

Genomic Imprinting in *Drosophila* has properties of both mammalian and insect imprinting

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Abstract Genomic imprinting is a process that marks DNA, causing a change in gene or chromosome behavior, depending on the sex of the transmitting parent. In mammals, most examples of genomic imprinting affect the transcription of individual or small clusters of genes whereas in insects, genomic imprinting tends to silence entire chromosomes. This has been interpreted as evidence of independent evolutionary origins for imprinting. To

investigate how these types of imprinting are related, we performed a phenotypic, molecular, and cytological analysis of an imprinted chromosome in *Drosophila melanogaster*. Analysis of this chromosome reveals that the imprint results in transcriptional silencing. Yet, the domain of transcriptional silencing is very large, extending at least 1.2 Mb and encompassing over 100 genes, and is associated with decreased somatic polytenization of the entire chromosome. We propose that repression of somatic replication in polytenized cells, as a secondary response to the imprint, acts to extend the size of the imprinted domain to an entire chromosome. Thus, imprinting in *Drosophila* has properties of both typical mammalian and insect imprinting which suggests that genomic imprinting in *Drosophila* and mammals is not fundamentally different; imprinting is manifest as transcriptional silencing of a few genes or silencing of an entire chromosome depending on secondary processes such as differences in gene density and polytenization.

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Introduction

Genomic imprinting is an epigenetic process that marks the parental origin of genomes causing a change in gene or chromosome behavior. Because imprinting allows the organism to distinguish their maternally and paternally derived genomes, genes or chromosomes can be treated differently depending on their parent of origin. This differential treatment can be manifest as silencing of a single gene, groups of adjacent genes, or entire chromosomes when derived from one, but not the other, parent.

Genomic imprinting has been found in a wide variety of organisms including mammals (Morison et al. 2005), fish (Martin and McGowan 1995a, b), many insects (Lloyd 2000; Goday and Esteban 2001; Bongiorno and Prantera 2003; Normark 2003), other invertebrates (Bean et al. 2004), and both higher and lower vascular plants (Alleman and Doctor 2000; Tourte et al. 1980). Despite this wide distribution, genomic imprinting has been generally categorized as either transcriptional imprinting, best described in mammals and plants, or whole chromosome imprinting, as found in many insects (de la Casa-Esperon and Sapienza 2003). The presence of imprinting in such a diverse array of organisms and the two general patterns by which it is manifest raises the issue of whether imprinting is an ancient process or has evolved independently in different groups (Hurst and McVean 1998; Lyko and Paro 1999; Pardo-Manuel de Villena et al. 2000; Feil and Berger 2007).

Evidence in favor of a single ancient origin for imprinting is that in organisms as disparate as mammals, plants and insects, imprinting is produced by the same suite of interrelated epigenetic processes: histone modification, DNA methylation and RNAi-dependent gene silencing, and the ensuing formation of heterochromatin (Lippman and Martienssen 2004; Grewal and Elgin 2007). Further, genes which regulate imprinting in *Drosophila*, including the *Su (var) 3–9* histone 3 methyl transferase and *heterochromatic protein 1 (HP1)* (Joanis and Lloyd 2002), are structurally and functionally conserved in mammals (Wreggett et al. 1994; Aagaard et al. 1999; Bultman and Magnuson 2000; Schotta et al. 2002; Ebert et al. 2004; Norwood et al. 2004). The strongest evidence for a single ancestral origin of genomic imprinting comes from experiments in which both mouse and human imprint control regions (ICRs), placed into transgenic *Drosophila*, silenced adjacent *Drosophila* genes (Lyko et al. 1997, 1998). ICRs are regions of DNA at which epigenetic processes act to impose an imprint on adjacent genes. Although in these experiments the silencing was not imprinted, these experiments show that mammalian ICRs are recognized by *Drosophila* boundary, histone modifying and *polycomb* group proteins (Lyko et al. 1997, 1998; Erhardt et al. 2003), proteins which are highly conserved and their interactions largely analogous to the action of the proteins which act at the endogenous ICR.

