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The Neo-X Neo-Y Sex Pair in Acrididae, Its Structure and Association

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Abstract. In most of the fifty described cases of neo-X neo-Y sex determining systems in *Acrididae* the pairing regions during meiosis are limited to distal regions. A comparative study on the structure and pairing mechanisms of *Dichroplus silveiraguidoi* (2n=8); *Dichroplus bergi* (2n=22) and *Dichroplus vittatus* (2n=20) has been undertaken. – The sex bivalents of these three grasshoppers are different: the neo-X centromere is associated with the neo-Y telomere in *D. silveiraguidoi*; in *D. bergi* the neo-X is related through the short arm telomere to the centromere of neo-Y and both members of the sex pair are associated by the telomeres in *D. vittatus*. Centromeric and telomeric C-band positive blocks are present in both members of the pair in the three species. *D. silveiraguidoi* also presents an interstitial block in the neo-X. These blocks are brightly fluorescent with quinacrine mustard and Hoechst 33258 at low concentration (0.05 μg/ml). The region of neo-X corresponding to the primitive X takes an intermediate staining during the early meiotic prophase with C-banding and Hoechst 33258. – The structure of the sex bivalent and the particular staining of the X region are discussed in relation to the available information on the presence of different types of DNA in this segment. The possibility that the neo-X interstitial block of *D. silveiraguidoi* plays a role in preventing the spreading of heterochromatinization along the chromosome is also discussed. The classical interpretation of the neo-X neo-Y association during meiotic prophase as the result of a terminalized chiasma is considered in the light of optic and electronmicroscopic data. Other possible mechanisms of relationship between both chromosomes are also presented by these three orthopteran species.

Introduction

Most of the orthopteran species present an XO sex determining mechanism. Nevertheless, more than fifty cases of neo-X neo-Y sex chromosome systems have been reported up to the present (White, 1973; Mesa, personal communica-

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tion). The new mechanism is the result of a fusion between the original X and one autosome (A). The homologous autosome (A′) becomes neo-Y and is limited to the male individuals.

White (1973) has differentiated two types of neo-X neo-Y systems according to the observed homology between neo-X and neo-Y during meiosis. In the first group the neo-Y pairs with the entire length of the autosomic part of the neo-X. Up to four chiasmata have been described in this type of sex mechanism (Mesa, 1961). However, in most of the described cases the pairing regions are limited to distal zones. It has been postulated (Saez, 1963; White, 1973) that both types are the extremes of a continuous spectrum in which the progressive heterochromatinization of the neo-Y would result in loss of homology.

Very few studies have been done regarding the structure and behavior of these sex determining mechanisms. Díaz and Saez (1968) have studied the DNA synthesis of these chromosomes by autoradiography and Cardoso et al. (1974) have presented information about C-banding and behavior under hypotonic solutions of C-band positive regions in the sexual bivalent during meiosis.

The genus *Dichroplus* presents several species with neo-X neo-Y sex system. In the present paper a comparative study on the structure and pairing mechanisms of three different neo-X neo-Y chromosome pairs corresponding to three species of grasshoppers (*Dichroplus silveiraguidoi, Dichroplus bergi* and *Dichroplus vittatus*) of this genus is reported.

Materials and Methods

Seven male individuals of *Dichroplus bergi*, 5 male specimens of *Dichroplus silveiraguidoi* collected in Cerro Batoví (Rivera, Uruguay) and 4 males of *Dichroplus vittatus* kindly provided by INTA (Prov. Buenos Aires, Argentina) were used.

The testes were submitted to hypotonic solution (KCl 0.07 M) for 20 min before fixation in 3:1 methyl alcohol acetic acid for at least 30 min. Each testis was disaggregated separately in 60% acetic acid and the cell suspension was transferred to 3:1 fixer. The cells were dropped and flame dried. C-banding technique was performed as previously described (Cardoso et al., 1974). For fluorescence technique, the slides were stained in quinacrine mustard (5 µg/ml, distilled water) for 10 min, washed in running water for 10 min and mounted in tris-maleate buffer (pH 5.6).

