

Patterns of replication in the neo-sex chromosomes of *Drosophila nasuta albomicans*

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Drosophila nasuta albomicans (with $2n = 6$), contains a pair of metacentric neo-sex chromosomes. Phylogenetically these are products of centric fusion between ancestral sex (X, Y) chromosomes and an autosome (chromosome 3). The polytene chromosome complement of males with a neo-X- and neo-Y-chromosomes has revealed asynchrony in replication between the two arms of the neo-sex chromosomes. The arm which represents the ancestral X-chromosome is faster replicating than the arm which represents ancestral autosome. The latter arm of the neo-sex chromosome is synchronous with other autosomes of the complement. We conclude that one arm of the neo-X/Y is still mimicking the features of an autosome while the other arm has the features of a classical X/Y-chromosome. This X-autosome translocation differs from the other evolutionary X-autosome translocations known in certain species of *Drosophila*.

1. Introduction

The basic chromosomal complement of the genus *Drosophila* consists of six acrocentric chromosomes ($2n = 12$) (Patterson and Stone 1952). Evolution has resulted in a variety of karyotypes that differ as a result of rearrangements of these chromosomes via translocations and fusions as well as inversions. At least 54 different chromosome translocations have been established from the ancestral to some very recent species. Nevertheless, the genetic content of the arms has been sufficiently conserved to allow construction of homology tables (Patterson and Stone 1952).

The *Drosophila nasuta albomicans* of the *nasuta* subgroup of the *immigrans* species group of *Drosophila* is one of the very few species, which have the lowest number of chromosomes with $2n = 6$. Ranganath and Hägele (1981) have convincingly argued that three centric fusions and a pericentric inversion occurred during the evolution of karyotype of *D. n. albomicans* from the ancestral karyotypic composition. In this species, nearly 60% of the genome is represented on the neo-sex chromosomes and nearly 50% of the neo-Y-chromosome is euchromatic (figure 1). This euchromatic part of neo-Y is trapped and

is restricted to the male genome only. In this paper, the patterns of replication of the neo-sex chromosomes (neo-X and neo-Y), products of sex chromosome-autosome fusion, have been examined.

2. Materials and methods

2.1 Autoradiographic procedure

The Okinawan strain of *D. n. albomicans* was used in the present study. Larvae were grown in standard *Drosophila* medium at $24 \pm 1^\circ\text{C}$. For the present study, salivary glands from 120–125 h larvae were excised in *Drosophila* Poels' solution and pulse labelled for 20 min with [^3H]thymidine (200 $\mu\text{Ci/ml}$; Sp. Act. 18000 mCi/mmol; BRIT, India). The labelled glands were fixed, squashed and processed for autoradiography with Amersham Nuclear Emulsion EM-1. The coated slides were exposed for 7 days at 4°C and then developed with D19b developer, fixed, washed and air-dried. The dried autoradiograms were stained with Giemsa, mounted with DPX and examined for different labelling patterns (Kalisch and Hägele 1976; Roy and Lakhotia 1981; Mukherjee and Chatterjee 1975).

Keywords. Centric fusion; *Drosophila albomicans*; neo-sex chromosomes; replication pattern

2.2 Sequencing of [³H]thymidine labelling patterns

Incubation of larval salivary glands in [³H]thymidine for a short period and subsequent autoradiography results in different patterns of labelling which are grouped into interband (IB), continuous (C) and discontinuous (D) types. Interband represents the initial stage of replication where only interbands and puffs are labelled, continuous type represents the middle stage of replication where the entire chromosomes are labelled and the discontinuous type represents the terminal stages of replication cycle wherein only the bands are labelled. These different patterns have been subgrouped into low-interband, mid-interband, heavy-interband, 2C, 3C, 3D, 2D and 1D types (Kalisch and Hägele 1976; Roy and Lakhota 1981; Das *et al* 1982).

