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ORIGINAL ARTICLE

Fluorescence *in situ* hybridization analysis of *hobo*, *mdg1* and *Dm412* transposable elements reveals genomic instability following the *Drosophila melanogaster* genome sequencing

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The genome of *Drosophila melanogaster* strain *y cn bw sp* has been sequenced and the transposable elements insertion sites have been determined. We hybridized fluorescence-labeled probes directed to the *hobo* transposon, *Dm412* and *mdg1* retrotransposons to polytene chromosomes and compared the observed sites to those published in the annotated genome sequence. We observed an almost twofold increase in the number of *hobo* hybridization sites (46 found as compared to 24 annotated sites). There was no evidence that the *hobo* transposition rate is slowing over the 10-year period. The patterns of *Dm412* and *mdg1* sites have changed less dramatically since the time of genome sequencing. Three novel *Dm412* hybridization sites were detected while 4 out of 30 annotated sites were missing. Only one additional *mdg1* site was found, while 1 out of 29 annotated sites has been lost.
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Keywords: strain *y cn bw sp*; *hobo*; *P*-element; *mdg1*; *Dm412*

Introduction

The presence of active transposable elements (TEs) in the genome is an important factor responsible for genetic instability. As much as 3.8% of euchromatin (Kaminker *et al*., 2002) and up to 22% of total DNA (Kapitonov and Jurka, 2003) in *Drosophila melanogaster* is composed of TEs. The spontaneous rate of TE transposition in *Drosophila* is about 10^{-3}–10^{-5} per site per generation depending on the genetic background and environmental conditions (Harada *et al*., 1990; Nuzhdin *et al*., 1996; Vieira and Biemont, 1997; Lampe *et al*., 1998; Maisonhaute *et al*., 2007).

P and *hobo* transposons invaded the *D. melanogaster* genome not earlier than 100 years ago (Blackman *et al*., 1987; Bazin *et al*., 1999). According to the hypothesis of Rouzic and Capy (2005), the transposition rate can vary by several orders of magnitude during the colonization process. They propose that the rate is high during the first generations following the horizontal transfer, but then decreases until an equilibrium value is reached corresponding to the known measured transposition rates (Rouzic and Capy, 2005).

TEs are now widely used to generate genome-wide mutant collections. *P* elements have been the vehicle most often employed to disrupt *Drosophila* genes because they transpose at the highest rates (Bellen *et al*., 2004). The minimal rate of transposition of another newcomer, *hobo*, was evaluated as 1.75×10^{-3} per site per generation in the strains studied by Harada *et al*. (1990). However, Aulard *et al*. (2004) show that a *hobo* copy artificially introduced into the fly genome previously free of *hobo* insertions transposes at the rate of 0.19–0.45 per site per generation.

The isogenic strain *y cn bw sp* of *D. melanogaster* is used by many researchers as the reference strain, in large part, because a complete genome sequence is available (Adams *et al*., 2000; Myers *et al*., 2000). This strain carries full-sized copies of *hobo*, *Dm412* and *mdg1* elements capable of inducing transpositions. It is, therefore, tempting to study the stability of TE patterns both for the newcomer *hobo* transposon and the ancient *Dm412* and *mdg1* retrotransposons in this strain, and determine transposition rates for those that are active. This may be of interest for understanding genome instability from an evolutionary perspective.

We found that the number of *hobo* hybridization sites on polytene chromosomes of the *y cn bw sp* genome is higher than that annotated in the database (http://flybase.bio.indiana.edu). In contrast, *Dm412* and *mdg1* hybridization sites largely coincided with those annotated *in silico*.

