The *Y* Chromosome of *Drosophila melanogaster* Exhibits Chromosome-Wide Imprinting

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ABSTRACT

Genomic imprinting is well known as a regulatory property of a few specific chromosomal regions and leads to differential behavior of maternally and paternally inherited alleles. We surveyed the activity of two reporter genes in 23 independent *P*-element insertions on the heterochromatic *Y* chromosome of *Drosophila melanogaster* and found that all but one location showed differential expression of one or both genes according to the parental source of the chromosome. In contrast, genes inserted in autosomal heterochromatin generally did not show imprint-regulated expression. The imprints were established on *Y*-linked transgenes inserted into many different sequences and locations. We conclude that genomic imprinting affecting gene expression is a general property of the Drosophila *Y* chromosome and distinguishes the *Y* from the autosomal complement.

GENOMIC imprinting was first discovered in the insect Sciara in 1925 due to the preferential elimination of paternally derived chromosomes (Crouse 1960). More recently it has become clear that imprinting is a widespread phenomenon, with many examples in the plant and animal kingdoms (Messing and Grossniklaus 1999). Genomic imprinting in mammals causes preferential chromosome inactivation, the unusual genetics of some human diseases (Lyon 1999), and possible complications for mammalian cloning (Dean *et al.* 2001; Reik *et al.* 2001). Loss of proper imprinting at specific loci has been correlated with some cancers (Tycko 1999).

Genomic imprinting is known to affect gene regulation at no fewer than 30 loci in mammals, manifesting as monoallelic expression (Beechey 1999; Moore 2001). Additionally, provisional imprinting has been hypothesized at dozens more loci (Pfeifer 2000; Sano *et al.* 2001). In fact, imprinting is estimated to affect hundreds of genes in humans, as calculated by estimation of disease loci with parent-specific origin (Barlow 1995). This is almost certainly an underestimate, as recent searches have shown potentially dozens of clusters in mice and humans (Beechey 1999; Mizuno *et al.* 2002).

Some genes under control of genomic imprinting may reflect control by discrete imprint-control centers with a range of influence over neighboring regions (Tilghman 1999). To date, three clusters have been identified in humans; at least two have syntenic imprinted regions in mice (Barlow 1995), and one has a syntenic imprinted region that has been the subject of selection by sheep breeders (Wylie *et al.* 2000; Charlier *et al.* 2001). Additional examples of imprinted genes in nonmammals come from insects (Crouse 1960; Golic *et al.* 1998; Lloyd *et al.* 1999; Haller and Woodruff 2000) and birds (Koski *et al.* 2000), as well as plants (Alleman and Doctor 2000). However, none of these studies include exhaustive searches for imprint-controlled regions of any genome.

The largest cluster of imprinted genes in humans is the *X* chromosome (Lyon 1999). In extraembryonic placental tissues of females, the paternal *X* chromosome is preferentially inactivated, leading to the conclusion that these cells are reading, and responding to, an as-yet-unidentified *X* chromosome imprint. Furthermore, imprint-controlled *X* chromosome inactivation is an even broader phenomenon in many noneutherian mammals, where the paternal *X* is often marked for inactivation in all tissues (Sharman 1971). Two reasonable alternative hypotheses are that (1) preferential *X* inactivation is an example of chromosome-wide imprinting and that the imprint is laid down along the length of the chromosome or (2) the imprint may be restricted to the *X*-inactivation center, which controls global dosage compensating inactivation of the *X* chromosome in females. The extent to which specific chromosomes or chromosome regions are subject to genomic imprinting is not known in any organism. For known imprinted gene clusters, the numbers and ranges of possible imprint-control regions are largely unknown.

Because the genes of both *X* chromosomes of Drosophila females are expressed, there is essentially no opportunity to observe specific inactivation of the type that is seen in mammals. Moreover, there is no evidence to suggest that autosomal genes in Drosophila show im-

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print-regulated behavior and, in fact, genes appear to express equally when inherited from males or females, which has led to the belief that imprinting does not occur in this insect. In mammals, uniparental diploids (androgenotes or gynogenotes) have a 2N complement of chromosomes, but the entire genome has been inherited from one parent. Uniparental diploid mammals typically die early in embryogenesis, likely due to misregulation of critical imprinted genes or chromosome regions. Uniparental diploidy of single chromosomes carrying imprinted gene clusters are common in some imprint-related diseases (Hall 1990).

Because uniparental diploids of *Drosophila melanogaster* are viable and have no visible phenotype (Lindsley and Grell 1969), very few cases of genomic imprinting have been described in this species, strengthening the erroneous argument that genomic imprinting is absent in Drosophila. However, a form of epigenetic inactivation is known to occur in Drosophila. Position-effect variegation (PEV) occurs when a euchromatic gene is juxtaposed to heterochromatin or when a heterochromatic gene is juxtaposed to euchromatin (Spofford 1976). PEV results in the clonal activation or inactivation of the gene, as well as genes closely linked to the new euchromatic/heterochromatic boundary. The state of gene inactivation is inherited epigenetically through multiple mitoses. Both males and females are capable of establishing and maintaining the epigenetic states of chromatin in the soma that are visualized by PEV. However, cases are known in other insects where males and females establish different epigenetic states in meiosis, termed imprints, resulting in parent-of-origin-dependent chromatin behavior. This genomic imprinting may result in parent-specific chromosome behavior (*e.g.*, chromosome condensation, inactivation, and elimination) or in the monoallelic expression of genes (Baker 1975; Sequeira *et al.* 1989; Zhang and Hawley 1990; Herrick and Seger 1999). The few cases of genomic imprinting in Drosophila that affect gene expression are due to chromosomal rearrangements that cause PEV (Golic *et al.* 1998; Haller and Woodruff 2000; Lloyd 2000), and are thus scored on the basis of the extent of PEV upon marker genes. Euchromatic genes transposed to the *Y* chromosome typically experience PEV and may be inherited maternally or paternally since the Drosophila *Y* chromosome does not determine sex (Bridges 1916). In two reported cases, genetically identical offspring with either a paternal or a maternal *Y* exhibit different levels of gene inactivation (Golic *et al.* 1998; Haller and Woodruff 2000). We investigated whether genomic imprinting in Drosophila applies generally to the *Y* chromosome and whether autosomal heterochromatin is similarly imprinted.

We selected 30 *P*-element transpositions to the *Y* chromosome and autosomal heterochromatin and assayed them for parent-of-origin-specific gene regulation. We found that 22 of 23 transpositions to the *Y* chromosome exhibited parent-specific gene regulation. These transgenes were inserted at a variety of locations on the *Y* chromosome and were embedded within a variety of DNA sequences, including satellite DNA, middle-repetitive, and apparently unique sequence DNA. A chromosomal aberration that appended the *yellow*$^+$ gene, in its regular genomic context, onto the tip of the *Y* chromosome was also subject to imprinting. In contrast, only one of seven *P* elements inserted into autosomal heterochromatin showed even marginal genomic imprinting, leading us to conclude that the *Y* chromosome receives a distinctive imprint that is generally not shared by regions of autosomal heterochromatin.

MATERIALS AND METHODS

Drosophila stocks: *P*-element stocks are described elsewhere (Roseman *et al.* 1995; Yan *et al.* 2002); chromosome aberration stocks are described in Lindsley and Zimm (1992) or at http://flybase.bio.indiana.edu/. The *y* stock is y^1 from the Karpen Laboratory; the *y w* stock is $y^1\ w^{1118}$; the *X^Y* stock is $Y^S X{\bullet} Y^L$, *In (1)EN, y*; the *Y, B* stock is $B^S Y$; the w^{m4} stock is *In (1)*w^{m4}; and the *X^X* stock is *C (1)RM, y v*. The $Y^S y^+$ chromosome was constructed by heat-shock-induced expression of I-CreI (Rong *et al.* 2002), which makes double-strand breaks in the genes encoding 28S rRNA. These genes are found in the heterochromatic *bobbed* (*bb*) loci of the *X* and *Y* chromosomes (Hawley 1989). *y w*/$B^S Y y^+$; *70I-CreI Sb*/+ males were heat-shocked at 36° for 1 hr during the first 3 days of development and crossed to *C(1)DX, y f*/*Y* females. The $Y^S y^+$ chromosome was recovered in a $y^+\ B^+$ female and consists of the *X* chromosome centromere joined to the short arm of the *Y* chromosome, with the tip of the *X* chromosome, including y^+, appended to the end (Figure 1). We refer to the distal half of the short arm of the *Y* as "Y^S" for ease, even though the Y^S material between the centromere and the *bb* locus is retained on the "Y^L" fragment. The reciprocal chromosome ($X{\bullet} Y^L B^S$) was recovered from *y B* males, and consists of the *X* chromosome attached to the long arm of the *Y* chromosome. These chromosomes were kept together in a stock as *C(1)DX, y f*/$X{\bullet} Y^L B^S / Y^S y^+$, assuring that they were isogenic for autosomes in our experiments.

