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A Quantitative Measure of the Mitotic Pairing of Alleles in *Drosophila melanogaster* and the Influence of Structural Heterozygosity

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ABSTRACT

In Drosophila there exist several examples of gene expression that can be modified by an interaction between alleles; this effect is known as transvection. The inference that alleles interact comes from the observations that homologous chromosomes pair in mitotically dividing cells, and that chromosome rearrangements can alter the phenotype produced by a pair of alleles. It is thought that heterozygous rearrangements impede the ability of alleles to pair and interact. However, because the existing data are inconsistent, this issue is not fully settled. By measuring the frequency of site-specific recombination between homologous chromosomes, we show that structural heterozygosity inhibits the pairing of alleles that lie distal to a rearrangement breakpoint. We suggest that some of the apparent conflicts may owe to variations in cell-cycle lengths in the tissues where the relevant allelic interactions occur. Cells with a longer cell cycle have more time to establish the normal pairing relationships that have been disturbed by rearrangements. In support, we show that *Minute* mutations, which slow the rate of cell division, partially restore a transvection effect that is disrupted by inversion heterozygosity.

THE phenomenon of homologous chromosome pairing in the mitotically dividing cells of Dipteran species is thoroughly demonstrated by classical and modern evidence. STEVENS (1908) first noted that the chromosomes from diploid cells of several species of Diptera, including *Drosophila melanogaster,* lie near their homologues in metaphase spreads. METZ (1916) examined the mitotic chromosomes from ~80 species of Diptera and in all cases observed that homologous pairs were associated in prophase, metaphase and telophase, with homologues showing the closest pairing at prophase and telophase. More recent studies establish what those results hinted at: that homologues are intimately synapsed during interphase. LIFSCHYTZ and HAREVEN (1982), KOPCZYNSKI and MUSKAVITCH (1992) and HIRAOKA *et al.* (1993) used *in situ* hybridization to show that in interphase cells of *D. melanogaster* the two alleles of the genes they examined (the *histone* gene cluster and the *Delta* locus) were so tightly paired that they could not be resolved with the light microscope. Mitotic recombination studies suggest that mitotic pairing can be quite precise (FRIESEN 1936; STERN 1969).

It is widely accepted that certain genetic phenomena owe their properties to the existence of mitotic pairing of homologues (TARTOF and HENIKOFF 1991). These include transvection [the complementation of certain allelic combinations at the Bithorax complex (LEWIS 1954), *decapentaplegic* (GELBART 1982), *eyes absent* (LEISERSON *et al.* 1994), *yellow* (GEYER *et al.* 1990), and *Sex combs reduced* (PATTATUCCI and KAUFMAN 1991)], the suppression of *white*$^+$ by mutations in *zeste* (GANS 1953; JACK and JUDD 1979), and the dominant inactivation of *brown*$^+$ alleles by the *bw*^{D} mutation (HENIKOFF and DREESEN 1989). These conclusions were arrived at largely on the basis that heterozygous chromosome rearrangements disrupt these interactions, and because the allelic interactions are, in general, restored in animals that are homozygous for a rearrangement. Some unusual effects on gene expression seen with certain transgenes have also been attributed to the pairing of alleles in mitotic cells, in part because some of the effects are seen when the insertions are homozygous, but not when hemizygous (KASSIS *et al.* 1991; HAZELRIGG and PETERSEN 1992; CHUNG *et al.* 1993; FAUVARQUE and DURA 1993; SCHOLZ *et al.* 1993; STURTEVANT *et al.* 1993; DORER and HENIKOFF 1994; KASSIS 1994; KAPOUN and KAUFMAN 1995). In one case it has been shown that the interaction of transgene alleles can also be disrupted by inversion heterozygosity (GINDHART and KAUFMAN 1995). Thus, the fact that homologues pair in nonmeiotic cells is of significant biological interest, yet we have little evidence as to the normal function of homologous pairing. An obvious way to approach this question would be to produce animals in which pairing is disrupted and examine them for abnormalities. But, flies that carry heterozygous chromosome rearrangements can appear quite normal.

A substantial difficulty in this field is the lack of a clear demonstration that heterozygous rearrangements disrupt pairing and the extent of that disruption. In none of the cases of transvection has it been directly shown that rearrangements disrupt the mitotic pairing

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of homologous chromosomes in diploid cells: disruption of pairing has been inferred from alterations in gene activity. This becomes troubling when the effects of rearrangements on different phenomena are compared: the answer to the question of whether a certain type of rearrangement disrupts pairing differs, depending upon which interaction is used as the measure of pairing. For instance, we can compare results obtained at Bithorax (BX-C), *decapentaplegic* (*dpp*), *eyes absent* (*eya*), *white* (*w*), and *brown* (*bw*). Rearrangements that disrupt transvection at BX, *dpp* or *eya* have one breakpoint in what has been termed the critical region. The critical regions at these loci are large, encompassing most or all of the euchromatic segment of the chromosome arm that lies proximal to each locus. These results lead to the conclusion that most rearrangements with one breakpoint between the centromere and the locus will disrupt the pairing of alleles at that locus. However, when either the *zeste-white* or the bw^D-bw^+ interactions are considered, only rearrangements with breakpoints in the immediate vicinity of the respective loci disrupt the allelic interactions (SMOLIK-UTLAUT and GELBART 1987; HENIKOFF and DREESEN 1989; DREESEN *et al.* 1991; HENIKOFF *et al.* 1995). These differences cannot be explained by supposing that there are chromosome-specific pairing differences. SMOLIK-UTLAUT and GELBART (1987) examined transpositions of *white*$^+$ to the BX critical region and to the *dpp* locus and showed that the *white* gene's restricted region of sensitivity to rearrangements is intrinsic to the gene and is not a property of its normal location at the tip of the *X* chromosome.

Further complicating the issue, MERRIAM and GARCIA-BELLIDO (1972) examined somatic clones produced by X-ray-induced mitotic recombination in heterozygotes for pericentric inversions of chromosome *3* and concluded that the chromosome *3* homologues were paired. GARCIA-BELLIDO and WANDOSELL (1978) used the same approach to examine the heterozygous effect of six inversions on *X* chromosome mitotic pairing. Their data indicated that pairing was reduced in only two cases; pairing was not reduced by four of the inversions. HIRAOKA *et al.* (1993) made a direct assessment of pairing at the histone gene complex in early embryos. They found that embryos carrying one allele on a translocation had a reduced frequency of pairing. However, only that single rearrangement was studied, and the number of embryos examined was quite limited.

The cytological examination of interphase pairing in diploid cells is technically difficult. In comparison, the examination of pairing in the interphase cells of salivary glands is straightforward, and there is no doubt that rearrangements can disrupt pairing of the polytenized homologues in these cells. The pairing of alleles at *dpp*, *bw* and *eya* has also been studied in the polytene chromosomes of animals heterozygous for rearrangements that disrupt the *trans* interactions at these loci. In these cases an increase in the frequency of asynapsis was observed (cited in SMOLIK-UTLAUT and GELBART 1987; HENIKOFF and DREESEN 1989; LEISERSON *et al.* 1994), but it is not clear that polytene chromosome pairing, which is measured in cells that have not divided for several days, necessarily bears a strict relationship to pairing in diploid cells that undergo cycles of chromosome condensation, segregation, and decondensation two or three times a day. For instance, heterozygosity for a chromosome rearrangement causes an increase in asynapsis of polytene chromosomes, not only for the pair of chromosomes involved, but also for chromosomes that are not involved in the rearrangement (LEFEVRE 1976).

In this work we set out to determine whether heterozygous chromosome inversions disrupt the pairing of alleles on those chromosomes in mitotically dividing cells. We did this without using an assay that depends on transvection. The approach we took uses the site-specific recombination system of the yeast 2μ plasmid. The *FLP* gene encodes the recombinase from this plasmid. When it is placed under the control of the Drosophila *hsp70* promoter (as is the case with the *70FLP* construct used in these experiments), it can be induced to high levels with a brief heat shock (GOLIC and LINDQUIST 1989). In the male germline, heat shock induces transcription of *hsp70*-promoted fusion genes in the mitotically dividing stem cells [BONNER *et al.* (1984); see MATERIALS AND METHODS; see LINDSLEY and TOKUYASU (1980) or FULLER (1993) for an overview of the male germline]. FLP efficiently catalyzes mitotic recombination between *FRT*s (FLP recombination target) that have been placed at allelic sites (GOLIC 1991). When a *70FLP*-bearing male is homozygous for an insertion of an *FRT*-bearing *P* element and heterozygous for flanking marker genes, the frequency of mitotic recombination between those allelic *FRT*s can be measured with a testcross. Normally there is no recombination in *D. melanogaster* males so the recombinants that are produced will result from FLP-mediated recombination between *FRT*s. FLP requires no yeast factors to function, and the purified protein catalyzes recombination between *FRT*s *in vitro* (MCLEOD *et al.* 1984; MEYER-LEON *et al.* 1984) in a reaction that requires that the recombining sites pair (SENECOFF and COX 1986) and proceeds through a Holliday structure intermediate (MEYER-LEON *et al.* 1990). Thus, the frequency of induced mitotic recombination will depend on the amount of FLP that is synthesized, the *FRT*s, and how frequently those *FRT*s pair. In the experiments described here, we generated males that carried the identical insertion of an *FRT*-bearing *P* element on both chromosome *3* homologues. These males were either homozygous for iso-sequential chromosomes *3* or heterozygous for a normal chromosome *3* and an inverted homologue. We carefully controlled the heat shock and