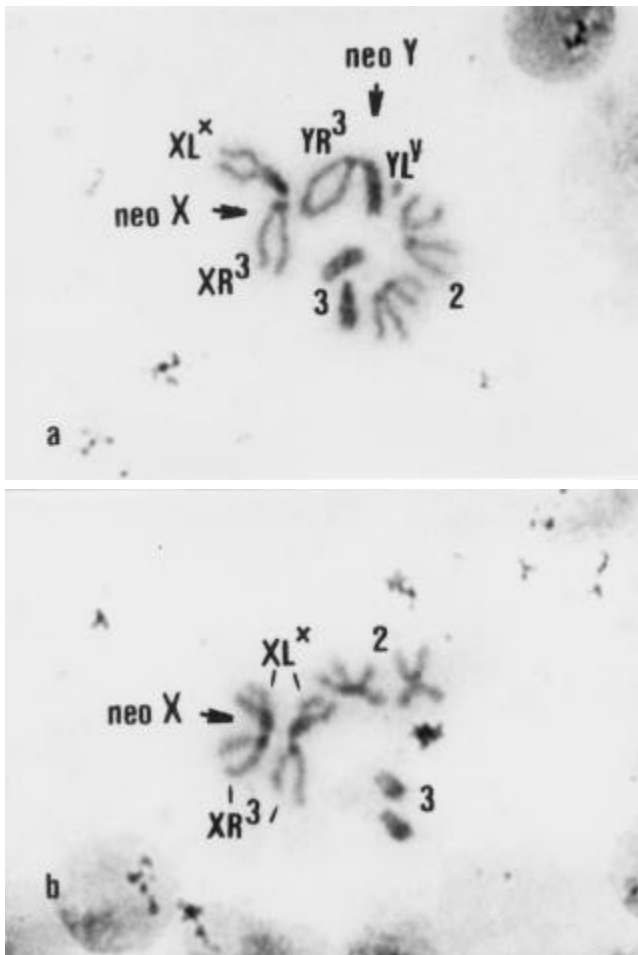


Figure 1. Metaphase chromosome complement of *D. n. albomicans* ($2n = 6$). It consists of a pair of metacentric autosomes (2), a pair of long dot chromosomes (3) and a pair of large metacentric neo-sex chromosomes (neo-X and neo-Y). XL^x and XR^3 are two arms of the neo-X-chromosome, YL^y and YR^3 are the two arms of neo-Y. (a) Male. (b) Female.

For a detailed analysis of the replication pattern, the labelling patterns of a part of the XL^x arm, a part of the XR^3 arm and a part of 2R arm were selected for comparisons. On the basis of maximum number of separable labelled sites, as resolved by the light microscope, these segments of the XL^x , XR^3 and 2R arms have been divided into 24, 31 and 22 independent replicating units, respectively (figure 2). To consider any site as labelled, a minimum of four grains was taken as the lower limit (Mutsuddi *et al* 1987). This information is recorded from the late S-stage, namely 3C-3D-2D-1D nuclei. Ten nuclei of each type were examined separately in both the sexes.

3. Results

3.1 Replication pattern of the neo-X-chromosome

The polytene nuclei of *D. n. albomicans* have four long and one short euchromatic arms. They are the two arms of the neo-sex chromosomes (in males $XL^x/-$; XR^3/YR^3 ; in females XL^x/XL^x ; XR^3/XR^3), 2L, 2R and 3 chromosome (dot chromosome) arms respectively. The [³H]thymidine labelling patterns in males have been examined and the replication of the two arms of the neo-X-chromosome, viz. the $XL^x/-$ arm and the XR^3/YR^3 arm, have been compared with each other and also with that of the reference segment on the 2R arm. The YL^y arm being heterochromatic is incorporated in the chromocentre.

Representative examples of labelling are shown in figure 3. In males, the $XL^x/-$ arm was the first to initiate replication (figure 3a). By the time XR^3/YR^3 arm entered the continuous phase (3C), the $XL^x/-$ arm was already in discontinuous phase (3D) (figure 3b). Further, as figure 3c depicts, when the $XL^x/-$ arm was in very late stage in the replication cycle (1D), the XR^3/YR^3 and the 2R arms were still in 2D stage of replication. Thus in males, the $XL^x/-$ arm was ahead of the XR^3/YR^3 arm or the autosome 2R in replication cycle. On the other hand, as shown in figure 3d, in females the XL^x/XL^x and the XR^3/XR^3 arms of the neo-X-chromosome as well as the autosome 2R arm showed high synchrony in their patterns of replication.