Genetic crosses and imprint assessment: Maternal *Y* chromosomes were inherited from *X*/*X*/*Y* females crossed to *X^Y*/0 males. This assures that, like males derived from *X*/*X* and *X*/*Y* parents, males bearing maternal *Y* chromosomes contain maternally inherited *X* chromosomes as well as sets of maternal and paternal autosomes. Exceptional classes from *X* chromosome nondisjunction in females (Bridges 1916) were easily discriminated from the balance of the offspring and were excluded from our analysis. These exceptions (*X^Y*/*Y*) were distinguished from the "100%" class by being fully white$^+$ and possessing a pseudopupil. Crosses to assay expression of a maternally inherited *Y* chromosome generated 50% *X*/0 sons, which were excluded from the "0%" class numerically (Golic *et al.* 1998) or by the complete absence of any *yellow*$^+$ or *white*$^+$ marker (for the *SUPorP* insertions this was a reliable method because the *SUPorP*-bearing males virtually always had some degree of *white*$^+$ or *yellow*$^+$ expression). In both cases, the assumption of 50% *X*/0 progeny was tested on a subset of flies by outcrossing scored males to test sterility (due to lack of a *Y* chromosome).

For *SUPorP*, we scored the occurrence of white$^+$ spots in eyes or yellow$^+$ patches on the dorsal abdominal cuticle as a measure of the degree of gene expression. When the ranges

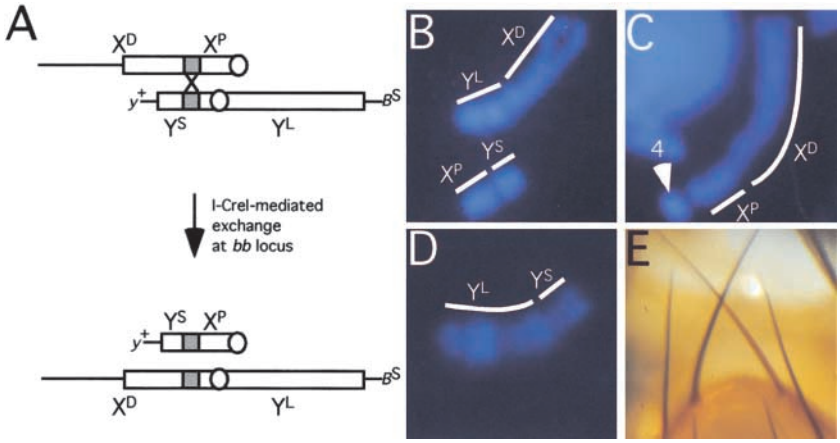


FIGURE 1.—Structure of $Y^S y^+$. (A) Schematic exchange between the *bobbed* (*bb*) loci (shaded boxes) of the *X* and *Y* chromosomes (top) to give the $Y^S y^+$ and the $X{\bullet}Y^L B^S$ chromosomes. (B) DAPI-stained neuroblast squash from an $X{\bullet}Y^L B^S / Y^S y^+$ male. (C) Neuroblast squash showing a wild-type *X* chromosome; proximal (X^P) and distal (X^D) portions of the *X* are indicated. An errant chromosome *4* is also visible. (D) Neuroblast squash showing a wild-type *Y* chromosome; long (Y^L) and short (Y^S) portions are indicated. (E) Example of variegation of *yellow*$^+$ in a *y w*/$Y^S y^+$ male. The crossed macrochaete emanating from the right is yellow$^-$, while the crossed macrochaete from the left is yellow$^+$.

of white$^+$ and yellow$^+$ were identical in range and dispersion, we considered the *P* element "not imprinted." In the case of *SUPorP*, at least 100 progeny from the crosses depicted in Figures 1 and 6 were compared, and initial ranges were determined by selecting the individuals with the most and the least expression. For flies with few white$^+$ or yellow$^+$ spots, this initial range was used as a "prior distribution" in Bayesian inference (IVERSEN 1984). We counted white$^+$ ommatidia on a smaller set of randomly selected individuals (10–20) and employed Bayesian inference *t*-tests to generate the ranges presented in Figure 2. For insertions exhibiting high levels of white$^+$ (> ~10% of the eye) or yellow$^+$ (> ~25% coverage of the abdomen) expression, quantitation was done by directly comparing groups of flies with either maternally or paternally inherited *Y* chromosomes and estimating ranges from such comparisons. For these cases, photographs of representative examples are shown in Figures 2 and 8. For *RSw*-10A, quantitation was done by assigning individual eyes to categories of expression: no *white*$^+$ ommatidia, <10% *white*$^+$ ommatidia, <50% *white*$^+$ ommatidia, <100% *white*$^+$ ommatidia, and 100% *white*$^+$ (GOLIC *et al.* 1998).

To test whether the autosomes of the *X^Y* stock used at the last cross of Figures 2 and 5A carried modifiers of variegation, we scored +/+ and +/*Balancer* offspring of females bearing *RSw*-10A crossed to *X^Y*/0; +/+, *X^Y*/0; +/*SM1, Cy*, or *X^Y*/0; +/*TM3, Sb* males, and the offspring of males bearing the elements crossed to *y w*/*y w*; +/+, *y w*/*y w*; +/*SM1, Cy*, or *y w*/*y w*; +/*TM3, Sb*. In no case did the +/+ offspring differ from the +/*Balancer* offspring, indicating that neither the *X^Y*/0 stock nor the *y w* stock had differences in autosomal modifiers of variegation. In a subset of cases, we generated fly stocks that were autosomally isogenic to the *y w* stock, but had *X^Y* and *X^X* for sex chromosomes. Comparison of the results of crosses with fathers of this genomic constitution did not differ from the balancer-containing fathers, further showing that the autosomal background did not contain modifiers of position-effect variegation.

Imprinting of *yellow*$^+$ on $Y^S y^+$ was quantitated by scoring the yellow phenotype of the scutellar macrochaete. A fly with one or more (of the four) yellow scutellar bristles (Figure 1E) was counted as "*yellow* variegating" (Table 1).

Statistics: Statistical analysis was done with Statistica 4.1 (Statsoft) or Excel 2001 (Microsoft) following the guidelines of SOKAL and ROHLF (1995) or IVERSEN (1984). Hypothesis testing was done using the Student's *t*-test with Bayesian inference (for Figures 3 and 8), the Kolmogorov-Smirnov two-sample test comparing frequency distributions (for Figures 5 and 6), or the chi-square contingency test (for Table 1). Significance was considered at $\alpha = 0.05$.

In Figure 5B, the Kolmogorov-Smirnov statistic $D_{max} = 0.72$ ($P < 10^{-3}$) for a comparison of offspring with *XXY vs. XY* parents; $D_{max} = 0.19$ ($0.05 < P < 0.1$) for comparison of offspring with *XY vs. XYY* fathers; $D_{max} = 0.13$ ($P > 0.1$) for comparison of offspring with *XXY vs. XXYY* mothers; H_0 states that expression distributions of *white*$^+$ in male offspring of both conditions will be drawn from identical populations. Numbers of *XY* offspring from various parents are as follows: *XY* (2079), *XYY* (868), *XXY* (877), and *XXYY* (1145). In Figure 5D, $D_{max} = 0.08$ ($P > 0.1$); H_0 states that *white*$^+$ expression from paternal *Y* chromosomes, with ($n = 1160$) and without ($n = 2079$) *Y,B* in the mother, will be indistinguishable. In Figure 6, $D_{max} = 0.14$ ($P > 0.1$); H_0 states that both paternal ($n = 398$) and maternal ($n = 342$) *Y* chromosomes will suppress variegation of w^{m4} equally.

