In addition, some interactions (*e.g.*, at BX-C, *dpp*, and *yellow*) require normal *zeste* function, while others do not (*e.g.*, the *zeste-white* interaction) (GANS 1953; KAUFMAN *et al.* 1973; GELBART 1982; GEYER *et al.* 1990).

In this study, we report striking interactions among certain alleles of *eya*. They are sensitive to similar kinds of transvection-disrupting rearrangements as BX-C and *dpp*, and are dependent on normal *zeste* function. These results indicate that transvection effects of a type similar to BX-C and *dpp* may be more general than previously thought.

MATERIALS AND METHODS

Fly strains: The alleles of *eya* used in this study are described in BONINI *et al.* (1993). All other mutations and balancer chromosomes are described in LINDSLEY and ZIMM (1992). The *zeste* mutation, $z^{ae(bx)}$, was obtained from E. B. LEWIS (California Institute of Technology).

Culture conditions: Flies were cultured on standard cornmeal medium at 25°. For cytological analysis of polytene chromosomes, larvae were grown at 19°, in a humidified atmosphere.

Screen for transvection-disrupting rearrangements: Males (either *al dp eya*^{2} or *eya*^{4}) were exposed to 4000 R, then mated to females mutant for the interacting allele. Approximately 2500 progeny from each combination were examined to select for individuals in which complementation was reduced, as judged by smaller eye size. Twenty of the reduced-eye progeny were rescreened by crossing to homozygotes of the interacting allele. From these, 11 lines were successfully established and analyzed, with the irradiated second chromosome balanced over *SM6a, al dp*^{lv2}*Cy pr sp*. These lines are referred to in the text as ETDs for *eya* transvection disruptor. Each line was tested over other *eya* alleles (*eya*^{3}, *eya*^{E1}, *eya*^{E4}, *eya*^{E11}, *eya*^{X8}, *eya*$^{E(P)10}$); and progeny scored for eye size and survival to adulthood.

Cytology: For cytological analysis of the salivary gland chromosomes, the second chromosome was balanced over *In(2LR) Gla, Gla Bc*. Males of this genotype were mated to CS or OR females. Salivary glands from *Bc*$^{+}$ crawling 3rd instar larvae were dissected, fixed in 45% acetic acid for 1–5 min, then treated in 1-2-3 solution (1 volume lactic acid, 2 volumes H_2O, and 3 volumes acetic acid) for 1–2 min, after which they were placed on a coverslip in a drop of lacto-acetic-orcein solution and allowed to stain for several minutes before squashing using standard techniques. To quantitate chromosomal pairing in ETD lines in *trans* to chromosomes of normal cytology or other ETD lines, two preparations of larval salivary glands were scored for each genotype. Every nucleus on the slide was examined; nuclei with scorable 2L chromosome arms ranged from 36 to 80% of the total nuclei on the slide. The total number of nuclei per slide ranged from 51 to 116.

Scanning electron microscopy: Flies, stored in 70% ethanol, were dehydrated for a minimum of 6 hr each in 85% and 95%, then three times in 100% ethanol. Next, the specimens were critical-point dried, mounted, and coated with gold-palladium 80:20. Microscopy was performed using 5 kV.

Quantitation of eye size: Ommatidia were counted either directly from scanning electron micrographs, or from nail polish replicas. Nail polish was brushed on the compound eye, allowed to harden, peeled off, then placed on a slide. The impressions left by the ommatidia were photographed through the microscope and counted.

RESULTS

The *eya* gene has been characterized for its function in eye development. Loss of function of the gene in the eye primordium, the eye portion of the eye-antennal imaginal disc, results in programmed cell death of the eye progenitor cells (BONINI *et al.* 1993). The amount of cell death in the eye disc is correlated with the degree of reduction in size of the adult compound eye. For most allele combinations, the phenotypes are consistent from individual to individual; the differences are readily observed in the stereo microscope.

Complementation at the *eya* locus: While the *eya* gene also has embryonic, ocellar and other functions (BONINI *et al.* 1993), we focus here on the compound eye phenotype, where a dramatic interaction between certain alleles can be observed. In Figure 1, this interaction is illustrated with two spontaneous alleles, *eya*^{2} and *eya*^{4}. Flies homozygous for *eya*^{2} are eyeless (Figure 1A); *eya*^{4} homozygotes have severely reduced eyes (Figure 1B). The penetrance of these phenotypes is complete: *eya*^{2} homozygotes are always eyeless; *eya*^{4} homozygotes always have fewer than 50 ommatidia. However, in the *eya*^{2}/*eya*^{4} *trans*-heterozygote, a roughly ¾ normal size eye is observed (Figure 1, C and D).