Although the mechanism of genomic imprinting appears to be conserved, the genes targeted by imprinting are not. In mammals, ICRs are scattered through the genome resulting in small islands of transcriptionally silenced genes. Imprinting of these genes functions as a form of transcriptional regulation necessary for normal development (Surani and Barton 1983; McGrath and Solter 1984; Feinberg 2007). In insects and other invertebrates, imprinting predominantly involves entire chromosomes (de la Casa-Esperon and Sapienza 2003; Normark 2003). Although

exceptions such as the imprinted inactivation of the entire X chromosome in marsupials and the extraembryonic tissues of mice (Sharman 1971; Takagi and Sasaki 1975; de la Casa-Esperon and Sapienza 2003) exist, these differences in the targets and size of the imprinted domain have been invoked as evidence that genomic imprinting is of different evolutionary origin, at least in insects and mammals (Killian et al. 2001; Feil and Berger 2007).

In *Drosophila*, imprinted silencing of both whole chromosomes and individual genes has been described (Fitch et al. 1998; Lloyd 2000; Loppin et al. 2005). Thus, analysis of imprinting in *Drosophila* might reveal how whole-chromosome and gene-specific imprinting are related. The *Dp(1;f)LJ9* mini-X chromosome has only about 150 genes but retains the endogenous ICR in the centric heterochromatin. We find that the transcriptional and the phenotypic impact of the imprint arising from this ICR extends over at least 1.2 Mb, a region encompassing over 100 genes. This transcriptional imprinting is also associated with reduced polytenization of the entire mini-X chromosome. This suggests that heterochromatin, formed in response to the imprint, blocks polytenization of adjacent regions to generate a large parent-of-origin dependent domain of differential gene expression. Selection against the silencing of so many genes is likely why all reported imprinted domains are confined to gene-poor heterochromatic regions in *Drosophila* (Lloyd 2000), whereas in coccids and *Sciara*, these large imprinted domains have been exploited as a sex determination mechanism (Goday and Esteban 2001; Bongiorno and Prantera 2003). Thus, a fundamentally similar type of parent-of-origin-dependent transcriptional silencing can affect only a few genes or an entire chromosome depending on species-specific properties such as gene density and somatic polytenization. These results, thus, imply that genomic imprinting is an ancient process, one flexible enough in its genetic targets that it can be adapted to serve different biological functions in different organisms.

Materials and methods

***Drosophila* culture and mutant strains** All fly stocks were maintained on a standard cornmeal/yeast/sugar medium supplemented with Tegosept (methyl 4-hydroxybenzoate; Sigma) as a mold inhibitor. Flies were reared at 20±5°C, unless stated otherwise. All crosses were conducted in vials with ten–15 females and five–ten males. These crosses were subcultured onto new food at 2–4-day intervals up to four times before the parents were discarded and each experiment was replicated two to six times separated by at least 1 week to control for environmental effects such as variation in media, temperature, or humidity. All mutations

used are described in Flybase (Crosby et al. 2007) and were provided by the Bloomington Drosophila Stock Center. The *Dp(1;f)LJ9* mini-X chromosome (FBab0003346), hereafter referred to as the mini-X, is derived from a full-length inverted *In(1)sc²⁹* X chromosome by X-ray-induced deletion to produce a large internal deletion (Hardy et al. 1984). This deletion removed most of the interstitial euchromatin leaving the telomere to 1B appended to 13A2–12A10, in reverse order as a result of the *In(1)sc²⁹* inversion, appended to the centric heterochromatin. The distal portion of the centric heterochromatin is likely also missing; complementation tests show that the proximal euchromatic genes *mst* and *fog* are deleted (data not shown).

Crosses Reciprocal crosses were performed for each target gene to create two sets of genotypically identical male progeny, each with a mutant allele of the target gene on the full-length X chromosome and the mini-X chromosome derived from either the female or male parent (mutant/mini-X^{Mat} and mutant/mini-X^{Pat}). To generate these genotypes, *Dp(1;f)LJ9/X⁺X* virgin females or *Dp(1;f)LJ9/X⁺Y* males were crossed to hemizygous or homozygous *y^{1a53d}*, *g^{50e}*, *mus101^{D1}*, *y¹*, *Flo-2^{KG00210}*, *na¹/Y*, *na¹/FM7c* males or females, respectively.