For the inactivation of *yellow*$^+$ on $Y^S y^+$ chromosomes in Table 1, $\chi^2 = 10.74$, d.f. = 1, and $P = 10^{-3}$; H_0 states that the fraction of *yellow*$^+$ variegating offspring will be the same if $Y^S y^+$ is inherited paternally or maternally.

Cytological localization of *P* elements and neuroblast cytology: Digoxygenin-labeled probe was made from the entirety of the *SUPorP* element using alkali-stable digoxigenin-11-2′-deoxyuridine-5′-triphosphate (Roche) and detected using anti-digoxigenin-rhodamine Fab fragment (Roche). Neuroblasts were isolated and squashed, and *in situ* hybridization and 4′,6-diamidino-2-phenylindole (DAPI) counterstaining were performed as described (YAN *et al.* 2002). The images in Figure 1 were obtained after squashing and DAPI staining. Images were collected from a Zeiss (Thornwood, NY) Axioplan microscope using a D1X camera (Nikon, Garden City, NY) or an Axiocam camera (Zeiss) and cropped on a G4 Macintosh computer (Apple) with Photoshop 6.0 (Adobe). Figure 1, B, D, and E were manipulated for brightness and contrast to show chromosome bands and the difference between yellow$^-$ and yellow$^+$ bristles more clearly.

Imaging of white$^+$ and yellow$^+$ phenotypes: Adult flies were submerged in heavy mineral oil and viewed under a Nikon SMZ1500 microscope. Images were captured on a D1X camera and cropped on a G4 Macintosh computer with Photoshop 6.0.

Inverse polymerase chain reaction and sequencing: Inverse PCR was done as described (DOBIE *et al.* 2001). PCR products were gel purified using Geneclean II or QIAGEN (Valencia, CA) gel purification columns and sequenced on a capillary sequencer (Beckman, Fullerton, CA). BLAST searches were done against the Berkeley *Drosophila* Genome Project Release 2.9 of the Drosophila genome at http://flybase.bio.indiana.edu/ and against the National Center for Biotechnology Information (NCBI) database at http://www.ncbi.nlm.nih.gov/blast/.

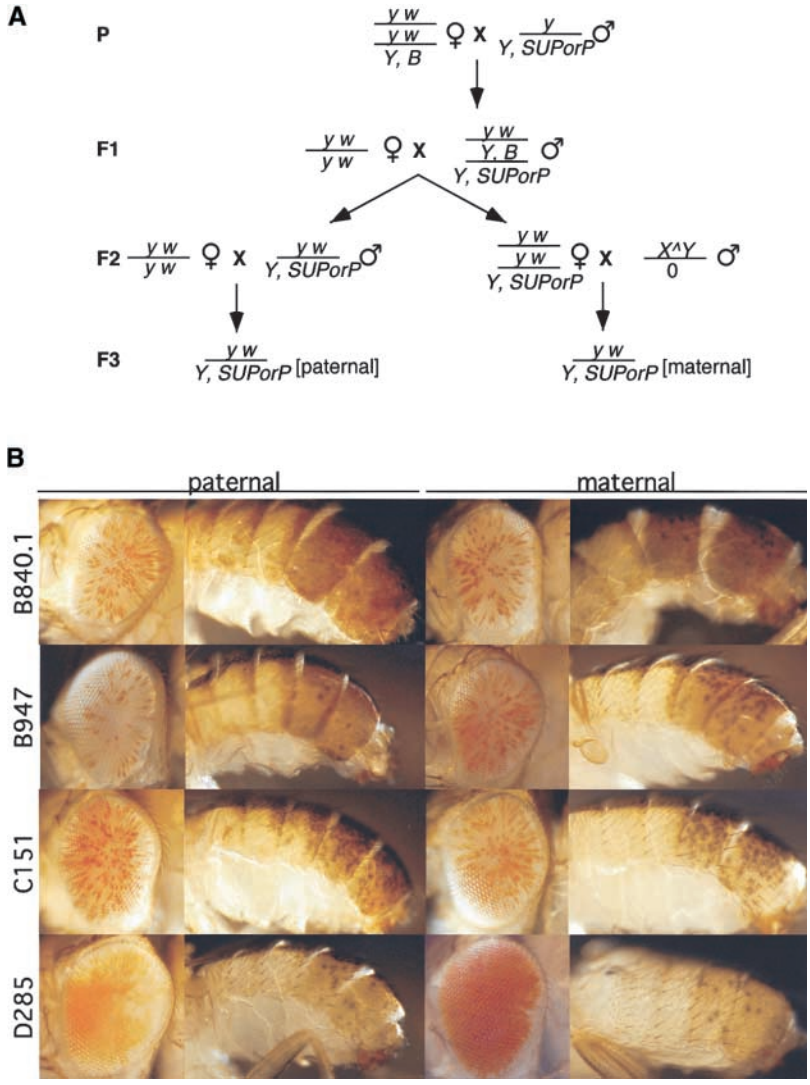


FIGURE 2.—Genetic scheme to detect imprinting of the *Y* chromosome. (A) Crossing scheme to generate flies with paternally derived *Y* chromosomes (left) or maternally derived *Y* chromosomes (right). P, F_1, F_2, and F_3 refer to generations. Genetic stocks are described in MATERIALS AND METHODS. (B) *white*$^+$ gene expression in eyes and *yellow*$^+$ gene expression on the abdominal cuticles of males with paternally inherited or maternally inherited *Y* chromosomes. Pictures of representative flies are provided for lines whose expression ranges were quantitated subjectively.

RESULTS

Genomic imprinting of *Y*-linked transgenes: DOBIE *et al.* (2001) generated a large number of autosomal and *Y* chromosome-linked *P* elements in an attempt to mutagenize the genome. Initially, >100 were identified as having variegated expression of at least one of the two marker genes carried by these *P* elements. Most were mapped genetically and cytologically to heterochromatin (YAN *et al.* 2002). We used the *Y*-linked members of this collection (18 members) and from other sources (3 from ROSEMAN *et al.* 1995 and 1 from S. Pimpinelli) to survey the *Y* chromosome for regions that are subject to imprinting. The *SUPorP* element used in this work carries two marker genes: *white*$^+$ and *yellow*$^+$. We assayed for parent-of-origin effects on the expression of both genes.

To test for imprint-regulated expression of the *Y*-linked transgenes, males carrying each *Y* chromosome were crossed to females with a supernumerary *Y* chromosome, as diagrammed in Figure 2A. Three generations later, groups of males were produced, differing in whether their *Y* chromosome was ultimately transmitted from their fathers or their mothers. To assay for genomic imprinting, we compared the levels of reporter gene expression, manifest as degree of variegation, in these flies.

All but one of the 22 *Y*-linked *SUPorP* transpositions that we tested showed imprint-regulated expression of *white*$^+$ or *yellow*$^+$ (or both) marker genes (Figures 2–4). In most cases (13 of 22), paternal inheritance of the marked *Y* chromosome led to reduced expression of *white*$^+$ and *yellow*$^+$, relative to maternal inheritance of the chromosome. We also retested the insertion *RSw*-10A, which was previously reported to be imprinted (GOLIC *et al.* 1998), and confirmed that flies inheriting this chromosome maternally show higher expression than do flies inheriting it paternally. Strong response to an imprint is exemplified by line 25-28-3, while a more subtle response is seen in line B947 (Figure 2). Notable