Two lines of evidence suggest that this partial complementation effect is not due to the genetic background: (1) recombinant lines, in which parts of the second chromosome were replaced and the other chromosomes were outbred, still show the effect; (2) other allele combinations show a similar effect. By testing a large

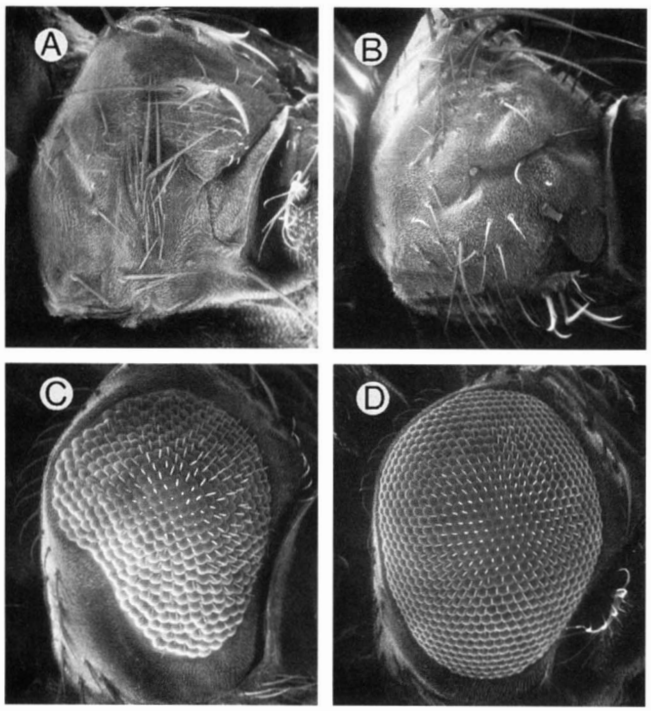


FIGURE 1.—Partial complementation by two alleles of *eya*. Scanning electron micrographs showing the adult eye phenotypes. (A) eya^2/eya^2, (B) eya^4/eya^4, (C) eya^2/eya^4, (D) Canton-S (wild type).

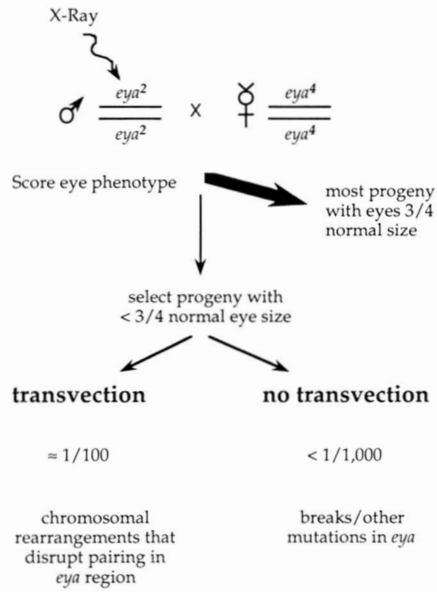


FIGURE 2.—X-ray screen to test for transvection. Two alternative outcomes are illustrated, depending on whether or not the interaction between eya^2 and eya^4 is altered by rearrangements of eya^2 to a different chromosomal position. The reciprocal screen was also performed, in which eya^4 males were irradiated and mated to eya^2 females.

number of alleles against each other, one can place eya^1 and eya^2 into one intragenic complementation group; eya^3, eya^4, along with others, form a second group. A third set of alleles shows no interaction with either group.

We considered the possibility that the complementation groups represent different genes, *i.e.*, that eya^1 and eya^2 are not allelic to the other group. Out of 40 mutations tested that fail to complement eya^1 or eya^2, all map to the *dumpy-spade* interval of chromosome 2L where the *eya* gene maps. Six breakpoint alleles of *eya* have been mapped at the molecular level to a 25-kb region at polytene bands 26EF. The six breakpoint alleles fail to complement any other alleles, suggesting that, if two different genes are involved, they both map within the 25-kb span of the breakpoints. The *eya* gene produces two alternatively spliced transcripts. However, since transformation with cDNAs of either type can rescue members of either group (LEISERSON 1994; N. M. BONINI and W. M. LEISERSON, unpublished), it seems unlikely that this alternative splicing can account for the interallelic interactions.

