CHROMOSOMAL BASIS OF DOSAGE COMPENSATION IN *DROSOPHILA*

III. Early Completion of Replication by the Polytene X-Chromosome in Male: Further Evidence and Its Implications

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ABSTRACT

Thymidine-^{3}H labeling patterns on the X (section 1 A to 12 E of Bridges' map) and 2 R (section 56 F to 60 F of Bridges' map) segments in the salivary gland chromosomes of *Drosophila melanogaster* have been analyzed in male and female separately. The observed patterns fit, with a few exceptions, in a continuous to discontinuous labeling sequence. In nuclei with similar labeling patterns on the 2R segment in both sexes, the number of labeled sites on the X in male is always less than in female X's. The labeling frequency of the different sites on the male X is considerably lower than those on the female X's, while the sites on the 2R segment have very similar frequency in the two sexes. The rate of thymidine-^{3}H incorporation (as judged by visual grain counting) is relatively higher in male X than in female X's. It is concluded that the model sequence of replication in polytene chromosomes follows a continuous to discontinuous labeling sequence, and that the single X in male completes its replication earlier than either the autosomes in male or the X's in female. This asynchronous and faster rate of replication by the polytene X-chromosome in male substantiates the hypothesis of hyperactivity of the single X in male as the chromosomal basis of dosage compensation in *Drosophila*.

INTRODUCTION

DNA replication patterns have been examined by thymidine-^{3}H (TdR-^{3}H) radioautography in a variety of biological systems, but the giant polytene chromosomes of Diptera provide a unique opportunity for visualization of the replication process at an elaborate scale. The resolution in such studies may extend to subgenic level even with light microscopic observations (see Arcos-Terán and Beermann, 1968). This is not generally possible with the usual somatic and meiotic chromosomes, though it is very likely that what is happening in the polytene chromosomes is also taking place in the ordinary chromosomes but is obscured by the compaction of the same during metaphase (see Mulder et al., 1968). It is generally agreed, following the original hypothesis of Keyl and Pelling (1963), that the replication in these giant chromosomes starts simultaneously in all the replicons and that it proceeds in a very ordered sequence with each replicon taking its characteristic time to complete. A short pulse of TdR-^{3}H given to an asynchronous population of cells produces various

types of labeling patterns ranging from continuous to discontinuous and unlabeled types. In continuous types all the chromosomes of a nucleus are labeled along their entire length, while in discontinuous types there are distinct labeled and unlabeled regions of chromosomes in a nucleus (see Rodman, 1968; Arcos-Terán and Beermann, 1968). According to the above scheme of DNA replication, continuously labeled nuclei are considered to be in the initial S phase and discontinuously labeled nuclei in the late stages of the S (see a recent review by Mulder et al., 1968).

Generally all the chromosomes of a nucleus show a similar type of labeling. However, Berendes (1966) for the first time noted that the single X in larval salivary glands of male *Drosophila* displays an asynchrony in its labeling with TdR-^{3}H: in many nuclei with an over-all continuous labeling, the X in male, but not in female, often was discontinuously labeled; in discontinuously labeled nuclei the male X had fewer sites labeled than the female X's. He interpreted this finding, on the basis of the above replication scheme, as an indication of an early completion of replication by the male X, and this has been considered by Berendes (1966) and by the present authors in their earlier works (Mukherjee et al., 1968, 1969; Lakhotia and Mukherjee, 1969; Lakhotia, 1970, *a,b*) to be related to the mechanism of dosage compensation in *Drosophila*.

Recently, Howard and Plaut (1968) have suggested that replication in *Drosophila* polytene chromosomes follows a discontinuous-continuous-discontinuous labeling sequence rather than a continuous-discontinuous sequence as generally believed. They have concluded that early completion of replication by the male X cannot be demonstrated on the basis of the criteria used by Berendes (1966).

It is the purpose of the present paper to discuss the model replication sequence and to provide further evidence to substantiate the early replicating property of the male X in polytene chromosomes of *D. melanogaster* and also to consider its implications on the dosage compensation mechanism in *Drosophila*.

MATERIALS AND METHODS

The wild type Oregon R+ stock of *D. melanogaster* was used in these experiments. Mature late, third instar larvae, developmentally synchronized by collecting eggs at 1 hr interval, were used for TdR-^{3}H labeling. All flies and the larvae were reared in the usual agar-cornmeal-brown sugar-yeast *Drosophila* food at 24° ± 1°C.

The excised salivary glands from two larvae of one sex at a time were incubated for 20 min in *Drosophila* Ringer's (Berendes et al., 1965) containing 3 μCi of TdR-^{3}H (sp. act. 5.7 Ci/mmole, Bhabha Atomic Research Centre, Trombay, India) and radioautographed with Kodak AR 10 stripping film. The radioautographic procedure was essentially the same as described earlier (Lakhotia and Mukherjee, 1969). The exposure time was 20 days.

From the radioautographs, TdR-^{3}H labeling patterns were recorded for a part (more than half of the total length) of the X (from section 1A to 12E of Bridges' map, [Lindsley and Grell, 1968]) in male and female separately, and also for the terminal region of 2R (from section 56F to 60 F of Bridges' map) from the same set of nuclei. The TdR-^{3}H labeling patterns for this region of 2R have already been described by Nash and Bell (1968), and their patterns in general have been confirmed in the present study. To consider any site as labeled, a minimum of three grains was considered as the lower limit.

For a comparison of the replicative behaviour of the X and 2R in male and female, several criteria have been used: (*a*) the number of labeled sites on the male and female X in relation to the number of labeled sites on the 2R in different labeled nuclei; (*b*) the frequency of labeling of different sites on the X and 2R among the labeled nuclei in male and female; (*c*) total number of TdR-^{3}H grains on the X and 2R in different labeling patterns in the two sexes.

OBSERVATIONS AND RESULTS

Labeling Patterns in Male and Female

Altogether 45 sites showing independent labeling in both male and female were delineated on the X-chromosome in the section 1A to 12E (Fig. 1 *a*). On the 2R segment, 20 sites were recognized (Fig. 1 *b*) after Nash and Bell (1968; see also Lakhotia, 1970 *a*). Labeling patterns were recorded for the X and 2R from the same nuclei in which the different chromosomal regions under study were clearly recognizable. Nuclei showing no labeling in the regions under observation were not recorded.

