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DIFFERENTIAL REPLICATION OF MALE AND FEMALE X-CHROMOSOMES IN DROSOPHILA

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Abstract. The replication patterns of larval salivary gland chromosomes of *D. hydei* and *D. melanogaster* were studied by autoradiography with tritiated thymidine injected in mid third instar larvae. The male X chromosome showed a different replication behavior in comparison to that of the female X chromosome and autosomes. It is concluded that the male X chromosome finishes its replication earlier than the female X chromosome. Moreover, the time needed for a complete replication cycle of individual identical replication units was found to be shorter in the male than in the female X chromosome. Although the whole X chromosomes behave different there were no differences observed in the sequence of the discontinuous labeling patterns of the two types of X chromosome. One autosomal replication unit was observed which showed a different replication behavior in males and females. The possible origin of the differential behavior of the two X chromosomes is discussed in terms of their difference in degree of polyteny.

Introduction

It is clear from autoradiographical studies on chromosomal replication that the chromosomes contain a variety of individual replication units arranged in linear order along the chromosome axis (TAYLOR, 1960; GAY, 1963; RODKIEWICZ and OLZEWSKA, 1963; PLAUT and NASH, 1964; EVANS, 1964; HSU, SCHMID and STUBBLEFIELD, 1964).

On the basis of their size and their structural differentiation the giant chromosomes offer favorable material for detailed analysis of the replication cycle of such individual replication units. In terms of DNA synthesis during replication, the replication units can be distinguished from one another by two criteria: 1. the moment at which the replication of the unit either starts or ends as compared with other units of the same chromosome complement. Replication units for instance, which are morphologically designed as, or possibly situated in heterochromatin tend to start replication later than units situated in euchromatin. Replication of such heterochromatic regions produces in general a discontinuous labeling pattern (LIMA DE FARIA, 1959; MORISHIMA, GRUMBACH and TAYLOR, 1962; SCHMID, 1963; NICKLAS and JAQUA, 1965; BAER, 1965). 2. the period of time which is necessary for complete repli-

cation of the unit. This means that within the chromosome complement of a given tissue each unit takes its characteristic period of time to fulfill its replication.

From detailed analysis of discontinuous labeling patterns in the giant chromosomes of *Drosophila melanogaster* and *D. funebris* (PLAUT, NASH and FANNING, 1966; ARCOS-TERAN, unpublished) and of *Chironomus thummi* (KEYL and PELLING, 1963) the coordination of replication of all different units of replication within the chromosome complement was reconstructed. The various discontinuous labeling patterns observed in the giant chromosomes could be arranged into an ordered sequence of decreasing number of labeled regions with as an initial stage a chromosome complement which showed all regions labeled. According to the results of double labeling experiments (KEYL and PELLING, 1963), it is most probable that discontinuous labeling patterns occur at the end of the replication cycle and that all regions start replication at the same time.

The two criteria mentioned above are sufficient to characterize exactly the behavior of each individual replication unit within the complex process of replication of the chromosome complement. However, these criteria must not necessarily remain constant under all conditions.

In the present investigation evidence will be provided that a group of identical replication units take a different period of time for their replication under different conditions. It will also be shown that individual replication units can change their behavior in this respect.

Material and Methods

Mid third instar larvae (136—140 hours after oviposition) of *Drosophila hydei* and late third instar larvae of *D. melanogaster* were injected with 1 µl Ringer's solution containing 1 mC/ml tritiated thymidine (Spec. Act. 14.5 C/mM; The Radiochemical Centre, Amersham). The effective thymidine concentration in the larvae of *D. hydei* was 0.4 µC/mg body weight. In different series of experiments the salivary glands of 10 larvae were dissected 10, 30, and 60 minutes respectively after thymidine injection. Also some experiments were performed where the larvae were injected twice with the same solution with an interval of two hours. The dissected glands were stained with aceto-orcein, squashed on gelatinized slides in 45% acetic acid and frozen on dry ice to remove the cover glass. After rinsing the slides in a series of methanol-formaldehyde mixtures with an increasing percentage of formaldehyde, the slides were kept overnight in a 9:1 mixture of formaldehyde and methanol. The slides were then washed in tap water, Kodak AR 10 stripping film was applied and they were exposed for seven days. The films were developed in Kodak D 19b. Post staining was performed with a solution containing 0.15 g gallocyanin in 100 ml 5% chromalum. The map positions of labeled regions in *D. hydei* recorded in this investigation are based on the cytological map of this species (BERENDES, 1963a).

