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DNA Synthesis in the Neo-X Neo-Y Sex Determination System of *Dichroplus bergi* (*Orthoptera*: *Acrididae*)*

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Abstract. DNA replication in the neo-X neo-Y sex determining system was studied by means of tritiated thymidine and autoradiography. Asynchronous replication was found in the X arm of the neo-X and the long arm of the neo-Y. In addition, striking asynchrony was also found for short isopycnotic homologous regions at the distal end of the autosomal arm of neo-X and the short arm of neo-Y to which pairing during meiosis is restricted. These short regions are asynchronous with respect to the heterochromatic segments as well as to the remaining proximal region of the autosomal euchromatic arm of neo-X. This difference in replication pattern within the same chromosome arm may be related to a differentiation between regions which are homozygous in both sexes and regions which are hemizygous in males (Summary see p. 15).

Introduction

During the evolution of sex determining systems, rearrangements produced by translocation between autosomes and sex chromosomes have played a role in some organisms. One type is represented by the so-called centric fusion (WHITE, 1954), an interchange between an acrocentric X chromosome and one member of a pair of acrocentric autosomes, with breaks close to the centromere in different arms resulting in the neo-Y sexual system. Evidence has been found that when the neo-X neo-Y system occurs, there is a differentiation in the chromosome sections incorporated in the system. The changes are noticed because the autosomal euchromatin is gradually transformed into heterochromatin. The heterochromatic regions formed that way particularly appear as positive heteropycnotic segments in the neo-Y chromosome during the meiotic prophase. In some orthopteran insects, the conversion of the euchromatin into heterochromatin takes place gradually and seems to be represented by the stages in different species constituting what we have called heterochromatinization gradient (SAEZ, 1963). In the Orthopteron *Dichroplus bergi*, the behaviour of the chromosomes involved in the neo-X neo-Y system is of especial interest with

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respect to this heterochromatinization process. As up to the present there is no information on the replication behaviour, special attention was paid to the study of this evolutionary transformation by means of this technique with tritiated thymidine.

Material and Methods

30 male individuals of *Dichroplus bergi*, collected in Cerro Batovi (Rivera, Uruguay) were injected with 4 μc of H^3 thymidine (11.6 c/mM) per individual. The injected locusts were kept in a constant temperature environment of 28—29° C with an artifical photoperiod of 12 hours.

Every 24 hours, during 30 days, one individual was dissected and the testes fixed in 3:1 ethanol-acetic acid after being treated in NaCl 0.5% for 10 min. The testes were stained by the Feulgen method (8 min of hydrolysis). Squashes were made in 45% acetic acid over gelatinized and albuminized slides. After freezing by solid CO_2 the cover-slips were detached and the squashes washed in H_2O and covered with strip-film (Kodak AR-10). The autoradiographies were developed after a 30 days exposure.

Results

As indicated in previous papers (Saez and Diaz, 1960; Saez, 1963), the metacentric arm corresponding to the original sex chromosome will

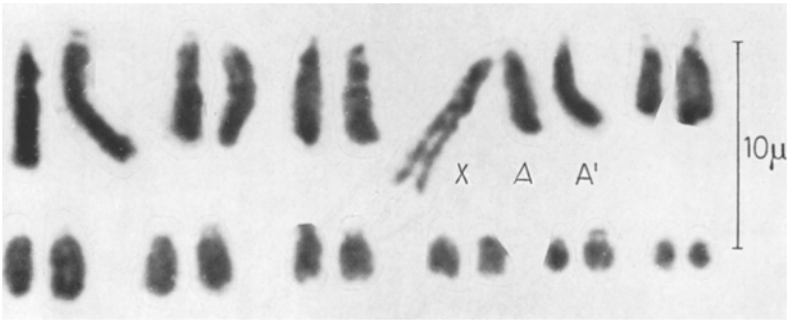
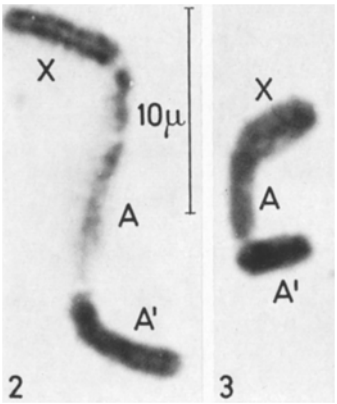


Fig. 1. The karyotype of *Dichroplus bergi* showing the chromosomes arranged in pairs according to their relative size. The fourth pair corresponds to neo-X and neo-Y elements

be called X; the fused arm corresponding to the translocated autosome will be called A and the free homologous autosome: A'. This means that the neo-X is composed by X and A, whereas A' constitutes the neo-Y (Fig. 1).