The frequency of labelling of a site was determined by the number of times a site was labelled among all the nuclei examined (Mutsuddi *et al* 1985). The sitewise analysis revealed that the labelling frequencies of almost all the replicating sites of the XL^x arm were significantly lower in male than the corresponding sites on the XL^x arm of female nuclei (figure 4). For instance, while the site 13 in male was not labelled in any of the nuclei, about 42% of cells in female glands showed labelling of this region. Likewise, the site 18 in male showed less than 10% labelling frequency, while in female, about 43% of nuclei had labelling in this region. A similar trend was seen for all the 24 sites of the $XL^x/-$ arms of males and females

(figure 4a). Figure 4b shows the frequency of labelling of the sites on XR^3/YR^3 and XR^3/XR^3 arms of males and females respectively. For all the 31 regions compared, the pattern of labelling remained almost similar, indicating lack of difference in the replicating pattern of these arms in male and female nuclei. A similar situation was seen

for the patterns of replication of different sites on 2R (figure 4c). Thus, the behaviour of replication of $XL^{x/-}$ arm in males is different from XR^3/YR^3 arm or 2R arm while the XR^3/YR^3 arm does not differ from that of 2R. These results suggest that in males, the $XL^{x/-}$ arm is faster replicating than the XR^3/YR^3 arm or an autosome.