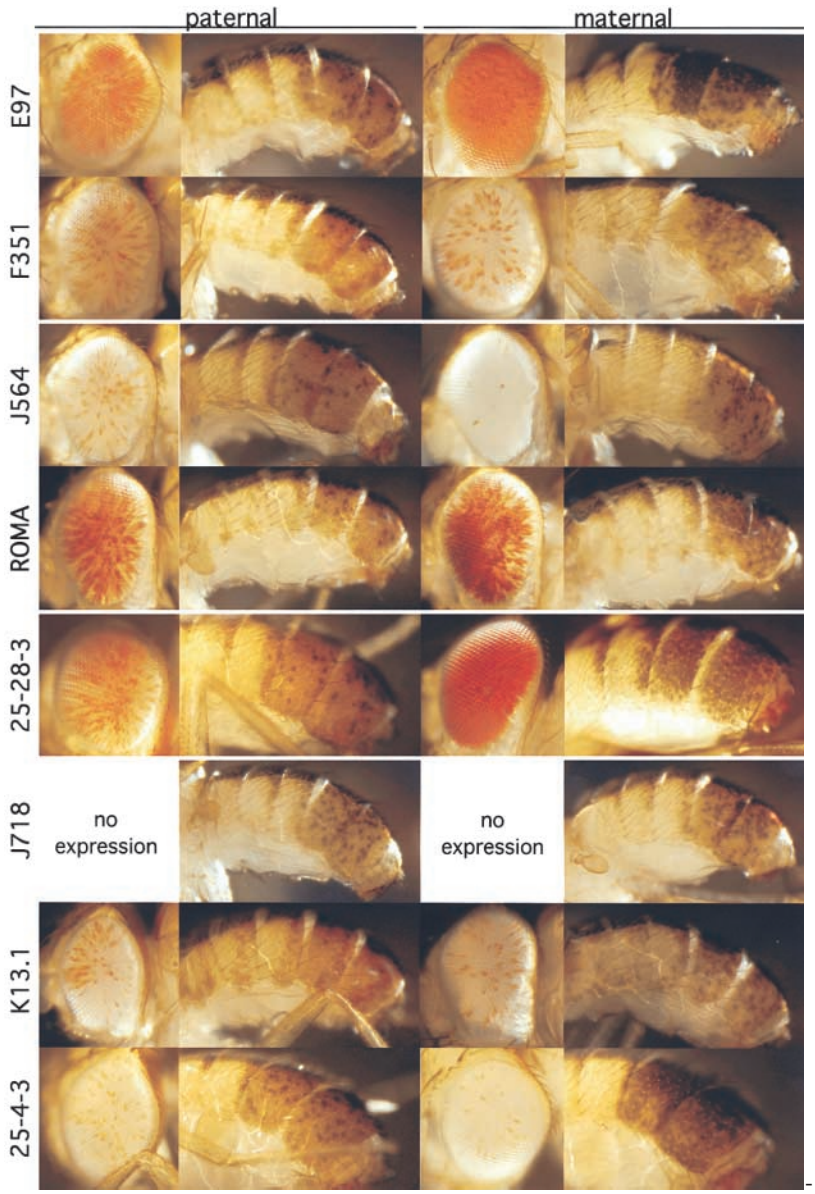


Figure 2.—*Continued.*

exceptions to this predominant pattern of high maternal/low paternal expression include four cases of the *white*$^+$ gene responding to the imprint in the opposite direction (lines C151, F351, J564, and 25-4-3). Another case of high paternal/low maternal expression was reported by Haller and Woodruff (2000).

In three cases, the *white*$^+$ gene was not appreciably imprinted (lines B840.1, J632.2, and K13.1), while the *yellow*$^+$ gene was. It may be significant that the four insertions that showed no imprinting of *white*$^+$ were found in cytological bands h10-h14 (Figure 3), despite a more uniform distribution of the rest of the insertions. This may reflect a characteristic of the sequence or structure of the heterochromatin in that region of the *Y* chromosome. Although the *white*$^+$ reporter gene showed a variable response to imprinting in this region, imprinting clearly does extend into this region because the *yellow*$^+$ genes of these elements reveal its presence. In 1 of the 23 transpositions, *white*$^+$ expression of both maternally and paternally derived transgenes was too low to detect a difference; however, the *yellow*$^+$ gene in that line was imprinted (Figure 4). Only one insertion showed no discernable difference between paternal and maternal inheritance (line 221-1).

Although there were clear exceptions, the dominant trend was for a maternally inherited *Y* chromosome to show high expression of the *white*$^+$ and *yellow*$^+$ transgenes, relative to the same chromosome inherited paternally. Fourteen of 23 *white*$^+$ gene insertions and 20 of 22 *yellow*$^+$ gene insertions showed this behavior.

Sequence and location of *P* elements on the *Y* chromosome: *P* elements from Dobie *et al.* (2001) have been

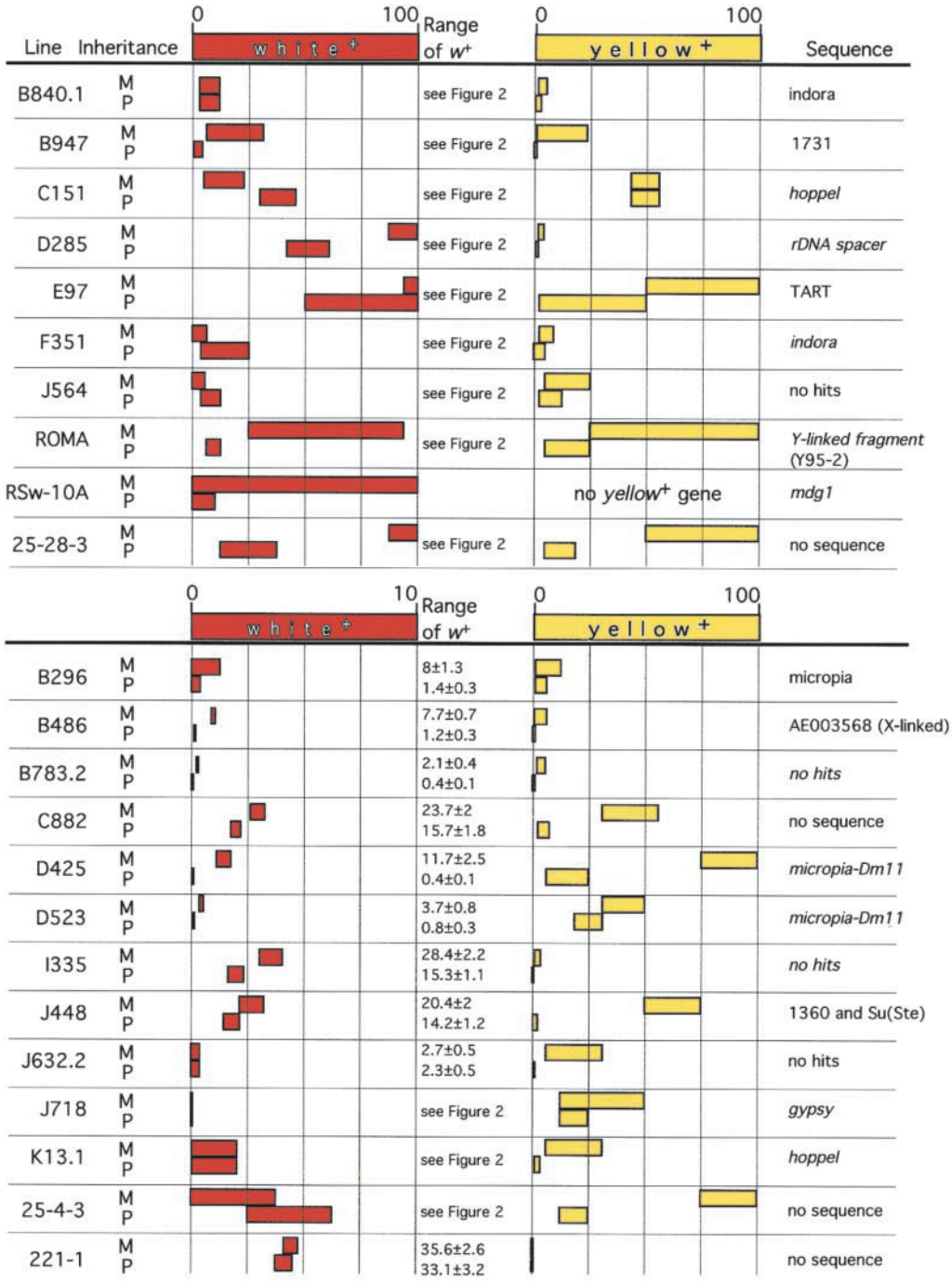


FIGURE 3.—Expression ranges for *white*$^+$ and *yellow*$^+$ in *Y*-linked lines. Line refers to the insert number (nomenclature from original laboratory). Inheritance indicates maternally (M)- or paternally (P)-derived *Y* chromosomes. Ranges for *white*$^+$ and *yellow*$^+$ are indicated by estimation of percentage of cells expressing the marker gene. Paternal and maternal groups were compared side by side and quantitation was performed where appropriate (see MATERIALS AND METHODS). Lower half of the figure has a rescaled *white*$^+$ expression (full range is 10% coverage of the eye). Sequence indicates results of a BLAST search with flanking DNA sequence to NCBI and FlyBase *D. melanogaster* genomic sequence databases. Sequence homologies in italics were generated in this study; the rest are from YAN *et al.* (2002).

localized cytologically using fluorescence *in situ* hybridization to mitotic *Y* chromosomes (YAN *et al.* 2002). We localized three *SUPorP* elements from the Geyer collection (ROSEMAN *et al.* 1995) and the one *SUPorP* element from S. Pimpinelli; our results confirmed previous localizations of two elements, ROMA and 25-4-3, done by S. PIMPINELLI (personal communication). The cytological localizations of these elements are shown in Figure 4.