Tables I and II show the labeling patterns observed on the X and 2R in 71 labeled nuclei in male and 65 nuclei in female larvae, respectively. The vertical columns in the tables represent the combined labeling patterns on X and 2R observed in a nucleus. The frequency with which any particular pattern was observed is not given

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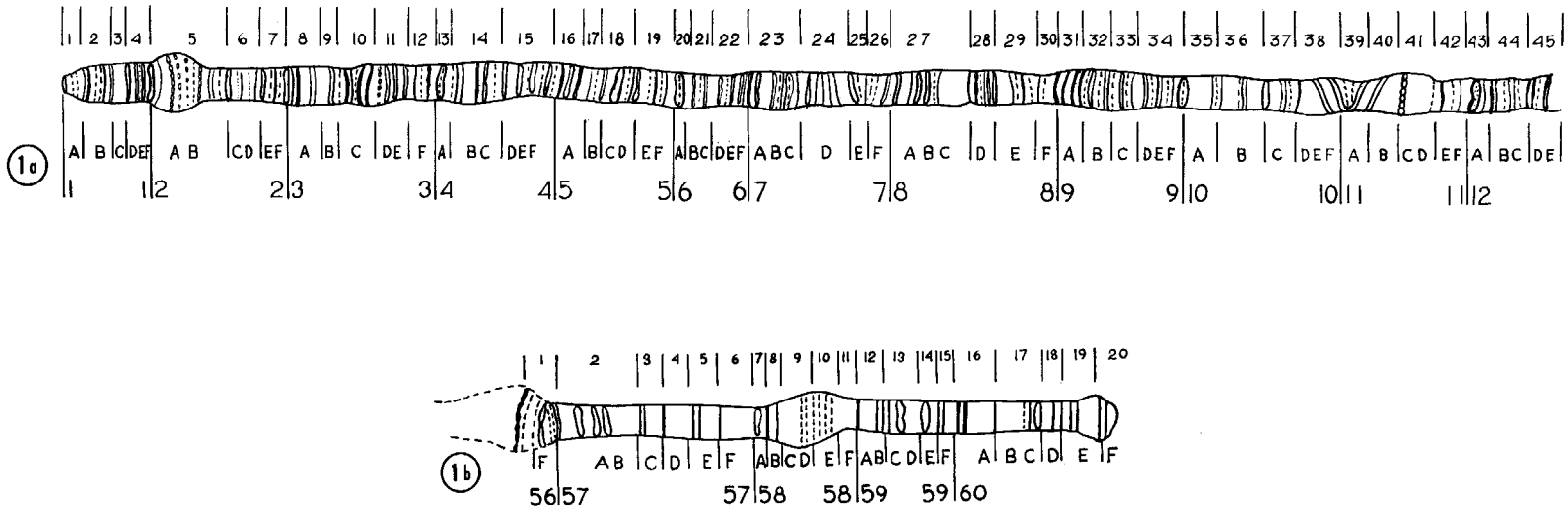

FIGURES 1 *a* and *b* The region of the X-chromosome (Fig. 1 *a*) and 2R (Fig. 1 *b*) observed in the study. The different labeling sites are shown corresponding to their respective cytological extent on the Bridges' map of salivary gland chromosomes of *D. melanogaster*.

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Table I

Ordered Sequence of Labeling Patterns in Different Male Nuclei

Labeling sites	Labeling patterns in different nuclei (Each vertical column represents one combined pattern on the X and 2R)																																	
56F	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
57AB	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
57C	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
57D	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
57E	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
57F	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
58A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
58B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
58CD	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
58E	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
58F	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
59AB	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
59CD	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
59E	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
59F	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
60A	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
60BC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
60D	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
60E	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
60F	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
1A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	+	+	+	+	0	+	-	-	-	#	-	-	-	-	-
1B	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1C	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1DEF	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2AB	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2CD	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2EF	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3A	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3B	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
3DE	+	+	+	-	-	#	#	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3F	+	+	+	+	+	0	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4A	+	+	+	+	+	0	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4BC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4DEF	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
5A	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5B	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5CD	+	+	+	+	+	+	0	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5EF	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6BC	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6DEF	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7ABC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-
7D	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7E	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7F	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8ABC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	#	-	#	-	#	-	#	-	#	-	-	-	-	-	-	-	-
8D	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8E	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8F	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9B	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9C	+	+	+	-	-	#	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9DEF	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10A	+	+	+	+	+	+	+	+	+	0	+	+	-	-	#	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10B	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10C	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10DEF	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
11B	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11CD	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11EF	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12A	+	+	+	+	+	+	+	+	+	+	0	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12BC	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12DE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-

+ indicates presence of labeling; –, absence of labeling; #, presence of unexpected labeling; 0, absence of expected labeling.

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TABLE II

Ordered Sequence of Labeling Patterns in Different Female Nuclei

Labeling sites	Labeling patterns in different nuclei (Each vertical column represents one combined pattern on the X and 2R)																																																					
56F	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
57AB	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0	+	+	+	-
57C	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
57D	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
57E	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	#	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
57F	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
58A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
58B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
58CD	+	+	+	+	+	+	+	-	-	-	-	#	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
58E	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
58F	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
59AB	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
59CD	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
59E	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
59F	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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60BC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	#	-	#	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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60E	+	+	+	+	+	+	+	+	+	+	0	+	+	-	-	#	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
60F	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
1A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
1B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	+	+	+	+	+	-	-	-	-	#	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1C	+	+	-	-	-	-	-	#	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1DEF	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	-	-	-	-	-	-	#	-	-	#	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2AB	+	+	+	+	+	+	+	+	+	+	0	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2CD	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2EF	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3A	+	+	+	+	+	+	+	+	+	+	0	+	+	+	0	+	+	-	-	#	-	-	-	-	-	-	-	-	-	-	-	-	-	#	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3B	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	+	+	-
3DE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	+	-	-	-	#	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3F	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	+	+	+	0	+	-	#	-	-	#	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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+ indicates presence of labeling; −, absence of labeling; #, presence of unexpected labeling; 0, absence of expected labeling.

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since the selection of labeled nuclei, though basically random, was limited by clear recognizability of labeled sites. It can be seen that almost all the patterns fit in an uninterrupted sequence with continuous labeling patterns at one extreme (left-hand columns of the tables) and highly discontinuous patterns at the other (right-hand columns). In a few nuclei one or two sites had either an unexpected presence (indicated in the table by #) or absence (0) of labeling. Expectation of labeling is based on the assumption of uninterrupted order of DNA synthesis in a particular site (see Howard and Plaut, 1968; Nash and Bell, 1968). An attempt to arrange these patterns in ordered arrays similar to those of Howard and Plaut (1968), however, also fails to give completely uninterrupted sequence. As such, the arrangement of the different combined patterns as followed in Tables I and II seems to be optimal. It may be noted that in female (Table II) the number of "exceptional" patterns is slightly higher than that in male. A possible reason for higher exceptionals in the former may be the fact that in any labeled nucleus the number of labeled sites on the X's in female is much higher than that in male, and this may lead to greater chances of technical and organizational "errors" (see Discussion).