Phenotypic assessment of yellow variegation The level of yellow (*y*) expression was assessed visually using an arbitrary scoring system. Each fly was assigned a score of 0, 1, 2, or 3 based on whether it expressed a complete mutant, severe mutant, moderate mutant, or wild-type phenotype when 2–5 days old. The value for the average phenotype was obtained by dividing the cumulative score by the total number of flies so is expressed as a proportion of full wild-type expression plus or minus the standard error of the mean. A fly was scored as “0” if its phenotype was identical to its *y¹/0* sibs, “1” if only the last segment was pigmented, “2” if it was lighter than wild-type but all segments were pigmented and “3” if pigmentation appeared wild-type. To control for variation in environmental conditions and subjectivity, only flies grown at the same time on the same media were compared; all flies were scored by one investigator (V. K. L.) in a single blind experiment.

RNA isolation Adult male flies were collected within 12 h of eclosion to ensure that all individuals were of similar age and were then held for 48 h. Flies 48–60 h old were frozen in liquid nitrogen and then stored at –80°C. Fifty milligrams, approximately 50 adults, of frozen flies were homogenized in liquid nitrogen and 1 ml Trizol (Invitrogen) with a ceramic mortar and pestle according to the manufacturer’s protocol. The precipitated dried RNA pellet was redissolved in 50 µl RNase-free water (Sigma Aldrich) and incubated briefly at 65°C to aid in resuspension and then stored at –80°C.

Real-time quantitative reverse transcriptase PCR To remove contaminating genomic DNA, 2 U of Turbo DNase (Ambion) in one times reaction buffer (Ambion) was added to each 18 µl RNA sample and incubated for 12 min at 37°C. Following the DNase treatment, the sample was extracted with phenol:chloroform, reprecipitated and redissolved in 20 µl RNase-free water (Sigma-Aldrich). Approximately 4 µl of the sample was used to check for RNA integrity by gel electrophoresis and to determine RNA concentration. Of RNA in 20 µl reaction volume, 0.5–2.0 µg of RNA was used to produce cDNA using the Omniscript RT kit (Qiagen) according to the manufacturer’s recommendations. Samples were incubated for 30 min at 37°C. Oligo dT primers were used for cDNA synthesis for *y*. For the other target genes, the following gene-specific reverse primers were used: *act88f*—5’CACAGCCACGACTCTTACGAT3’, *Flo-2*—5’AAATGATTAACGAGGCAAAACA3’, *g*—5’TTTGTATGTGGGCTTGTGTG3’, *mus101*—5’ACAAAA CAATGGCTGGCACT3’, and *na*—5’TCCGTTGCTGAC TGGTGATA3’. All primers were obtained from AlphaDNA (Montreal, Canada). Following cDNA production, the reaction mix was diluted ten times and 1 µl of the cDNA product was used for each quantitative real-time polymerase chain reaction (PCR) reaction. Primers for the quantitative real-time PCR reaction were 18–24 nucleotides long with melting temperatures between 58°C and 60°C, spanned an intron or gave different product sizes for cDNA and genomic DNA, and were as follows: *act88f* (forward) 5’ATCCGC AAGGATCTGTATCG3’ and (reverse) 5’CAGGGCAGT GATCTCCTTCT3’, *Flo-2* (forward) 5’CGAGATTGAGT CGCAGGAA3’ and (reverse) 5’CGATGGTCTGACATT GCTTG3’, *g* (forward) 5’GGACTCGGACCAGAACTT GA3’ and (reverse) 5’CTCTTCGGGTGCGTCTTTAG3’, *mus101* (forward) 5’AAGAGGGCAAGGATTGTGTG3’ and (reverse) 5’TGCTGGTTCGTTGATGCTTAC3’, and *na* (forward) 5’GGCGAGGATGTTACCTTTCA3’ and (reverse) 5’CCTCCTCAACCAGGTA CTG 3’. Quantitative real-time PCR was performed using the iQ SYBR Green kit (Bio-Rad) with a final reaction volume of 20 µl and the iQ iCycler (Bio-Rad). Melt curve analysis and gel electrophoresis was used to verify the specificity of the amplification. Minus reverse transcriptase controls were performed to establish the lowest detection limit and ensure effectiveness of DNase treatment. All C_t values for minus RT controls were at least four cycles higher than their respective samples. The relative gene expression in maternal versus paternal samples was calculated using the formula: $\text{Expression ratio} = \frac{E(\text{target})^{\text{DCT target}(\text{mean maternal} - \text{mean paternal})}}{E(\text{reference})^{\text{DCT reference}(\text{mean maternal} - \text{mean paternal})}}$ where E is the primer efficiency. Primer efficiency was calculated with LinRegPCR (Ramakers et al 2003). The *actin88f* gene, the expression of which is not expected to differ depending on the parental origin of the *Dp(1;f)LJ9* mini-X chromosome as it is