Results

In the salivary glands of mid third instar larvae of *D. hydei* 37—46% of the cells are replicating. In the labeled nuclei of this species, as in other Dipteran species, three different types of labeling patterns can be distinguished. Most of the labeled nuclei (93%) show weak continuously labeled chromosomes, 2% of the nuclei showed heavy continuously labeled chromosomes and 5% show discontinuously labeled chromosomes. The distinction between the first two types of labeling pattern is rather subjective and in a number of nuclei an overlap of the two types can be observed. A certain overlap was also observed between heavy continuous and heavy discontinuous labeling patterns. An increase in the period of incubation neither significantly altered the number of labeled nuclei nor the distribution of the different patterns of labeling. Small variations in the distribution of the various labeling patterns in different individuals of the same experiment occur.

Usually no apparent differences between labeling patterns of individual chromosomes within the same nucleus can be observed. In *D. hydei* and in *D. melanogaster* however, a number of nuclei of the heavy continuously labeled type revealed a striking difference in labeling pattern of the X chromosome as compared with the autosomes. A critical analysis of discontinuously labeled nuclei made clear that such pictures are due to differences in the number of labeled regions in X chromosome and autosomes. When labeling patterns in males and females were compared separately it was found that in males 55% of the heavy *continuously* labeled nuclei showed a heavy *discontinuously* labeled X chromosome (Fig. 1a). In females, however, a difference of this kind was never observed (Fig. 1b). Correspondingly, in discontinuously labeled nuclei the number of labeled regions in the male X chromosome as compared with the autosomes was obviously smaller than in female nuclei (compare Fig. 1c with 1d).

In order to provide a reference for the difference in labeling pattern between the single (male) X and the double (female) X chromosome in relation to that of the autosomes, location patterns of labeled regions in the second chromosome and in the X chromosome of the same nucleus were established. Twenty-five of such patterns were made from female nuclei and 15 from male nuclei. Among these patterns, those which showed an identical pattern of labeled regions of the second chromosome in males and females were used for a comparison of the pattern in the X chromosomes. From Fig. 3a it is apparent that there exists a definite difference in the pattern of labeled regions of the X chromosomes in males and females in nuclei which reached an identical stage of repli-

cation of the second chromosome. When the pattern of labeled regions in the second autosome is considered to represent a definite time point during the replication cycle in each type of cell, then it is clear that at this particular moment the male X chromosome shows definitely less regions labeled than the female X chromosome. It must be pointed out however, that although the number of labeled regions in the male X is smaller than in the female X, the regions which are labeled in the male X are labeled too in the female X when compared at the same stage of autosome replication. A comparison of replication stages which occur previous to or later the one presented in Fig. 3a gave confirming evidence for this. For instance, a pattern was observed where only one region in the male X chromosome was labeled together with ten regions in the second chromosome. A similar stage in a female nucleus showed 4 regions labeled in the X chromosome among which the one (7C1) also observed in the male X chromosome.

One exception of this rule was observed. In male nuclei, region 2—45 C 5,6 was found to be labeled more frequently than in females. Its frequency of labeling in relation to five neighboring regions was determined in 52 female and in 20 male nuclei. The Table indicates that

Table. *Differential sequence of labeling in region 2—45C5,6 in male and female giant chromosomes of D. hydei, as compared with neighboring regions*

	Region						No. of observations
	43C1—3	44C1,2	44D45A	45C5,6	46D	47B3	
Frequency of labeling							
female	1.00	0.41	0.68	0.20	1.00	0.36	52
male	1.00	0.35	0.80	0.60	0.95	0.40	20

the replication of this region in relation to the other regions is different in males and females. Assuming that the discontinuous labeling pattern is a final stage in the replication process, this would mean that region 2—45 C 5,6 finishes its replication earlier in females than in males.