The fluorescent banding with Hoechst 33258 was done using two different concentrations: 0.05 and 0.5 µg/ml dissolved in Hanks' salt solution (pH 7.0) during 10 min. The slides were rinsed in water and mounted in citrate buffer (pH 5.6).

The following modifications were introduced to the silver staining technique described by Howell (1977) for nucleolar organizer regions: 1) Incubation in 50% aqueous silver nitrate at 65° C for 18–20 h; 2) three changes of distilled water; 3) air drying and counterstaining in 3% phosphate buffered solution (pH 7.0) of Giemsa (Merck).

Results

a) Dichroplus silveiraguidoi

This grasshopper species presents the lowest number of chromosomes described up to the present in Acrididae (2n = 8). As described by Saez (1957) the karyotype consists of one metacentric pair, one submetacentric and two telocentric. One

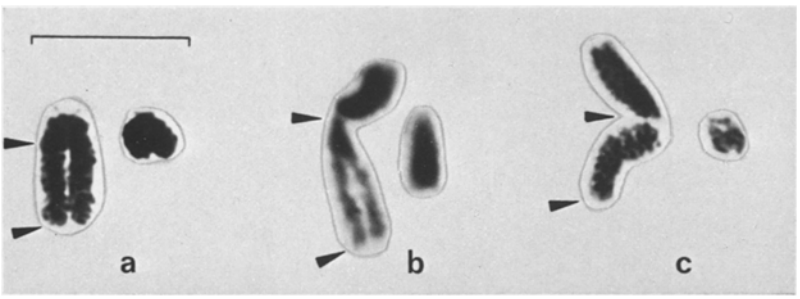

Fig. 1a–c. Neo-X neo-Y sex pair of the three species. The arrow heads point to the extremes of the heterochromatic region corresponding to the original X chromosome. **a** *Dichroplus silveiraguidoi* (2n=8); **b** *Dichroplus bergi* (2n=22); **c** *Dichroplus vittatus* (2n=20). The bar represents 10 μm

of the two telocentric pairs is heteromorphic and corresponds to the sexual one. The neo-X is the largest chromosome and presents a negative distally heteropycnotic region during mitosis (Fig. 1a). This new type of sex determining mechanism originated by the fusion of the primitive X chromosome to an autosome (Fig. 2a, A). The homologous one (Fig. 2a, A′) becomes neo-Y. During meiosis both chromosomes are only distally related during prophase I as has been previously reported (Cardoso et al., 1974). The neo-X centromere is associated with the neo-Y telomere (Fig. 2a) and the bivalent takes a singular L-shape at the end of diakinesis and metaphase I (Fig. 3a).

Four C-band positive blocks corresponding to the centromeres and telomeres are present in the bivalent. An additional block limits the proximal extreme of the primitive X region which stains during the early meiotic prophase in an intermediate form (Fig. 2a). This special stain affinity is lost at diplotene due to the packing of the chromatin and thus the zone becomes homogeneously positive (Fig. 4a). All these results confirm the findings previously presented by us (Cardoso et al., 1974). The association of the Neo-X centromere to the Neo-Y telomere is so tight that, very often it is possible to observe both blocks as a large mass with only a slight medial constriction (Fig. 4b). By means of quinacrine mustard it was not possible to demonstrate a similar pattern. The C-band positive blocks show a bright fluorescence but the primitive X region is uniformly bright during all of prophase I. The A part of the neo-X and the neo-Y present a dull fluorescence (Fig. 4b) At metaphase I, all the sex bivalent shows an intense and homogeneous fluorescence.

The results obtained with the fluorochrome Hoechst 33258 depend on the concentration of the dye (Gatti et al., 1976). At high concentration (0.5 μg/ml) the bivalents stain with a pattern similar to quinacrine mustard. At early stages and low concentration (0.05 μg/ml) the region of the neo-X corresponding to the original X presents a moderately intense fluorescence.

This zone behaves in a similar manner as already described for C-banding, the difference in the staining intensity being lost at diplotene. The localization of the bright fluorescent locks overlaps with the results described for C-banding and quinacrine (Fig. 4a–c).