autosomal, was used as a reference gene. These calculations were performed using the Relative Expression Software Tool (REST; Pfaffl et al. 2002). In all cases, amplifications from each cDNA sample were replicated three times and the results of the technical replicates averaged and treated as a single data point. For each gene and parental origin (maternal or paternal), two or three cDNA samples were obtained from independently raised groups of flies; the results of the amplifications of these independently obtained cDNA samples were averaged and the standard error calculated. The pairwise fixed reallocation randomization test included in the REST software (Pfaffl et al. 2002) was used to assess the statistical significance of the differences between maternal and paternal C_t values for each gene.

Cytology Salivary chromosomes were prepared essentially as described by Kennison (2000). Salivary glands were dissected from well-fed third instar $y^1z^ag^{53d}/Dp(1;f)LJ9$ male larvae into phosphate buffered saline. The larvae had either maternally or paternally derived mini-X chromosomes. The glands were fixed in acetic acid for 3 min and then stained for 10–20 min with 2% Orcein (Sigma) in a 1:1 solution of lactic and 45% acetic acid. After staining, the glands were manually squashed. Chromosome spreads in which the chromocenter with the small fourth and the mini-X chromosome were visible were photographed with a Sony DSC-S70 digital camera mounted on a Zeiss Axiovert 25 compound microscope. Using Adobe Photoshop 7.0 imaging software, chromosome images were cropped to a 2.5-in.² image containing the mini-X chromosome and fourth chromosome. To assess the relative endoreplication of the mini-X chromosome, the width of the mini-X and fourth chromosomes were measured directly on the screen. The fourth chromosome was used to control for differences in magnification, degree of replication, and degree of chromosome spreading and stretching during preparation. The mini-X chromosome was measured at the level of the most proximal doublet (12D1–2) and the fourth chromosome measured at the 102F distinct doublet as these bands were easily identified in all photographs. The width of the mini-X chromosome was expressed as a percentage of the width of the fourth chromosome and this percentage compared between groups with maternally versus paternally derived mini-X chromosomes. The band/interband contrast was assessed using Adobe Photoshop imaging software (Adobe). Images were converted to gray scale, selected band and interband areas selected using the Magic Wand Tool and their darkness quantified using the “Histogram” option. The most proximal doublet, 12D1–2, and the corresponding proximal interband was used for the mini-X chromosome and the 102F band and its proximal interband region was used for the fourth chromosome. Contrast was calculated as the ratio of the

difference in band to interband staining between the mini-X chromosome, maternally or paternally derived, and the fourth chromosome from the same nucleus. The fourth chromosome was used to control for differences in staining during polytene chromosome squash preparations. Student’s *t* test was used to assess the statistical significance of differences between maternal and paternal measurements.