Materials and methods

Fluorescence *in situ* hybridization (FISH) experiments were performed with *y[1] oc[R3.2]; cn[1] bw[1] sp[1]; LysC[1] MstProx[1] GstD5[1] Rh6[1]* strain (hereafter

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referred to as *y cn bw sp*) obtained from Bloomington Drosophila Stock Center (USA) in summer 2004 and summer 2005. Flies of the isogenic strain *y cn bw sp* of *D. melanogaster* are suitable for the analysis of mobility because this stock has been made isogenic and labeled with recessive phenotypic markers. Thus, all heterogeneity should be due to novel instability as opposed to drift of preexisting polymorphism or contamination.

The cytological localization of *hobo* element, *Dm412* and *mdg1* in the nearly completely sequenced genome was performed with the use of FlyBase Insertions query results (http://flybase.net/transposons). Information concerning *hobo* (or *H*-element), *Dm*412 (or 412) and *mdg1* (or mdg1) in sequenced genome is designated as H{}# (2003), hobo{2005}#, 412{}# and mdg1{}#, respectively in the database.

FISH was performed on the squashed preparations of larval salivary glands. We used 4–5 squashed preparations for every type of probe. Slides were heated at 60°C for 1 h and denatured in 0.07 M NaOH for 3 min. The completely cloned copies of *hobo* transposon (provided by J Lim), *Dm 412* and *mdg1* (provided by N Fedorova) were used as a probe. The probe was labeled by nick translation with biotinylated dUTP (Medigen, Novosibirsk, Russia), Cy3-UTP (Roche oligolabeling kit) or digoxigenin-dUTP (Molecular Probes, Invitrogen, Eugene, OR, USA). The *in situ* hybridization solution was as follows: 50% formamide, 10% dextransulfate, 4 × SSC, 1 × Denhardt solution, 0.1 M phosphate buffer (pH 7.6) and 10–20 ng labeled DNA per slide. After hybridization, the squashed preparations were washed with 2 × SSC three times for 5 min. The detection of biotin was performed with avidin-fluorescein isothiocyanate (avidin-FITC), digoxigenin was detected by anti-digoxigenin-Cy3 (Molecular Probes). Vectashield with 4′,6-diamidino-2-phenylindole (DAPI; Vector Laboratories, Inc., Burlingame, CA, USA) was used as an antifading solution.

Following the FITC-DNA hybridization and FITC/Cy3-DNA hybridization, the preparations were examined with an Axioskop-2 Plus microscope equipped with a black-and-white CCD VC44 camera (PCO). The images were processed with the ISIS program (METSYSTEMS GmbH). The result of Cy3-labeled *Dm412* DNA hybridization was analyzed on Zeiss Axiophot2 microscope equipped by Zeiss Axio Cam HRm. The images were processed with the AxioVision version 3.1.

Results

We compared the *in silico* localization sites of *hobo, Dm412* and *mdg*1 TEs in the *y cn bw sp* strain of *D. melanogaster* to our FISH data. Since the correspondence between genome sequence and the cytological map is known (with a polytene chromosome bandwidth accuracy) *in silico* data can be used to localize a mobile element on the cytological map, to allow this comparison.

By 2003, as many as 24 *hobo* sequences had been annotated in the database for *y cn bw sp* strain. One of these annotated sequences represents the full-sized copy (2959 bp) encoding the active transposase (accession number M69216), while the other *hobo* copies carry deletions in the central part (http://flybase/). A 1406-bp-long variant is a predominantly truncated version with high homology to a full-sized *hobo*. According to the database, the *hobo* element is distributed non-uniformly on the chromosomes. Thus, the X chromosome contains five copies while 2L, 2R, 3L, and 3R carry 10, 2, 1 and 6 *hobo* hybridization sites, respectively.

In comparison to the database localizations, the number of FISH *hobo* sites is almost twofold higher (46 sites against 24); X chromosome has 7; 2L, 13; 2R, 3; 3L, 6; 3R, 17 *hobo* hybridization sites (Figure 1 and Table 1). For example, out of five annotated X-linked sites, two sites from chromocenter region were lost, while we were able to determine four novel sites in the regions 12EF, 13EF, 16A and 18EF (Figure 2). Some sites from 3R chromosome are polymorphic (Table 1).