A comparison of the number of labeled sites on the X in different labeling patterns in male and female reveals a striking difference in the two sexes. It is seen from Tables I and II that none of the combined labeling patterns in male and female are similar to each other. Only the patterns in which all the 20 sites on the 2R and the 45 sites on the X-segments are labeled (vertical columns 1 in Tables I and II) are similar in the two sexes. A comparison of the total number of labeled sites on the male and female X's in relation to the total number of labeled sites on 2R has been made in Fig. 2. In this figure, the different labeling patterns have been connected by an arbitrary line in both sexes to illustrate the ordered sequence of replication. Since many more intermediate patterns are possible, the ordered sequence may differ in detail from the one presented here. It is also to be noted that a few patterns—in both male and female—do not fit the expected sequence. It is clear, however, that, except in some of the continuously labeled nuclei (i.e., with all the 20 sites labeled on the 2R segment), the number of labeled sites on the X is invariably less in the male than in the female, although the 2R segment shows similar patterns. It is worth mentioning that, with one particular type of labeling pattern on 2R, one may have several labeling patterns on the X in both the sexes, but these patterns on the X are *always different* (as regards the number of labeled sites) in the two sexes. There is no overlapping at any stage. For example, with the five sites labeled on 2R (56F, 57AB, 58A, 59CD, and 60F), the range of labeled sites on the X in female is from 20 (Fig. 3 *a*) to 9 (Fig. 3 *c*) sites; in male, on the other hand, with the same five sites labeled on the 2R, the range of sites labeled on X varies from only seven (Fig. 3 *b*) to three (Fig. 3 *d*) sites. In Fig. 3, photomicrographs of these two types of labeling patterns are presented to emphasize the point that, with similar labeling on the 2R of the two sexes, the number of labeled sites on the X in male is always less than that in female. From the data, it is also evident that the sites that are labeled on the male X are also labeled on the female X's from nuclei having similar autosomal labeling patterns, except in very discontinuous patterns (i.e., with very few sites showing labeling). In male, the nuclei having labeling only at the sites 3C, 11A, and 12DE of the X are most frequent; in female, no such pattern has been observed. In fact, only one nucleus was observed in the female in which only three sites were labeled on the X; but the sites that were labeled in this nucleus were 4DEF, 9A, and 11A (last vertical column in Table II).

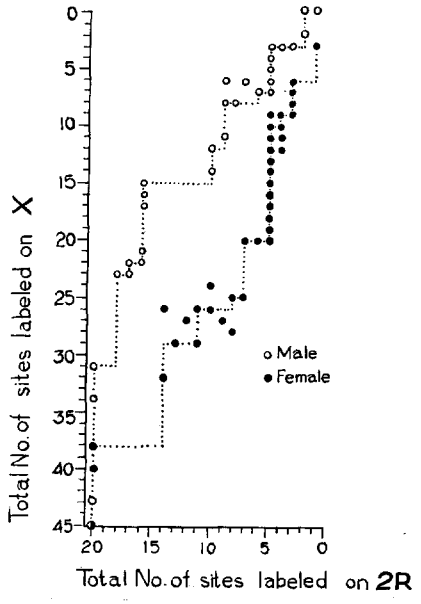

FIGURE 2 Graphical representation of the number of labeled sites on the X and 2R segments in different labeled nuclei in male and female. For further explanations, see text.

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Labeling Frequency of Different Sites on X and 2R in Male and Female

Figs. 4 *a* and 4 *b* show the labeling frequency in the two sexes of the various sites on the X and 2R, respectively. It is apparent that the frequency of labeling of the 20 sites on 2R is remarkably similar in male and female nuclei examined. But a comparison of the sites on the X in the same set of labeled nuclei in male and female reveals that in male X the frequencies of labeling of all the 45 sites are considerably lower than those in the female X's except for the sites 3C, 11A, and 12DE. The sites 11A and 12DE (Nos. 39 and 45, respectively, in Fig. 4 *a*) have relatively similar labeling frequency (nearly 100%) in male and female. But the 3C site (No. 10 in Fig. 4*a*), which is one of the "late" replicating sites in the male X (see Arcos-Terán and Beermann, 1968), exhibits a comparatively low labeling frequency in female (89% in female and 96% in male). This difference may be incidental or, more likely, may reflect a real difference in the relative time taken by the 3C site in male and female to complete its replication.

Rate of TdR-^{3}H Incorporation in X and 2R in Male and Female

A comparison of the total number of TdR-^{3}H grains on the X and 2R in different labeling patterns in male and female also indicates a difference in the kinetics of replication in the two sexes. In Table III the number of grains scored on X and 2R in the two sexes in relation to the labeling patterns on X is presented. The grouping of different labeling patterns on the X into five labeling classes based on the number of labeled sites is largely arbitrary. However, these criteria are suggestive enough for the differential replication of the X in male and female. It should be noted here that the single X in male has half the amount of DNA as compared to the paired X's in female (Aronson, Rudkin, and Schultz, 1954). As a best approximation to compensate for the difference in the DNA content in the two sexes, the mean number of grains on the single X in male has been doubled to be directly comparable to that on the paired two X's of female. It is clear from the data that the degree of incorporation of TdR-^{3}H in the male X at any stage of replication is relatively higher than that in the female X's. It may, however, be noted that the degree of increase of the grain numbers on the male X over that on the female X's is not uniform throughout the entire part of the replication cycle: in the 31–45 sites-labeled class the male X has nearly twice the number of grains that the female X's have; in the 21–30 sites-labeled class the difference is small (ratio of grains on male X to that on female X's = 1.13), while in the remaining three labeling classes the male X (corrected value) has nearly three times as many grains as the female X's. In the case of 2R, the mean number of silver grains in any labeling class of the X-chromosome is much higher in male. This is to be expected, since with a similar number of labeled sites on the X in male and female, the number of labeled sites on male 2R is always higher (see columns 6 and 7 in Table III, and also Fig. 2). In Table III, the mean 2R/X grain ratios in different labeling classes of the X in male and female are also given (the last two columns in the table). The 2R/X grain ratios in female nuclei are more or less constant throughout the S. On the other hand, while the 2R/X grain ratio in the 31–45 sites-labeled class in male is very similar to that in female, in more discontinuous patterns (i.e., with fewer and fewer sites labeled on the X) the 2R/X grain ratio in male shows a consistent and remarkable increase.