Results and discussion

Goal and experimental design The goal of this study was to examine the expression of genes on an imprinted mini-X chromosome in *Drosophila*. The mini-X chromosome is a supernumerary chromosome, and thus, it can be present in a single copy, either maternally or paternally inherited, which simplifies analysis of the expression of the genes on this chromosome. However, as the mini-X contains only a small subset of X chromosome genes, at least one normal X chromosome must be present for viability. Thus, the comparisons in this study are between X/mini-X^{PAT} and X/mini-X^{MAT} males (where PAT and MAT indicate paternal and maternal transmission, respectively, of the wild-type alleles on the mini-X chromosome). The genotypes of these individuals are identical, differing only in the parental origin of the mini-X. In order to assess the expression of the allele on the mini-X chromosome, it was necessary to have a null or severe allele of that gene on the regular X chromosome, greatly restricting the choice of genes analyzed. The expression of six genes, located proximally, medially, and distally on the mini-X chromosome spanning a 1.2-Mb region adjacent to the centric X chromosome ICR (Fig. 1a), was assessed following maternal or paternal transmission.

Transcriptional silencing of genes on the mini-X chromosome The *Dp(1;f)LJ9* mini-X chromosome provides one of the most thoroughly characterized examples of transcriptional imprinting in *Drosophila*. Parent-dependent expression of the *garnet* (*g*), *narrow abdomen* (*na*), and *tiny* (*ty*) genes has been previously reported; all are paternally silenced in mosaic pattern (Lloyd et al. 1999a). As *yellow* (*y*) is the furthest gene from the centric ICR with a readily visible phenotype, we assessed its expression when it was present on either a maternally or paternally derived mini-X chromosome. Expression of the y^+ allele on the mini-X chromosome in $y^1/Dp(1;f)LJ9$ males is more evidently variegated and weaker when the mini-X chromosome is transmitted paternally than maternally. Using a phenotypic assay (see “Materials and methods”), $y^1/Dp(1;f)LJ9$ males with a paternally derived mini-X chromosome have a score of 1.97 ± 0.05 ($n=167$), corresponding to 66% wild-type *y* gene expression, versus genotypically identical flies with a

maternally inherited mini-X chromosome with a score of 2.57 ± 0.07 ($n=99$), corresponding to 86% wild-type *y* gene expression. These differences are statistically significant ($p \leq 0.001$). Thus, four genes, spanning the mini-X chromosome, are all phenotypically paternally imprinted.

To confirm the phenotypic results and extend this analysis to genes without easily assessed visible phenotypes, we performed real-time quantitative reverse transcriptase PCR to assess transcript abundance of genes on the mini-X chromosome following maternal or paternal transmission.

For the *y* gene, paternal inheritance resulted in decreased *y* transcript abundance; transcript abundance was 1.868 ± 0.2481 fold lower for paternal versus maternal inheritance of the mini-X chromosome ($p=0.0325$; Fig. 1b). For the *g* gene, paternal transmission of the mini-X chromosome also results in a 2.135 ± 0.1099 -fold decrease in transcript abundance, relative to *g* transcription in genotypically identical flies with a maternally derived mini-X chromosome ($p=0.017$; Fig. 1b) when the g^{50e} allele is on the normal X chromosome. These data are consistent with previous phenotypic analysis of this gene (Lloyd et al.

1999a). The g^{50e} allele is the most severe of the visible *g* alleles (Lloyd et al. 1999b) and when the g^{53d} allele, which only affects *g* expression in some tissues, was present on the normal X chromosome, the decrease in transcript abundance was only 1.305 ± 0.107 fold upon paternal transmission, a nonsignificant difference ($p=0.267$). This result emphasizes the importance of having a strong allele on the normal X chromosome to eliminate or minimize expression of the target gene in most or all tissues.

Similarly, the narrow abdomen (*na*), *flotillin 2* (*Flo-2*), and *mutagen-sensitive 101* (*mus101*) genes on the mini-X chromosome showed a 8.620 ± 0.080 ($p=0.008$), 2.933 ± 0.1247 ($p=0.0370$), and 1.066 ± 0.104 ($p=0.3245$) decrease in transcript abundance, respectively, when paternally versus maternally transmitted (Fig. 1b). The values for the *na* and *Flo-2* genes are statistically significant ($p < 0.05$) whereas, although the *mus101* gene showed decreased expression when paternally transmitted, it was not statistically significant, likely due to masking of altered transcription from the mini-X allele by the hypomorphic allele on the normal X chromosome. The values obtained for the fold differences in