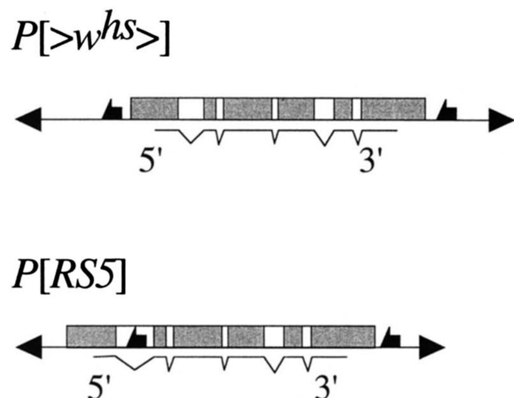

FIGURE 1.—The *FRT*-bearing *P* elements. Small solid arrowheads indicate the *P*-element terminal inverted repeats. Solid half-arrows indicate *FRT*s. The shaded boxes indicate the w^{hs} gene. Transcripts are indicated below the genes. In $P[>w^{hs}>]$ the *FRT*s flank the w^{hs} gene. In *P*[*RS5*] one of the *FRT*s is located in the first intron of w^{hs}: it does not interfere with w^{hs} function. In each case, excision of the *FRT*-flanked portion causes loss of *white* function.

culture conditions used for the induction of FLP and then measured the frequencies of FLP-mediated recombination that occurred in the germlines of these males. Under these circumstances the relative frequencies of recombination between homologues must reflect the relative pairing efficiencies of the allelic *FRT*s in animals with different chromosomal organization.

MATERIALS AND METHODS

All flies were raised at 25°. Descriptions of mutations not specifically discussed below are given by LINDSLEY and ZIMM (1992).

***P*-element constructs:** Two *FRT*-bearing *P*-element constructs were used in this work: *P*[*RS5*] and $P[>w^{hs}>]$. The construction of $P[>w^{hs}>]$ is described by GOLIC and LINDQUIST (1989). The construction of *P*[*RS5*] will be described elsewhere. Their structures are diagramed in Figure 1. Each carries two directly repeated *FRT*s and a functional *white* gene (designated w^{hs}). The insertions *P*[*RS5*]1A, $P[>w^{hs}>]$1A and *P*[*RS5*]2A are located at cytological loci 65B, 67A and 88B, respectively. The *70FLP* gene construct is similar to the *hsFLP* construct described by GOLIC and LINDQUIST, except that the 3′ noncoding sequences from the *hsp70* gene have been substituted for the yeast *FLP* 3′ sequence (R. PETERSEN, personal communication). Its construction will also be described elsewhere. The *70FLP* gene has a high level of constitutive activity in the eye. In the crosses of Figure 2 this constitutive activity provides the ability to select G_2 flies that carry *70FLP* by looking for eye-color mosaicism, even in flies that have not been heat-shocked. *70FLP*3F is an insertion of this construct on the *X* chromosome. The suffix 3F is an arbitrary isolate designation, not a cytological location.

Inversion stocks: The following inversion (*In*) stocks were obtained from the Drosophila Stock Center in Bloomington, Indiana: *In*(*3L*)*D*/*Gl*: the inversion breakpoints are 69D3-E1;70C13-D1; *pn2*; *In*(*3LR*)*224*: the inversion breakpoints are 69E7-F1;83B7-C1; *pn2*; *In*(*3LR*)*C190*/*TM6*, *Ubx*: the inversion breakpoints are 69F;89D; *In*(*3LR*)Ubx^{101}/*Sb*: the inversion breakpoints are 71B-C;89D9-E1; *pn2*; *In*(*3LR*)*225*/*TM3*, *Sb*: the inversion breakpoints are 77E1;88E2-3.

Each of the above inversion stocks was crossed to w^{1118}; *cu* kar^2 *Sb*/*TM6*,*Ubx* e^s (or in the cases of *In*(*3L*)*D*/*Gl* and *In*(*3LR*)Ubx^{101}/*Sb*, the flies were crossed to w^{1118}) to cross in the w^{1118} null mutation. The w^{1118} inversion stocks were then crossed to w^{1118}; *ru h* $P[>w^{hs}>]$1A *th cu* e^s and to w^{1118}; *ru* *P*[*RS5*]1A *h th cu* e^s flies. *In224* was also crossed to w^{1118}; *ru h th cu* *P*[*RS5*]2A e^s. Females heterozygous for each insertion and each inversion were backcrossed to w^{1118}; *ru h th cu* e^s males. Recombinant progeny that carried the inversion and one of the insertions on the same chromosome were recovered by screening for offspring with pigmented eyes that carried the mutant alleles of the marker genes distal to the *P*-element insertion and the wild-type alleles of the marker genes that lay in the inverted region of the chromosome (see Figure 5). In the case of *In*(*3L*)*D* the *D* mutation was also followed, and in the case of *In*(*3LR*)Ubx^{101} the *Ubx* mutation was also followed. Stocks were made by crossing to w^{1118}; *cu* kar^2 *Sb*/*TM6*,*Ubx* e^s. Each inversion was verified cytologically by examining polytene chromosome cytology of *ru* *P*[*RS5*]1A *In*/+ animals. Our determination of *In*(*3LR*)Ubx^{101} does not match that reported in LINDSLEY and ZIMM, but the inversion we received under this name quite clearly involves both arms with the breakpoints specified above. We also constructed *P*[*RS5*]1A, $P[>w^{hs}>]$1A and $P[>w^{hs}>]$2A-bearing chromosomes that were marked reciprocally from each inversion-bearing chromosome. Only one of these inversions, *In224*, survives as a homozygote, and these homozygotes survived only with the *P*[*RS5*]1A and the *P*[*RS5*]2A insertions: the $P[>w^{hs}>]$1A *In224* homozygotes did not survive. To measure the frequency of recombination in homozygous males, we also constructed *In224* chromosomes that carried *P*[*RS5*]1A or *P*[*RS5*]2A and that were reciprocally marked to the right and to the left of the *P* elements.

Measurement of recombination: An example of a crossing scheme to determine mitotic recombination frequencies in males homozygous for the chromosome *3* insertion *P*[*RS5*]1A and heterozygous for an inversion (represented as *In*) is given in Figure 2. For each recombination experiment the same scheme was used. In the G_1 cross, females ≤ 7 days old were crossed to males carrying the marked *FRT In* chromosome. Typically, two to four vials of 10 females by five males were set up for each experiment. The males were either balanced *In*/*Sb* (*InUbx*^{101}) or *In*/*TM6* (*InD*, *In224*, *In225*, *InC190*). Three days after the G_1 cross was started, the flies were transferred to fresh vials and allowed to lay eggs for 24 hr. (The old vials were discarded.) Three more 24-hr collections were made by transferring the parents to new vials every 24 hr. After the fourth 24-hr collection the parents were discarded. Six hours after each transfer, the vial from which the parents had been removed was heat-shocked (or not) for 1 hr at 38° in a circulating water bath. The four vials were treated such that two heat-shocks (+HS) were alternated with two no heat-shock (−HS) controls. The $TM6^+$ G_2 males were testcrossed 1–4 days after they eclosed. The mosaic or white-eyed males (which therefore carried *70FLP*3F) were crossed to females that were between 2 and 10 days old as one male by two females. Parents were discarded on the seventh day after setting up the cross, and the progeny were scored on the 14th and 18th days.

For the measurement of recombination in *In224* homozygotes, the G_0 male carried one of the *P*-element *In224* chromosomes; the G_1 male carried the reciprocally marked *In224* chromosome with the same *P*-element insertion. Measurement of recombination frequencies in males without inversions used the same scheme except that the G_0 and the G_1 males carried reciprocally marked *P* element-bearing chromosomes without inversions.

It might seem that these crossing schemes could be shortened by constructing stocks that carry *70FLP* and each of the