It might be assumed that the difference in labeling pattern between male and female X chromosomes is a consequence of the single state of the male X chromosome. In order to test whether or not the single state of a chromosome always brings about a change in its replication behavior, the pattern of labeling in asynapsed chromosomes was compared with that in normal chromosomes. However, no obvious differences were observed (Fig. 2). To obtain better information about the correspondence of the labeling pattern of asynapsed chromosomes and the pattern

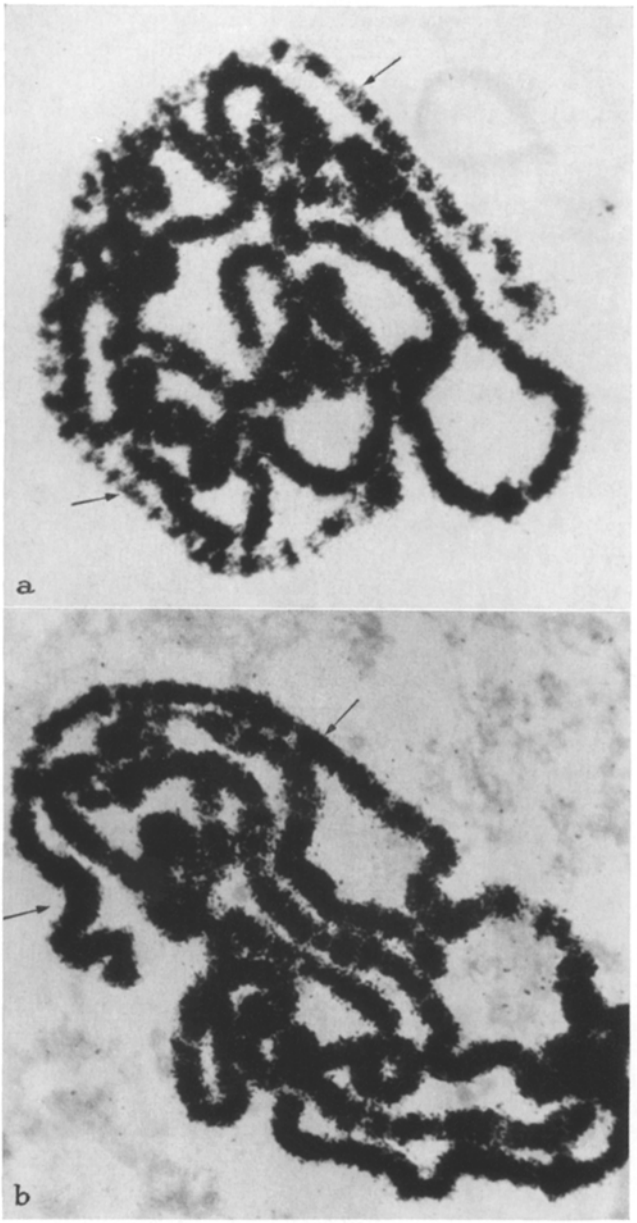
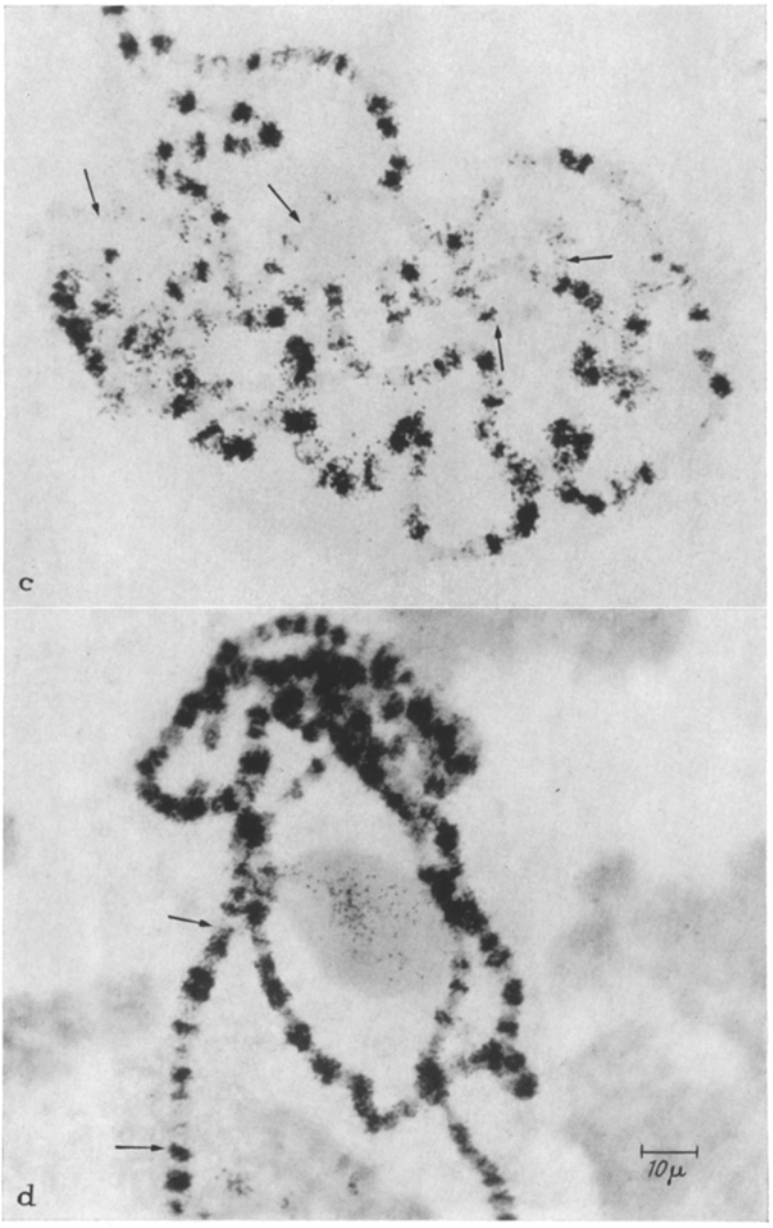