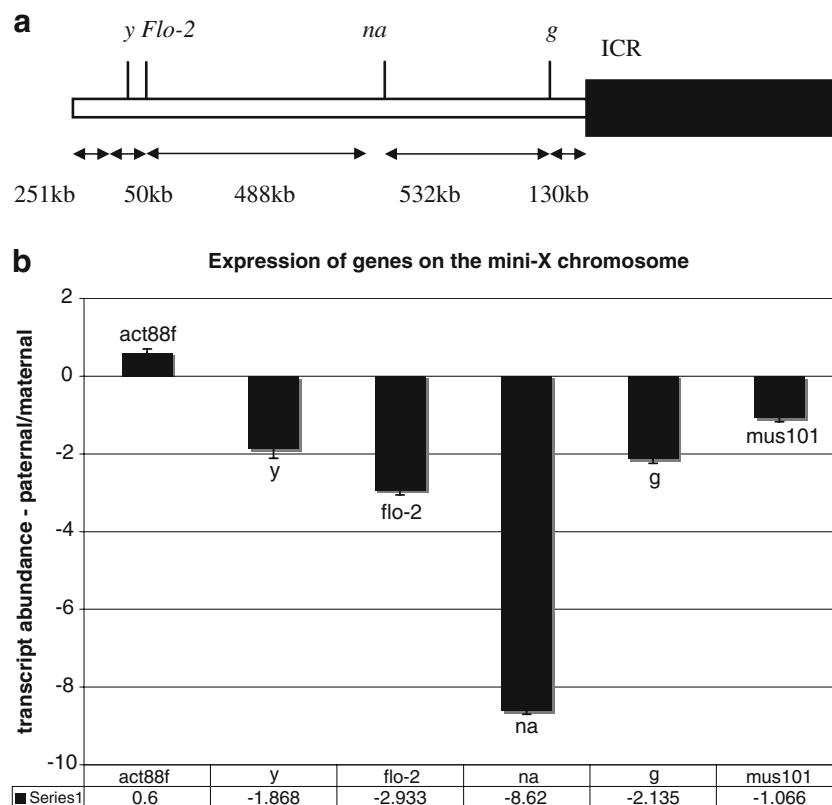


Fig. 1 Imprinted genes on the *Dp(1;f)LJ9* mini-X chromosome. **a** The distance in kb is shown between the imprinted *yellow* (*y*), *flotillin 2* (*Flo-2*), *narrow abdomen* (*na*), and *garnet* (*g*) genes and the ICR which is near the heterochromatin–euchromatic boundary. The centric heterochromatin is depicted as a *black bar*, euchromatin as an *open bar*. **b** The difference in transcript abundance following paternal versus maternal transmission. The *y*-axis shows the fold difference

between genotypically identical individuals differing only in the parental origin of the mini-X chromosome and with a mutant allele of the given gene on the normal X chromosome (mini-X^{PAT}/mutant versus mini-X^{MAT}/mutant). Decreased paternal transcript abundance is indicated by values below 0. The *Actin 88F* (*Act88F*) gene is used as a control as it is autosomal and not expected to be influenced by the parental origin of the mini-X chromosome

transcription upon paternally inheritance of the mini-X chromosome for the *na*, *Flo-2*, and *mus101* genes are conservative. The mini-X chromosome undergoes a high rate of nondisjunction in the female but not male parents so that approximately 10% of the progeny from the maternal cross will not have the mini-X chromosome (Lloyd et al. 1999a). As these X/0 individuals are not distinguishable from the X/mini-X^{MAT} genotype, they would lower the apparent level of maternal transcription from these genes.

Thus, six genes appended to the centric ICR of the mini-X chromosome, which together span 1.2-Mb region, are all paternally imprinted. While only a subset of genes on the mini-X chromosome were sampled, it seems unlikely that these genes are independently imprinted. Imprints causing maternal silencing are as frequent as those causing paternal silencing in *Drosophila* (Lloyd 2000), so independent imprinting events would be expected to produce both maternally and paternally imprinted genes, interspersed with nonimprinted genes. The data are more consistent with a single imprinted domain extending the length of the mini-X chromosome from its ICR in the centric heterochromatin.