YAN *et al.* (2002) used the inverse polymerase chain reaction to determine the DNA sequences that flanked several of the *P* elements. Our analysis continued their study by generating sequences for more of the elements in our survey. These sequences are summarized in Figure 3. In total, these elements were inserted in eight different transposable elements as well as in four sequences without homology in the Drosophila database that are presumably repetitive sequences, which are underrepresented in the Drosophila sequencing scheme. There were no apparent commonalities between types

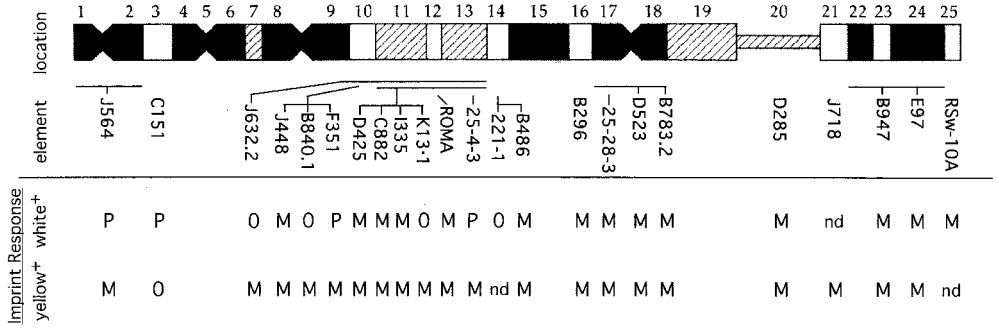


FIGURE 4.—Map and imprinting of *Y* chromosome. A cytological map of the *Y* chromosome (GATTI and PIMPINELLI 1992) is shown with locations of elements indicated beneath. Horizontal bars indicate cytological localization within that region. The relative locations of elements within a given band or region are not meaningful. Responses to the imprint are shown separately for *white*$^+$ and *yellow*$^+$; M, the maternally derived gene is expressed higher than the paternally derived gene; P, the paternally derived gene is expressed at a higher level; 0, both are expressed at approximately the same level; nd, determination was not possible due to low gene expression (for 221-1 and J718) or the absence of the marker gene (for *RSw*-10A). Localizations of ROMA and 25-4-3 have been confirmed by S. Pimpinelli (University of Rome).

of insertions and imprint characteristics. Elements that exhibited a difference in *yellow*$^+$ and not *white*$^+$ expression (B840.1, J632.2, and K13.1) had transposed to three different types of sequence. The four lines that showed a strong high paternal expression of *white*$^+$ response to imprinting (C151, F351, J564, and 25-4-3) had transposed to three different DNA sequences. Two elements that behaved quite differently with respect to imprinting (*e.g.*, C151 and K13.1) were found to lie within homologous sequences at different sites on the *Y* chromosome. There is no apparent correlation between location of insertion, flanking DNA sequence, and the nature of the response to the genomic imprint that affected the *white*$^+$ and *yellow*$^+$ genes within the transposed *SUPorP* element, apart from the aforementioned cluster in h10-h14.

Spreading of an imprint: The results of this *Y* chromosome survey lead us to conclude that imprinting is a general, chromosome-wide feature of the *Y* chromosome. Dosage compensation of the *X* chromosome is also a chromosome-wide feature in Drosophila. On the *X*, dosage compensation appears to be controlled by sequence elements spread across the chromosome, and the imposition of dosage compensation spreads only a short distance in cases where these elements are moved to autosomal locations (KELLEY *et al.* 1999). In contrast, mammalian *X* inactivation may proceed along an entire chromosome arm in transgenic mice that move the *X*-inactivation center to an autosome (LEE *et al.* 1996).

To determine whether an imprint can spread from *Y* heterochromatin to euchromatin, a Y^Sy^+ chromosome was generated that carries ~300 kb of the *X* chromosome including the *yellow*$^+$ gene appended to the short arm of the *Y* chromosome (Figure 1; see MATERIALS AND METHODS). The *yellow*$^+$ gene is ~20 kb from the euchromatic/heterochromatic breakpoint (MAGGERT and KARPEN 2001). This element was transmitted maternally and paternally to X/Y^Sy^+ males, and the extent of *yellow*$^+$ variegation (assayed by yellow bristles on the notum) was assayed (Table 1). Although the *yellow*$^+$ gene on B^SY^+, from which Y^Sy^+ was derived, does not normally variegate, it does in this context. This is likely because a large amount of heterochromatin (Y^L) that is present in normal males is absent in this genotype, enhancing PEV. We observed that inactivation of the *yellow*$^+$ gene was more pronounced if the Y^Sy^+ was inherited paternally ($P = 10^{-3}$; Table 1), demonstrating that the *Y* chromosome imprint can spread for a distance of at least 20 kb into adjacent non-*Y* sequence (as in LLOYD *et al.* 1999).

Analysis of maternal effects: The *Y* chromosome is known to be the most potent suppressor of PEV. NOUJDIN (1944) reported that the effect of a *Y* chromosome on PEV can have a maternal effect, such that genetically identical offspring from *X^X/0* or *X^X/Y* mothers show different levels of suppression of PEV. In this case, maternal effect is taken to mean a nonchromosomal or cytoplasmic effect. Our experiments to detect genomic imprinting rely on using *X/X/Y* mothers as a source of maternal *Y* chromosomes. To abrogate the possibility that *X/Y* offspring of *X/X/Y* mothers differ from *X/Y* offspring of *X/Y* fathers not simply because the *Y* was inherited maternally, but because of a *Y* chromosome maternal effect, we analyzed the offspring of *X/X/Y*, *RSw*-10A/*Y*, *B* mothers (Figure 5A). We reasoned that

TABLE 1

***yellow*$^+$ variegation on Y^Sy^+ transmitted maternally or paternally**

Transmission	Phenotype: yellow$^+$	Phenotype: yellow^{var}	Frequency
Paternal	453	75	0.14
Maternal	241	16	0.06

The male progeny of *y w/y w* × $X^{\bullet}Y^LB^S/Y^Sy^+$ (paternal inheritance) were compared to the male progeny of *C(1)DX, y f/*Y^Sy^+ × *y w/Y* (maternal inheritance).