The same FISH experiments performed a year later (in 2005) revealed the additional *hobo* pattern changes in X and 3R chromosomes, namely, the loss of 18EF site and generation of a *de novo* X-linked site in the 16EF region. Novel 9A and 14C *hobo* sites were observed in a single slide out of five analyzed (Figure 3 and Table 1). The loss of four annotated sites and generation of one new site occurred in 3R chromosome (Figure 4 and Table 1). In addition, we observed the case of *hobo* localization polymorphism in 93 region of 3R chromosome (Figure 5). Gray arrows show *hobo* positions in different homologs on the different sides of *mdg1* in the 93rd region. No *hobo*-pattern changes were observed in the other chromosomes in 2005 experiments as compared to 2004 (Table 1). In 2005, the database was supplemented with 39 additional defective *hobo* elements that had less homology with full-sized *hobo* and were much shorter than those annotated in 2003 (most of them being 100–300 bp derivatives). However, those additional potential *hobo* hybridization sites on X chromosome were annotated to the regions 11B, 20B, 20D1 and 20D2, whereas in our experiments, hybridization was observed in the other

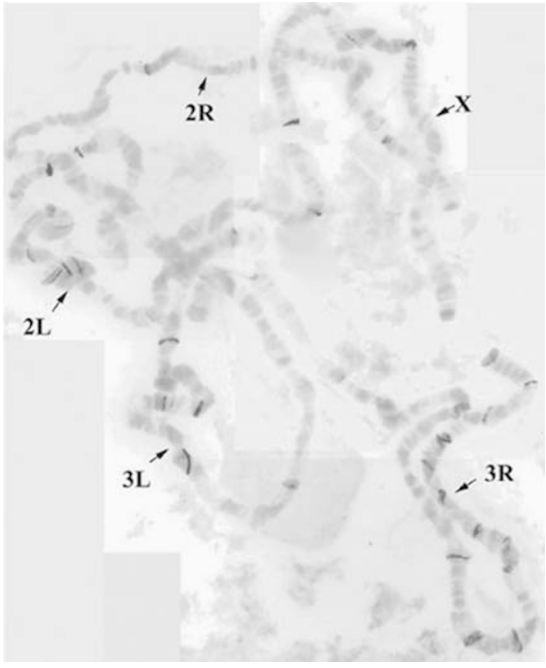

Figure 1 Fluorescence *in situ* hybridization (2004) of fluorescein isothiocyanate-labeled *hobo* DNA on the salivary gland polytene chromosomes from *y cn bw sp* strain. Arrows show chromosome arms.

Table 1 Distribution of *hobo* transposable element on *y cn bw sp* polytene chromosomes according to FISH (2004 and 2005) and *in silico* data

In silico	*FISH (2004)*	*FISH (2005)*
X 7E1, 9D3, 19C5, 19E5, 20B1	7E, 9D, 12EF, 13EF, 16A, 18EF, 20	7E, 9A(1/5), 9D, 12EF, 13EF, 14C(1/5), 16A, 16F, 20
2L 34A2 (two sites), 35D3 (two sites), 35D4 (two sites), 36C2, 36D2, 36E2, 38C2	26AB, 26F, 28CB, 29CD, 30BC, 31A, 33AB, 33D, 34CD (two sites), 35D (two sites), 38C	26AB, 26F, 28CB, 29CD, 30BC, 31A, 33AB, 33D, 34CD (two sites), 35D (two sites), 38C
2R 45E1, 45E1	50A, 55AB, 59AB	50A, 55AB, 59AB
3L 67A1	61E, 67A, 67DE, 70E, 71A, 74A	61E, 67A, 67DE, 70E, 71A, 74A
3R 84B5, 84D11, 90D1, 95E1, 99B9, 99D5	84B, 84D(2/3), 86A, 86D(3/4), 89A, 90D, 92A, 93E, 94A(2/4), 95A(2/4), 96B(2/4), 96F, 97A, 97C(1/4), 98B, 99F(2/4), 100F	84B, 84D, 86A, 86D, 89A, 90D, 92A, 93E, 95A, 96B, 97C, 98B, 100C, 100F
Total 24	46	45