Fig. 1a—d. Labeling patterns in male (a, c) and female nuclei (b, d) of *D. hydei*. a and b show heavy continuously labeled autosomes together with a discontinuously labeled X chromosome in the male nucleus (arrows in a) and a continuously labeled X chromosome in the female nucleus (arrows in b); c and d represent discontinuously labeled stages. It is evident that in the male nucleus (c) the X chromosome (arrows) contains less labeled replication units than the female X chromosome (d) as compared with the autosomes



in a normal situation, the pattern of labeled regions in asynapsed female X chromosomes was compared with normal female X chromosomes in nuclei with a similar pattern of labeled regions in the second chromo-

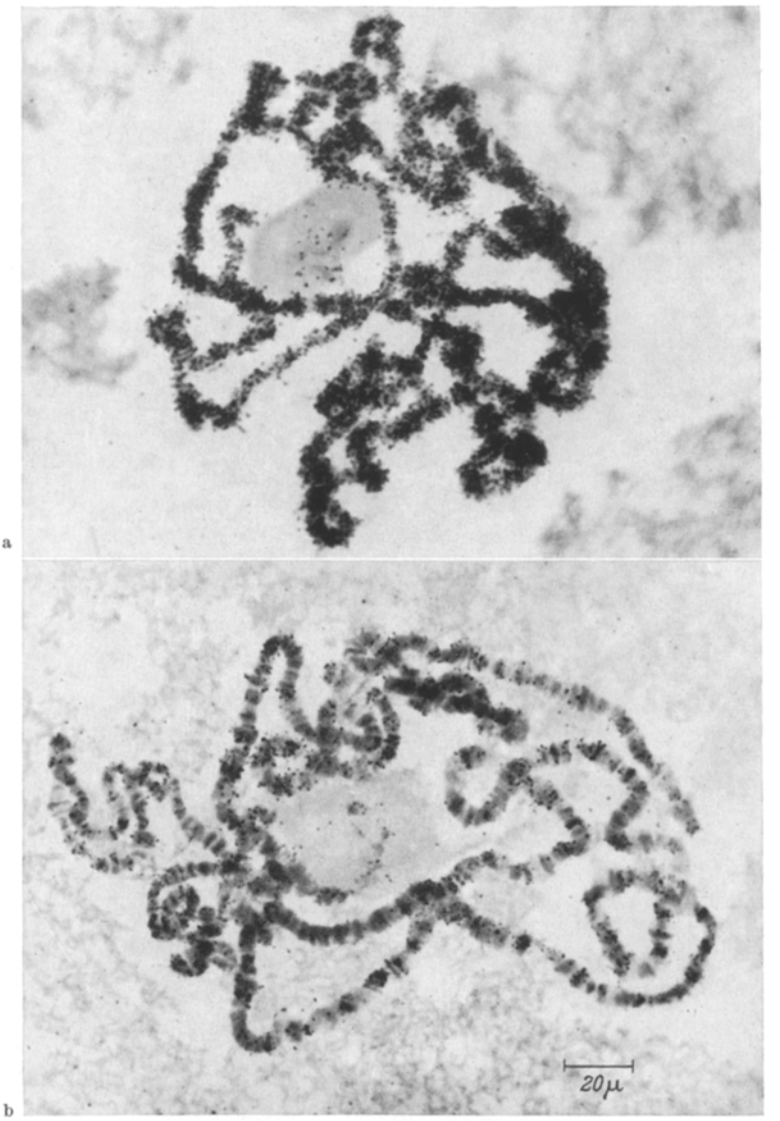


Fig. 2a and b. Labeling patterns in asynapsed female X chromosomes of *D. hydei*. a, heavy continuously labeled chromosomes; b, discontinuously labeled chromosomes. The number of labeled regions in the two asynapsed female X chromosomes as compared with the autosomes is not reduced to the same extent as in the male X chromosome (see Fig. 1c)

some. Although the number of asynapsed X chromosomes is rare (BERENDES, 1963b), a few suitable stages could be found, one of which is presented in Fig. 3b. From a comparison of Fig. 3b with Fig. 3a it is evident