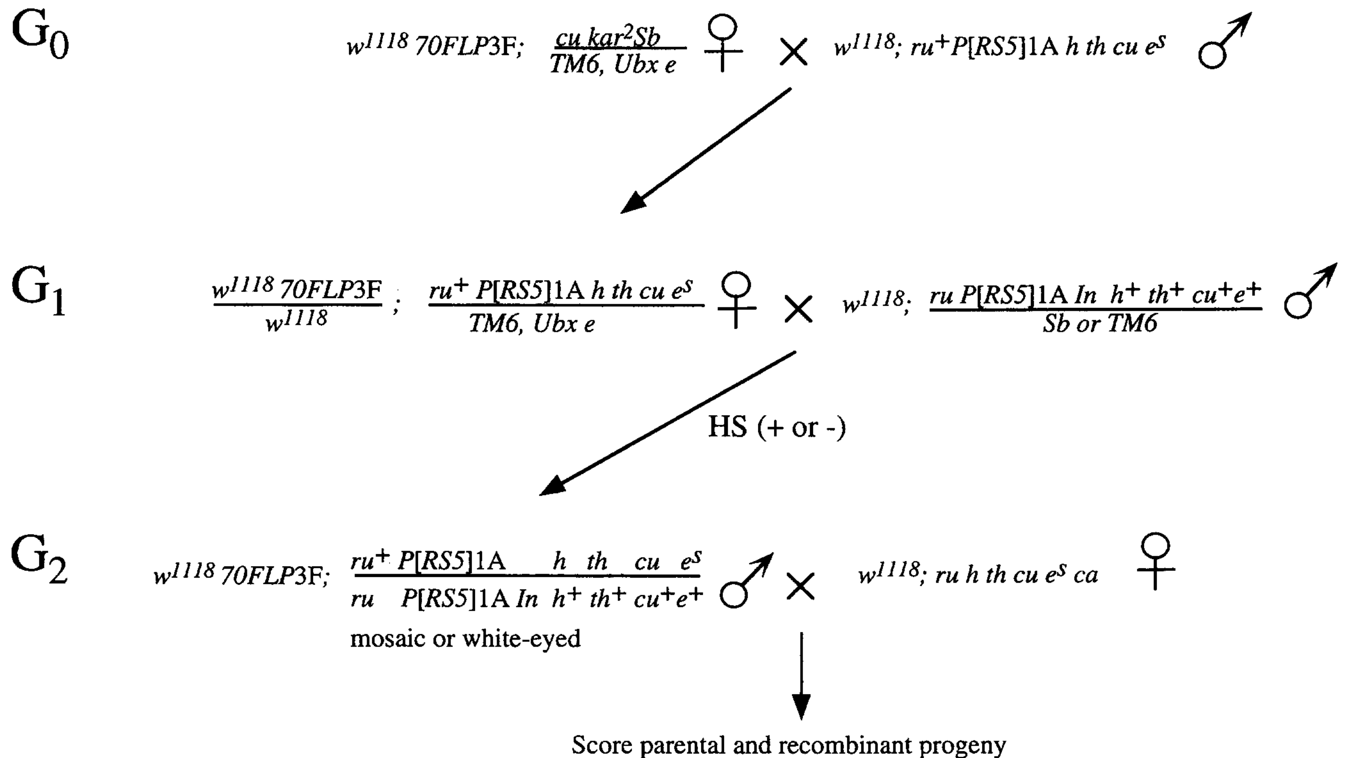

FIGURE 2.—Crosses used to measure mitotic recombination. Details are provided in MATERIALS AND METHODS.

P-element insertions, and then using females from these stocks in place of the G_1 females from our scheme (Figure 2). We specifically avoided this because when *P* elements with direct *FRT* repeats are maintained in the presence of *70FLP*, the constitutive level of FLP that is present, although low, causes recombination between the two *FRT*s and produces chromosomes with just a single *FRT*. The chromosomes that carry these remnants of excision will tend to accumulate in a stock that carries a *FLP* gene, and the single *FRT* remnants may recombine with a different frequency than the original double *FRT* insertions (GOLIC 1991). By keeping *70FLP* and the *FRT*-bearing *P* elements separate until the experiment is performed, we avoid introducing this additional variation. It is still true that there may be some constitutive *70FLP* activity in the germline of G_1 females, which will produce G_2 males that carry a chromosome with a single *FRT*. We assayed the frequency of such events by mating G_1 females (Figure 2) to w^{1118} males and treating these crosses as described previously except that none of the vials were heat-shocked. The Ubx^+ progeny from each series of vials were then scored for eye color. Excision of the *FRT*-flanked DNA is detected by the presence of offspring that are completely white-eyed. Mosaic offspring are scored as $white^+$ since they must have received an intact element from their mothers. *P*[*RS5*]1A- or *P*[*RS5*]2A-bearing females produced no white progeny among 1457 and 1094 total Ubx^+ progeny, respectively; *P*[>w^{hs}>]1A females produced only three white progeny among 1393 total Ubx^+ offspring. Thus, the frequency of *FRT-FRT* recombination in G_1 females is so low as to be of negligible concern in our experiments.

Controls: *−FLP control:* Mitotic recombination frequencies were measured without the presence of *70FLP* in males homozygous for either *P*[*RS5*]1A, *P*[>w^{hs}>]1A or the *P*[*RS5*]2A insertions. The −FLP control crosses were carried out as in Figure 2 except that the G_0 females did not carry *70FLP* and the G_1 males were homozygous for a marked chromosome 2 that carried the relevant *P*-element insertion but no inversion.

−FRT control: Mitotic recombination frequencies were measured in males that were hemizygous for the *P*[*RS5*]1A, *P*[>w^{hs}>]1A or *P*[*RS5*]2A insertions. The *−FRT* control crosses were carried out as in Figure 2 except that the G_1 males were homozygous for a marked chromosome *2* with no inversion and no *P*-element insertion.

Rationale for timing of heat shocks: In *D. melanogaster* the process of spermatogenesis requires ~10 days to complete. This process is ongoing throughout the life of a male and continuously generates new sperm (LINDSLEY and TOKUYASU 1980). Because cells in different stages of spermatogenesis respond quite differently to heat shocks, the dynamics of spermatogenesis must be considered when measuring recombination in the male germline. When males that carried an *hsp70-Adh* fusion gene were heat-shocked, the production of Adh in the testis was limited to cells in the earliest stage of spermatogenesis (BONNER *et al.* 1984). We confirmed this observation using *hsp70-lacZ* (LIS *et al.* 1983) and *hsp70-GFP* (Green Fluorescent Protein: CHALFIE *et al.* 1994) transgenes (results not shown). If adult males are heat-shocked, one can anticipate that only after an interval of 9–10 days will such males transmit sperm that are derived from cells that induced an *hsp70* fusion gene.

This supposition was verified by measuring the frequency of *FRT-FRT* recombination induced in *hsFLP*-bearing flies by heat shocks given at different stages of development. Males and females that were homozygous for the chromosome *3* insertion *P*[>w^{hs}>]2A and that also carried either *hsFLP*1 on their *X* chromosomes or *hsFLP*2B balanced over a S^2 *CyO, cn bw* chromosome *2* were allowed to breed as several pairs per vial. When pupae appeared on the side of the vials, the parents were removed and the vials were heat-shocked at 38° for 1 hr. When adults began to eclose (after 5 days), males and virgin females were collected each day and immediately mated individually to two w^{1118} virgin females or males. Progeny were scored for eye color on the 14th and 18th days after the cross was started. *P*[>w^{hs}>]2A normally confers an orange

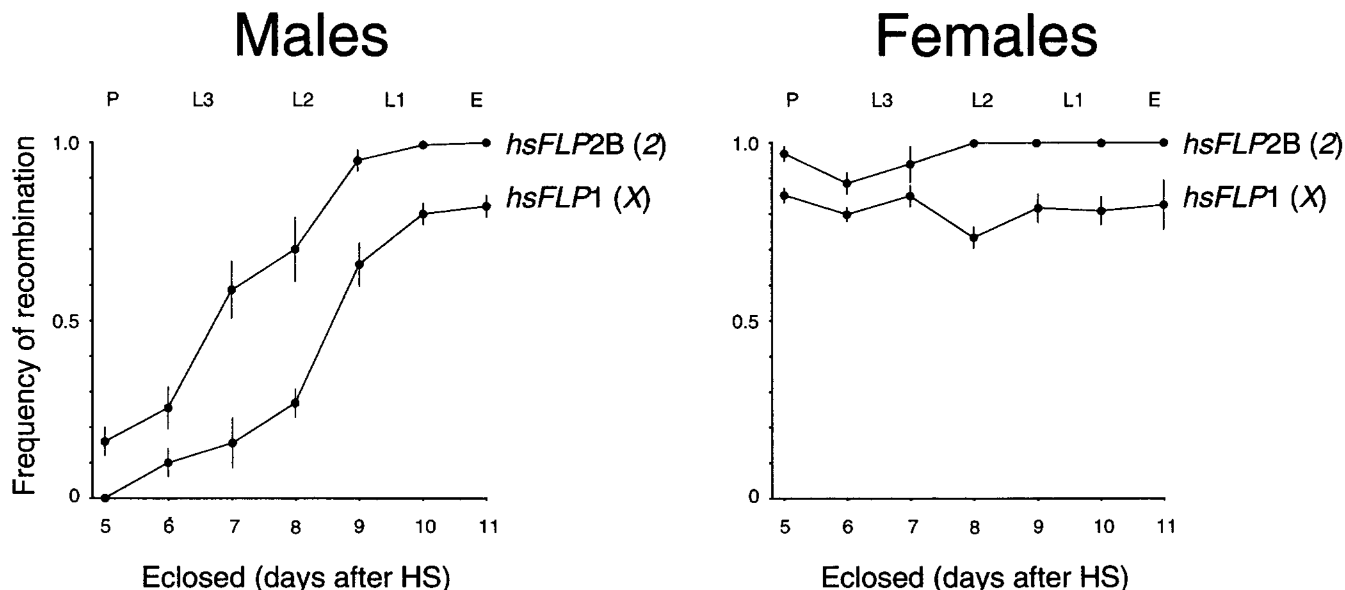

FIGURE 3.—Germline activity of *hsFLP* genes. Recombination was measured in *hsFLP*-bearing males and females that were heat-shocked at different stages of development by test-crossing flies that eclosed on successive days after the heat shock. The approximate stage of heat shock is indicated across the tops of the graphs: E, embryonic; L1, L2, L3, larval instars; P, pupal. Vertical bars represent ±1 SE. See text for further details.

eye color. When FLP recombines the *FRT*s that flank w^{hs}, the gene is either excised from the chromosome (most frequently) or amplified (much less frequently, <1% in these crosses). When these recombinant products are transmitted through the germline, they can be recognized because white- or red-eyed progeny are the result. The frequency of FLP-induced intrachromosomal recombination was calculated for each parent as (white-eyed + red-eyed)/total progeny. A mean recombination frequency was calculated for the flies that eclosed on a given day by calculating the average of the individual frequencies. The results are presented in Figure 3. These graphs show that maximal levels of recombination were obtained in males that eclosed 10–11 days after the heat shock. This corresponds roughly to heat shocks applied during the embryonic or first larval instar stages of development. No such restriction was observed for females. The results indicate that males should be heat-shocked very early in development to avoid sampling sperm derived from cells in which *hsp70-FLP* fusion genes were not induced. In this experiment the males heat-shocked in later stages also produced some recombinants. It is likely that these arose from rematings that occurred a few days after the start of the cross, because the 14-day counts showed a much lower frequency of recombinants than the 18-day counts. However, these results do not exclude the possibility that cells in later stages of spermatogenesis may respond to heat shock, but to a lesser degree than stem cells. There was virtually no recombination in the −HS controls: males that carried *hsFLP2*B had a mean frequency of recombination frequency of 0.00 (SE = 0.001, N = 14), males with *hsFLP1* had a mean frequency of 0.02 (SE = 0.02, N = 13), *hsFLP2*B females had a mean frequency of 0.00 (SE = 0.00, N = 3), and *hsFLP1* females had a mean frequency of 0.00 (SE = 0.00, N = 7).