Transvection-disrupting rearrangements reduce *eya* allelic interactions: If transvection underlies the described interactions, then one should be able to isolate rearrangements that disrupt these effects (*i.e.*, give smaller eyes). In similar screens (LEWIS 1954; GELBART 1982) approximately 1% of the progeny of X-irradiated males had rearrangements that disrupted transvection. A comparable value was anticipated for rearrangements with breakpoints between the *eya* gene, located at 26EF, and the centromere. To test whether *eya* alleles undergo transvection, we irradiated eya^2 or eya^4 males and crossed them to females homozygous for the interacting allele (Figure 2). The majority of the progeny should have eyes that are ¾ the size of wild type, the phenotype ordinarily seen in eya^2/eya^4 heterozygotes. If the alleles complement by transvection, approximately 1% of progeny should show reduced eyes due to transvection-disrupting rearrangements. This frequency is substantially higher than the frequency expected for new lesions in the *eya* gene, or dominant modifier mutations, both of which could cause a reduced eye phenotype.

A total of approximately 5,000 progeny were scored from the reciprocal screens. A broad spectrum of eye sizes was observed, ranging from 3/4 wild type (the typical eya^2/eya^4 phenotype) to eyeless. Over 30 flies had eyes that were reduced by a factor of 2 or more. Twenty of those were bred for further analysis, from which 11 independent lines were successfully established (Table 1). These are referred to as "*eya* transvection disruptor" (ETDs).

Each line was tested genetically with various *eya* alleles to determine whether any chromosomes had suffered a lesion in the *eya* gene itself. Based on earlier screens, the predominant phenotype of X-ray induced lesions in the *eya* gene is lethality. Since both eya^2 and eya^4 are viable alleles, lethality was a convenient assay for secondary lesions in these alleles. Of the eleven lines, only one (ETD4.6) failed to complement lethal *eya* alleles, and thus appeared to have an induced lesion of the *eya* gene.

TABLE 1

Analysis of *eya* transvection-disrupting lines

Line[a]	No. of ommatidia[b]	Rearrangement	Remarks
ETD4.2	225 ± 31 (n = 12)	*T(2; 3) 28D; 67A* and *In(3L) 61A; 65E*	
ETD4.3	87 ± 45 (n = 11)	*T(2; 3; 4) 30A; 101; 98D*	Cyclical translocation: tip 2L to *4*; tip *4* to *3*R; tip *3*R to 2L
ETD4.4	338 ± 44 (n = 15)	*26D; 40; 58; 81*	Complex
ETD4.5	164 ± 26 (n = 15)	*In(2LR) 26EF; 51CD*	
ETD4.6	0	*Df(2L) 25E; 26F*	
ETD4.8	181 ± 18 (n = 9)	*T(2;Y) 28F*	
ETD2.1	231 ± 34 (n = 11)	*T(2;3) 35; 40; 41; 94A*	Complex
ETD2.2	204 ± 20 (n = 12)	*In(2LR) 29C; 41*	
ETD2.3	278 ± 34 (n = 12)	*Tp(2;2) 33B-E; 40-41*	
ETD2.4	224 ± 19 (n = 13)	*T(2; 3) 28E; 90C*	
ETD2.5	182 ± 12 (n = 8)	*T(2; 3)27F; 80*	

[a] Nomenclature: ETD (*eya* transvection disruptor); ETD2 lines were derived from eya^2 chromosomes; ETD4 lines were derived from eya^4 chromosomes.

[b] Eye phenotype of flies with the chromosome in *trans* to the interacting allele (eya^2 or eya^4, as appropriate). The number of ommatidia in a wild-type eye is 755 ± 46 (n = 4).

The other ten lines were candidates for transvection-disrupting rearrangements.

All lines were analyzed for chromosomal rearrangements by examining squashes of salivary gland polytene chromosomes. The results are listed in Table 1. As anticipated, the ETD4.6 line has a deficiency spanning the *eya* region, confirming that the secondary mutation is intragenic. The other ten lines have rearrangements with breakpoints on 2L proximal to *eya*, such that the distal portion of 2L bearing the *eya* locus is rearranged to another chromosome arm (Figure 3). This fits the two criteria cited above for transvection-disrupting rearrangements of the type described by LEWIS.

The rearrangements are plotted in Figure 3. Judging from the breakpoint locations on chromosome 2L, a critical region extends from *eya* at chromosome division 26 to at least division 33. The breakpoint in ETD4.4 at division 40 is not necessarily in this critical region, since the disruption of pairing observed in that line may depend in part on the distal breakpoint in 26D. Such rearrangements were recovered as disruptors of transvection at *dpp*, but were excluded from the identification of the critical region, because rearrangements with such proximal breakpoints were always found to also have breakpoints distal to the transvecting gene (GELBART 1982). Examples of reduced eyes resulting from the transvection-disrupting rearrangements are shown in Figure 4, B and C (compare to A). Eye sizes were quantified by counting the number of ommatidia (Table 2). The eyes shown in Figure 4B and 4C are typical for most of the lines obtained, ranging from ⅛ to ¼ of normal size. Two lines (ETD4.4 and ETD2.3) showed slightly larger eyes, ranging from ¼ to ½ of normal. Based on the experience of LEWIS (1954) and GELBART (1982) who observed that approximately 1% of the progeny of X-irradiated males has rearrangements that disrupted transvection, the probability of obtaining 10 such lines at random for *eya* would be exceedingly small (10^{-20}).