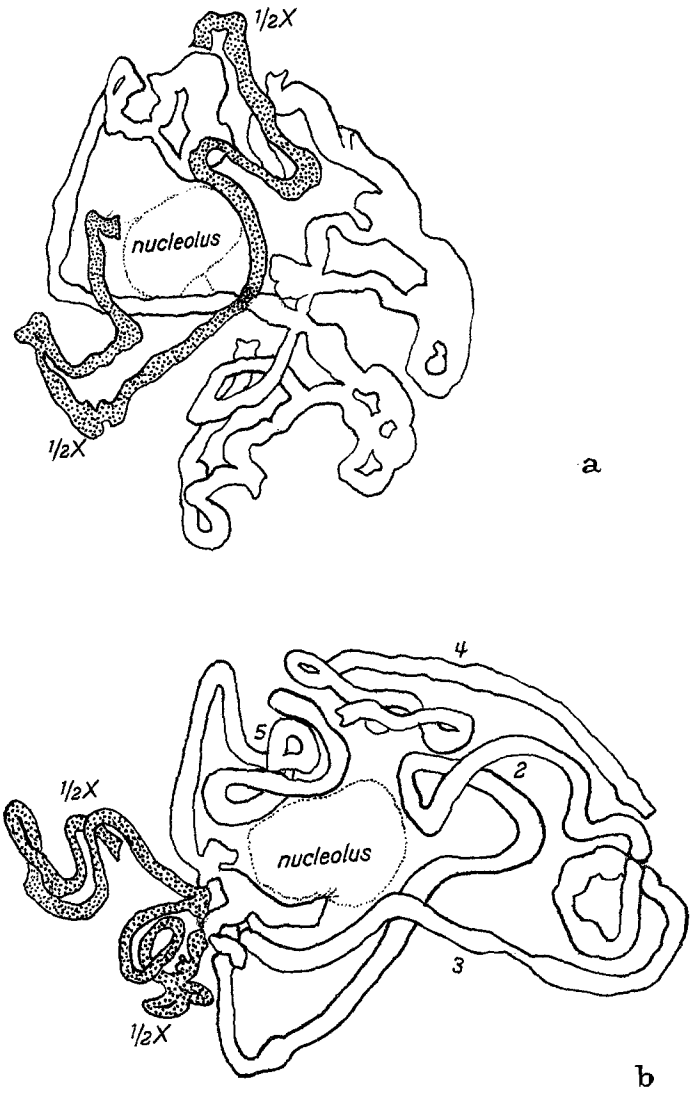


Fig. 2

that the pattern of labeled regions in the asynapsed state of the double X chromosome corresponds more closely with that of the female X than with that of the male X chromosome, although the second chromosomes are slightly different in their stage of replication. It therefore can be concluded that the single state of the female X chromosome as caused by asynapsis does not bring about a similar difference in the pattern of labeling as observed in the single male X chromosome.