Effect of *Minute* mutations on transvection: Two *Minute* mutations on chromosome *2* ($M(2)S\text{-}7$ and $M(2)c^{33a}$) were tested for their effect on transvection in *Cbx Ubx*/In^{+} and *Cbx Ubx*/*In* heterozygotes. Flies that carry either of these mutations are easily recognized by the dominant bristle phenotype that they confer. These were obtained from the Mid-America Drosophila stock center as *M(2)S-7/SM1* and $Df(2R)Mc^{33a}$, *c px* $M(2)c^{33a}$/*SM1* stocks. The *Cbx Ubx* chromosome was obtained from JILL HENDRICKSON (University of Utah) as p^{P} *Cbx Ubx* gl^{3}/*TM3, Sb Ser*. The w^{1118} mutation was crossed into these stocks by mating each to w^{1118} females, crossing the F_1 together, and selecting white-eyed F_2 that carried the desired chromosomes. Recessive mutations carried by the stocks were not followed during these crosses. The inversion we used in these experiments is *In(3LR)*75C-D;88B, w^{hs} *ru h th cu e*. This inversion carries w^{hs} at one of the inversion breakpoints, therefore, progeny that receive *In* or In^{+} are easily distinguished (unpublished data).

The crosses shown in Figure 4 were used to produce flies that were *M* or M^{+} and carried a *Cbx Ubx* chromosome *3* heterozygous with the normal chromosome *3* or the inversion. Although *Cbx* and *Ubx* are very close and recombination would rarely separate them, the presence of *Ubx* along with *Cbx* in the G_1 males was verified by examination of the halteres. G_1 crosses were set up as two males by two females in standard vials. They were allowed to mate and lay eggs for 2 days and then transferred to fresh vials. These transfers were repeated three more times. Progeny were collected 13–15 days after a vial was first started and again on the 18th day. Stubble progeny were discarded, and the remaining *Cbx Ubx* offspring kept for scoring. Collection was done by etherization. After all progeny eclosing through day 18 were collected, all the *Cbx Ubx* flies from a single vial were scored: first for the severity of the Cbx phenotype, and then for Minute or Minute^{+} and white (In^{+}) or white^{+} (*In*). Flies were scored for the severity of Cbx by ranking them with respect to the other flies from the same vial. The most severe phenotype was given a rank of one and the least severe had the highest rank in that vial. There are significant advantages to this method of scoring. First, it allows for a very high degree of resolution of phenotypes because the exact flies under comparison are in view in the microscope at the same time. Second, vial to vial environmental variation is controlled for internally, and third, this procedure controls for any effects of recessive mutations segregating in the background. The extreme phenotypes found in any vial from each series were very similar.

The three factors that were considered in scoring the sever-

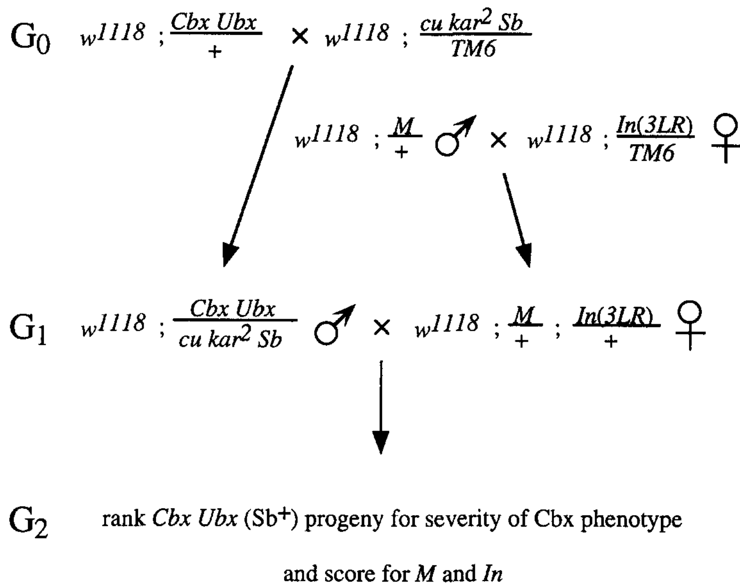

FIGURE 4.—Crosses to test the effect of *Minute* mutations on transvection. Details are given in MATERIALS AND METHODS.

ity of the Cbx phenotype (and their relative importance) were as follows: first, wing size; second, wing shape (the degree of cupping); and third, wing posture (angle with respect to the body axis). The flies with the lowest ranks had small wings that were cupped with the concave surface ventrally and were angled down. Mid-ranked phenotypes had larger wings with lesser degrees of cupping (sometimes with a reversed direction), and they were spread horizontally away from the body or sometimes held sharply up. The highest ranks had wings of fairly normal size and shape that were slightly spread horizontally. The alula was not used for ranking, however, it was strongly reduced in all flies. It is unlikely that the wing phenotypes that we scored were affected by the recessive *cu* allele carried by the *In* chromosome because the *cu kar^2 Sb/In$^+$* flies from this cross had normal wings irrespective of whether they were M or M$^+$, and the *cu kar^2 Sb/In* flies had the expected curled wings whether or not they were M. During the course of this experiment we discovered that the original *Cbx Ubx* chromosome carried an allele of *ru* because occasional ru progeny appeared in some vials [although not all (*ru* is located far from *Cbx* and would segregate independently in females)]. The *ru* mutation was also carried by the *In(3LR)* chromosome. These ru progeny were found in all classes (*ru* is not tightly linked to the *In*) and did not obviously affect the wing phenotype under consideration here.

For purposes of analysis each fly was then assigned a rank value that ranged from one up to the number of flies in the vial (in accord with standard practice for nonparametric statistics). In tied groups, each fly was given the average ranking of the group. For instance, if there were five flies in a vial that were scored as rank one, then each of those flies was assigned a rank value of three for analysis. If the group of flies scored as rank two comprised five more flies, they were all assigned a rank value of eight. After all flies were assigned rank values in this fashion, a mean rank value was calculated for each genotype.

Statistical analyses: Recombination frequencies are reported as unweighted means. A recombination frequency was calculated for each male, and the average of the individual frequencies is reported for each experiment. (However, the tables show only the total progeny counts.) Only males that produced ≥ 10 progeny were included. In all the experiments reported here there were altogether only 14 males that produced fewer than 10 offspring. The recombination frequencies from inversion heterozygotes (In^+/In) or homozygotes (In/In) were compared with the frequencies from control males (In^+/In^+) that carried the same FRT insertion using a randomization test of the individual male frequencies (FISHER 1935). The randomization test calculates the probability that two groups are drawn from the same population by pooling the frequencies associated with the individuals in those two groups, randomly assigning those individual frequencies to two groups of the same sizes as the experimental groups, and then calculating the difference between the summed frequencies of each group. This procedure was repeated for a total of one million trials for each comparison. The number of trials in which the two trial groups differ by an amount greater than that of the original experiment is divided by one million to give an accurate approximation of the probability (P) that the two groups are drawn from the same population. The one-tailed P values were used for determination of significance levels. The same procedure was used to compare the frequencies of recombination in the −FLP and the −*FRT* controls of Table 2. Macintosh software for performing these randomization tests was supplied by BILL ENGELS. Correlation statistics were obtained using StatView statistical software (Abacus Concepts, Berkeley, CA) for Macintosh. The *Cbx* transvection experiment was analyzed using a paired sign test to compare the difference between the mean ranks of the *M* and *M$^+$* classes from the *In$^+$* genotype to that difference in the *In* genotype.