For a comparison of the replicative behaviour of the 2R segment in the two sexes, the mean numbers of grains in male and female for similar labeling patterns of 2R have been presented in Table IV. Here again, the different labeling patterns on 2R have been arbitrarily grouped into three labeling classes based on the number of labeled sites on the 2R. The data show that while in the 11–20 sites-labeled class the rate of incorporation of TdR-^{3}H by the male and female 2R is similar ($2R^f/2R^m$ grain ratio = 0.96), in the later discontinuous patterns the relative incorporation is higher in the male 2R than in female 2R ($2R^f/2R^m$ grain ratio in 1–5 sites-labeled class = 0.60).

DISCUSSION

Model Sequence of DNA Synthesis in Polytene Chromosomes

Most of the earlier workers (Keyl and Pelling, 1963; Berendes, 1966; Plaut, Nash, and Fanning, 1966; Nash and Bell, 1968; Mulder et al., 1968; Rodman, 1968; Acros-Terán and Beerman, 1968; Lakhotia, 1970 *a,b*) believe that a continuous labeling of chromosomes observed after a short pulse of TdR-^{3}H indicates that these nuclei are in the beginning of the S period, while a discontinu-

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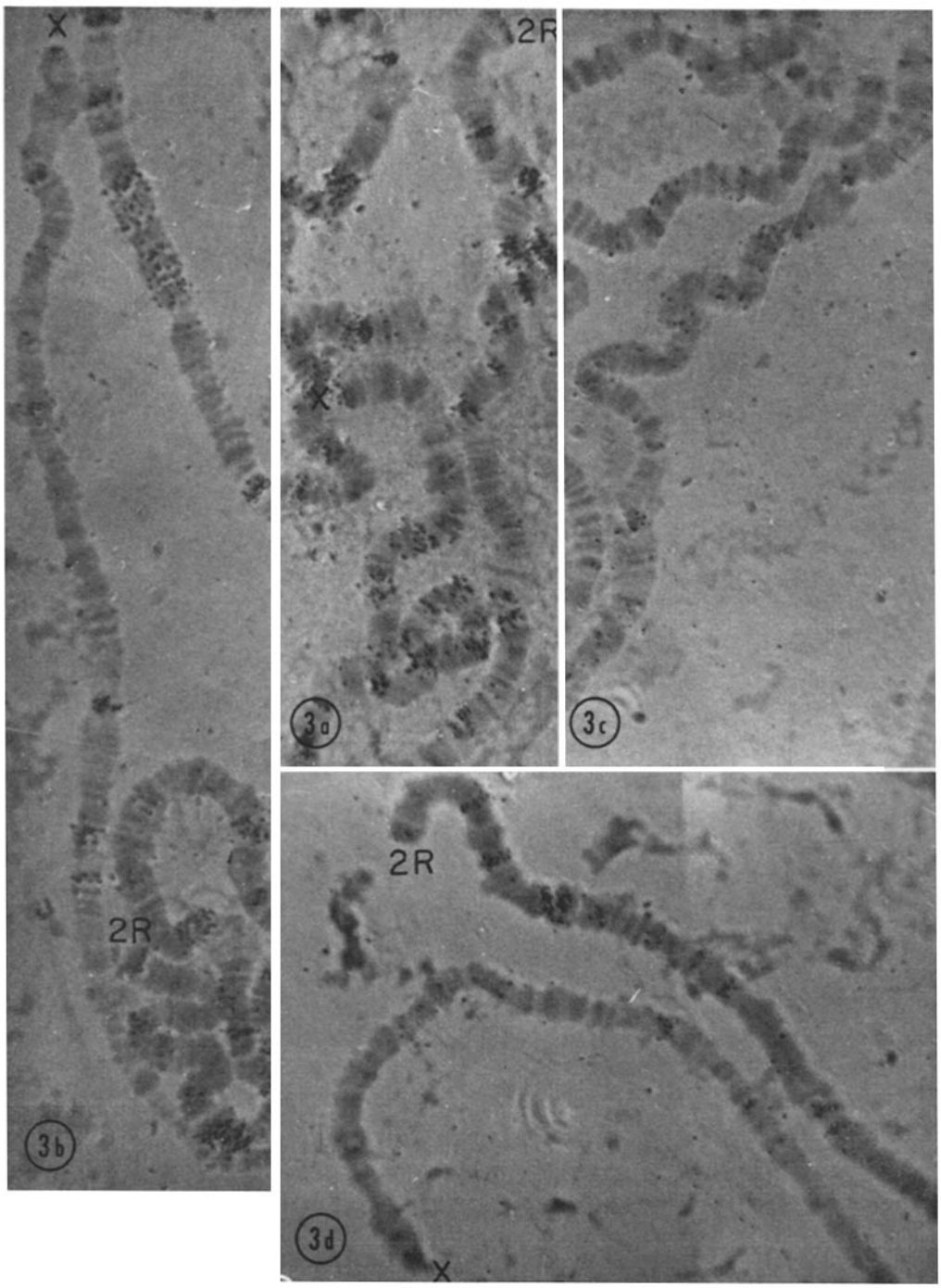

FIGURES 3 *a–d* Photomicrographs of representative TdR-^{3}H labeling patterns in male and female. (Fig. 3 *a*) A female nucleus with five sites labeled on 2R and 18 sites on the X; (Fig. 3 *b*) A male nucleus with five sites labeled on 2R and seven sites on the X; (Fig. 3 *c*) A female nucleus with five sites labeled on 2R and nine sites on the X; (Fig. 3 *d*) A male nucleus with five sites labeled on 2R and three sites on the X. Magnification about 1400 ×.