Abbreviations: FISH, fluorescence *in situ* hybridization; *y cn bw sp*, *y[1] oc[R3.2]; cn[1] bw[1] sp[1]; LysC[1] MstProx[1] GstD5[1] Rh6[1]*.
In case hybridization patterns are polymorphic, ratio of the slides with hybridization to the total number of slides analyzed is given in parentheses. Not less than three genomes per slide were analyzed.

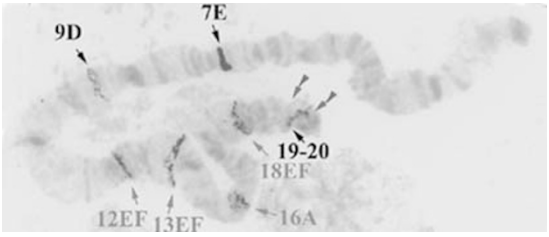

Figure 2 Fluorescence *in situ* hybridization (2004) of fluorescein isothiocyanate-labeled *hobo* DNA on the salivary gland X chromosome from *y cn bw sp* strain. Black arrows show annotated positions; gray arrows, new sites; double arrows, lost sites.

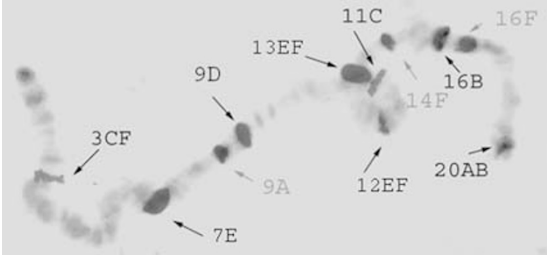

Figure 3 Fluorescence *in situ* hybridization (FISH; 2005) of fluorescein isothiocyanate-labeled *hobo* DNA (black arrows) and Cy3-labeled *mdg1* DNA (3CF and 11C) on the X chromosome from *y cn bw sp* strain. Gray arrow shows the position of the new *hobo* sites in comparison with FISH (2004).

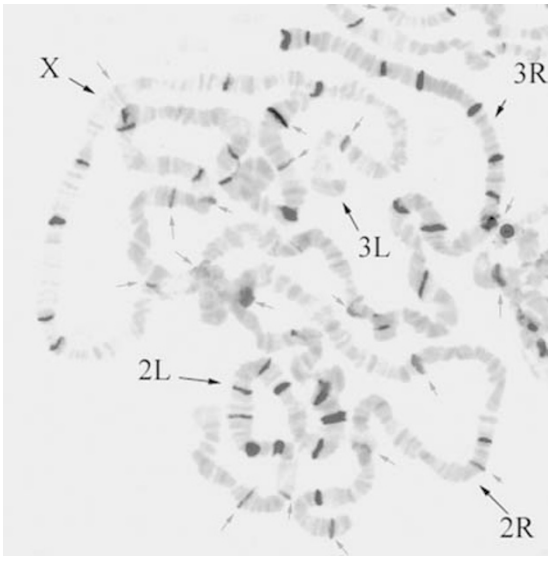

Figure 4 Fluorescence *in situ* hybridization (2005) of fluorescein isothiocyanate-labeled *hobo* DNA and Cy3-labeled *mdg1* DNA on the salivary gland polytene chromosomes from *y cn bw sp* strain. Black arrows show chromosome arms, gray arrows show *mdg1* hybridization sites.