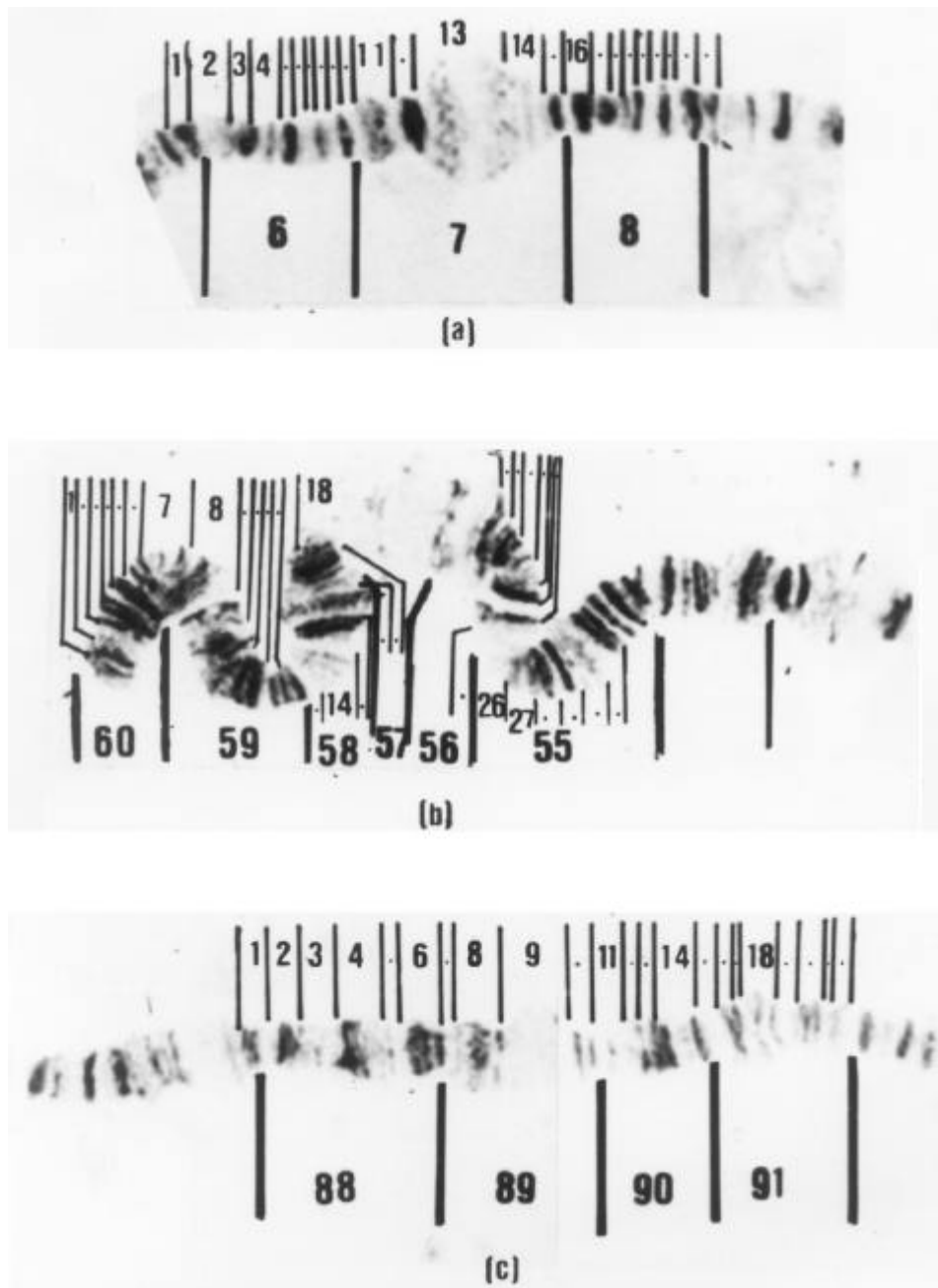


Figure 2. Replication units identified in polytene chromosomes of *D. n. albomicans*. (a) Section 6–8 of the XL^x arm with 24 replicating units. (b) Section 55–60 of the XR^3 arm with 31 replicating units. (c) Section 88–90 of the 2R arm with 22 replicating units.

4. Discussion

It has been shown that in all the *Drosophilids*, the polytene nuclei follow a regular pattern of replication, that is from a dispersed discontinuous (interband) pattern to continuous pattern to discontinuous pattern (Kalisch and Hägele 1976; Roy and Lakhotia 1981; Mutsuddi *et al* 1985, 1987). The present study also has revealed a similar pattern in *D. n. albomicans*, thus strengthening the idea of conserved nature of replicative pattern in *Drosophila* species.

It has been shown by earlier studies that the pattern of replication is one of the parameters to document dosage compensation in *Drosophila*. It has been demonstrated that the X-chromosome in males of other *Drosophilids*, viz. *D. melanogaster*, *D. hydei*, *D. kikkawai*, *D. bipecti-*

nata, *D. n. nasuta* and *D. pseudoobscura* is faster replicating (Berendes 1966; Lakhotia and Mukherjee 1970, 1972; Chatterjee and Mukherjee 1973; Mukherjee 1973; Hägele and Kalisch 1974; Mukherjee and Chatterjee 1975; Roy and Lakhotia 1981).

As pointed out by Lucchesi (1978), the neo-X- and neo-Y-chromosomes of certain members of the genus *Drosophila*, which are formed by centric fusion between an autosome and a basic X- or Y-chromosome, provide an excellent material for studying intermediate stages of the evolution of Y-chromosome and of dosage compensation. In *D. miranda*, a second X-chromosome is in the process of evolution. The C element has become translocated to the Y-chromosome and is in the process of degeneration, while the homologous autosomal element is presumably becoming dosage compensated. Evidence is provided by