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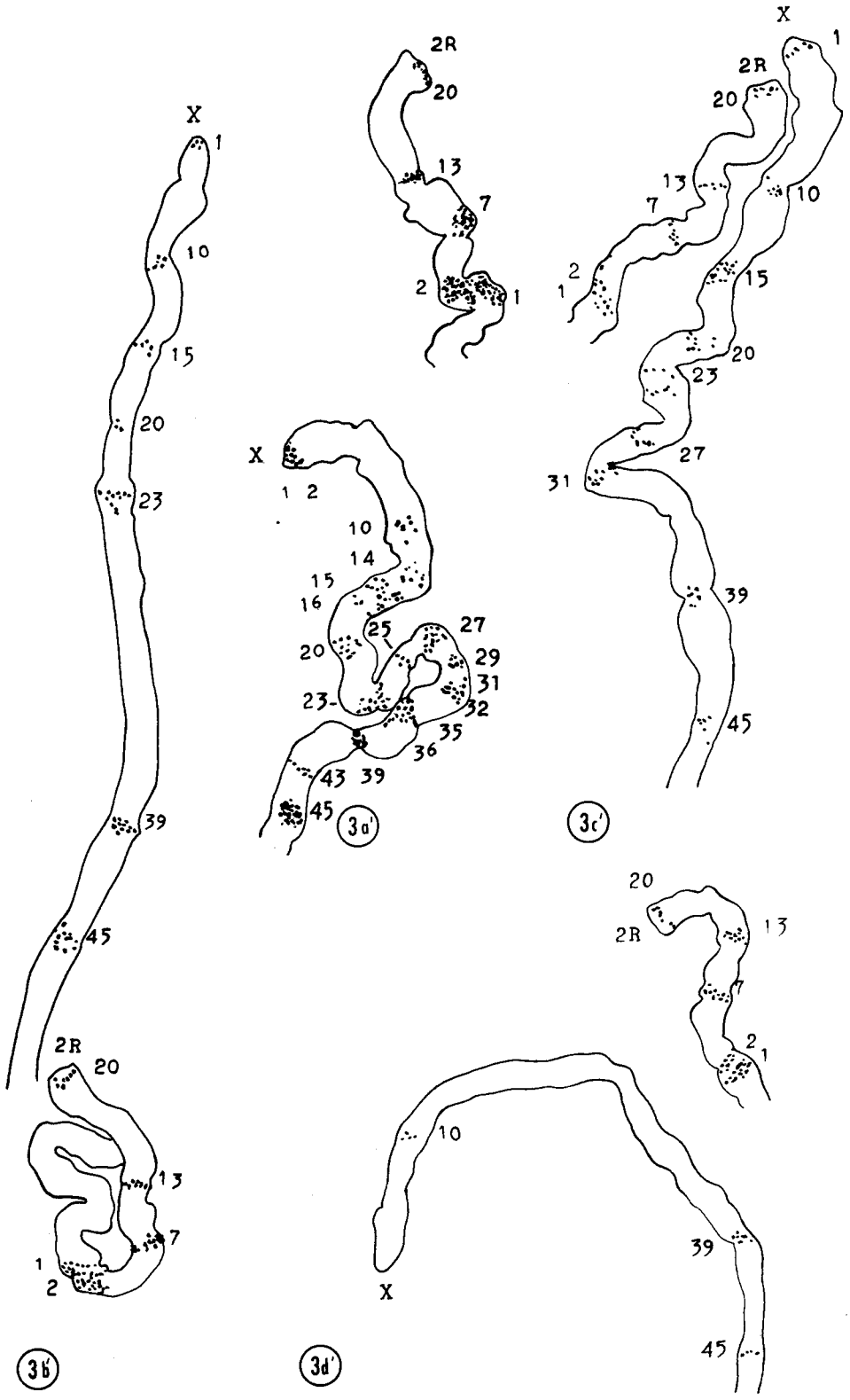

FIGURES 3 *a'–d'* Diagrammatic representation of sites labeled in the X and 2R segments in each nucleus shown in Figs. 3 *a-d*.

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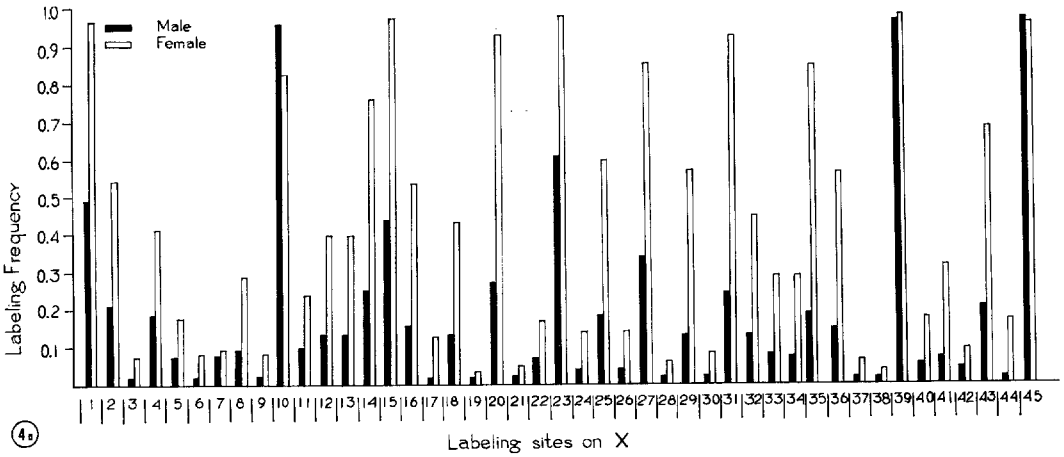

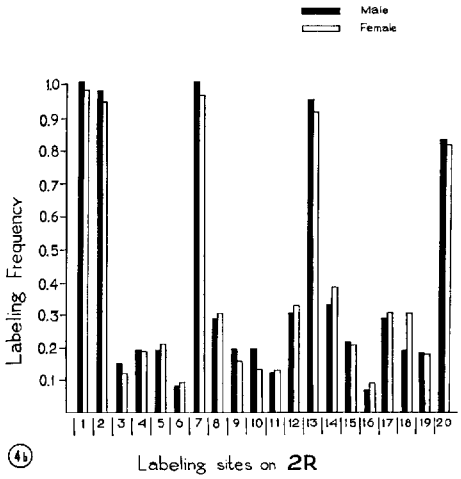

FIGURES 4 *a* and *b* Frequency of labeling of the various sites on X (Fig. 4 *a*) and 2R (Fig. 4 *b*) segments in male and female labeled nuclei.

ous labeling indicates the terminal stages of DNA replication.