We attempted to restore chromosomal pairing of rearranged alleles by putting two rearrangements with similar breakpoints in *trans*. If chromosomal pairing is the determining factor, then restoring the pairing between rearranged chromosomes should restore the interaction. Results published for *dpp* suggested that the ETD lines with rearrangements involving heterochromatin would be likely to pair in *trans* to one another (GELBART 1982). The ETD4.3 line was crossed to all the lines bearing rearranged eya^2 chromosomes. Three lines with rearrangements involving heterochromatin (ETD2.2, ETD2.3 and ETD2.5) all gave progeny with eyes larger than controls of the genotype $R(eya^2)/eya^4$. One such example is shown in Figure 4D (compare with Figure 4B and 4C; Table 2). In contrast, two lines (ETD2.1 and ETD2.4) gave progeny with smaller eyes than controls (data not shown). One of these lines (ETD2.4) has a rearrangement involving euchromatin, not heterochromatin.

Examination of polytene chromosomes from larvae of the genotype ETD2.5/ETD4.3 revealed that the arms of chromosome 2L were indeed paired in many of the nuclei [Figure 5, E–H; percent paired 82 ± 7% (mean ± range)]. In contrast, the 2L arms are more frequently unpaired in controls, ETD4.3/eya^2 (Figure 5, A–D; percent paired 36 ± 3% in ETD4.3/+, and 32 ± 8% in ETD2.5/+). The difference in pairing of the *eya* region between the genotypes demonstrates that the allelic complementation is increased in combinations of rearrangements in which the *eya* regions are more likely to undergo pairing.

Normal *zeste* function is required for *eya* transvection: Transvection at the BX-C and *dpp* has been shown to be sensitive to loss-of-function mutations of *zeste* (KAUFMAN *et al.* 1973; GELBART and WU 1982). The *zeste* gene appears to provide several functions that can be differentially mutated. In addition to supporting transvection, the *zeste* gene increases transcription of the *white* gene and has been proposed to provide some vital function (reviewed in PIRROTTA 1991). The *zeste* protein