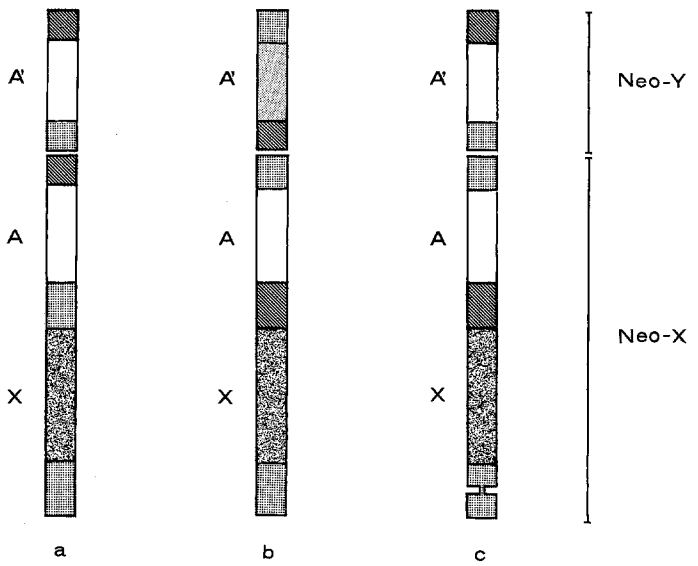

Fig. 2a–c. Schematic bivalent configuration of the sex pair during the meiotic prophase. The centromeres are indicated by an oblique striping. The C-band positive blocks are shadowed darker than the X region. **a** *Dichroplus silveiraguidoi*; **b** *Dichroplus bergi,* in this species the neo-Y is also heterochromatic; **c** *Dichroplus vittatus.* Note the secondary constriction in the telomeric neo-X block

The silver staining shows the presence of the nucleolus only up to mid pachytene (Cardoso et al, in manuscript). During the previous stages no regular association between nucleolus and sex bivalent was observed.

b) Dichroplus bergi

The diploid set of *Dichroplus bergi* is 2n=22. The karyotype was described by Mesa (1962) and consists of 10 pairs of autosomes all of them telocentric and an heteromorphic neo-X neo-Y sexual pair. The neo-X is submetacentric and its whole long arm presents a negative heteropycnosis during mitosis (Fig. 1b) which corresponds to the original sex chromosome. The short arm is euchromatic and corresponds to the autosome (Fig. 2b, A) fused to the primitive X chromosome. The homologous autosome (Figs. 1b and 2b, A′) which becomes neo-Y is clearly telocentric and eupycnotic during the mitotic cycle.

During meiosis the sex bivalent is composed of two more condensed and positive heteropycnotic regions, corresponding to the X region of the neo-X and to the neo-Y (Fig. 4d–f), and one eupycnotic. Both members of the sex pair are associated in a strictly terminal form during the first meiotic prophase. No other type of relationship between the two chromosomes was observed. The neo-X is related through the short arm telomere to the centromere of

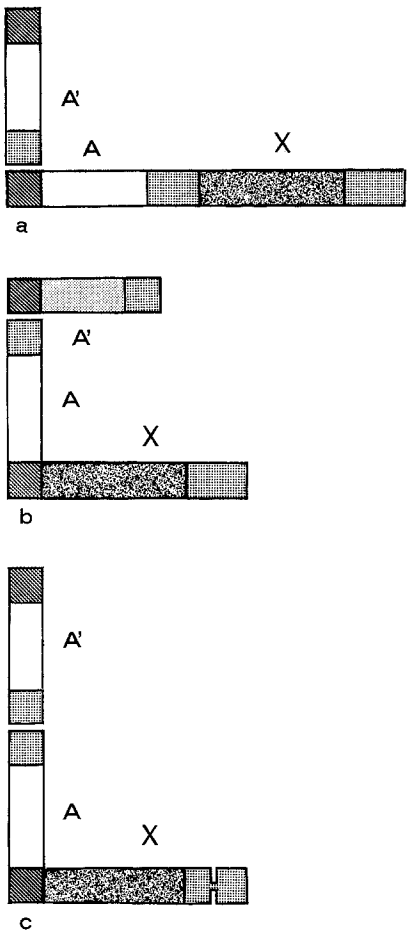

Fig. 3a–c. Schematic drawing of the neo-X neo-Y bivalent at metaphase I. **a** *Dichroplus silveiraguidoi*; **b** *Dichroplus bergi*; **c** *Dichroplus vittatus*

neo-Y. At the end of diplotene the bivalent takes its characteristic C-shaped configuration due to the localization of both centromeres (Fig. 3b).