RESULTS AND DISCUSSION

Mitotic pairing and structural heterozygosity: To set a baseline for assessing the effect of inversions on pairing, we first measured the frequencies of FLP-mediated mitotic recombination in males that were homozygous for an *FRT*-bearing *P*-element insertion (either *P*[*RS5*]1A, *P*[>*w^{hs}*>]1A or *P*[*RS5*]2A) on structurally normal chromosomes. The constructs are diagramed in Figure 1; the locations of each insertion are shown in Figure 5. In the experiments where *70FLP* was induced with a heat shock applied early in development, 20.6% recombi-

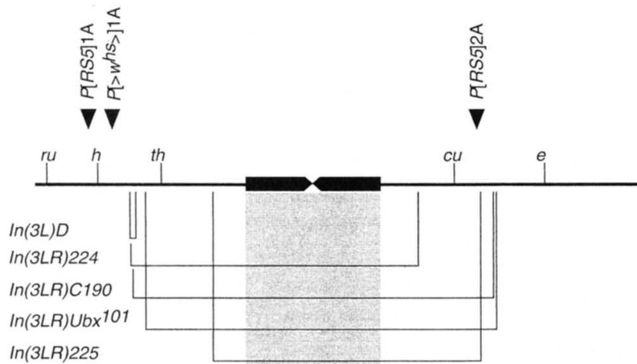

FIGURE 5.—Map of chromosome *3*. The metaphase length of chromosome *3* is indicated by the thick horizontal line. Centric heterochromatin is indicated by the filled box, and the centromere is indicated by the constriction. The locations of the three *FRT* insertion constructs and the marker genes used are indicated above the chromosome. The extent of each inversion is indicated below the chromosome. See MATERIALS AND METHODS for exact positions and breakpoints.

nants were produced by *P*[*RS5*]1A homozygous males, 20.3% by *P*[>w^{hs}>]1A homozygous males, and 15.6% by *P*[*RS5*]2A homozygous males (Table 1).

This high frequency of recombination requires FLP and a homozygous insertion of *FRT*s. In the absence of FLP (−FLP control) there were only 30 recombinants (arising from 10 males) among 16,250 progeny. There were instances of exchange in each of the marked intervals among these recombinants (Table 2). In males that carried *70FLP* but that carried *FRT*s on only one homologue (−*FRT* control), we also observed a low level of recombination (60 recombinants from 21 males were found in 18,597 progeny), and among these recombinant chromosomes there were also instances of exchange in each marked interval (Table 2). In the −*FRT* controls, wherein males did carry the *70FLP* gene, the level of recombination was significantly higher in the males that had been heat-shocked ($P = 2.3 \times 10^{-5}$). On the other hand, there was no significant difference between the heat-shocked and non-heat-shocked males in the −FLP control ($P = 0.32$). This implies that the higher level of recombination seen in the heat-shocked −*FRT* controls is not merely a consequence of heat shock but results from the presence of FLP. This further suggests that there are sequences in the Drosophila genome that FLP can recognize and recombine, albeit inefficiently relative to the *FRT* from the yeast 2μ plasmid. These sequences may be somewhat dissimilar to the canonical *FRT* sequence, as FLP can tolerate some base substitutions in the minimal *FRT* sequence (SENECOFF *et al.* 1988). Along similar lines, SAUER (1992) has shown that the yeast genome harbors sequences that are inefficiently utilized as substrates by the bacteriophage P1 Cre recombinase.

The measurement of recombination in males that carry a homozygous insertion of *FRT*s provides, nonetheless, an accurate assessment of the pairing of those allelic *FRT*s because the great majority of exchanges occur only with both FLP and homozygous *FRT*s. In the experiments of Table 1, the small number of recombinants that arose from exchange at sites other than the *FRT* insertions are enumerated in the footnotes but are not included in the recombinant or total counts.

In these experiments our aim is to compare the frequencies of recombination that are produced from a standard amount of FLP. If the inversion genotypes induced some level of constitutive stress response, thereby inducing *70FLP*, then there would be justification to make an adjustment for the −HS recombination frequencies before comparing the +HS frequencies. If the −HS results do reflect constitutive levels of stress response resulting from the genotypes, then those results should follow two patterns: (1) there should be a correlation between the mitotic recombination frequencies obtained with *P*[*RS5*]1A and *P*[>w^{hs}>]1A in each inversion genotype, and (2) there should be a correlation between the excision frequencies of *P*[*RS5*]1A and of *P*[>w^{hs}>]1A in each inversion genotype. In neither of these comparisons of −HS data is there a significant correlation (the coefficients of correlation are 0.49, $P = 0.35$ and 0.14, $P = 0.81$, respectively). We conclude that the −HS levels of mitotic recombination cannot be attributed to the inversion genotype, and we have chosen not to subtract the −HS results from each genotype before normalizing. In a subset of the progeny of each −HS cross there is a strong relationship between excision and mitotic recombination: the frequency of excision is much higher among the recombinants than among the progeny as a whole (with only three exceptions). This merely reinforces the fact that a common agent, FLP, underlies both excision and mitotic recombination.

When recombination was measured in heat-shocked males that carried *70FLP* and homozygous *FRT*s, the presence of a heterozygous inversion (Figure 5) reduced the frequency of mitotic recombination in every case. Table 3 presents the +HS frequencies normalized to the frequency of the +/+ control. Unlike the −HS results discussed above, there is a strong correlation between the mitotic recombination frequencies obtained with *P*[*RS5*]1A and *P*[>w^{hs}>]1A in each chromosomal genotype. (The coefficient of correlation is 0.88, $P = 0.02$.)

A closer examination of the data shows that the observed reductions in mitotic recombination frequencies do not result from a general inhibition of *70FLP* induction or FLP-mediated recombination. In these data, a second assay of FLP activity is the frequency of intrachromosomal recombination that excises the *FRT*-flanked DNA and causes loss of w^{hs} function. The frequency of excision can be calculated as white-eyed/total progeny. The numbers of progeny that had white eyes are given in parentheses in Table 1. In every case but one, the frequency of excision was >99%. In the re-

TABLE 1

Mitotic recombination frequencies

Chromosomal genotype	HS	*FRT* insertions: *P*[*RS5*]1A				*P*[>w^{hs}>]1A				*P*[*RS5*]2A			
		n	Recombinants	Total	Recombination	*n*	Recombinants	Total	Recombination	*n*	Recombinants	Total	Recombination
+/+	+	38	733 (730)	3514 (3505)	20.6 ± 2.1	18	323 (323)	1580 (1580)	20.3 ± 2.9	35	472 (472)[j]	3134 (3141)	15.6 ± 2.0
	−	36	54 (3)	3322 (67)	1.9 ± 0.6	27	38 (11)	2013 (101)	2.0 ± 0.6	39	9 (0)[k]	3555 (29)	0.3 ± 0.1
D/+	+	32	427 (427)[a]	2375 (2371)	17.8 ± 1.6	25	254 (254)[e]	1602 (1601)	14.7 ± 2.5				
	−	40	135 (21)[b]	2887 (65)	5.4 ± 1.5	34	85 (11)[f]	2633 (79)	3.5 ± 0.7				
224/+	+	39	261 (256)	2965 (2959)	9.3 ± 1.3	36	248 (248)	2711 (2664)	9.1 ± 1.6	39	281 (281)	3306 (3281)	8.8 ± 1.4
	−	33	56 (19)	2625 (74)	2.1 ± 0.5	37	11 (1)	2438 (72)	0.5 ± 0.2	36	11 (1)	3065 (36)	0.6 ± 0.3
C190/+	+	24	281 (281)	1810 (1810)	14.8 ± 2.4	36	331 (331)	2963 (2959)	10.7 ± 1.3				
	−	36	67 (16)	2503 (110)	2.5 ± 0.8	38	30 (0)	2893 (58)	1.1 ± 0.3				
Ubx^{101}/+	+	39	453 (453)	2889 (2889)	15.0 ± 1.6	35	332 (332)[g]	2800 (2800)	11.7 ± 1.7				
	−	28	27 (1)	2348 (31)	1.1 ± 0.3	41	86 (7)[h]	3449 (51)	2.4 ± 0.6				
225/+	+	26	308 (308)[c]	2055 (2044)	15.9 ± 1.7	32	198 (198)[i]	1786 (1786)	11.0 ± 1.7				
	−	25	66 (3)	1625 (18)	3.5 ± 1.5	36	36 (0)	3267 (35)	1.3 ± 0.4				
224/*224*	+	38	643 (643)	3036 (3030)	21.6 ± 1.9					32	381 (381)	2052 (2050)	18.9 ± 2.3
	−	29	36 (2)[d]	2284 (24)	1.5 ± 0.5					32	29 (2)	2475 (24)	1.2 ± 0.4

n, number of males tested; HS, heat shock. The numbers in parentheses represent the number of progeny in that group that had white eyes. The frequency of recombination given is the unweighted mean and is expressed as a percentage ± SE.

Recombinants at sites other than *FRT*s: [a]one single in *cu-e* interval, two doubles in *ru-h* and *cu-e*; [b]one *cu-e*, three *th-e*; [c]five *cu-e*, one *th-e*; [d]two *h-cu*; [e]seven *th-cu*, two *th-e*; [f]30 *th-cu*; [g]one *cu-e*; [h]one *th-e*; [i]six *th-cu*; [j]15 *ru-h*, four *th-cu*; [k]nine *ru-h*.

TABLE 2

Frequencies of recombination in control crosses

Control	HS	*FRT* insertions: *P*[*RS5*]1A				*P*[>w^{hs}>]1A				*P*[*RS5*]2A			
		n	Recombinants	Total	Recombination	*n*	Recombinants	Total	Recombination	*n*	Recombinants	Total	Recombination
−FLP	+	30	4 (0)[a]	2290 (2)	0.2 ± 0.1	30	0	2459 (0)	0.0	40	13 (0)[e]	3773 (1)	0.5 ± 0.3
	−	30	0	2302 (0)	0.0	30	0	2313 (6)	0.0	30	13 (0)[f]	3113 (0)	0.4 ± 0.2
−*FRT*	+	39	33 (23/23)[b]	3028 (1785/1785)	1.2 ± 0.5	38	17 (11/11)[d]	3430 (1990/1992)	0.5 ± 0.2	36	8 (4/4)[g]	2609 (1553/1553)	0.5 ± 0.5
	−	36	2 (0/1)[c]	3027 (62/1876)	0.1 ± 0.1	36	0	3508 (46/2164)	0.0	39	0	2995 (13/1801)	0.0

n, number of males tested; HS, heat shock. The numbers in parentheses represent the number of progeny in that group that had white eyes. In the −*FRT* control only a portion of the progeny would be expected to receive the w^{hs}-bearing chromosome. In parentheses are given the numbers of white-eyed progeny as a fraction of those which received that chromosome. The frequency of recombination given here is the unweighted mean and is expressed as a percentage of ± SE.