Gabrusewycz-Garcia (1964), working with *Sciara*, demonstrated that there are two types of discontinuous labeling patterns in *Sciara*: P-patterns (prepuff and puff sites labeled) and C-patterns (hererochromatic regions labeled) in addition to a continuous labeling pattern. It was proposed that the P-patterns represent the initiation, the C-patterns the terminal stages, and the continuous labeling the intermediate stages. In *Drosophila*, no patterns directly comparable to the P- and C-patterns of *Sciara* have been observed. However, Howard and Plaut (1968) have proposed a model sequence in *D. melanogaster* according to which more or less similar discontinuous patterns both precede and follow the continuous labeling in a cycle. They have come to this conclusion on the basis of observed labeling patterns on small terminal segments of three chromosomes (X, 2R, 3L) of *D. melanogaster*. Several objections limit the acceptance of the replication sequence proposed by these authors, and we would consider them at some length since the model sequence of DNA synthesis in polytene chromosomes is important for a correct interpretation of the observed asynchrony in the replication of the polytene X-chromosome in male *Drosophila*.

Howard and Plaut (1968) have not indicated whether they have analyzed labeled nuclei from male and female separately or together. A careful analysis of their data on the combined labeling patterns on the three chromosomes presented in their Table VIII shows that they have apparently pooled the labeling patterns from male and female together. Thus one of their labeling patterns, with their sites 1 and 3 on 2R and the site 7 on X (corresponding to 58A, 59CD on 2R and 3C on X) only labeled, was observed in our study only in the male and never in the female. On the other hand, some of their other patterns have been observed in the present study only in the female nuclei. We have shown in the present study that the combined patterns for X and 2R in male and female differ considerably from each other and, as such, a pooling of the combined patterns from the two sexes together would lead to very heterogenous data. This in fact seems to be mainly responsible for Howard and Plaut's inability to build an uninterrupted sequence in which discontinuous patterns are expected to be at only one end of the S. Indeed our data on the combined labeling patterns for *larger regions* of both X and 2R when analyzed separately in male and female *do fit* in a sequence in which the continuous patterns are at one end

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TABLE III

TdR-^{3}H Incorporation by X and 2R in Male and Female in Different Labeling Classes of X-Chromosome

No. of sites labeled on X	Mean No. of TdR-^{3}H grains				No. of sites labeled on 2R		Mean 2R/X grain ratio	
	X		2R					
	Female	Male*	Female	Male	Female	Male	Female	Male*
31–45	689 (4)	1332 (2)	290	473	14–20	20	0.41	0.37
21–30	470 (14)	532 (4)	185	271	5–13	16–17	0.38	0.45
11–20	135 (31)	366 (7)	52	218	4–7	9–16	0.38	0.60
6–10	51 (15)	128 (13)	23	112	3–5	5–9	0.44	0.87
1–5	19 (1)	58 (40)	6	63	1	2–5	0.32	1.15

* The number of grains on the single X of male have been doubled to be directly comparable to those on female's two X's.
The numbers in parentheses in columns 2 and 3 indicate the number of nuclei in different labeling classes in the two sexes.

TABLE IV

TdR-^{3}H Incorporation by 2R in Male and Female in Different Labeling Classes of 2R

No. of sites labeled on 2R	Mean No. of TdR-^{3}H grains on 2R		$2R^f/2R^m$ ratio
	Female ($2R^f$)	Male ($2R^m$)	
11–20	291 (10)	305 (9)	0.96
6–10	110 (11)	160 (10)	0.69
1–5	41 (44)	68 (47)	0.60

The numbers in parentheses indicate the number of nuclei in different labeling classes in male and female.

and the discontinuous at the other. A few "exceptional" or "unconventional" patterns were, however, also observed here as well as by Nash and Bell (1968). Nash and Bell (1968) have attributed the exceptionals mainly to the technical errors of radioautography. While this is entirely possible, the role of cellular physiology and the state of DNA condensation in chromosomes are also likely to be important factors in influencing the relative time taken by each replicon for its replication. Since each replicon is considered to be an autonomous unit of DNA synthesis (Pelling, 1966), changes in puffing or other factors may influence a particular site in such a way as to cause a comparatively earlier or delayed completion of replication by the site. As the exposure to TdR-^{3}H is very short compared to the long S-period (about 8–14 hr in *D. melanogaster* salivary glands, see Plaut and Nash, 1964), such a change in the replication of any site may cause production of unconventional patterns. A similar interpretation was forwarded by Nash and Bell (1968) that a mixed population of cells would produce the results obtained by Howard and Plaut (1968).

More conclusive evidence for considering the continuous labeling as representing the initial phase of replication is provided by Mulder et al. (1968). They have combined TdR-^{3}H radioautography with Feulgen cytophotometry. Their observed labeling patterns also fit in a sequence with discontinuous labeling patterns at one end. Furthermore, their results show that, in all discontinuously labeled chromosomes examined, "the labeled bands had not yet finished replication. No chromosome had labeled bands with relatively higher DNA content than the unlabeled bands." These data lead to the only conclusion that the unlabeled bands had completed their replication for that cycle, and that in polytene chromosomes all the replicons of a nucleus start DNA synthesis simultaneously but end it at different times (Mulder et al., 1968). Similar conclusions based on different criteria have been arrived at by Rodman (1968) and Nash and Bell (1968). The works of Arcos-Terán and Beermann (1968) and unpublished work done in this laboratory (Dutta Gupta, 1969), have also provided evidence for considering the discontinuous labeling patterns as the terminal events in the S, by identifying the sites labeled in such patterns on the polytene chromosomes with the sites showing high frequency of ectopic pairing. These ectopic pairing sites are considered to represent loci of intercalary heterochromatin (Kaufmann and Iddles, 1963; Dutta Gupta, 1969), and heterochromatin is generally late replicating (Lima-de-Faria and Jaworska, 1968).

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In an independent series of experiments, observations on the effects of X-irradiation of *D. melanogaster* larvae on DNA synthesis patterns of the male X-chromosome at different postirradiation time intervals (Lakhotia, 1970 *b*) have thrown some light on the probable order of the replication patterns. Since the average S-period in the polytene nuclei is prolonged (see Plaut and Nash, 1964) and the replication in different nuclei of a gland is not synchronized, it is obvious that at any one time interval after X-irradiation one would have a population of replicating nuclei which were irradiated at different stages in their replication cycle. Hence by analyzing the replication patterns at different time intervals, one may expect to find a difference in the response of different nuclei depending upon their stage in the S at the time of irradiation. It has been observed that during the 1–2 hr postirradiation interval a greater effect (e.g., a higher frequency of exceptional patterns on the male X) is appreciable in continuous and heavy discontinuous patterns (3D and 2D of Rodman, 1968), while the nuclei with fewer sites labeled (late discontinuous) are least, if at all, affected since they are expected to have completed, the major part of their DNA synthesis. During the 2–4 hr postirradiation interval, in addition to the continuous and heavy discontinuous patterns, some of the late discontinuous patterns too are affected, while in the 6–7 hr postirradiation interval discontinuous patterns with still fewer sites are seen to be affected (Lakhotia, 1970 *b*). These results are best explained on the basis of the proposition that a continuous labeling of chromosomes of a nucleus after a short pulse of TdR-^{3}H represents the beginning of S-period of the nucleus, and the discontinuous patterns the terminal stages.