In the material stained for constitutive heterochromatin the centromeric and telomeric regions can be recognized by the large C-band positive blocks at early stages (Fig. 2b). The autosomic part of the neo-X is negatively stained with this technique, but the neo-Y and the X region appear moderately postitive (intermediate) (Fig. 4d). At diplotene the region becomes homogeneously positive and the centromeric and telomeric blocks become indistinguishable. A similar behavior is observed in the neo-Y (Fig. 4d–f).

A structure with the same characteristics can be observed in the material stained with quinacrine mustard. A bright fluorescence is demonstrated for the X region of the neo-X and for the neo-Y. No clear intermediate staining similar to C-banding results was obtained. With this fluorochrome only on very few occasions can the centromere and telomere regions be detected as distinct zones. The autosomic part of the neo-X shows a dull fluorescence (Fig. 4e).

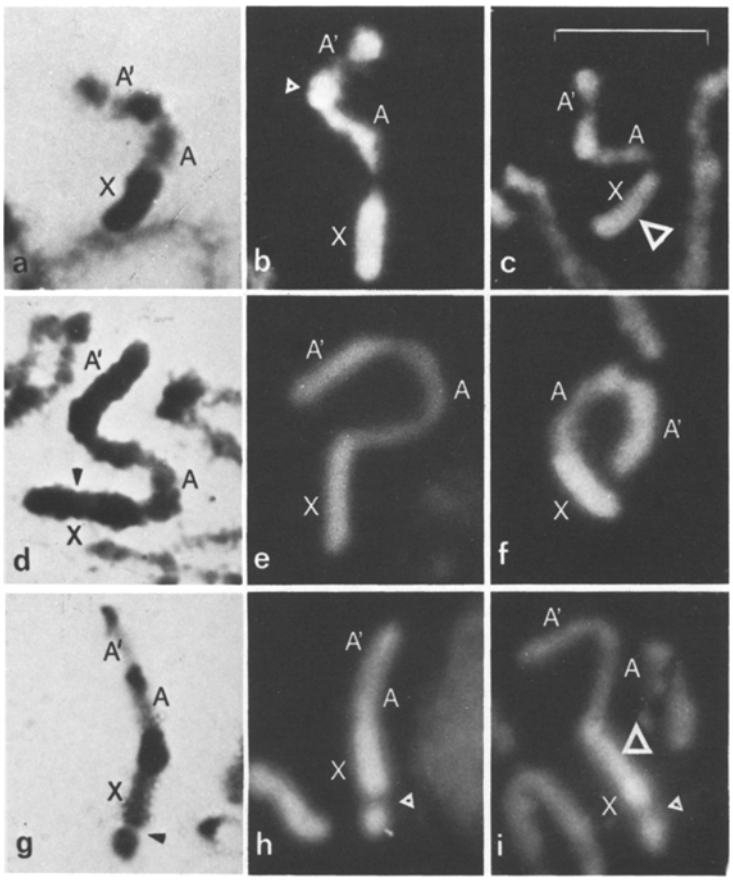

Fig. 4a–i. Sex bivalent of the three species at late pachytene-diplotene. **a–c** *Dichroplus silveiraguidoi.* **a** C-banding, the intermediate staining of the X segment is not observed. **b** Quinacrine mustard, the small arrow head points to the synaptic region between both members of the pair. **c** Fluorescent staining with Hoechst 33258 (0.05 μg/ml) the arrow head indicates the intermediate staining corresponding to the X. **d–f** *Dichroplus bergi.* **d** C-banding, the arrow head points to the intermediate stained region, the A′ segment of the bivalent is already homogeneously positive. **e** Quinacrine mustard, the X and A′ segments are homogeneously positive. **f** Hoechst 33258 (0.05 μg/ml), X and A′ are shown as bright regions. **g–i** *Dichroplus vittatus.* **g** C-banding, the small arrow head points to the secondary constriction. **h** Quinacrine mustard, the secondary constriction is indicated. **i** Fluorochrome Hoechst 33258 (0.05 μg/ml) the big arrow head shows the intermediate stained region, the small one points to the secondary constriction in the telomeric block of neo-X. The bar represents 10 μm