In *Dichroplus bergi*, the sex bivalent composed of a long isopycnotic segment showing positive heteropycnotic ends is found during pachytene and diplonema (Mesa, 1962; Saez, 1963). The union between XA and A' is strictly terminal in these stages. Probably, pairing and the formation



Figs. 2 and 3. The neo-X neo-Y bivalent. Fig. 2. Diplotene showing terminal pairing of the elements and heteropycnosis of *X* and *A'* arms. Fig. 3. First metaphase showing perpendicular orientation of neo-Y in relation to the spindle axis due to pericentric inversion

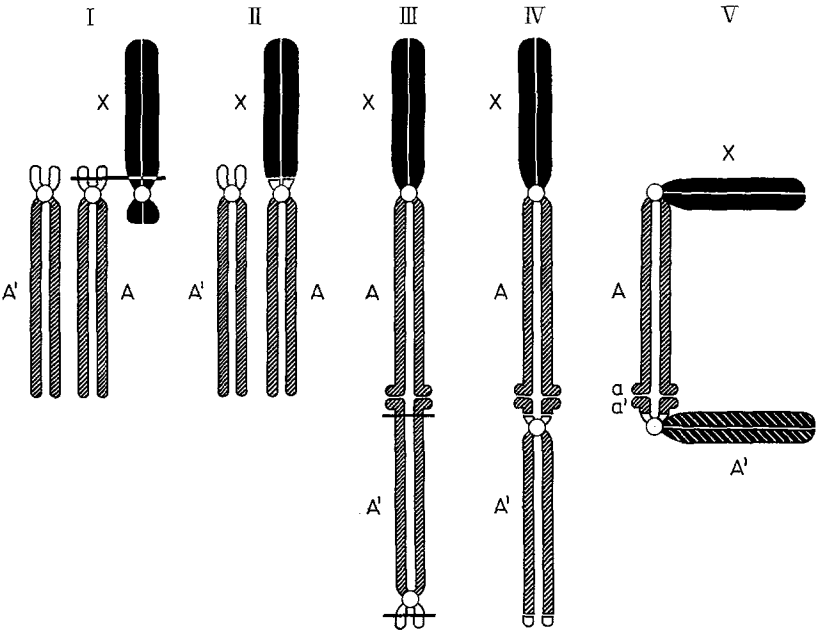
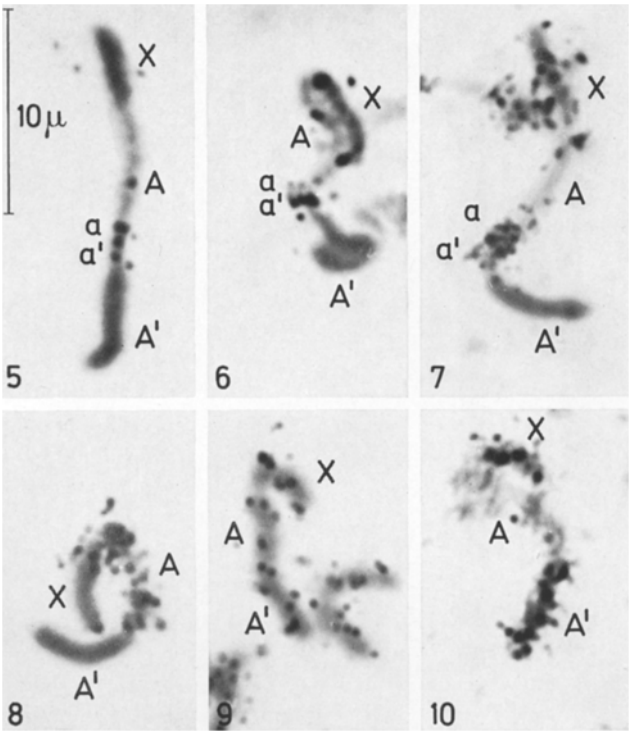


Fig. 4. Diagrammatic representation of the different steps of the rearrangement and heterochromatinization process of the neo-X neo-Y system in *Dichroplus bergi*: I. breakages of one autosome (A) and the X chromosome; II. centric fusion between *X* and the *A* autosome; the homologue *A'* remains free; III. the neo-X neo-Y structure during diplotene showing places at which *A'* chromosome is broken before the inversion; IV. configuration after the pericentric inversion; V. configuration of the sex bivalent such as seen at the end of diplotene and diakinesis, showing heterochromatinization of *A'* and restriction of pairing to the homologous regions *a* and *a'*



Figs. 5—10. Autoradiographs of the neo-X neo-Y bivalent. Fig. 5. Diplotene showing labelling pattern I. Figs. 6 and 7. Labelling pattern II with higher density of grains over a and a' regions. Fig. 8. Diplotene showing labelling pattern II with uniform labelling of A, a and a' regions. Fig. 9. Metaphase I showing labelling pattern III. Fig. 10. Labelling pattern IV

of a chiasma have taken place in the homologous regions adjacent to the contacting ends (Figs. 2, 3). Two rearrangements must have originated this system: 1. a centric fusion between X and the A autosome, and 2. a pericentric inversion in the A' autosome (Fig. 4). Pairing between homologous regions of the autosomal segments has become restricted to the distal end of the translocated autosome and its homologous region transposed to the short arm of the free autosome by the pericentric inversion. Thus, the sex bivalent is composed of five segments (Fig. 4V): the heteropycnotic X segment of the neo-X, its euchromatic segment A with its heteropycnotic end, a, which is paired with the homologous region a' in neo-Y or A'; and the remaining heteropycnotic segment A'.