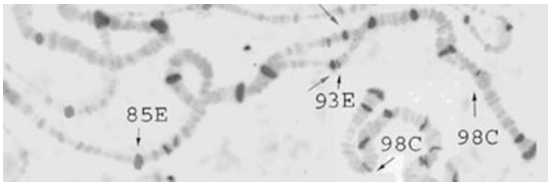

Figure 5 Fluorescence *in situ* hybridization (2005) of fluorescein isothiocyanate-labeled *hobo* DNA and Cy3-labeled *mdg1* DNA on 3R chromosome from *y cn bw sp* strain. Arrows show positions of *mdg1* hybridization sites (85E, 93F, 98C). The polymorphic *hobo* hybridization pattern is shown in 93 regions.

locations indicated above. Only three out of six annotated *hobo* sites (in regions 84B, 84D and 90D) are present in the 3R chromosome in our FISH data. Fourteen novel *hobo* hybridization sites also do not colocalize with those annotated additionally *in silico* in 2005 (Tables 1 and 2). In addition, those six sequences annotated *in silico* in 2005 represent short *hobo* derivatives with a low homology, thus, probably, being below FISH sensitivity (Table 2). We, therefore, believe that the novel *hobo* hybridization sites detected by FISH most likely appeared as a result of *de novo* transpositions.

We were able to detect 20 *mdg1* hybridization sites as compared to 29 annotated sites (Figure 4 and Table 3). It is noteworthy that *mdg1* sites are found more often then *hobo* in the same band or neighboring bands of the polytene chromosome. In FISH experiments, this results in a single visible hybridization site instead of two sites actually present. In such twin cases, the loss of one copy might go unnoticed. We observed only one additional *mdg1* site compared to the *in silico* data, 11C (Figure 3), while one site from the 27C7 region was lost (Table 3).

FISH analysis of *Dm412* retrotransposon distribution also yielded a better correspondence to *in silico* data (Figures 6a and b) than the *hobo* case. Out of 30 annotated insertions of *Dm412*, four sites (1E1, 41E3, 41E5 and

Table 2 Size and cytological localization of *hobo* sequences in 3R chromosome of *y cn bw sp* strain according to *in silico* data

Annotated in silico *H (2003)*, hobo *(2005)*	hobo *size (bp)*	*Cytological map position* (in silico)
hobo{}2835	275	86C10
hobo{}4793	433	87C1
H{}1380	1406	90D1
hobo{}5686	98	91F2
hobo{}4815	552	92D1
H{}1414	1406	95E1
hobo{}2368	35	96D6
hobo{}2918	80	98A10
H{}1453	1406	99B9
H{}1456	1406	99D5

Abbreviation: *y cn bw sp, y[1] oc[R3.2]; cn[1] bw[1] sp[1]; LysC[1] MstProx[1] GstD5[1] Rh6[1].*

Table 3 Distribution of *mdg1* transposable element on the *y cn bw sp* polytene chromosomes according to FISH and *in silico* data

In silico	*FISH (2005)*
X-chromosome *3C1-3C2, 3F1*, 16B4, *20A1, 20B1*	3CF, 11C, 16B, 20AB
2L 23D6, 25A1, 27C7, 40F7	23D, 25A, 40F
2R *41C5, 41D2, 41D3, 41E1, 47B1, 47B4,* 51D8, 56F2, 60D1	41DE, 47B, 51D, 56F, 60D
3L 61E1, 63A2, *74F3, 75A1,* 75F8, *80C1, 80D5, 80F9*	61E, 63A, 74F/75A, 75F, 80CF
3R 85E11, 93F9, 98C2	85E, 93F, 98C

Abbreviations: FISH, fluorescence *in situ* hybridization; *y cn bw sp, y[1] oc[R3.2]; cn[1] bw[1] sp[1]; LysC[1] MstProx[1] GstD5[1] Rh6[1].* Closely located *mdg1* insertions that cannot be resolved by FISH are marked in italics.