The fluorochrome Hoechst 33258 showed a banding pattern depending on the dye concentration; at low concentration the X part of the neo-X and the neo-Y present a moderately intense fluorescence. As in the case of *D. silveiraguidoi*, the differentiation is lost at late diplotene.

In this species the nucleolus is identifiable up to diakinesis as described elsewhere (Cardoso et al., in manuscript) but it appears only occasionally in the vicinity of the sex bivalent; no permanent relation was observed.

c) Dichroplus vittatus

The karyotype of this species (2n=20) was partially described by Cosen and Saez in 1974. It is composed of a pair of large submetacentric chromosomes, 2 pairs of large acrocentrics, 2 pairs of medium telocentrics, 2 pairs of acrocentrics, 2 pairs of small telocentrics and the sex heteromorphic pair. The neo-X neo-Y sex determining systems of this species is composed of a metacentric chromosome (neo-X) and a telocentric medium sized neo-Y (Fig. 1c). The same figure shows that one of the arms presents a negative heteropycnosis in somatic metaphases. During the meiotic prophase both chromosomes are related in a terminal fashion as in the other two previously described species, but in this case the autosome fused to the original X (A) and the neo-Y (A′) are associated by the telomeres (Fig. 2c). At metaphase I the bivalent takes an L-shape where the vertical part is longer than the horizontal one (Fig. 3c). During the meiotic prophase the neo-X presents a positive heteropycnotic arm in which a secondary constriction next to the telomere is present (Figs. 2c, 4g–i).

The C-banding technique demonstrates positive blocks in telomeric and centromeric regions. The secondary constriction is included in the distal heterochromatic block of the neo-X (Figs. 2c and 4g).

The primitive X region stains in an intermediate form during the early prophase. Quinacrine mustard staining of the sex bivalent shows a similar distribution pattern of intense fluorescent blocks. No intermediate staining is differentiated with this fluorochrome in the X zone (Fig. 4h). By contrast with this result this type of differential pattern can be elicited with low concentrations of Hoechst 33258 during the early prophase (Fig. 4i).

The presence of the nucleolus during meiosis can only be demonstrated during the early stages; at diplotene the nucleolus is not detected by the silver nitrate technique. Only occasional relationships between nucleolus and sex bivalent were observed in spite of the existence of a secondary constriction on the neo-X.

Discussion

Structure of Neo-X Neo-Y Bivalent

It is interesting to point out that these three sex determining systems, which probably originated independently, present a similar structure. Also the distribution pattern of blocks along the bivalent is the same for the three techniques used by us. The C-band positive blocks, the bright fluorescence regions with quinacrine mustard and Hoechst 33258 are located in centromeric and telomeric positions in both members of the sex pair. The only exception is the interstitial block of neo-X in *D. silveiraguidoi*; but, the position of this block is probably related with the fusion of the primitive X to the autosome, in other species such as *D. elongatus* with an XO system the telocentric X chromosome also shows centromeric and telomeric positive zones (unpublished data). These blocks are not distinguishable with current staining procedures.

Such an interstitial block can be interpreted as originating either from the centromeric block of the X, the telomeric block of the autosome or from both together. If part of the X centromeric block is involved, the microtubules are clearly not inserted in such a region during metaphase as can be shown by the bivalent configuration during this phase (Fig. 3a).

The distal two-thirds of the neo-X in *D. silveiraguidoi*, the neo-Y of *D. bergi* and a complete arm of the metacentric neo-X of *D. bergi* and *D. vittatus* are positively heteropycnotic during meiosis with current staining procedures (Saez, 1957; Díaz and Saez, 1968; Cosen and Saez, 1974).