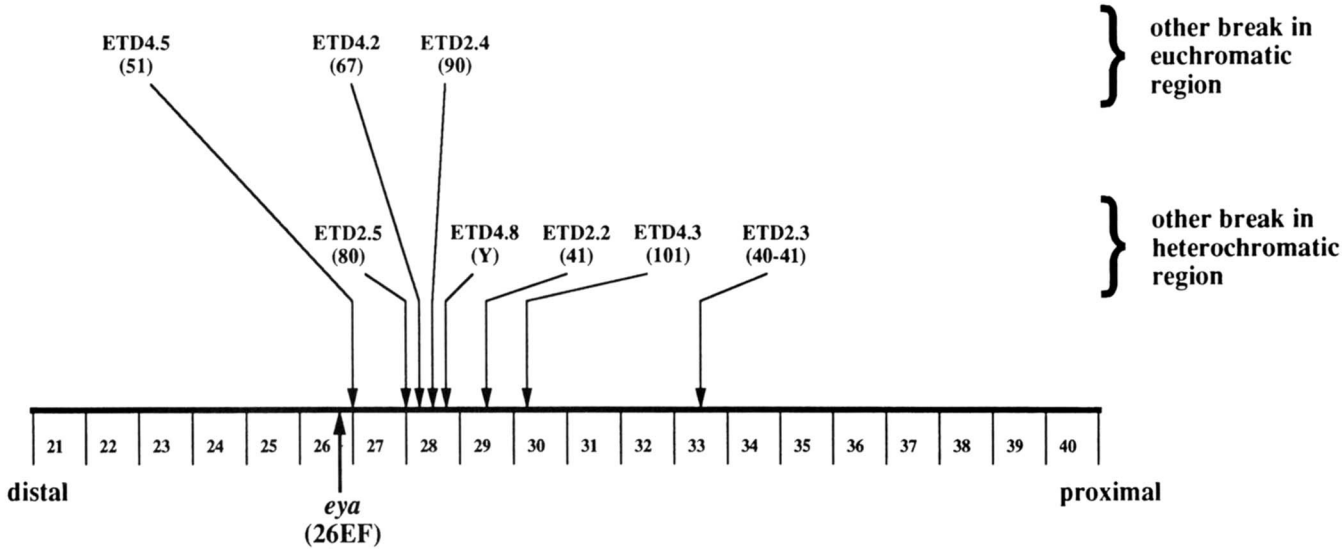


FIGURE 3.—Distribution of eya transvection-disrupting breakpoints. The *x* axis represents the segments of polytene chromosome 2L. The breakpoint on 2L of each mutant strain is indicated. In parentheses is shown the location of the other breakpoint to which the distal portion of 2L has rearranged. The upper level corresponds to rearrangements having their second breakpoint in euchromatic regions, the lower level to ones in heterochromatic regions. The critical region spans from *eya* to division 33. ETD2.1 and ETD4.4 are not represented because they have two breakpoints on 2L, and therefore the breakpoints do not necessarily fall within the critical region.

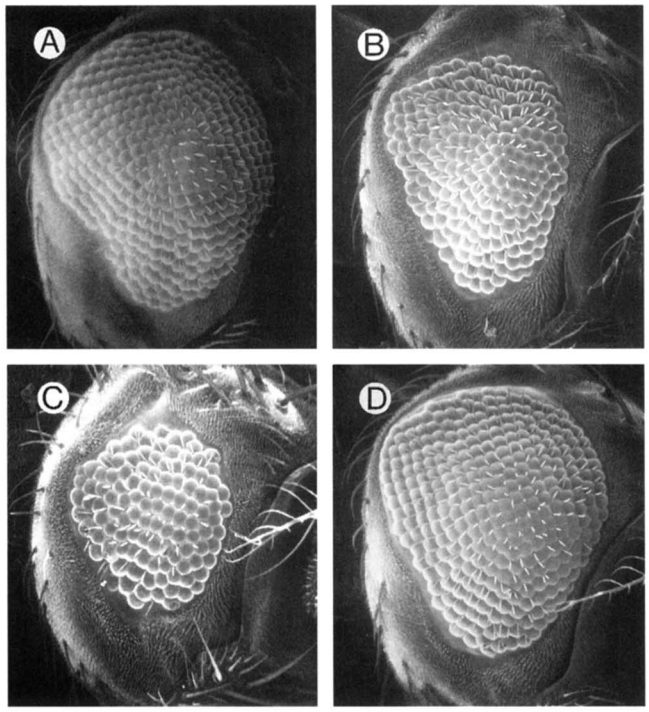


FIGURE 4.—Disruption and restoration of transvection between alleles of *eya*. (A–D) Scanning electron micrographs showing the eye phenotypes of mutants bearing different combinations of *eya* transvection-disrupting rearrangements. Flies bearing similar rearrangements of the eya^2 and eya^4 alleles show a restoration of the transvection effect. (A) eya^2/eya^4; (B) ETD2.5, eya^2/eya^4; (C) ETD4.3, eya^4/eya^2; (D) ETD2.5, eya^2/ETD4.3, eya^4.

is made up of 575 amino acid residues. Normally found as a multimer, it can bind DNA at specific sites. The z^1 mutation encodes a neomorphic function that represses, instead of activating, transcription of *white*. The z^a series of alleles, on the other hand, are hypomorphic, and, along with others, have been shown to reduce the transvection effect at BX-C and *dpp*.