77D4) were lost, whereas three novel sites (21CD, 39CD and 59B) were detected (Figures 6a and b). In region 60 of 2R chromosome, we sometimes observe only three *Dm412* sites instead of four annotated, which may be explained by a close localization of 60C and 60D *Dm412* sites, poorly resolvable by FISH. No changes in *Dm412* pattern were detected by FISH performed in 2005 as compared to the FISH results obtained a year earlier (not shown), thus testifying a lack of its transposition activity over this period.

Discussion

We observed clear differences in the distribution of the three TEs between the *in silico* records and our FISH data for the *y cn bw sp* strain**.** This difference is most prominent for the *hobo* element, apparently indicating a high transposition activity. The frequency of *hobo* insertions is higher than that of excisions, as confirmed by the numbers of hybridization sites appearing *de novo* and disappearing (Table 1).

Assuming that the DNA used for sequencing the complete *D. melanogaster* genome was isolated 10–15 years ago (Smoller *et al*., 1991; Tamkun *et al*., 1992; Shaffer *et al*., 1994; Adams *et al*., 2000) and that the fly life span under laboratory conditions is approximately 1 month, at least 100–150 generations have passed over that period. During that time, 10 *hobo* sites (out of the 24 annotated) have been lost, while 36 new sites have been generated (Table 1).

Since we are unable to follow all intermediate events of appearance/disappearance of sites, the minimal rate of *hobo* element transposition is 3×10^{-3} per site per generation for excision $(10/24 \times 150)$ and 10×10^{-3} per site per generation for insertion $(36/24 \times 150)$. The transposition frequency calculated for both excisions and insertions is 13×10^{-3} per site per generation $(46/24 \times 150)$. We cannot distinguish closely located hybridization sites by FISH, which means that we have probably underestimated the *hobo* transposition rate.

Assuming that all *hobo* elements transpose at the same rate, the data suggest that at least 1 *hobo* copy out of nearly 50 found in *y cn bw sp* strain will transpose in every second generation; this rate translates into six transpositions per genome per year. Over the period of 12 months (2004–2005), we have observed 4 novel *hobo* sites out of 46, while 5 sites were lost. The average frequency of transposition over this period is 16×10^{-3} $(9/46 \times 12)$ or 12×10^{-3} $(6.4/46 \times 12)$ taking into account the polymorphic nature of some sites. Comparing the average transposition rate over 1-year and the 10-year period, we can conclude that *hobo* transposition rate did not diminish with time.

Dm412 and *mdg1* transposition rates are an order of magnitude lower than that of *hobo* element in *y cn bw sp* strain, being about 2×10^{-3} $(7/30 \times 150)$ per site per generation for *Dm412* and 5×10^{-4} $(2/29 \times 150)$ per site per generation for *mdg1*.

D. melanogaster has nearly 100 different TEs in its genome (Kaminker *et al*., 2002). Assuming that every type of transposable element has an average of 10 copies per genome (Rouzic and Capy, 2005), and that the average transposition rate is in the range 10^{-3}–10^{-5} (Nuzhdin *et al*., 1996; Vieira and Biemont, 1997), we can conclude that the total rate of TE transposition will be close to the transposition rate of *hobo* obtained in our study.

In comparing the transposition rates of transposons (*hobo*) and retrotransposons (*Dm412* and *mdg1*), we should also consider the difference in the mechanism of transposition. Retroelements transpose via an RNA intermediate by a 'copy and paste' mechanism while transposons transpose by 'cut and paste' process in the DNA form. The 'copy and paste' process might be more time-consuming than the 'cut and paste', since it involves the reverse transcription step in cytoplasm, while the 'cut and paste' mechanism is completely localized to the nucleus. Possibly, retroelements are less active than the tranposons simply due to the different mechanism of transposition.