Early Replication of the X-Chromosome in Male

Berendes (1966) noted that in *D. melanlogaster* and *D. hydei* the single male X-chromosome completes its DNA synthesis earlier than the female X's. The conclusion was based on detailed observations in *D. hydei* that, in relation to a particular pattern of labeling on an autosome—chromosome 2—, the labeled sites on the male X were fewer than on female X's. A similar situation has been noted in the present study in *D. melanogaster* and also in *D. ananassae* (Dutta Gupta, 1969). Howard and Plaut (1968) have objected to the use of these criteria for demonstrating the early completion of replication by the male X. Suprisingly, these authors have identified their data on the labeling patterns of *2R in D. melanogaster* with the labeling patterns on the *chromosome2 of D. hydei* observed by Berendes (1966). However, the basic argument of Howard and Plaut (1968) is that the time periods during which the 2R exhibits some particular pattern are extended relative to the periods during which the X exhibits some particular patterns. We do agree with this observation, but, as pointed out earlier (in Observations and Results), the corresponding patterns on the X in male and female are *always* different. In male, the number of labeled sites on the X in relation to a particular autosomal pattern is invariably less than the number of labeled sites on the X's in female, except in *some* continuously labeled nuclei. Even in many of the continuously labeled nuclei (i.e., with the autosomal segment showing labeling in all the sites), the number of labeled sites on the male X is less than in female. Thus, although we agree with Howard and Plaut's contention that an autosomal labeling pattern does not represent a particular *moment* in the S, the X in male has decidedly fewer sites labeled at any time in the S period.

Asynchrony in the replication of the single male X is not the result of the unpaired condition or haploid DNA content per se (Berendes, 1966). Berendes has shown that the labeling patterns on the asynapsed single X's of female, having the same DNA content as the single male X, are very similar to the patterns seen on the paired two X's of female and not to those on the single male X. In the present study, too, a similar situation was observed and the results of Lakhotia (1970 *a*) also show that haploid condition of a chromosome (due to asynapsis) does not alter the labeling patterns (also, see Plaut and Nash, 1964). In nonpolytene chromosomes, too, it is known that alteration in the DNA content does not cause any change in the time or the pattern of replication (see Cameron and Stone, 1964; Graham, 1966) as long as the organization of the chromatin material is maintained intact and unaltered.

An analysis of the labeling frequency and the relative rate of TdR-^{3}H incorporation also attests to the existence of a faster replicating X in male. The frequency with which any particular site is seen to be labeled after a pulse of TdR-^{3}H is obviously a function of the relative time taken by the site to complete its replication. The relative labeling frequency of different sites on 2R was similar in the two sexes, while the labeling frequency of the different sites on the X-chromosomes of the same set of nuclei was consistently lower in

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the male than in female. This comparatively low labeling frequency of the various sites on the male X would indicate the less time spent by these sites in completing their DNA synthesis.

In the present study, the grain density was found to be higher in the male X as well as the 2R (in late discontinuous patterns). Since the total amount and period of radioactive precursor availability were practically the same in both male and female, and since the chromosomes compared are in the same labeling classes, a higher incorporation in the male would indicate a higher rate of DNA synthesis. However, the present data do not rule out the possibility of differences in the permeability and internal pool sizes of the radioactive precursor in the two sexes, and this may be thought to explain the higher label density in the male. Nevertheless, several considerations suggest that such differences, if any, may not sufficiently explain the observed higher rate of incorporation of TdR-^{3}H in the male, especially the X. First, it was seen that the 2R segment has a similar label density in male and female in the nuclei that are in the early part of their S (i.e., with 11–20 sites labeled on 2R), while the nuclei in the late stages (i.e., with 1–10 sites labeled on 2R) had a grain density higher on male 2R. Secondly, in the X-chromosome, too, the increase in the grain density in the male over that in female was not uniform throughout the S, and thirdly, the degree of increase in the label density on the male X over that on female X's is much higher than that for the male 2R. Since all the types of labeling patterns may be found within a pair of glands from one larva, it is obvious that a simple variation in the pool sizes in the two sexes is not sufficient to explain this differentially higher rate of DNA synthesis by the male X and 2R. It seems logical, therefore, that the observed differences in the rate of TdR-^{3}H incorporation in female and male represent differences in the relative kinetics of replication in the two sexes as well as in the two chromosomes (namely the X and 2R in male).

A higher rate of incorporation of TdR-^{3}H by the male X suggests a faster rate of DNA synthesis, which would be necessary for early completion of replication. Analysis of the mean 2R/X grain ratios in male and female indicates that there is a progressive decline in the relative rate of incorporation by the male X in later discontinuous patterns, although the absolute labeling intensity is still higher than in female X's of corresponding labeling patterns. If the X in male, after a synchronous beginning of replication, proceeds faster with its DNA synthesis, then, at any subsequent time in the S period, more units on the male X would have completed their replication as compared to the autosomes and the female X's. This would cause comparatively less incorporation of TdR-^{3}H by the male X in comparison to 2R as the replication cycle advances (also see Lakhotia, 1970 *a*) and hence a higher 2R/X grain ratio in male nuclei with fewer and fewer sites labeled on the X.

There is some suggestion from the data obtained in the present study that the replication of the 2R segment studied may also be faster in the later parts of the S in male than in female. The rate of incorporation of TdR-^{3}H, as judged by visual grain countings, was higher in the male 2R in late patterns. It is not clear from the present data whether this higher rate of incorporation applies to all the autosomes in male. Probably it does, because the label density is apparently uniform over all the autosomes in male as well as in female. This apparent faster replication of the autosomes as well in male may seem to be in contradiction to earlier observations in *Drosophila* by Rodman (1967) and Pettit et al. (1967). These authors have shown that the polytene values in male salivary glands are consistently less than in female larvae of corresponding age. This would mean that in male the chromosome duplication is, in general, lagging behind. A possible explanation for these contradictory results may be that, in male, while the S period may be shorter, the intersynthetic period is more prolonged. This apparent faster replication of the autosomes in male does not, however, undermine the differential replication of the X chromosome in male. If the autosomes are indeed faster replicating than the female autosomes—as the present data indicate—, the X in male would be still faster replicating than in female.