TABLE 2

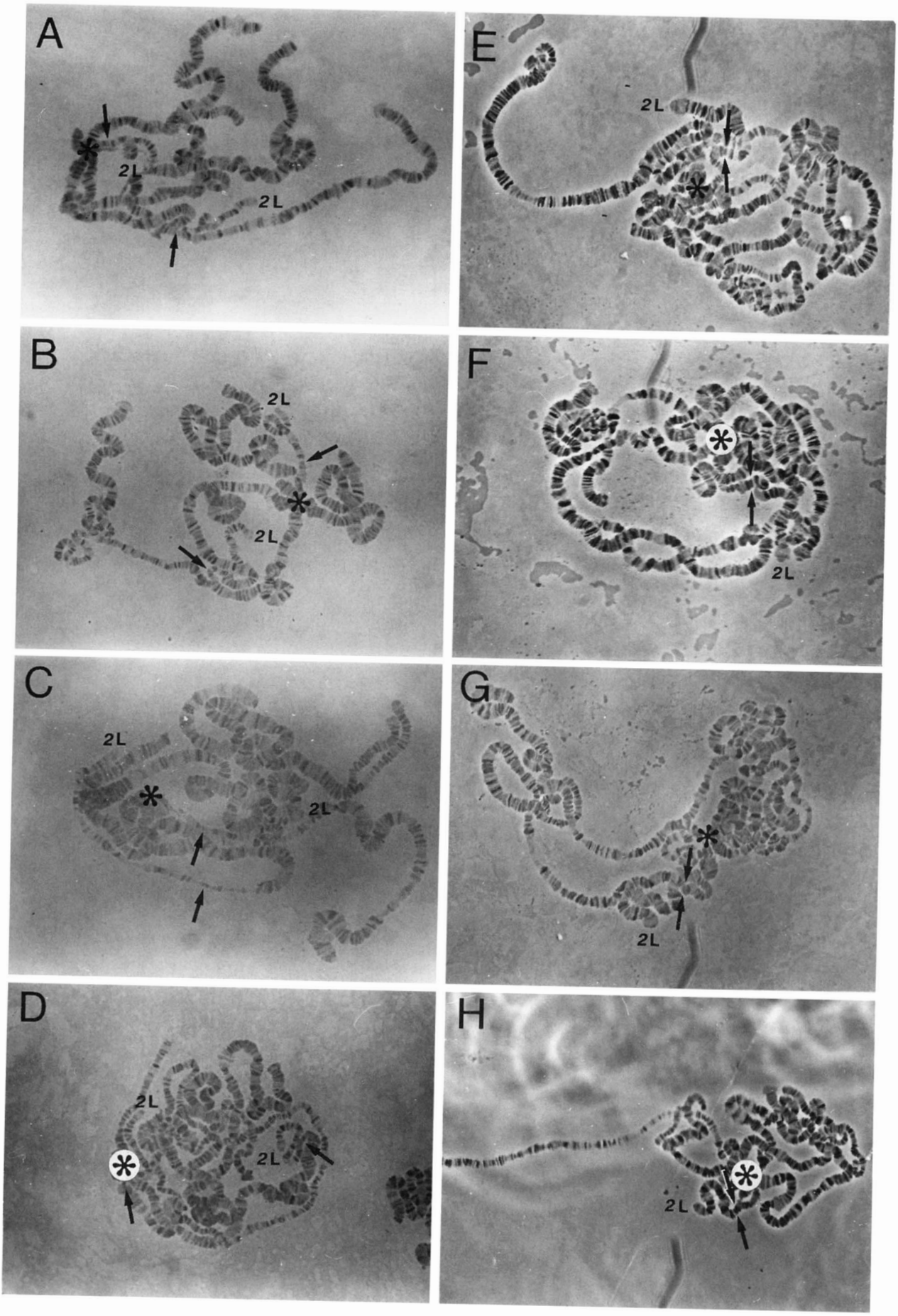
Quantitation of transvection-disruption illustrated in Figures 4 and 6

Figure	Genotype	No. of ommatidia
4A, 6A	+; *al dp* eya^2/*al dp* eya^4	494 ± 42 (n=11)
4B	+; ETD2.5, eya^2/eya^4	182 ± 12 (n= 8)
4C	+; ETD4.3, eya^4/eya^2	87 ± 45 (n=11)
4D	+; ETD2.5, eya^2/ETD4.3, eya^4	319 ± 30 (n=11)
6B	$z^{ae(bx)}$; *al dp* eya^2/*al dp* eya^4	178 ± 29 (n=10)

For this study, we used the $z^{ae(bx)}$ allele, which was recovered by Lewis in a screen for mutations that enhance the bx^{34e}/Ubx phenotype. This allele contains an inversion that breaks in the coding region, truncating the protein at position 310 of the amino acid sequence, thereby impairing its ability to complement the z^1 allele or to support transvection (PIRROTTA 1991). Flies of genotype $z^{ae(bx)}$; eya^2/eya^4 (Figure 6B) have eyes that are roughly half the size of control flies (z^+; eya^2/eya^4) (Figure 6A). This shows that the complementation at *eya* depends on *zeste* function.

DISCUSSION

The interaction of *eya* alleles is a transvection effect mediated by chromosomal pairing. Three lines of evidence support this assertion. First, X-ray-induced transvection-disrupting rearrangements occurred in all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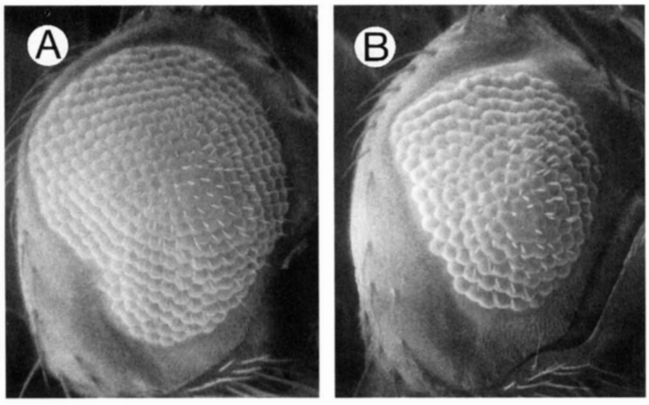

FIGURE 6.—A loss of function zeste mutation suppresses transvection at *eya*. (A and B) Scanning electron micrographs show eye phenotypes. (A) z^{+}; eya^{2}/eya^{4}. Transvection occurs. (B) $z^{ae(bx)}$; eya^{2}/eya^{4}. Transvection is reduced.