The recombinant chromosomes that arose in these experiments were as follows: [a]two recombinants in the *ru-h* interval, two in the *h-th* interval; [b]four *ru-h*, one *h-th*, one *th-cu*, 27 *cu-e*; [c]two *th-e*; [d]four *ru-h*, nine *h-th*, four *cu-e*; [e]two *h-th*, eight *th-cu*, three *th-e*; [f]four *th-cu*, nine *th-e*; [g]eight *cu-e*.

TABLE 3

Relative frequencies of mitotic recombination

Chromosomal genotype	*FRT* insertion		
	P[*RS5*]1A	*P*[>w^{hs}>]1A	*P*[*RS5*]2A
+/+	1.0	1.0	1.0
D/+	0.86	0.72*	
224/+	0.45***	0.45***	0.56***
C190/+	0.72**	0.53***	
Ubx^{101}/+	0.73**	0.58***	
225/+	0.77*	0.54***	
224/*224*	1.05		1.2

One-tailed probability levels for the comparisons with the +/+ control for the respective insertions are indicated as follows: *$P \leq 0.10$; **$P \leq 0.05$; ***$P \leq 0.01$.

maining cross (*P*[>w^{hs}>]1A *In224*) the frequency of excision was only slightly less (98.3%). It is true that the ability to resolve differences in FLP activity is not great when excision is nearly complete, nevertheless, there is no suggestion in these results that the inversions suppress *70FLP* expression or FLP function.

Finally, we measured the frequency of recombination in males that were homozygous for *P*[*RS5*]1A or *P*[*RS5*]2A and *In224*. (Only these two combinations survive as homozygotes.) If the reduction in recombination frequencies is caused by structural heterozygosity in *In*/+ animals, then the frequency of recombination should be restored to the +/+ levels in the *In*/*In* homozygotes. This is the result we obtained (Tables 1 and 3). We conclude that the reduced frequencies of mitotic recombination exhibited by *In*/+ males reflect a reduced degree of mitotic pairing.

Our results demonstrate that heterozygous chromosome inversions inhibit the mitotic pairing of alleles that lie distal to the inversion breakpoints. The inversions that we tested do not, however, completely eliminate pairing. The allelic *FRT*s on structurally different homologues still recombine with substantial frequency. *FRT*s that lie far apart on structurally normal chromosomes recombine infrequently, at a rate of ~0.1% or less under similar circumstances of *70FLP* induction (our unpublished results). The much higher rate of recombination between allelic *FRT*s on structurally rearranged homologues indicates that these alleles still pair to a significant extent in mitotically active cells. Inversion heterozygosity may inhibit pairing by reducing the portion of the cell cycle during which alleles are paired or by completely eliminating pairing in some fraction of cells, or perhaps by a combination of both.

When Lewis (1954) first described the phenomenon of transvection some 40 years ago with the study of chromosome rearrangements that disrupted *Ubx*/*bx*^{34e} complementation, his results allowed him to infer several characteristics of mitotic chromosome pairing. He found that rearrangements with one breakpoint proximal to BX disrupted complementation, from which he concluded that pairing starts at the centromere and spreads distally. He also observed that not all rearrangements disrupted complementation (or pairing) to the same degree; he concluded that breakpoints close to the BX locus disrupted pairing by virtue of their proximity, but that breakpoints farther away only disrupted pairing if they also were asymmetrical, symmetrical interchanges of arms having relatively little effect. Rearrangements that disrupt transvection of *dpp* and *eya* alleles follow similar patterns (Gelbart 1982; Leiserson *et al.* 1994).

In this work we measured pairing with an assay that requires physical contact between alleles, and our results are very similar to those that came from the more indirect assay of *Ubx*/*bx*^{34e} transvection. The inversions we tested reduced the pairing of *P*[>w^{hs}>]1A alleles to a greater extent than *P*[*RS5*]1A alleles. These two elements are very similar in structure and they show almost identical frequencies of recombination on structurally normal homologues. It is probable that their differential response reflects their proximity to the inversion breakpoints: *P*[>w^{hs}>]1A lies closer to the inversions and as a consequence is more sensitive to pairing inhibition. We also found that three inversions that have almost identical left-hand breakpoints (*In*(*3L*)*D*, *In*(*3LR*)*224*, *In*(*3LR*)*C190*), inhibit pairing to differing degrees. It is not surprising that *In*(*3L*)*D* should have the least effect considering its small size. Indeed, the effect of *In*(*3L*)*D* is only marginally significant in our experiments. However, *In*(*3LR*)*224* caused a greater reduction in pairing than *In*(*3LR*)*C190*, although it is substantially smaller. This difference may owe to the asymmetry of *In*(*3LR*)*224*: *In*(*3LR*)*C190* has breakpoints in each arm that lie similar distances from the centromere of chromosome *3*, but the left-hand breakpoint of *In*(*3LR*)*224* is farther from the centromere than the right-hand breakpoint. *In*(*3LR*)*225* is similarly asymmetrical but reduces pairing to the same degree as *In*(*3LR*)*C190*. In this instance the effect of asymmetry may be counterbalanced by the fact that the breakpoint of *In*(*3LR*)*225* lies farther from the *FRT*s. *In*(*3LR*)*Ubx*^{101} is structurally very similar to *In*(*3LR*)*C190* and has a similar effect.

Mitotic pairing and cell-cycle length: In light of the results presented above, it is entirely reasonable to imagine that transvection at BX, *dpp*, and *eya* requires the pairing of homologous chromosomes, and that heterozygous rearrangements disrupt transvection because they disrupt pairing. One must then ask why the *zeste-white*$^+$ interaction and the *bw*^{D}-*bw*$^+$ interaction are apparently insensitive to many rearrangements that should interfere with pairing. The answer may lie in their time of action: BX and *dpp* function in cells that divide two or three times a day [Kaufman *et al.* (1973); Masucci *et al.* (1990); see Postlethwait (1978) for a discussion of the dynamics of cell division during devel-

opment], while *white* and *brown* function in cells that completed their final mitotic division several days earlier (STELLER and PIRROTTA 1985; ZACHAR *et al.* 1985; DREESEN *et al.* 1988; HENIKOFF and DREESEN 1989). It seems sensible to suppose that the ability of cells to accomplish full pairing of homologous chromosomes, especially in the face of impediments such as structural heterozygosity, may well depend on the time they are allotted before mitosis ensues. In other words, the ability of cells to fully pair homologous chromosomes might depend on the length of their cell cycle.

In the present study we induced recombination in cells that divide approximately every 10 hr (LINDSLEY and TOKUYASU 1980). The efficiency of the pairing that is measured in our experiments should be comparable to that achieved at the BX or *dpp* loci in imaginal disc cells that divide every 8–12 hr. This would account for the similarities between our results and those of LEWIS (1954) and GELBART (1982). The resistance of *white* and *brown* alleles to disruption of pairing by structural heterozygosity could be explained because they have a much longer time to achieve pairing before the stage of their peak expression. Only those rearrangements with the very strongest effects (with the closest breakpoints) would prevent the pairing of their alleles. In fact, it is conceivable that some of the rearrangements that do interfere with the *zeste-white* or *brown^D-brown$^+$* interactions may do so precisely because pairing is virtually complete in these postmitotic cells. This could put a mechanical strain on the chromosomes at the rearrangement breakpoint that disrupts the ability of nearby alleles to pair in a normal fashion. GUBB *et al.* (1990) suggested that homologous chromosome pairing was responsible for disrupting a particular form of *zeste-white* interaction that they studied. EPHRUSSI and SUTTON (1944) placed a similar emphasis on the forces of homologous pairing in their consideration of position effects. Studies done by TALBERT *et al.* (1994) and HENIKOFF *et al.* (1995) provide further evidence that rearranged homologues are substantially paired in the postmitotic cells of the eye during the pupal stage.