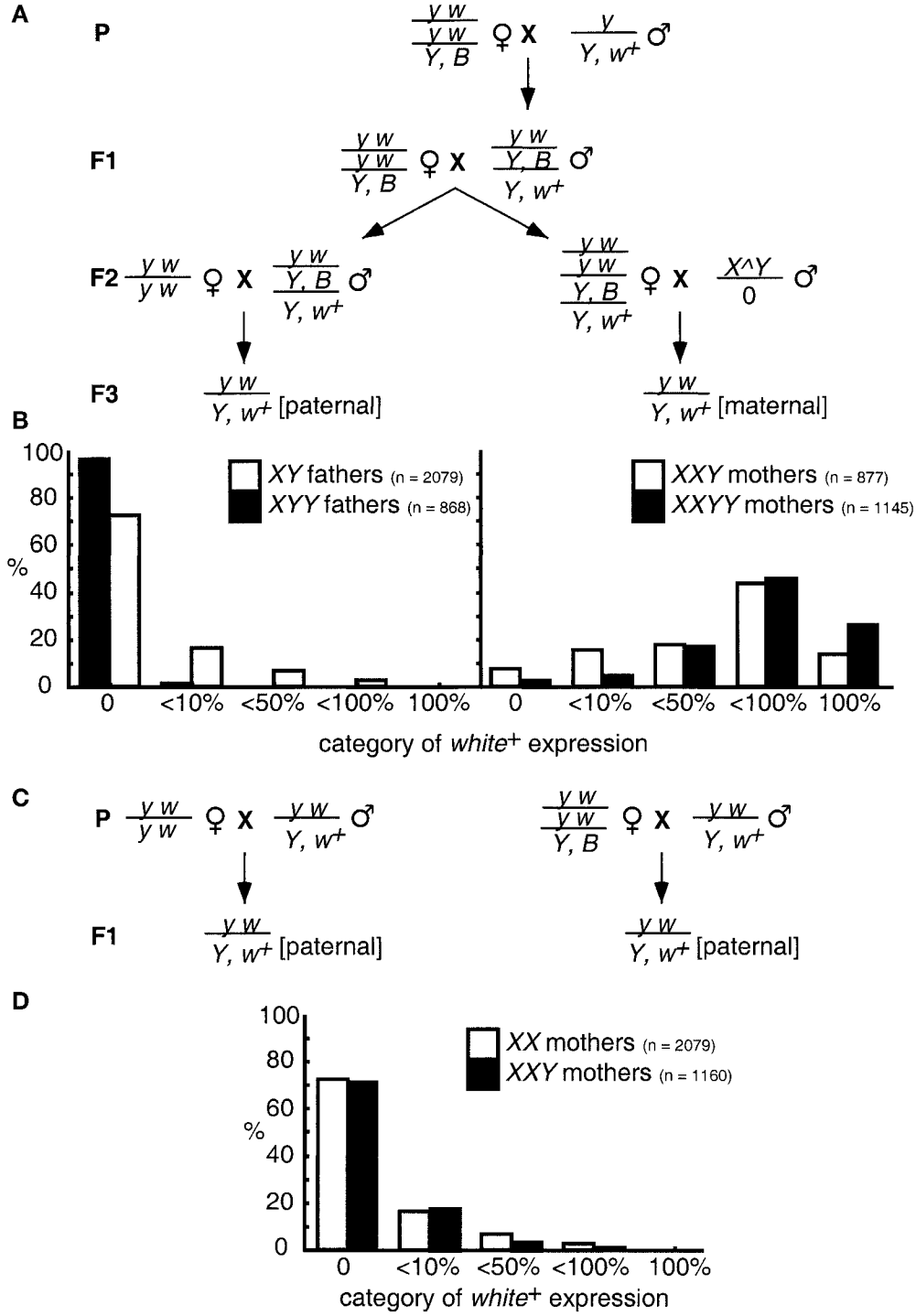


FIGURE 5.—Testing paternal and maternal effects of supernumerary *Y* chromosomes. (A) Crossing scheme to test offspring from parents with extra *Y* chromosomes with paternally derived *Y* chromosomes (left) and maternally derived *Y* chromosomes (right). (B) Results of the comparison between flies having parents with different numbers of *Y* chromosomes. Left-hand graph shows *white*$^+$ expression in sons of *XYY* fathers *vs.* sons of *XY* fathers. In the right-hand graph, expression from sons of *XXYY* and *XXY* mothers is compared. Data for flies with *XY* and *XXY* parents were derived from crosses similar to those of Figure 2A. *n*, total eyes counted. (C) Crossing scheme to compare offspring from *XX vs. XXY* mothers and (D) the results of that comparison. In all cases, the *Y*, w^+ is *RSw*-10A.

if an extra *Y* chromosome in the mother acts to suppress PEV in the offspring, then two additional *Y* chromosomes (*X/X/Y/Y*) would be expected to suppress PEV to a greater extent, increasing the expression of a *Y*-linked transgene even more. The offspring of *X/X/Y/Y* mothers were not statistically distinguishable from the offspring of *X/X/Y* mothers ($P > 0.1$, Figure 5B), indicating that additional *Y* chromatin in a mother is not sufficient to explain the disparity in gene expression from maternal and paternal alleles.

To confirm this result, we crossed *X/X/Y, B* mothers, which have an extra *Y* chromosome, to *X/Y, RSw*-10A males (Figure 5C). The Bar$^+$ male offspring (*y w/Y*, w^+) had a canonically "paternal" level of expression ($P > 0.1$, Figure 5D), confirming that the imprint is carried by the chromosome alone and not by the ooplasm. Our experi-

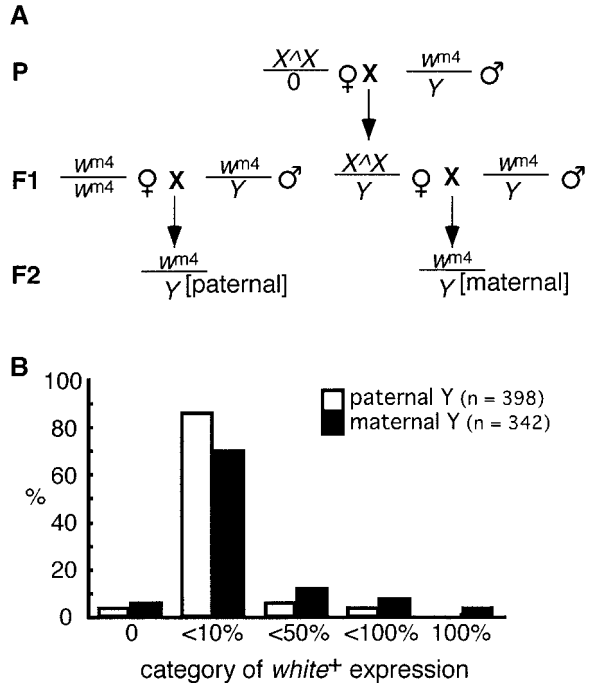


FIGURE 6.—Testing maternally and paternally inherited *Y* chromosomes for their ability to suppress position-effect variegation. (A) The crossing scheme. (B) The results of the comparison. *n*, total eyes counted.