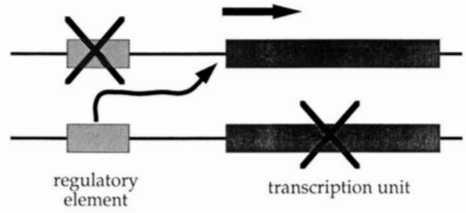

FIGURE 7.—Model for transvection. Simplified view of a gene, consisting of a regulatory region which can act on an associated transcription unit. The interacting alleles, shown paired on homologous chromosomes, have different kinds of mutations. The upper one has a defective regulatory region, while the lower one has a defect in the transcription unit. Either allele, when homozygous, would produce little gene function. In *trans*, however, the normal regulatory region of one allele can act on the normal transcription unit of the other, resulting in enhanced activity. Disruption of chromosomal pairing prevents this interaction by removing the regulatory region from the vicinity of the transcription unit (after PIRROTTA 1990).

10 lines examined. (The 11th was a hit in the *eya* gene.) The probability of obtaining this result by chance is indeed small. Second, pairing of rearrangements to restore chromosomal synapsis restores the interaction. Third, $zeste^{+}$ function is required for these interactions. By these criteria, the interaction at *eya* is similar to transvection effects described for BX-C and *dpp*.

Pairing of alleles as a crucial requirement for transvection effects has been suggested from examination of the salivary gland polytene chromosomes (see Figure 5; LEWIS 1954; GELBART 1982). This is relevant to other cells because the chromosomes of all somatic cells are paired in Diptera; when condensing chromosomes become visible in early prophase, they are already paired with their respective homologs (METZ 1916). Sophisticated imaging of probes hybridized *in situ* in embryonic nuclei has also shown alleles to be paired (HIRAOKA *et al.* 1993).

Although all the ETD lines we recovered disrupted transvection at *eya*, none completely eliminated pairing and none completely eliminated the allelic complementation. The amount of complementation in eya^{2}/eya^{4} structural heterozygotes, as measured by the eye phenotype, correlates with the degree of pairing observed in the salivary gland chromosomes (see Figure 5 and Table 2). This observation, which has also been reported for the BX-C and *dpp*, is shown here to stand the test of quantification. Therefore, any proposed mechanism underlying transvection at *eya* must take into account that the response of gene activity is commensurate with the degree of pairing.

Molecular mechanisms: For alleles of *Cbx* (of BX-C) it has been shown that expression of one allele is dependent on its being paired with the other (CASTELLI-GAIR *et al.* 1990); the same is true of *Sgs-4*, a salivary gland secretion gene (KORNHER and BRUTLAG 1986). Several models have been proposed; one with substantial experimental support is a *trans* effect of *cis*-regulatory enhancer elements (PIRROTTA *et al.* 1985; ZACHAR *et al.* 1985) (Figure 7). It has been proposed that enhancers can interact with the transcriptional machinery at the promoter by looping out intervening DNA (PTASHNE 1986). This view is bolstered by experiments demonstrating that enhancer elements need not reside on the same DNA molecule from which transcription is initiated (DUNAWAY and DRÖGE 1989; MÜLLER *et al.* 1989; MÜLLER and SCHAFFNER 1990; WEDEL *et al.* 1990).

The *trans* effect of *cis*-regulatory elements appears to explain a subset of transvection effects. For example, *Cbx* is a regulatory mutation that misexpresses *Ubx* in the wing disc (WHITE and AKAM 1985; CABRERA *et al.* 1985). It has been demonstrated that, in *trans*, *Cbx* can misregulate *Ubx* (CASTELLI-GAIR *et al.* 1990). Regulation in *trans* is also consistent with the analysis of *dpp* (ST. JOHNSTON *et al.* 1990) and *yellow* (GEYER *et al.* 1990), where defects of transcriptional regulation appear to be complemented by a *trans* allele that has a defective transcription unit but an intact enhancer element.