Arcos-Terán and Beermann (1968) have used the labeling frequency and labeling intensity (i.e., the total number of silver grains) to characterize the replicative behaviour of *white* region in *D. melanogaster*. With some reservations, their data suggest that in general the frequency and intensity are functions of the amount and organization of DNA involved in a replicating unit. Our results show that, while the labeling frequency is lower, the labeling intensity of any site is higher in the male X than the female X's. These two replicative attributes of the male X are not the function of the amount of DNA involved, since as seen above, the asynapsed X in female (with the same amount of DNA as the single male X) does not exhibit these properties. The simultaneous

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presence of low labeling frequency and high label density in the single male X indicates a difference in the organization of the DNA in male and female X's.

Taken together, these data demonstrate that the replication of the male X in *D. melanogaster* larval salivary glands is faster than that of the autosomes in male and female or that of the X-chromosomes in female. There are two possibilities regarding the temporal order of the male X-chromosome replication: the X-chromosome either may begin replication simultaneously with the autosomes and end earlier, or may begin replication later but end simultaneously with the autosomes. The first alternative is preferred, since we believe, as discussed above, that replication begins simultaneously in all the replicating sites of a nucleus, and this implies that the male X-chromosome also initiates its DNA synthesis together with the rest of the autosomes but completes it earlier.

Implications of Early Completion of Replication by the Male X on the Dosage Compensation Mechanism in Drosophila

Earlier works (Mukherjee and Beermann, 1965; Mukherjee, 1966; Mukherjee et al., 1968, 1969; Lakhotia and Mukherjee, 1969; Lakhotia, 1970 *a,b*; Kaplan and Plaut, 1968) have indicated that, in *Drosophila*, dosage compensation, i.e. an equality of expression of X-linked genes in the two sexes (Muller, 1950; Stern, 1960), is achieved by an hyperactivity of the male X. This hyperactivity is manifested at the chromosomal level by an enlargement of the single X in male larval salivary glands, and there is a corresponding enhanced rate of RNA synthesis by the male X (see Lakhotia and Mukherjee, 1969). The present data show that the male X-chromosome in *Drosophila* polytene nuclei is hyperactive with respect to DNA synthesis too.

This faster replication of the male X seems to be related to the mechanism of dosage compensation operative in *Drosophila*. Evidence for this presumption is provided by a consideration of the general relationship between the chromosome condensation, genetic activity, RNA synthesis, and the replication patterns. DNA replication is very intimately related to the functional state of chromosomes and thereby to the genetic activity. All the available evidence indicates that, in general, condensed chromatin or heterochromatin is genetically inactive, lacks RNA synthesis, and also shows characteristic late replication of DNA (see Schultz, 1965; Brown, 1966, 1969; Lima-de-Faria and Jaworska, 1968). A direct demonstration of correlated changes in the transcriptive and replicative activities with changes in the condensation of the X-chromosome in grasshopper spermatogonia has been made by Nicklas and Jaqua (1965).

In the Dipteran polytene chromosomes, too, a relationship between the uncoiling of chromatin fibrils (puffing), active RNA synthesis, and faster replication has been suggested by earlier workers. Studies by Keyl and Pelling (1963) and Fujita (1965), and also by the present authors, indicate that, in polytene chromosomes, puffed loci, the sites of active RNA synthesis, complete their DNA synthesis comparatively earlier than the nonpuffed loci. All these observations are in agreement with the idea that the early completion of replication by the polytene male X-chromosome is related to its inflated appearance and higher rate of transcription and hence to the mechanism of dosage compensation in *Drosophila* (see Berendes, 1966; Lakhotia and Mukherjee, 1969; Lakhotia, 1970 *a,b*).

In *Rhynchosciara angelae*, Pavan and Frota-Pessoa (1964, quoted in Muller and Kaplan, 1966) observed that, after TdR-^{3}H radioautography, the paler X in male, but not the female X's is less labeled than the autosomes. Although the authors apparently did not comment about the differential replication, the reported data suggest that the single X in male *Rhynchosciara*, too, is early replicating. Interestingly, in this species the male X, like that in *Drosophila*, is hyperactive in RNA synthesis (Pavan, personal study).

More direct evidence for relating the faster replication of the male X to the dosage compensation mechanism in *Drosophila* has been obtained in experiments, referred to earlier, on the effect of X-irradiation and other agents on the transcriptive and replicative activities of the male X in larval salivary glands of *D. melanogaster*. As reported earlier (Mukherjee, et al., 1968), X-irradiation causes a selective reduction in the width of the male X-chromosome, and recent studies have revealed that this treatment selectively alters the replication pattern of the male X, causing it to be more female-like, and also selectively lowers the transcriptive activity of the normally hyperactive male X. More or less similar results have been obtained with certain other chemicals that disturb the normal nucleoprotein metabolism of a cell and also cause a selective reduction of the width of the male X-chromosome (Lakhotia, 1970 *b*). These

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results show that the architectural organization of the polytene X-chromosome in male larvae is different from that in female larvae and, as suggested by Berendes (1966), that the "DNA complex" in the bands of a male X-chromosome may be more loosely packed to facilitate faster transcription and replication.

The early completion of replication by the male X and the synchronous replication by the two X's of female *Drosophila*, thus, justify the hypothesis that dosage compensation in *Drosophila* is achieved by a hyerpactivity of the X in male (Mukherjee, 1966; Lakhotia and Mukherjee, 1969). Muller's theory of "piecemeal" control of dosage compensation in *Drosophila* (Muller, 1950; Muller and Kaplan, 1966) laid more stress on the repression in female rather than enhancement in male. However, recent studies (see Dobzhansky, 1957; Rudkin, 1964; Mukherjee and Beermann, 1965; Mukherjee, 1966; Mukherjee et al., 1968, 1969; Lakhotia and Mukherjee, 1969; Lakhotia, 1970 *a,b*: Kaplan and Plaut, 1968) on the salivary gland chromosomes in *Drosophila* have indicated that compensation is the result of enhanced activity of the single X in male, since the two X's in female are typically similar, in their functional morphology and transcriptive and replicative activities, to each other and to the autosomes, while the single X in male is more enlarged and also transcribes and replicates faster than the autosomes.

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