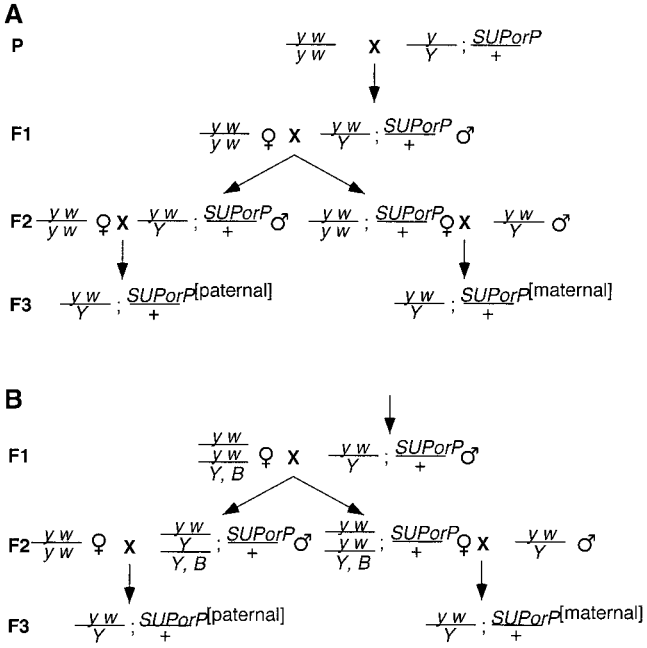


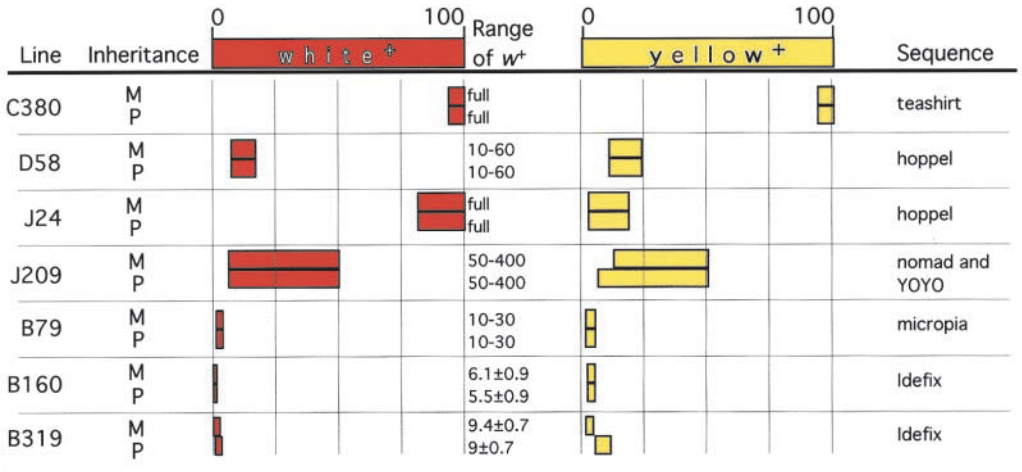
FIGURE 7.—Genetic scheme to detect imprinting on the autosomes. (A) Crossing scheme for autosomes transmitted paternally (left) or maternally (right). (B) The crossing scheme for autosomes transmitted paternally (left) or maternally (right), where mothers of the F_3 offspring contain an extra *Y* chromosome. P generation was the same as in A.

ments provide no support for the idea that the *Y* chromosome has a maternal effect on PEV (*cf.* NOUJDIN 1944; *q.v.* SPOFFORD 1976). This confirms our previous results from the examination of the *RSw*-10A imprint (GOLIC *et al.* 1998).

A *P* element transposed to the *Y* chromosome is subject to PEV because of its proximity to heterochromatin. It is, however, also subject to alleviation of PEV by virtue of the fact that the *Y* chromosome in which it is embedded can act as a suppressor of variegation. It has been previously reported that the suppression-of-variegation activity of a *Y* chromosome may be heritably modified in a particular genetic background (DORN *et al.* 1993). Similarly, a *Y* chromosome imprint could represent an alteration either in the chromatin of the transposed gene or in the ability of that *Y* chromosome to act as a suppressor of variegation. We tested this by assaying the suppression-of-variegation activity of *Y* chromosomes inherited from males and from females. Males and females carrying unmarked *Y* chromosomes were crossed to flies with a rearranged *X* chromosome showing *white*$^+$ variegation, *In (1)* w^{m4} (Figure 6A). Male offspring differed in only the maternal or the paternal origin of their *Y* chromosomes. Comparison of the extent of suppression of the white-mottled phenotype revealed no difference between males with paternally derived *Y* chromosomes and those with maternally derived *Y* chromosomes ($P > 0.1$, Figure 6B). We find no evidence that, in our experiments, the potency of the *Y* chromosome as a suppressor of variegation is altered by the parental source. We conclude that the altered levels of *Y*-linked transgene expression derive more directly from the chromosomal imprint.

Analysis of paternal effects: We noted a small enhancement of variegation of *white*$^+$ when the *Y*, w^+ chromosome was inherited from an *X/Y/Y* father rather than from an *X/Y* father (Figure 5B), but the difference was slight. Although a difference in expression based on paternal genomic constitution may exist, our assay is not sufficiently powerful to detect it ($0.05 < P < 0.1$). In any event, our assay for imprinting did not rely on assaying variegation in sons of *X/Y/Y* fathers.

Assaying for imprinting in autosomal heterochromatin: We wished to know whether transposons inserted into heterochromatin would show imprint-regulated expression in general or whether this property is specific to the *Y* chromosome. We obtained seven *SUPorP* transpositions into autosomal heterochromatin and tested them for imprint-regulated expression of the variegating reporter genes within *SUPorP*. The crosses are shown in Figure 7A. The expression of genes carried by *SUPorP* did not differ appreciably whether transmitted by females or males (Figure 8). In only one case (B319) did the *yellow*$^+$ gene of the *P* element show a parental source effect, where the maternally transmitted allele was expressed at a lower level than that of the paternally inherited allele.



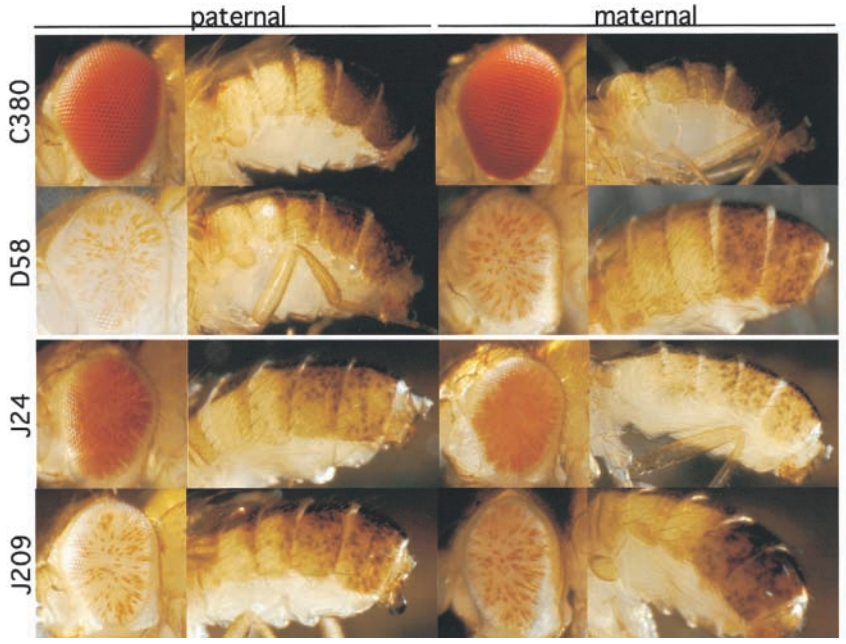


FIGURE 8.—Expression ranges for *white*$^+$ and *yellow*$^+$ in autosomal lines. Results are indicated as in Figure 3. Lines C380, D58, J24, and J209 are inserted into chromosome *2* centric heterochromatin; B79, B160, and B319 are inserted into chromosome *3* centric heterochromatin. Numerical ranges of expression (and standard deviation) are given to the right of the graphical representation. Pictures of representative flies are provided for elements that were quantitated subjectively.

We also considered the possibility that an imprint is triggered only in organisms with supernumerary *Y* chromosomes. We tested the expression of the autosomally linked transpositions from *X/X/Y*, *B* and *X/Y/Y*, *B* parents (Figure 7B). The expression of *white*$^+$ and *yellow*$^+$ did not differ between the offspring of these males and females (with the exception of B319) or differ from the offspring of the *X/X* and *X/Y* parents (data not shown). Thus, the presence of an extra *Y* in a parent does not impose an imprint on autosomal *SUPorP* transgenes that is sufficient to create a detectable difference between paternally and maternally inherited elements. We conclude that, in general, autosomal heterochromatic insertions are not subject to genomic imprinting.

DISCUSSION

Thirty transposon insertions bearing *white*$^+$ and *yellow*$^+$ markers were assessed for genomic imprinting and parent-of-origin-specific gene inactivation. Twenty-two of the 23 transpositions to the *Y* chromosome were imprinted. In contrast, only 1 of the 7 transpositions to autosomal heterochromatin was imprinted.

Imprinting is a general feature of *Y*-linked heterochromatin: To date, about a dozen examples of genomic imprinting in Drosophila have been reported, affecting half as many genes (LLOYD 2000). Of those genes, *white*$^+$ and *yellow*$^+$ are common markers for germline-mediated transformation of Drosophila, and yet the phenomenon of their imprinting, when inserted in *Y* heterochromatin, has only rarely been commented upon. This is likely due to the normally sex-limited inheritance of the *Y* chromosome, as well as the extreme variegation of most *Y*-linked transpositions. In this study, we have shown that the preponderance of *P* elements on the *Y* chromosome becomes imprinted. Indeed, no cytological band tested is immune to imprinting. This collection of *SUPorP* transpositions to the *Y* chromosome is likely to be biased in

two ways. First, insertions of the *P* element were scored genetically for variegated gene silencing, and so insertions to the *Y* chromosome that underwent extreme PEV would not have been collected. Second, since the screen to recover transposition events included passage of *Y* chromosomes through males, any transposon insertion that affected one of the six male fertility loci on the *Y* chromosome would have been lost due to male sterility. Nonetheless, this collection appears to provide a broad sampling of *Y* chromosome insertion sites. *P* elements were inserted in approximately half of 25 *Y* chromosome bands, including insertions into four of the regions that contain fertility factors and one into the rDNA locus (Figure 4), and into a wide range of DNA sequences (Figure 3).