Cytological analysis of the mini-X chromosome when maternally versus paternally inherited The mini-X chromosome is a small chromosome approximately 75% of the

size of the “dot” fourth chromosome and in polytene chromosome squashes is found near the chromocenter, the underreplicated region containing the fused centric heterochromatin of all the chromosomes (Fig. 2). The cytology of the mini-X chromosome differs dramatically according to parental origin. When maternally inherited, the morphology and banding pattern was consistently clear (Fig. 2a). The maternally inherited mini-X chromosome was $97\pm 4\%$ of the width of the fourth chromosome ($n=8$), which was used to control for differences in magnification, replication, and stretching during preparation. When maternally inherited, the ratio of the mean contrast in the band and interband areas between the mini-X chromosome and the fourth chromosome was 0.579 ± 0.050 ($n=8$). In contrast, when inherited paternally, the chromosome morphology varied considerably. In many cases, the chromosome appeared elongated, thinner, “wispy” with a lighter interband area and poorly defined banding (Fig. 2b). The paternally inherited mini-X chromosome was $76\pm 6\%$ of the width of the fourth chromosome ($n=10$). The difference in the width of the maternally and paternally inherited mini-X chromosomes were statistically significant ($p=0.013$). When paternally inherited, the interband regions also appear lighter leading to higher contrast between the band and interband regions. The ratio of the mean contrast in the band and interband areas between the mini-X chromosome and the fourth chromosome was 0.819 ± 0.071 ($n=10$, $p=0.018$). This suggests that paternal transmission causes increased chromatin compaction and underreplication of the mini-X chromosome. Importantly, the changes to the chromosome morphology were uniform along the length of the chromosome. In no case were interspersed regions of normal and abnormal morphology observed.

Collectively, these data show that paternal transmission of the mini-X chromosome caused decreased transcript abundance of genes spanning at least 1.2 Mb and this decreased transcription is associated with reduced somatic replication of the entire 1.5 Mb mini-X chromosome. Other similarly large imprinted domains have been reported in *Drosophila*. Prokofyeva-Belgovskaya (1947) observed altered chromosome morphology in a segment of the imprinted *In(1)sc⁸* X chromosome corresponding to 1 Mb. This region also correlates with DNA underreplication extending at least 100 kb from the ICR (Karpen and Spradling 1990). Likewise, the paternally imprinted domain of the *T(1;2)dor^{var7}* chromosome and the maternally imprinted domain of the *Dp(1;3)w^{m264-58a}* chromosome correspond to at least 1.5 (Demakova and Belyaeva 1988) and 0.8 Mb (Spofford 1961), respectively, and in early in *Drosophila* development, parent-specific histone modifications encompass entire chromosomes (Loppin et al. 2005). In humans and mouse, imprinted gene clusters can encompass 1–2 Mb regions as well (Verona et al. 2003).

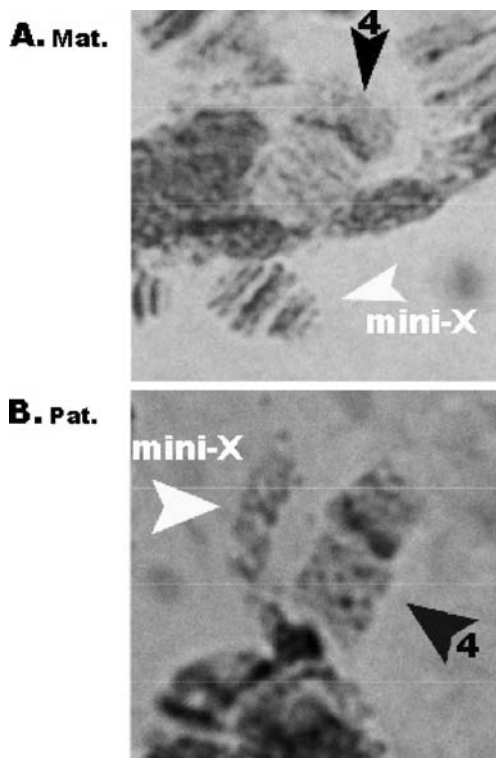


Fig. 2 Morphology of the *Dp(1:f)LJ9* mini-X chromosome in salivary glands when maternally (a) or paternally (b) inherited. The mini-X chromosome is indicated by a white arrow and the fourth chromosome is indicated by a black arrow