When these regions are treated for C-banding or stained with low concentrations of Hoechst 33258 an intermediate staining can be observed during the early phases of meiosis. Similar results have been obtained with C-banding in sex chromosomes for other cases: the Y chromosome of the mouse (Hsu et al., 1971), the Y chromosome of the Bovidae (Hansen, 1973) and the Y chromosome of the guinea pig (Bianchi and Ayres, 1971).

No differential staining can be observed in any of the three species with quinacrine or higher concentrations of H 33258. The variations in the observed fluorescence pattern in accordance with the concentration of H 33258 has been demonstrated in *Drosophila* by Gatti et al. (1976). The same authors recognize different types of heterochromatin according to their reactivity to fluorescent dyes. In our case the three neo-X neo-Y systems present two types of heterochromatin: a) the centromeric, telomeric blocks of sex bivalents and the interstitial block of *D. silveiraguidoi* with positive C-band, quinacrine positive and H 33258 (0.5 and 0.05 μg/ml) positive; b) the X region of the three bivalents and the neo-Y of *D. bergi* which stain in an intermediate form with C-band, quinacrine positive and Hoechst 33258 (0.05 μg/ml) intermediate.

In our material such differences disappear with the chromosome condensation which occurs during the meiotic prophase. Similar results were reported in mitotic chromosomes of some species of *Drosophila* (Holmquist, 1975).

It has been postulated that the intermediate staining is the result of the presence of low repetitive DNA in such regions (Bianchi and Ayres, 1971). Our previous interpretation in *D. silveiraguidoi* (Cardoso et al., 1974) was in accordance with such a view, but extensive DNA studies in *D. silveiraguidoi* did not demonstrate highly repetitive DNA and the rDNA was the only low repetitive DNA isolated in this grasshopper (Cardoso and Wettstein, unpublished data). Therefore, we are now inclined to believe that this weakly positive staining would rather be the result of differences in chromatin packing. Although the association of at least part of the proteins to DNA might be determined by the nucleotide sequence and some of them related to the condensation process in each region, such staining would not necessarily reflect a difference in chromatin structure.

White (1973, p. 612) has postulated that the centromere would prevent the spreading of the special type of heterochromatinization which is characterized by a negative heteropycnosis in spermatogonia. This author based his hypothesis upon the observation of a complete heterochromatic neo-X chromosome which originated by a tandem fusion in a morabine (warramaba) grasshopper. A centric fusion followed by a pericentric inversion has been proposed (Saez and

Pérez Mosquera, 1977) as the origin of the *D. silveiraguidoi* neo-X. In this case the heterochromatic regions are restricted to the primitive X, no centromere is between eu- and heterochromatin. Not enough evidence has been produced until the present which strongly supports the hypothesis of a gradual heteropycnosis except for the results presented at the ultrastructural level by Solari (1971) in the autosomal part of the Searle translocation. But if this were the case our data could be interpreted as the result of a fresh pericentric inversion which has occurred in *D. silveriraguidoi* without enough elapsed time for the "spreading" of the heterochromatinization. The lack of pairing along the formerly homologous regions (A and A') would be against such an interpretation.

An alternative more in accordance with our data would be that the centromere itself would not prevent the "spreading" of the heterochromatinization but the C-band positive pericentromeric block would play such a role.

Neo-X Neo-Y Association

Classically, it has been described that in these neo-X neo-Y systems both chromosomes are related during late meiotic prophase through a terminalized chiasma (White, 1973; Saez, 1957; Díaz and Saez, 1968; John and Lewis, 1965). John (1976) has questioned the possibility that terminal association always means a terminalized chiasma. Several other mechanisms have been postulated which would keep homologous and non homologous chromosomes together during meiosis. It would be interesting to consider the possibilities of some of these mechanisms in relation to the neo-X neo-Y sex determining systems presented by these three orthopteran species.

a) Terminalized Chiasma

The homology between the autosomic part of the neo-X and the neo-Y is clearly demonstrated by the pairing of both segments during the early prophase in several species (White, 1973; Mesa and Mesa, 1967). Although, John and Freeman (1976) have observed in grasshoppers and increase of distal chiasma frequency and a decrease of proximal ones in the autosomes involved in the fusion. In most cases of neo-X neo-Y, however, both chromosomes are related only through distal regions during the meiotic prophase.