Genes placed near known imprint-control regions in mammals have been shown to come under the control of those regions, and the introduced genes may exhibit monoallelic expression (Ripoche *et al.* 1997; Jones *et al.* 1998; Brenton *et al.* 1999; Kaffer *et al.* 2000). In Drosophila, transposed *P* elements have been utilized because of their ability to easily adopt regulatory features of the chromosomes near where they insert (Hazelrigg *et al.* 1984). Taken together, these results suggested that the *P* elements that exhibit imprint-controlled expression have inserted in or near regions of the *Y* that are subject to imprinting. Since these insertions span most of the *Y* chromosome, this imprint is apparently applied to the *Y* chromosome as a whole during gametogenesis. The observation that the imprint can spread into attached non-*Y* material (in the case of $Y^{s}y^{+}$) strengthens the case that the imprint is laid down across the length of the *Y* chromosome, without respect to the sequence being imprinted. This is not to say that there are not specific sequence elements that trigger the imprint, analogous to a mammalian imprint center. If such elements do exist, at least one must be on the short arm of the *Y* chromosome to account for the imprinting of *yellow*$^{+}$ on $Y^{s}y^{+}$. In contrast, most autosomally inserted elements fail to exhibit a response to an imprint, and it follows that any such imprint-controlling regions are largely absent from the autosomes.

In humans, *Y* chromosome-specific repeats have been shown to be hypermethylated in mutants that compromise global chromatin remodeling, while other CpG-containing motifs are hypomethylated, suggesting that discrimination between the *Y* and other chromosomes also exists in mammals (Gibbons *et al.* 2000). A mechanism to make this distinction could rely on the unique activity of the *Y* chromosome during spermatogenesis. Alternatively, the distinction may depend on specific sequences that are found only on the *Y* (Ganguly *et al.* 1992; Danilevskaya *et al.* 1993; Aguso *et al.* 1999).

Parallels between mammalian and Drosophila imprinting: Most of the imprinted insertions show relatively high maternal and low paternal expression. However, some of the insertions show higher expression of *white*$^{+}$ when paternally inherited, in contrast to the behavior of the *yellow*$^{+}$ gene inserted at the same location. Such a dichotomy is not unprecedented. Within mammals, a single genomically imprinted gene cluster can contain some loci that are expressed only when inherited paternally and others that show expression only of the maternal allele (Beechey 1999; and at http://www.mgu.har.mrc.ac.uk). These genes may be closely linked and alternate in their chromosomal position. Moreover, despite the general conservation of imprinting in clusters, individual genes within a cluster may not exhibit parent-specific expression in some organisms or may fail to respond to an imprint in certain individuals or tissues or at certain times in development (Xu *et al.* 1993; Jinno *et al.* 1994; Jouvenot *et al.* 1999). This variability in response may be similar to our observation that, in four insertions, only one of the two genes showed a detectable response to the imprint.

Another similarity between mammals and Drosophila is that the response to an imprint need not be an absolute on/off expression. Polymorphisms in the establishment or interpretation of an imprint may also cause individual cells or organisms to fail to respond to an imprint altogether (Xu *et al.* 1993; Jinno *et al.* 1994; Jouvenot *et al.* 1999). This latter phenomenon appears to be a case of individual genes within an otherwise imprinted cluster failing to respond to the imprint, rather than the failure of the cluster to be imprinted (Jouvenot *et al.* 1999), and may be likened to the variegated expression of the *Y*-linked insertions assayed here.

A complex series of determinants may affect a gene's ability to respond to an imprint, perhaps similar to mammalian *X* chromosome inactivation. In *X* inactivation, the entirety of the *X* chromosome is cytologically condensed, and most genes are inactivated. However, some loci escape inactivation and are expressed at wild-type levels (Carrel and Willard 1999). The escape of some mammalian *X*-linked genes and the escape of Drosophila *Y*-linked transgenes from chromosome-wide regulation are, at least on the surface, similar phenomena.

The role of imprinting in Drosophila: Since the *Y* chromosome is not normally inherited through females, any evolutionary role for this imprint must be based on the relative silencing observed with normal paternal inheritance. The *Y* chromosome is thought to have arisen through inactivation and deterioration of an ancestral *X* chromosome, abetted by transposable element accumulation. Elements that invaded the *Y* chromosome may have been retained there by global inactivation of the evolving *Y* chromosome through a mark that is generally inconsistent with gene activity (Steinemann and Steinemann 1992, 2000; Junakovic *et al.* 1998). Once transposons accumulated to a significant degree, an additional advantage may have accrued in animals that were able to inactivate the *Y* as a whole because inactivation would silence and neutralize the expanding transposon load, similar to the proposed role of DNA

methylation in mammals (Yoder *et al.* 1997; Bestor 1999).

The male fertility loci of the *Y* chromosome have clearly circumvented this inactivation. A large portion of the *Y* chromosome is highly transcribed during spermatogenesis. And, since *Y* fertility factors are heterochromatic in nature, the *Y* chromosome may normally be regulated to facilitate heterochromatic gene expression, to the detriment of the expression of euchromatic genes transposed therein (Spofford 1976). Moreover, it is known that gene activity may play a role in the establishment of epigenetic states (Cavalli and Paro 1998; Ahmad and Henikoff 2001). Thus, an alternative rationale for the *Y* imprint is that it is both a consequence of and a prerequisite for proper expression of the fertility factors in primary spermatocytes. One implication of this view of *Y* chromosome imprinting is that males with a maternal *Y* should experience impaired fertility. However, as has been known for years, Drosophila males with only a maternal *Y* chromosome are still fertile. We have not undertaken a quantitative assessment of their fertility, and it is possible that such experiments might reveal a difference in fecundity of males with paternal or maternal *Y* chromosomes.

It might also be imagined that transcription of the *Y* in the soma has deleterious consequences and that the paternal imprint and response has evolved to eliminate such expression. But, there is no obvious phenotypic effect on males or females that receive a maternal *Y*.

A current hypothesis for the role of imprinting in some mammals and plants is the parental conflict hypothesis (Spielman *et al.* 2001). This proposes that imprint-controlled gene expression is the logical consequence of an evolutionary struggle between a paternal attempt to maximize the use of maternal resources and a maternal response to limit and evenly distribute her own contribution (Haig and Trivers 1995). Although this model predicts the fetal overgrowth seen in mutant analyses of approximately half of the imprinted genes tested in mammals, it cannot explain the broad existence of imprinting in organisms that provide no maternal care and whose contribution to offspring is fixed before fertilization occurs. Drosophila would be included in such a group.

It has also been suggested that mammalian genomic imprinting serves as a block against parthenogenesis, a possibility underscored by some defects observed in mammalian cloning. Although parthenogenic Drosophila have not been produced, viable uniparental diploids show that the reason is likely not due to imprinting, and many insects commonly reproduce by parthenogenesis.

It may not be possible to find a common role for imprinting in chordates, insects, and plants. But, even if examples of imprinting are not teleologically related, it is possible that genomic imprinting in the strictest sense, meaning the differential marking of genetic material according to the sex of the parent, has evolved but once. When such a mark was available it was perhaps inevitable that it would be used, potentially in different ways by different species. Since the exact nature of the parental mark is not definitively known for any species, this common explanation cannot be ruled out at this time. A comprehensive assessment of the genes involved in imprint establishment, maintenance, and interpretation must be made to understand why organisms with disparate lifestyles would appear to go to lengths to remember from where each of their chromosomes came. The ease of genetic manipulation in Drosophila promises that this system will be of central importance in answering these concerns.

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