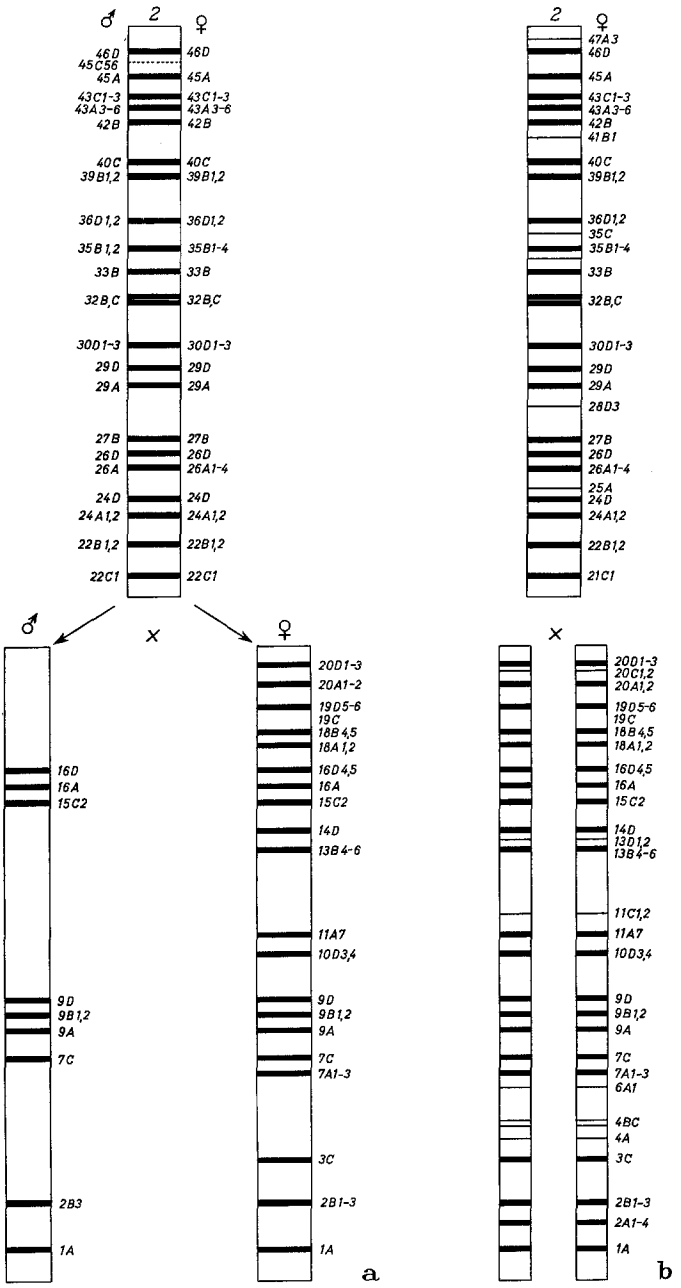


Fig. 3a and b. Schematical representation of a discontinuous labeling pattern in the second chromosome together with the pattern of labeled regions occurring at this stage in a male X chromosome and in a female X chromosome of the same nucleus. In b the pattern of labeled regions of the second autosome is shown together with the pattern of an asynapsed female X chromosome. Although the pattern of the second chromosome in a and b is not completely identical (in b there are a few more regions labeled which are drawn as thin bands) it is obvious that the pattern of labeled regions in the asynapsed X chromosome is more likely that of the female X than that of the male X chromosome in Fig. a

Discussion

From detailed studies of the replication of mitotic chromosomes (TAYLOR, 1960; HSU, SCHMID and STUBBLEFIELD, 1964; EVANS, 1964, and others), and of Dipteran giant chromosomes (KEYL and PELLING, 1963; GABRUSEWYCZ-GARCIA, 1964; PLAUT, NASH and FANNING, 1966) by autoradiographic techniques, it has been concluded that discontinuously labeled chromosomes represent late stages of the replication cycle of these chromosomes. Provided that this relation between the pattern of labeling and the stage of replication holds true also for the giant chromosomes of *D. hydei*, it can be concluded that the single X chromosome in the male completes its replication earlier than the double X chromosome in the female and also earlier than the autosomes. Assuming that the continuous labeling pattern precedes in each case the discontinuous pattern and the fact that there are no observations of nuclei containing continuously labeled autosomes together with another type of labeling of the X chromosome except that which is thought to be characteristic for the end of the replication process, suggests that all chromosome regions start replication at the same moment. This is in agreement with conclusions obtained from a number of other studies on chromosomal replication (see: PELLING, 1966). This would therefore mean that the differential behavior of the male X chromosome compared to the female X chromosome is due to the fact that the period of time for complete replication is shorter in the single male X than in the double female X chromosome.