Saez (1963) and White (1973) have postulated that both types of behavior are extremes of a same process. The second type would be the evolutionary result of a restriction of proximal homology probably originating by heterochromatinization of these zones and a distally located chiasma and a posterior terminalization would held the pair together. Therefore, this hypothesis implies the existence of small homologous (and pairing) regions near the chromosome ends and distal lateral pairing at early stages. The material studied by us behaves in the same way although in the three cases the segments which are kept joined during meiosis are different: In *D. silveiraguidoi* the neo-X centromere is associated to the neo-Y telomere; in *D. bergi* a neo-X telomere is related to the

neo-Y centromere and the neo-X telomere of *D. vittatus* is joined to the neo-Y telomere (Fig. 2a–c).

The results obtained in *D. vittatus* can be interpreted as a result of a loss of proximal homology with the bivalent being held together through the association of a distal chiasma between small homologous regions. The same interpretation cannot be so clearly applied to the remaining species. Díaz and Saez (1968) postulated in *D. bergi* that a pericentric inversion which followed the pairing restriction was the mechanism giving rise to this particular sex bivalent configuration. However, the neo-Y is clearly telocentric and the remaining homologous material, if any, would have to be small enough to be below the optical microscope resolution or have fused to the centromere. In *D. silveiraguidoi* a pericentric inversion was proposed for the neo-X as an interpretation of the meiotic figures. But, again we have the problem that no short arm was observed in this chromosome. The existence of chiasma terminalization has been recently questioned in rye (Jones, 1978). DNA labelling of meiotic chromosomes has demonstrated a coincidence between chiasmata and points of crossover (Taylor, 1965; Jones, 1971). BrdU substitution techniques seem to confirm this result, at least in locust (C. Tease, cited by Jones, 1978). Also detailed analysis of chiasma distribution did not demonstrate chiasma movement at diplotene in the locust *Schistocerca gregaria* (Henderson, 1963; Fox, 1973) and at diakinesis in human spermatocytes (Hultén, 1974).

The absence of other types of association between sex chromosomes besides the observed end to end would led us to postulate also here the absence of terminalization.

Recently, Solari and Ashley (1977) have found the regular formation of a sex vesicle in *Psommomys obesus* without the formation of a synaptonemal complex; in this case the absence of chiasmata was accompanied by a variable type of connection between both chromosomes. No synaptonemal complex was found in serial sectioning reconstructions of the sex bivalent at late paquitene of *D. silveiraguidoi* (R. Wettstein, unpublished data). If the presence of synaptonemal complexes is necessary for crossing over and chiasmata (Moens, 1974) these data do not support the existence of a chiasma in such a region. Therefore, the available information at present would not incline us to postulate the existence of an almost terminal chiasma and its posterior shift to the chromosome extremes as a general explanation for this special type of terminal association.

b) Terminal Chiasma

As both members of the sex bivalent in the three species are related through C-band material during all the meiotic prophase, the existence of a localized chiasma in such region is another possibility to be taken into consideration. But, chiasmata and crossing-over are rare or absent between heterochromatic regions of plants and animals (Hyde, 1953; Linnert, 1955; John and Lewis, 1965; Natarajan and Gropp, 1971). John (1976) has pointed out that although chiasmata occur in some heteropycnotic regions, no chiasma has been found in zones which are heterochromatic at the time of chiasma formation. The

centromeric and telomeric blocks involved in the chromosome association are C-band positive through all the meiotic prophase and probably no chiasma can be present between such regions. The already cited results of the serial sectioning are also in accordance with this interpretation.

c) Nucleolus Mediated Association

Non chiasmatic association between sex chromosomes has been described in neuropteran insects by Suomalainen (1952) and Makino and Kanô (1947). In these species a small nucleolus was found connecting both chromosomes. Later, John and Lewis (1960) have described an achiasmatic pairing system in beetles. In this mechanism a large metacentric X is associated by its extremes to the small Y giving at metaphase I a parachute appearance. The nucleolus is associated with the sex bivalent from pachytene to metaphase I and would be responsible for this Xy_p association.