Our present knowledge of the *eya* gene supports this view of *trans* action. One class of alleles (eya^{1}, eya^{2}) affect only the eye while the interacting alleles (eya^{4} and others) affect additional structures. Both eya^{1} and eya^{2} are spontaneous in origin, and appear to be specifically defective in expression of the *eya* gene in the eye disc, as judged by *in situ* hybridization and antibody staining (BONINI *et al.* 1993; data not shown). A simple interpretation is that both alleles have defects that eliminate activity of an eye disc-specific enhancer element. This interpretation is consistent with the molecular analysis:

FIGURE 5.—Correlation of transvection with chromosomal pairing. Squashes of polytene chromosomes bearing transvection-disrupting rearrangements. The *eya* gene location is indicated by the arrows. The chromocenter is marked by the asterisks. (A–D) ETD2.5/eya^{4}. In this combination, the *eya* region is frequently unpaired; transvection is disrupted. (E–H) ETD2.5/ETD4.3. Here the *eya* region is often paired; transvection occurs.

both alleles appear to be small deletions outside the coding region (NANCY BONINI, unpublished). The alleles that partially complement them may have lesions in the *eya* coding region (see Figure 7). In the eya^4 complementation group, the eya^4 allele is of spontaneous origin; no information is currently known about its molecular defect. Many of the alleles were induced with the mutagen ethylmethane sulfonate, however, and show normal chromosomal cytology, consistent with the idea that they are point mutations. It seems likely that the interacting groups of *eya* alleles represent regulatory enhancer mutations on the one hand, and mutations of the protein on the other.

Different classes of transvection: Some transvection effects appear to be less sensitive to reduction of allele pairing than BX-C, *dpp* and *eya*. An example is the *zeste-white* interaction. This was shown by using transposon insertions of the *white* gene in *cis* to *dpp* or BX-C (SMOLIK-UTLAUT and GELBART 1987). Rearrangements that disrupt transvection at *dpp* and BX-C have no effect on the *zeste-white* interaction. In screens for rearrangements that disrupt the *zeste-white* effect, only breakpoints very close to *white* are recovered (GANS 1953), while for *eya*, breakpoints at a considerable distance are recovered. Breakpoints located very near *eya* do not differ much, with respect to the disruption of transvection, from breakpoints located farther away. For the *zeste-white* interaction, the disruption of the effect appears to be all-or-none, while transvection *eya* varies, *pari passu*, with the amount of pairing of the *eya* alleles.

One intriguing possibility is that these differences may depend upon how the genes are regulated. Interactions at "sensitive" loci might respond quantitatively to chromosomal synapsis (*e.g.*, BX-C, *dpp* and *eya*) requiring constant *trans* interaction to maintain maximum gene expression. Other types of transvection, such as *zeste-white*, might need only a short period of *trans* interaction, after which the transcriptional state of the gene remains imprinted. This view is consistent with emerging evidence for a dynamic state of synapsis in the nucleus (HIRAOKA *et al.* 1993). "Amount of pairing" may actually translate into "fraction of time paired." This dynamic view is quite different from the static impression derived from examining synapsis in fixed squashes of polytene chromosomes.

In view of the different classes of transvection, it is striking that the three examples of "sensitive" transvection operate in large genes (over 30 kb) with complex expression patterns and large transcriptional regulatory regions. All three genes, BX-C, *dpp* and *eya*, have vital functions and involve one allele that affects a specific adult tissue.

Implications for mammals: There is evidence that mammalian chromosomes in somatic tissue also can pair; *in situ* hybridization to human chromosomes in interphase suggests that certain regions may be paired in some tissues and unpaired in others (ARNOLDUS *et al.* 1991; MARASCHIO *et al.* 1992; LEWIS *et al.* 1993; WU 1993). Somatic pairing has been postulated as a mechanism for mitotic recombination in mammals (HENSON *et al.* 1991; TARTOF and HENIKOFF 1991).

The basic framework of transcription regulation is shared by eukaryotes, as seen in the homology among *cis*-acting regulatory sequences and the *trans*-acting factors that interact with them (JOHNSON and MCKNIGHT 1989; PABO and SAUER 1992; KINGSTON and GREEN 1994). This homology has been extended by discovery of the "chromo" domain in Drosophila and mouse proteins, which may link chromatin structure to control of transcription (PARO 1990; WU 1993). Transgenic studies have shown that many gene regulatory elements can function in heterologous species [*e.g.*, "insulators" in mice and Drosophila (CHUNG *et al.* 1993)]. BX-C and *dpp* loci have known mammalian homologs, the Hox and TGF-β gene families, respectively (AKAM 1989; GELBART 1989). Transvection in Drosophila may provide insights into gene regulation and chromosome structure applicable to mammals.

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