Isogenization does not rescue the *Drosophila* strains from instability. Moreover, during the process of isogenization the genome instability might be increased. One cannot rule out the possibility of *hobo*-mediated hybrid dysgenesis, when crossing the flies of *hobo*-carrying strains with the individuals free of this element, thus inducing the genome instability (Blackman *et al*., 1987; Bazin *et al*., 1999). Strain *y cn bw sp* of

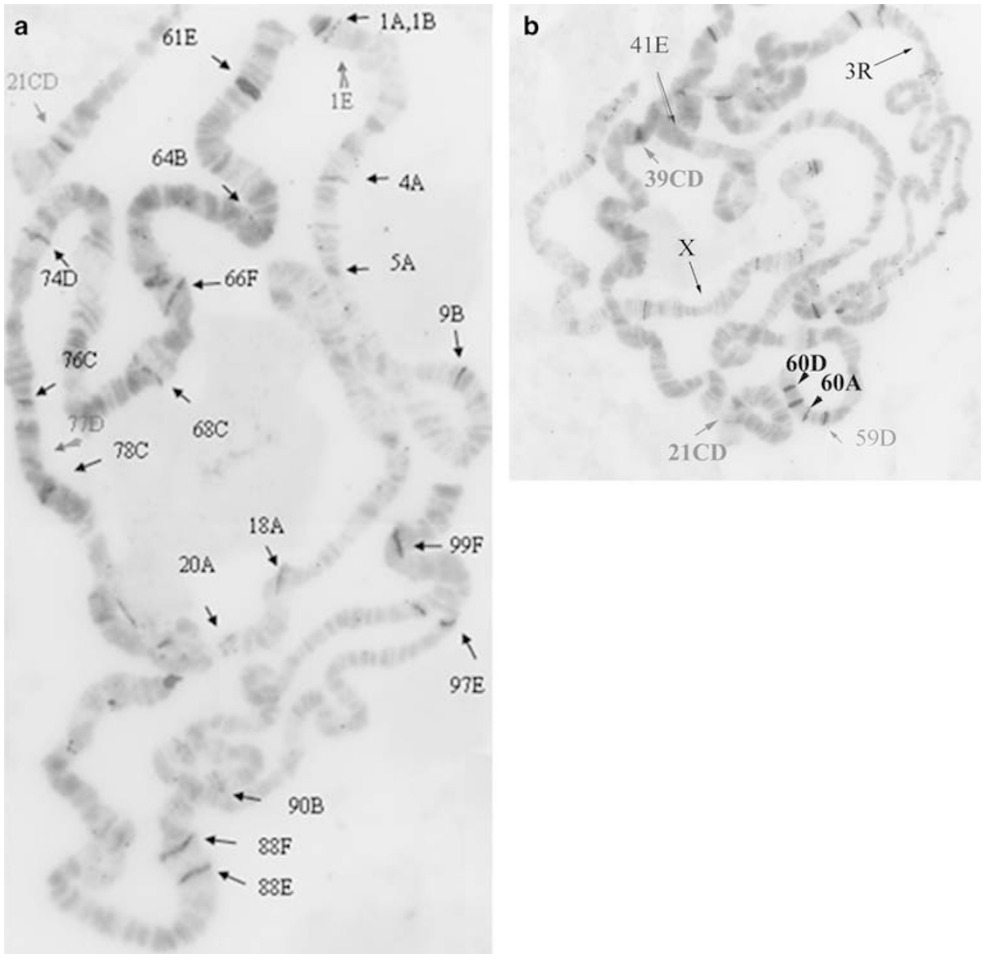

Figure 6 Fluorescence *in situ* hybridization (2004) of Cy3-labeled *Dm412* DNA on the salivary gland X chromosome and third chromosome (**a**) and X and second chromosome (**b**) from *y cn bw sp* strain (black arrows—annotated positions; gray arrows—new sites; double arrows—lost sites). Two closely located lost sites are annotated to region 41 (41E3 and 41E5).

D. melanogaster was isogenized before DNA separation for sequencing, which may account for the high *hobo* instability.

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