However, gene density is typically lower in mammals than in *Drosophila*; the *H19/Kcnq1ot1* and PWS/AS imprinted gene clusters contain only ten–20 genes (Verona et al. 2003) compared to the over 100 genes subject to the mini-X chromosome imprint. Imposition of parent-dependent expression on such a large number of genes would be expected to have appreciable consequences for the organism.

The large chromosomal domains subject to imprinting in *Drosophila* could be explained if this organism lacks boundary elements that can limit the spread of the chromatin domains resulting from an imprint. However, this seems unlikely as boundary elements capable of delimiting chromatin domains with distinct transcriptional properties have been well characterized in *Drosophila* (Valenzuela and Kamakaka 2006). A more likely explanation is that somatic polytenization acts, secondarily to the imprint, to extend the range of the imprint. We postulate that the ICR imposes a parent-dependent heterochromatic chromatin structure on the immediately adjacent region, as it does in mammals (Verona et al. 2003). However, unlike mammals, in *Drosophila* and many other insects, heterochromatin induces underreplication of large 3–40 Mb regions during somatic polytenization (Nagl 1978; Hoskins et al. 2002). This parent-dependent decrease in the copy number would affect the expression of hundreds of adjacent genes. In mammals, some genes within imprinted clusters can be imprinted in only some tissues or at some stages in development and those that are secondarily imprinted by their proximity to ICRs may be able to escape the imprint (Verona et al. 2003). However, large underreplicated domains do not offer much opportunity for gene-specific regulation or the fine tuning of the transcriptional effects. Thus, in insects, the combination of high gene density and the secondary underreplication of imprinted domains would amplify the effect of the imprint, resulting in the silencing of hundreds of functionally unrelated genes. Silencing of so many genes makes imprinting an unwieldy mechanism for parent-dependent gene regulation. As a result, ICRs would only persist in regions of the genome with low gene densities or if the aneuploidy resulting from the silencing of whole chromosomes could be tolerated. Insects tolerant of aneuploidy, such as the cockid scale insects and the fungus gnat *Sciara*, have co-opted imprinting of entire chromosomes as their mechanism of sex determination (Goday and Esteban 2001; Bongiorno and Prantero 2003; Normark 2003), whereas in *Drosophila*, which is not tolerant of aneuploidy, imprinted domains are confined to gene-poor heterochromatic regions of the genome. These regions are generally centromeric (Lloyd 2000) and the sequestering of imprinted domains near the centromere could affect chromosome stability in mitosis and meiosis. This may explain why imprinting in insects is often associated with parent-of-origin-specific chromosome elimination.

Parent-of-origin-dependent silencing of single or small groups of genes is a common manifestation of imprinting in mammals whereas in insects imprinting characteristically affects large chromosomal segments (de la Casa-Esperon and Sapienza 2003). Imprinting of the *Dp(1;f)LJ9* mini-X chromosome exhibits properties of both manifestations of imprinting; genes are transcriptionally silenced yet the entire chromosome is affected. This suggests that the two types of response to the parental origin of a gene or chromosome are not fundamentally distinct. One corollary of this supposition is that genetic analysis of *Drosophila* imprinting may provide insights into the mechanism of mammalian imprinting. Equally, the sophisticated understanding of mammalian imprinting could shed light on outstanding issues in insect imprinting. Thus, even though imprinting in insects can affect entire chromosomes rather than small groups of genes, the spread of the imprint is due to species-specific differences in gene density and the extensive somatic polytenization typical of insects. The imprint itself is formed by similar epigenetic processes (Singh 1994; Lippman and Martienssen 2004; Delaval and Feil 2004) and results in similar parent-of-origin specific transcriptional silencing. In this way, a fundamentally similar imprinting mechanism can be adapted to serve different functions in different organisms.

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