The hypothesis that pairing ability is governed by the length of the cell cycle could be tested if a means were available to lengthen cell cycles. *Minute* mutations can significantly slow the rate of cell division in imaginal discs (FERRUS 1975; MORATA and RIPOLL 1975). Accordingly, we examined the effect of two *Minute* mutations on transvection at BX. The *Contrabithorax* (*Cbx*) mutation causes inappropriate activation of *Ultrabithorax* (*Ubx*) in wing discs, resulting in a partial transformation of wing to haltere (LEWIS 1955). When the *cis* copy of *Ubx* is inactivated by mutation, it can be seen that *Cbx* will *trans* activate *Ubx$^+$* on the homologue (CASTELLI-GAIR *et al.* 1990). This transvection is disrupted by rearrangements with one breakpoint in the BX critical region. Thus, the wings of a *Cbx Ubx/+* fly are obviously deformed, while *Cbx Ubx/In* flies can have wings that are near normal. We examined whether *Minute* (*M*) mutations could restore transvection in *Cbx Ubx/In* flies by setting up a cross that produced *Cbx Ubx* flies with four different genotypes: *M$^+$* or *M* on chromosome *2*, and *In$^+$* or *In* on the chromosome *3* homologue of the *Cbx Ubx* chromosome. The right arm breakpoint of the pericentric inversion used is in the BX critical region. All *Cbx Ubx* flies were ranked according to the severity of their Cbx phenotype, with rank one corresponding to the most severe phenotype. Thirty vials that used the *M(2)S-7* mutation were scored. Figure 6 graphically presents the results for the eight vials with at least four *M In* progeny. The rankings show that, as expected, the inversion disrupts transvection: *Cbx Ubx/In* flies (*M$^+$ In*) have a much milder phenotype than *Cbx Ubx/+* flies (*M$^+$ In$^+$*). It has been reported that *Minute* mutations suppress the *Cbx* phenotype (GONZALEZ-GAITAN *et al.* 1990), and this effect is also seen in our results (compare *M$^+$ In$^+$* to *M In$^+$*). The critical comparison for our purpose is *M In vs. M$^+$ In.* In every vial, the Minute flies have a more severe Cbx phenotype than the non-Minute. This is especially striking considering that in the *In$^+$* controls the *M* mutation has the opposite effect. The results for all 30 vials are given in Table 4. In 29/30 vials the mean rank of *M In$^+$* flies is greater than *M$^+$ In$^+$* flies, and in all 30 vials the mean rank of *M In* flies is less than *M$^+$ In* flies. The probability of obtaining these results if the phenotypic variation between *M* and *M$^+$* flies were solely a result of random sampling would be $(1/2)^{30}$, or 9×10^{-10}.

We also examined the effect of the *M(2)c^{33a}* mutation on transvection. Figure 7 shows the results for the eight vials with six or more *M In* progeny. In these vials the effect of the *M* on transvection is not as obvious, however when the results are tabulated (Table 5), it is seen that in all 14 vials the *M In* flies have a lower mean rank than the *M$^+$ In* flies, while in the controls the *M* has the opposite effect. The probability of obtaining these results by chance is $(1/2)^{14}$, or 6×10^{-5}.

These experiments show that *Minute* mutations enhance the Cbx phenotype in *Cbx Ubx/In* flies. This result is consistent with the idea that lengthening the cell cycle allows cells to overcome pairing impediments. There are two caveats concerning these experiments. First, *Minute* mutations are pleiotropic and it is possible that the appearance they give of restoring transvection comes about through some unanticipated and as yet undiscovered mechanism. Second, one of the critical genotypes, *M In,* is greatly underrepresented. If flies with the least severe Cbx phenotype in this genotype were selectively dying, then the difference between the two *In*-bearing groups could be artifactual. We think it unlikely that the flies that exhibit less of the mutant phenotype would die more frequently; the opposite seems more likely. We also found that the Stubble offspring of this cross (the siblings of the flies that were scored) showed a strong reduction in survival of the *M*

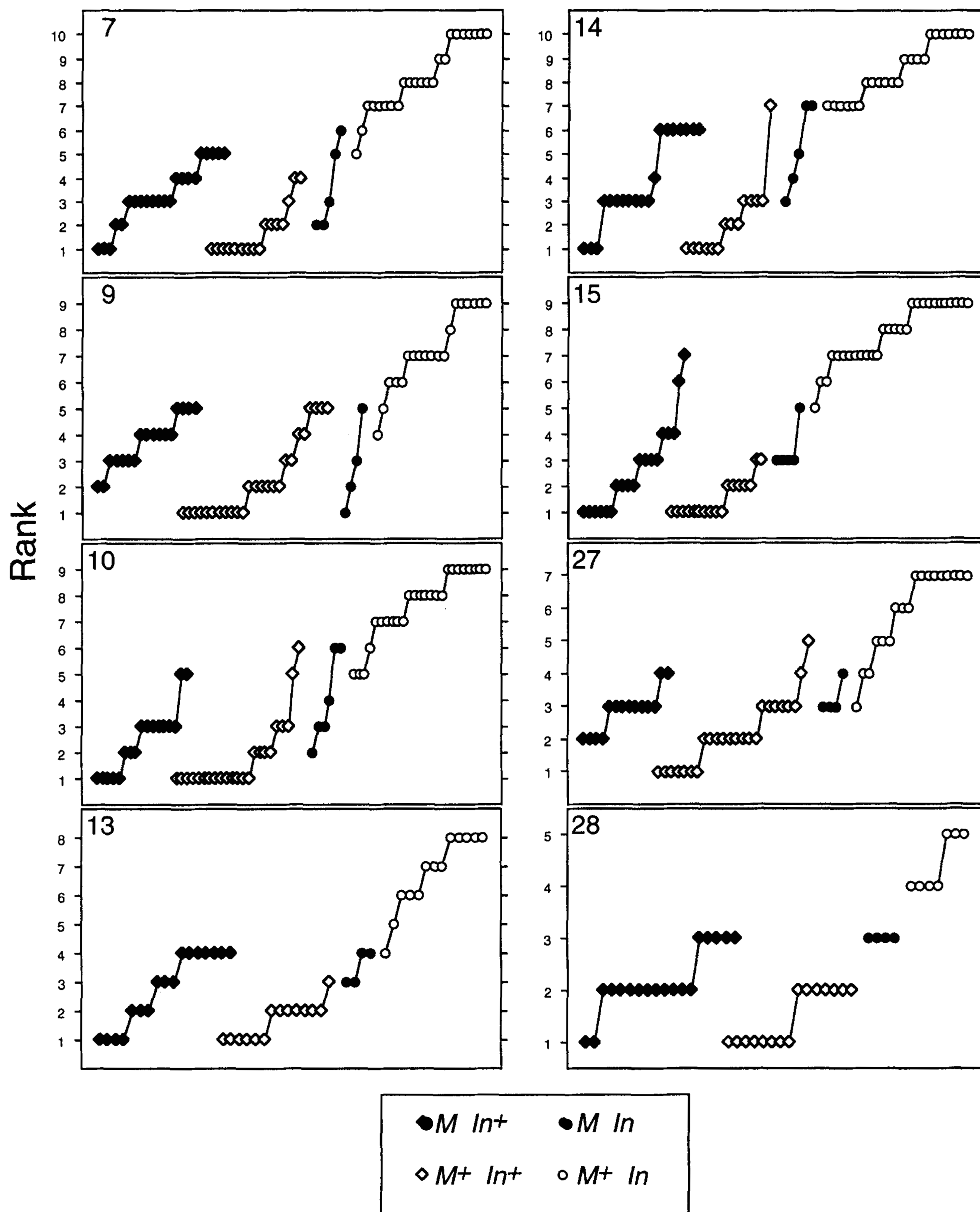

FIGURE 6.—Graphic depiction of rank scores for the effect of *M(2)S-7* on *Cbx* transvection. These graphs show the Cbx phenotype rank assignments for all four *Cbx Ubx* genotypes. Each point represents one individual. Rank 1 is the most severe phenotype. The number in the upper left corner of each graph indicates the number of the vial (Table 4) that corresponds to that graph.

TABLE 4

The Effect of *M(2)S-7* on *Cbx* transvection

		n				Mean rank			
		In+		*In*		*In+*		*In*	
Vial	*N*	*M*	*M+*	*M*	*M+*	*M*	*M+*	*M*	*M+*
1	55	13	29	1	12	32.9	16.6	44.0	49.0
2	52	17	13	1	21	24.2	9.2	34.5	38.7
3	48	13	14	1	20	20.0	10.7	9.0	37.8
4	25	6	9	2	8	10.4	6.6	16.0	21.4
5	65	16	25	1	23	28.8	17.4	8.0	54.0
6	70	16	25	1	28	29.4	16.5	36.5	55.9
7	66	22	16	5	23	26.8	13.6	28.6	54.8
8	64	16	19	3	26	23.2	18.6	27.3	48.9
9	65	17	25	4	19	31.7	18.7	23.1	55.1
10	71	17	23	6	25	26.0	18.5	37.9	58.4
11	76	12	31	1	32	34.4	18.0	34.0	60.1
12	74	18	31	2	23	32.3	22.4	27.0	62.9
13	48	17	14	4	13	21.1	11.8	27.5	41.7
14	61	19	14	5	23	22.0	12.8	30.7	49.6
15	69	19	17	5	28	24.1	14.8	32.3	55.1
16	32	11	6	1	14	10.2	6.9	17.5	25.5
17	48	12	23	2	11	20.0	17.1	36.5	42.6
18	59	15	21	2	21	25.1	14.9	28.5	48.8
19	72	12	30	2	28	32.8	17.8	40.8	57.8
20	66	16	27	2	21	27.0	19.9	32.0	56.0
21	44	15	13	1	15	18.9	10.7	24.5	36.2
22	55	18	18	1	18	25.1	12.3	33.5	46.3
23	60	22	17	2	19	25.8	18.7	21.5	47.4
24	35	8	12	1	14	10.8	11.3	9.5	28.5
25	64	16	21	2	25	29.0	14.8	33.5	49.5
26	42	9	9	2	22	11.0	8.5	26.3	30.7
27	60	14	24	4	18	26.8	17.5	32.5	50.3
28	44	18	15	4	7	21.7	12.0	33.0	41.0
29	26	8	9	1	8	11.8	7.1	14.5	22.4
30	32	5	16	1	10	14.2	10.3	18.0	27.4

N, total number of *Cbx Ubx* progeny; *n*, number of progeny of each genotype.