During the meiotic prophase, the X segment corresponding to the primitive sex chromosome, is strongly positive heteropycnotic, while the A' segment shows positive heteropycnosis during pachytene and diplotene.

The A, a and a' segments remain isopycnotic in relation to the autosome. In the diplotene the segments (X, Aa, $A'a'$) can be clearly distinguished from each other presenting good images to study each segment's labelling.

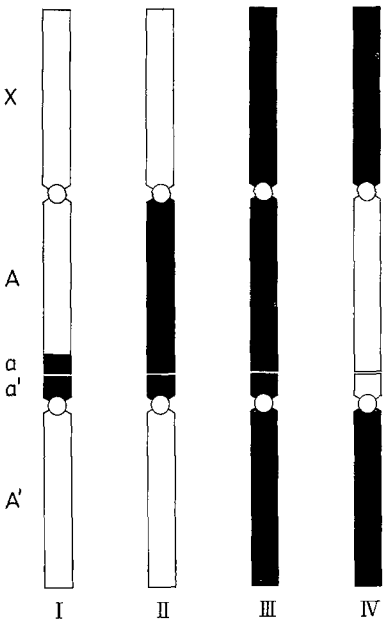


Fig. 11. Diagrammatic representation of labelling patterns observed in the neo-X neo-Y bivalent during diplotene

In the 28 days' slides the first labelled diplotene nuclei were observed. These nuclei have incorporated the precursor in the premeiotic interphase excluding the possibility of interchange and segregation of the labelled material during the preceding gonial divisions (Taylor, 1965).

The sex complex shows different labelling patterns in the diplotene, which always coexist with labelled autosomes.

The patterns can be classified into four groups (Fig. 11):

I. Only a and a' segments labelled (Fig. 5).

II. A, a and a' segments uniformly labelled or with more intense labelling of a and a'; segments X and A' not labelled (Figs. 6—8).

III. The five segments uniformly labelled (Fig. 9).

IV. A, a and a' segments with a very weak labelling; X and A' intensely labelled (Fig. 10).

Discussion

The information obtained from the described experiments is in good agreement with the known fact of the heterochromatinization of the neo-Y and allows us to trace this process up to the preleptotene period in which the first manifestation of the same appears: the asynchronic replication of DNA. If we generalize the data obtained by Lima-de-Faria (1959) for *Melanoplus differentialis*, to the whole *Acrididae* family, the asynchronic synthesis of DNA in X and A' segments in *D. bergi* would be a late replication. In our case, we have not observed any nucleus with exclusive labelling of the positive heteropycnotic regions (X and A') but we have observed nuclei in which these regions appear without label in presence of the labelled isopycnotic regions.

This can be interpreted as due to a late initiation of the duplication of the heteropycnotic regions.

Besides the facts on the duplication of A', foreseeable by its heteropycnosis, we can deduce from the autoradiographs that a differentiation different from the one observed in A' has taken place in segment A. This segment presents an asynchronous duplication in relation to segment a, from which only its heterozygotic condition in the male can distinguish it. It is also asynchronous in relation to the A' segment which is genetically isolated by heterochromatinization and the pericentric inversion of the latter. The A segment cannot be morphologically distinguished by its behaviour in the chromosome condensation cycle of the a segment which constitutes its distal end. We call the distal end of the isopycnotic arm of neo-X a, because we know that it contains the homologous region a' but we cannot define clearly a from A for lack of structural differentiation between both. However, the autoradiographies show us a different behaviour of this distal region. As in the diplotene, the association between the homologous regions a and a' is strictly terminal, the larger density of labelling in this region cannot be attributed to the presence of a pairing region with superposition of the four chromatids. Anyway, the repulsion of the homologous ends in this stage would prevent such overlapping. The chronological sequence in which the duplication of these different segments takes place cannot be determined from the data obtained in our experiment. Some chronological relations between different segment replication periods can be established: a and a' segments are isochronous in replication. Its replication period partially overlaps with that of A. Few overlapping is observed between replication periods of a or a' and X or A'. An overlap occurs in the X or A' replication periods.

These facts suggest a sequential replication of the different segments, beginning by a and a', followed by A and ending with X and A' or in the reverse sequence. The direction of the sequence cannot be established from the data of our experiments. Anyway, if we admit X and A' being late replicating segments, the proposed sequence should be the correct one.

New experiments are in progress to obtain complementary information about this process.

Summary

The DNA replication in the different segments of the neo-X neo-Y sex determination system of *D. bergi (Orthoptera, Acrididae)* has been studied by means of autoradiographic techniques with H^3 thymidine. The results are discussed in relation to the heterochromatinization process of neo-Y and differentiation of the A segments of neo-X.

A special pattern of replication relates the X to the neo-Y region which is genetically restricted to the male line and has become heterochromatic. The short homologous regions of the autosome part of the neo-X and neo-Y which are not isolated and pair during meiosis, show a reverse pattern of replication.

The period of replication of the autosome part of the neo-X overlaps partially with the two previously described patterns.

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