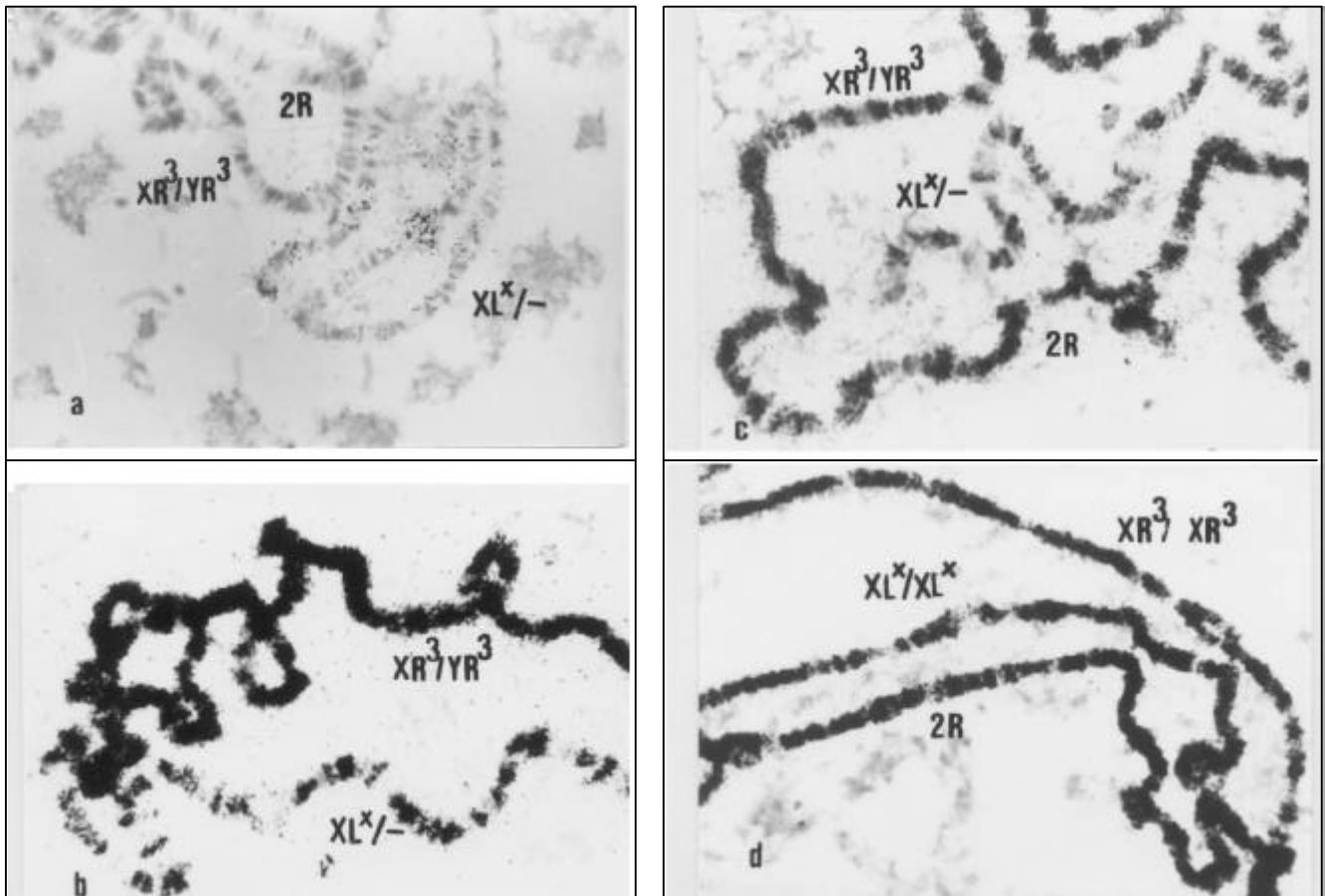


Figure 3. Comparison of labelling patterns in the two arms of the neo-X-chromosome in male (a–c) and female (d) polytene nuclei of *D. n. albomicans*. (a) The $XL^x/-$ arm is at low interband stage while the XR^3/YR^3 and the 2R arm are yet to initiate replication. (b) The $XL^x/-$ arm is at 3D stage while the XR^3/YR^3 arm is at 3C stage of replication. (c) The $XL^x/-$ arm is at 1D stage while the XR^3/YR^3 and the 2R arm are at 2D stage. (d) Chromosomes of a female nucleus with XL^x/XL^x , XR^3/XR^3 and 2R arms at the same phase (3D).