Solari and Ashley (1977) have proposed that the presence of a nucleolus-like structure associated with the XY pair in the sand rat would play the role of a segregation body (John and Lewis, 1965). In this rodent which presents different types of associations in the achiasmatic XY pair, this body would allow a normal segregation of sex chromosomes.

The presence of the nucleolus during meiosis is demonstrated by the silver nitrate technique described by Howell (1977) for nucleolar organizer regions as modified by us (Cardoso et al., unpublished). None of the species studied by us consistently presents a regular relation between the sex chromosomes and the nucleolus, since only sporadic associations with different sex bivalent regions were observed. This includes *D. vittatus* in which a clear secondary constriction is observed in the telomeric block of the neo-X. No nucleolar organizer regions seem to be located on the sex chromosomes of these three grasshoppers. The lack of a regular relationship between the neo-X neo-Y bivalent and the nucleolus makes it very difficult to consider this mechanism as a really possible one.

d) Terminal Association

Different types of association have been observed between homologous and non-homologous chromosomes during mitosis and meiosis (Benavente and Wettstein, 1977; Cardoso et al., 1974; Drets and Stoll, 1974; John and Lewis, 1965; Murray, 1977; Schmid et al., 1975; Solari and Ashley, 1977; Solari and Bianchi, 1975). These reported chromosome associations have been studied both at the ultrastructural and the optical level.

A specialized structure has been found at the ultrastructural level in part of these materials. Wettstein et al. (1974) and Solari and Bianchi (1975) have found that one of the axial extremes of each sex chromosomes (X and Y) is joined to a differentiated zone of the nuclear membrane in two marsupials. Benavente and Wettstein (1977) have demonstrated by an electron microscopic

study the existence of a particular laminar structure connecting both non homologous sex chromosomes (X_1, X_2) in *Lycosa malitiosa.*

Differing with these observations, Solari and Ashley (1977) reported in a rodent the lack of an ordered structure between the sex chromosomes. At present we have information on this matter only in one of the three species (*D. silveiraguidoi*) which does not present any connecting structure between the sex pair. Serial sectioning reconstructions of sex bivalents of the two other species will probably clarify this point.

Studies carried out at the optical level showed that C-band positive material is present in the associating regions in different species during mitosis (Murray, 1977; Schmid et al., 1975) and meiosis (Cardoso et al., 1974; Drets and Stoll, 1974; Schmid et al., 1975). It has been postulated that heterochromatin (repetitive DNA) has a role in chromosome pairing and regular segregation (Yunis and Yasmineh, 1971). The terminal association of the bivalents described during meiosis in haploid plants has been interpreted by John (1976) as the possible result of the homology due to similar DNA content. Nevertheless Miklos and Nankivell (1976) have postulated that the heterochromatin would be implicated in meiotic recombination rather than chromosome pairing. In *Drosophila melanogaster* (Yamamoto and Miklos, 1977, 1978) the heterochromatically deleted X chromosome segregates in a manner similar to the normal one and in salivary gland nuclei the satellite deficient X is able to participate in the chromocenter formation. According to such data they conclude that if the repetitive DNA were necessary for chromosome pairing, its amount must be extremely small. Therefore, the hypothesis proposed by Yunis and Yasmineh (1971) could not be generalized. But, it has been suggested by Commings and Riggs (1971) that some proteins would bond to particular DNA regions and to each other. Consequently, these proteins would be involved in chromosome pairing. Such a type of speculation has been proposed by Drets and Stoll (1974) as responsible for the non-homologous association observed from pachytene to diakinesis in a cricket.

Although the available information is in some aspects contradictory, it is clear that more complementary information at the ultrastructural and biochemical level is needed for supporting any of the probable mechanisms proposed here. But, it seems to be also clear that at least in these three orthopteran species the classical interpretation of neo-X neo-Y association through a terminalized chiasma must be reviewed.

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