In class, and these flies were Cbx^+ Ubx^+. For M(2)*S-7* we counted the Stubble progeny from nine vials and found 108 *M* In^+, 154 M^+ In^+, 43 *M In*, and 125 M^+ *In*. For $M(2)c^{33a}$ the numbers (from four vials) were 33, 83, 29, and 73 for the respective classes. It seems that most of the viability reduction is not related to *Cbx* but results from an interaction between the *M* alleles and the *In* chromosome.

The effect of temperature on BX transvection has been previously characterized. Lower temperatures, which lengthen the cell cycle, enhance transvection (Kaufman *et al.* 1973). The effect of temperature is obviously also pleiotropic, but the observation is consistent with our hypothesis.

Three recent cases of transvection or allelic interaction have been described in which the effect of heterozygosity for chromosome rearrangements has been studied. These cases provide additional tests of the hypothesis that a cell's ability to accomplish pairing is dependent on the length of the cell cycle. The *eyes absent* gene functions in the same tissue as *white* and *brown*, yet it shows a sensitivity to rearrangements that is similar to BX and *dpp*. In the eye, *eya* functions in cells just anterior to the morphogenetic furrow (Bonini *et al.* 1993), and these cells are either actively dividing or have very recently divided (Ready *et al.* 1976; Wolff and Ready 1993). Accordingly, transvection at *eya* should be easily disrupted by chromosome rearrangements, and indeed it is (Leiserson *et al.* 1994).

Gindhart and Kaufman (1995) describe a case of *white* transgene expression that exhibits an allelic interaction. They studied a *white* mini-gene with variegated expression that is more severe when the inserted gene is homozygous than when it is hemizygous. The severity of variegation in the homozygote is lessened when one of the homologues carries a chromosome rearrangement, even though in this case the rearrangement breakpoint lies far from the site of insertion. The variegation of *white* in this instance owes to the presence of DNA from the regulatory region of the *Sex combs reduced* (*Scr*) gene in the same *P* element that is carrying the *white* mini-gene. In these experiments the *white* gene

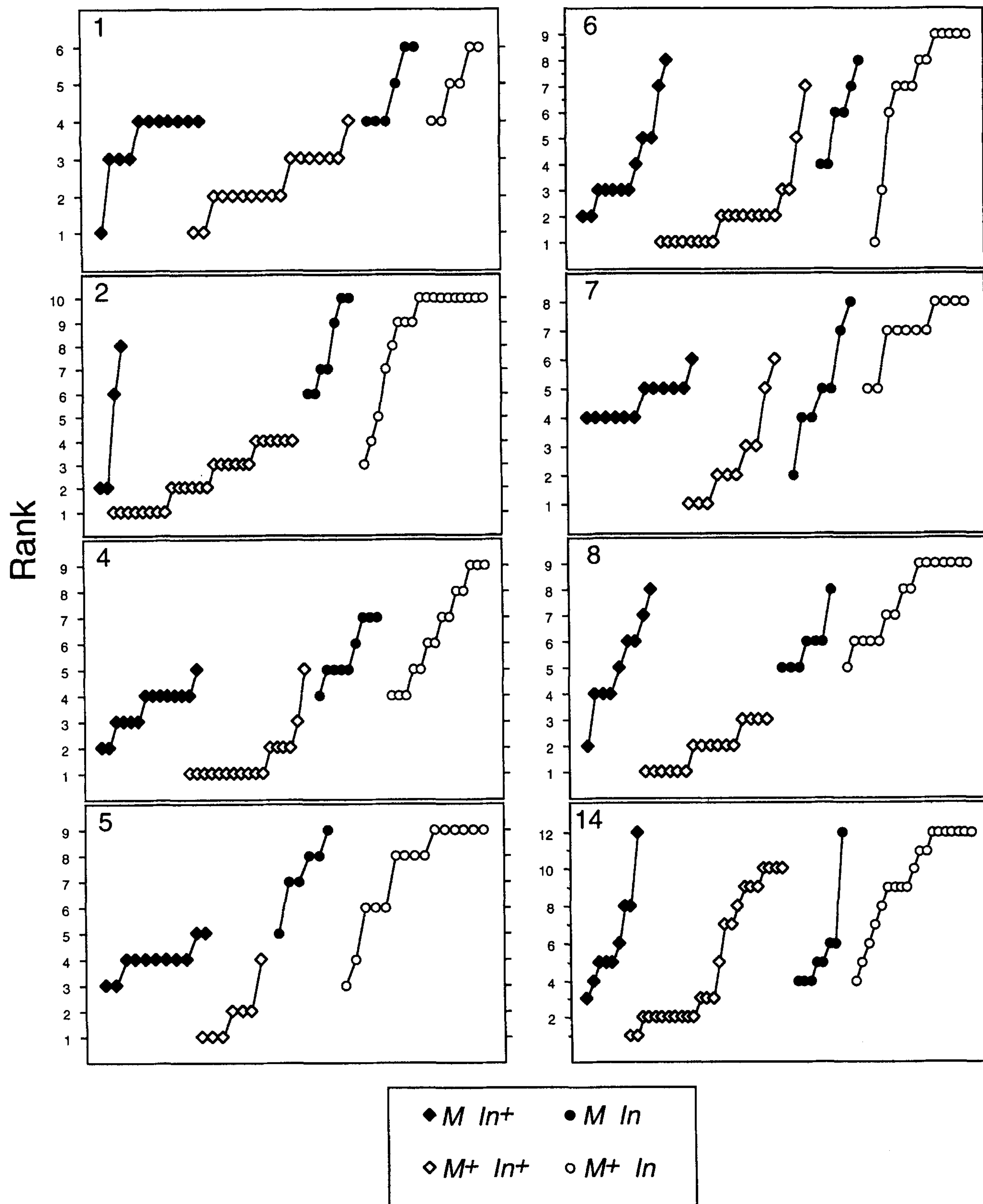

FIGURE 7.—Graphic depiction of rank scores for the effect of $M(2)c^{33a}$ on *Cbx* transvection. These graphs show the Cbx phenotype rank assignments for all four *Cbx Ubx* genotypes. Each point represents one individual. Rank 1 is the most severe phenotype. The number in the upper left corner of each graph indicates the number of the vial (Table 5) that corresponds to that graph.

TABLE 5

The effect of $M(2)c^{33a}$ on *Cbx* transvection

		n				Mean rank			
		In+		*In*		*In*+		*In*	
Vial	*N*	*M*	*M*+	*M*	*M*+	*M*	*M*+	*M*	*M*+
1	40	11	17	6	6	21.7	11.0	32.2	33.5
2	55	4	26	7	18	24.1	15.1	39.8	42.9
3	53	13	15	5	20	22.4	11.4	32.8	40.2
4	54	14	17	9	14	24.4	10.7	40.2	42.8
5	39	11	7	6	15	14.0	5.0	27.7	28.3
6	51	12	20	6	13	27.0	13.7	35.3	39.7
7	40	12	10	7	11	18.5	9.1	21.1	32.7
8	48	9	16	7	16	25.1	8.9	28.4	38.1
9	40	8	15	3	14	21.8	8.5	23.0	32.1
10	74	7	37	3	27	38.1	21.1	38.2	59.8
11	35	5	19	1	10	21.3	10.6	29.0	29.3
12	31	11	9	2	9	14.3	7.0	17.0	26.8
13	24	1	9	2	12	10.0	5.0	14.0	18.1
14	61	9	25	8	19	29.2	22.0	27.3	45.2

N, total number of progeny; *n*, number of progeny of each genotype.

apparently acts as a reporter of *Scr* regulation, as shown by the sensitivity of this variegation to mutations in genes that normally control *Scr* expression. The normal pattern of *Scr* expression is established early in development and is then maintained through the action of the *Polycomb* and *trithorax* groups of genes (PIRROTTA 1995). Because the differential expression of *Scr* is a regulatory step that occurs in mitotically active cells, we expect that the pairing-dependent aspect of this regulation should have a large critical region. The *white* gene's unusual sensitivity to rearrangements is, in this case, to be expected.

Finally, HOPMANN *et al.* (1995) and HENDRICKSON and SAKONJU (1995) have recently shown that the *Abd-B* locus of the Bithorax complex exhibits a *trans* interaction that is extremely difficult to disrupt by chromosome rearrangement. In this case complementation between two reciprocally defective loci is observed even when one of the alleles is transposed to the *Y* chromosome as a small duplication. The adult phenotype that was monitored for this interaction occurs in structures that arise, at least for the most part, from the abdominal histoblasts. These cells do not divide throughout the 4 days of larval development. If the critical allelic interaction occurs during the larval stages (this has not yet been determined), then the ability to overcome virtually all tested impediments to pairing may be attributable to the lengthy period that these cells have to accomplish pairing.

The question of the normal biological function of mitotic pairing remains. In all the phenomena where mitotic pairing has been implicated, it has been through effects on gene activity. In addition to the examples already discussed, in two cases synapsis-dependent gene activity in the salivary gland has been demonstrated (ASHBURNER 1967; KORGE 1977; KORNHER and BRUTLAG 1986). Pairing of homologues is first observed at the time of general zygotic gene activation (HIRAOKA *et al.* 1993). It seems likely that mitotic pairing may play a role in the proper regulation of genes. The fact that animals with heterozygous rearrangements can apparently be completely normal might be explained because rearrangements, at least simple inversions such as those examined here, do not completely eliminate mitotic pairing but only reduce its efficiency.

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