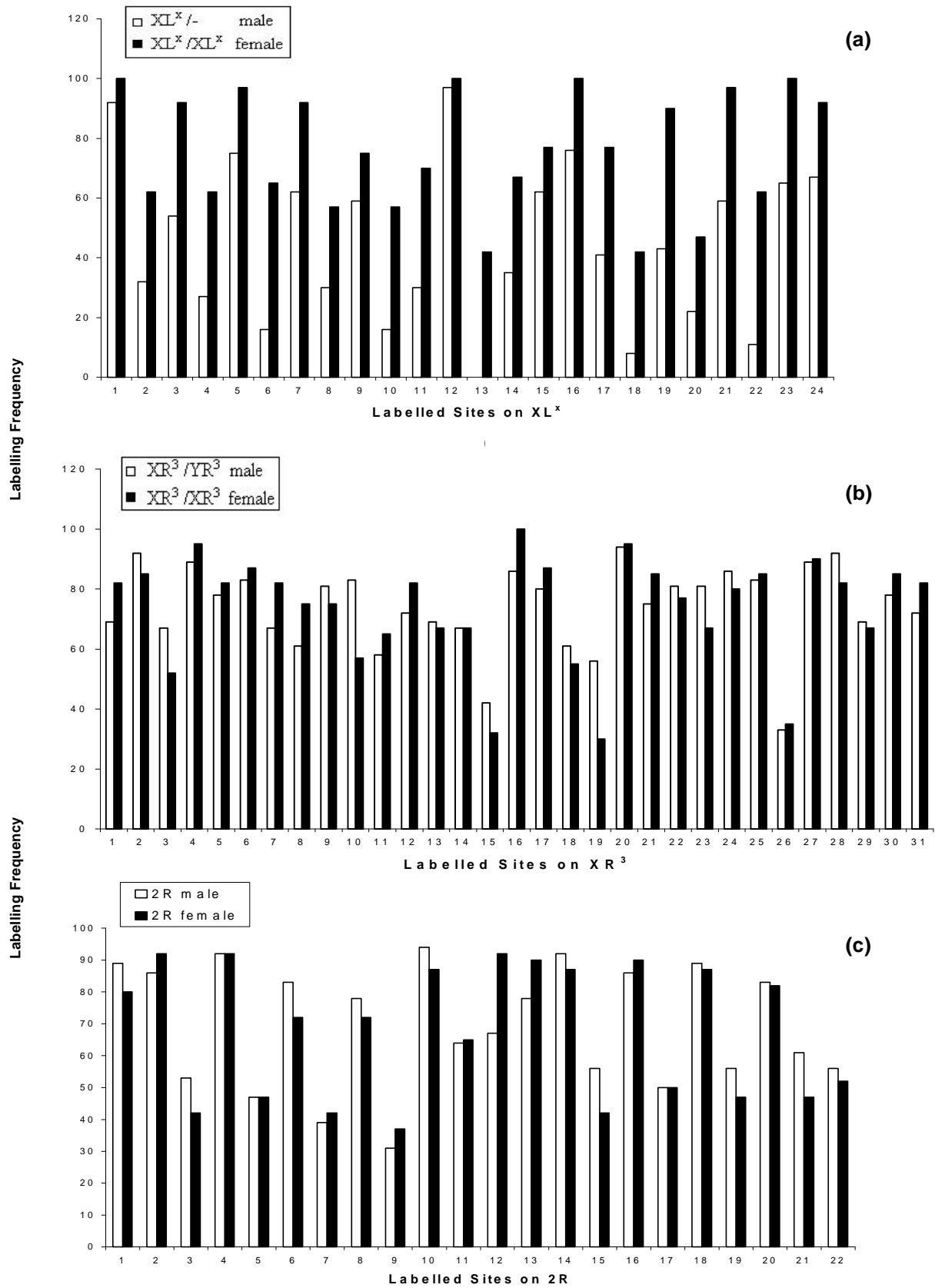


Figure 4. Frequency of $[^3\text{H}]$ thymidine labelling of the 24 replicating units on XL^x (a), 31 replicating units on XR³ (b) and 22 replicating units on 2R (c) of *D. n. albomicans*.

segmental incomplete early completion of replication i.e. the X_2 is heterogeneous in its activity (Das *et al* 1982).

The present study provides an insight into the replication of neo-Y-chromosome in *D. n. albomicans*. The data revealed that in males there is asynchrony in the replication between two arms of the neo-sex chromosomes, that is, the $XL^{x/-}$ arm is faster replicating than the XR^3/YR^3 arm, which is replicating synchronously with the autosomes. These results indicate that the YR^3 arm of the neo-Y-chromosome is still behaving like an autosome. The early completion of replication of the $XL^{x/-}$ arm indicates dosage compensation for this arm and this is similar to other species of *Drosophila* which once again confirms the conserved nature of dosage compensation. The euchromatic arm of the neo-Y-chromosome of *D. n. albomicans* shows little signs of degeneration. This is consistent with unpublished results obtained by Ranganath and Kalisch. In a mutant strain of *D. n. albomicans* all females are purple (*pr*) eyed while all males are with normal eyes. This recessive mutation is on the autosomal part of the neo-X (XR^3) arm and its corresponding wild type allele is on the euchromatic YR^3 arm of the neo-Y-chromosome. Similarly, *sepia* (*se*) and *cardinal* (*cd*) genes have been localized on the euchromatic arm of the neo-Y-chromosome. Ranganath and Kalisch (unpublished) have shown lack of meiotic recombination in males of *D. n. albomicans*.

The present observations on differences in the replication patterns of the two arms of sex chromosomes of *D. n. albomicans* suggest that the act of centric fusion between the autosome (namely chromosome 3) and the sex chromosomes has not so far changed the replicative behaviour of these components in the neo-sex chromosomes. Even though the YR^3 is a part of Y-chromosome, it has still retained the features of euchromatic autosome. Therefore it is felt that the neo-Y-chromosome of *D. n. albomicans* with euchromatic and heterochromatic arms is in the initial stages in the evolution of Y-chromosome.

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