One possible explanation for the origin of this differential behavior might be a relation of the replication process with a difference in DNA content of the two types of X chromosome (ARONSON, RUDKIN and SCHULTZ, 1954). The difference in replication behavior of the two types of X chromosome could depend simply on their difference in degree of polyteny. However, as single autosomes and single female X chromosomes in cases of asynapsis have an identical degree of polyteny as the male X chromosome, they should show a similar differential replication behavior as the male X. This however, could not be found.

Morphologically, single male X chromosomes are far from identical with single asynapsed female X chromosomes. The asynapsed female X chromosome does not show a similar increased diameter as occurs in the male X chromosome, although both have the same DNA content. Diameter measurements on *D. hydei* chromosomes gave a ratio of 1.2:1.0:0.7 for paired female X, male X and asynapsed single female X chromosomes respectively (see also OFFERMANN, 1936). The relatively swollen appearance of the male X chromosome indicates the presence of definite differences in the secundairy structure of the DNA in the

same band between male and female X chromosomes. It could be imagined that the DNA complex in the bands of a male X chromosome is more losely packed. Such a property could facilitate the penetration of nucleic acid polymerase and precursors in the DNA complex, which also could have some bearing on the RNA synthesis of the male X chromosome as was suggested in the form of a dosage compensation effect which is shown by most of the genes in this chromosome (MULLER, 1932; STERN, 1960; MUKHERJEE and BEERMANN, 1965). Genetic analysis of factors which might be responsible for the differential replication behavior are not yet known.

Structural differences may also play a role in the determination of the length of the replication period of certain special regions. For example, when a replication unit constitutes a puff the period necessary to replicate might be shorter than in a non-puffed situation. The observed difference in time at which region 2—45 C 5.6 ends its replication in male and female cells could be based on this principle. It must be pointed out however, that the differences in time necessary for a complete replication cycle observed so far seem to be very small. It is likely to suppose that all phenomena reported in this investigation originate from differences in structural configuration on the molecular level of the chromosomes.

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