Chromosome Polymorphisms and Chiasma Frequency in two Populations of *Staurorhectus longicornis* (Orthoptera-Acrididae)

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Chromosome rearrangement in Acrididae are often associated with racial differentiation and speciation processes (White 1957a, 1978, Sáez and Pérez Mosquera 1971, Shaw 1976). On the other hand, polymorphisms for the presence of heteromorphic bivalents, translocations, centric fusions and numerical variations in karyotype have been described in numerous species (White 1973a, Hewitt 1979). The study of chromosome rearrangements occurring in natural populations is considered by many authors very important for the understanding of the influence of such phenomena on recombination and some other processes.

There are many studies dealing with the influence of different chromosome rearrangements on chiasma formation in several species of Orthoptera (Hewitt and John 1965, John and Hewitt 1966, Sannomiya 1968, Shaw 1971a, Hewitt 1979). Sáez (1956) and Mesa (1956) have described the haploid number of *Staurorhectus longicornis* (G. Tos) studying only a few individuals. In the present study karyotype, meiotic behaviour and chromosome polymorphisms are studied in two populations of this species. With this material it was analyzed whether the structural alterations are or are not related with chiasma frequency and terminalization, stressing methodological aspects of this type of study.

Material and methods

The individuals studied were sampled from two populations in La Rioja Province (Argentine Republic). One of them was composed of 35 males, and located near La Rioja City Airport (population AR). This locality is situated East of La Rioja City. Approximately 30 km north-west of this site, on the other side of the city, the second population was found, at Puerta de la Quebrada. This second sample contained 25 males, caught at both sides of the road from La Rioja City to Los Sauces Dam. Only males were used for cytological studies, though some females were also caught to help in the taxonomic recognition. The systematic identification was made by Prof. R. A. Ronderos, specialist on the taxonomy of this group.

The hypotonic shock was accomplished by making a cut at the abdomen of every individual and dipping it in 0.5% KCl solution during 30 min. Fixation was made...
with 3:1 (methanol: acetic acid) fixative solution. Later the testis were dissected and used for the cytological preparations.

Most of the slides were made using the dispersion technique in 60% acetic acid, and some were prepared by squashing in propionic haematoxylin (Saez 1960). Some of the dispersion slides were stained with Carbol-Fuchsine, according to Carr and Walker (1961), others with 5% Giemsa stain in phosphate buffer pH 7.6, and the remaining were assayed for C-banding. The banding technique consisted of Ba (OH)$_2$ treatment during 20 min. at room temperature, rinsing with distilled water, and 2× SSC at 60°C during 30 min.

Results

Karyotype

The karyotype was determined by studying cells in gonial division, MI and AI. In most of the males the karyotype was composed of 2n=23, with a sexual mechanism X0 ♀ / XX ♂♂. The autosomes may be grouped in three large (L1-L3), six medium (M4-M9) and two small (S10-S11) (Fig. 1A). X chromosome length varies respect to that of autosomes according to the stage analyzed, since their condensation-decondensation cycles are different, but, on the average is similar to pair L3. All of the chromosomes have terminal centromeres, and no short arm was visible at the light microscope level. Therefore, they are $t$, according to Levan et al. (1964), or telocentric, if their existence is accepted, as John and Hewitt (1968) and Hewitt and John (1971) assure. The X chromosome presents a secondary constriction near the centromeric region that determines a linear satellite which constitutes most of the chromosome arm (Fig. 1B). This constriction was most clearly visible in meiotic prophase, specially at diplotene, while at MI, MII and gonial metaphase the high chromosome contraction made it less evident. The individuals having this karyotype had regular meiosis forming invariably 11 bivalents+X (Fig. 1B).

Centric fusion

One out of 35 individuals analyzed from population AR had a submetacentric (sm) chromosome, its karyotype being composed of 2n=21 AA+X and nF=23. The submetacentric chromosome of this specimen would have arisen through fusion between one L2 chromosome and one of pair L3 (Fig. 2A). During meiosis the individual formed invariably 1 III+9 II+X (Fig. 1C). The study of centromere co-orientation, observing 10 cells at each stage, indicated that at AI the non homologous centromeres of the free chromosomes L2 and L3 were orientated always towards the same pole, while the fusesd L2-3 was to the opposite one (Fig. 2B), even though the trivalent orientation at MI was linear or alternate with approximately the same frequency. This type of segregation would lead to the formation of balanced gametes, and this seems to be true on the basis of five cells observed at MII (Fig. 2C).

Supernumerary segment

In three individuals from AR and two from PQ the pair L2 was heteromorphic for the presence of a proximal heterochromatic segment, immediately close to the
centromeric region which remains terminal. This segment was clearly visible at pachytene in every cell analyzed (Fig. 3A). At diplotene, the heteromorphism is

Fig. 1. A, basic karyogram of the species *S. longicornis* at gonial prometaphase. B, diplotene of an individual with normal chromosome complement; the arrow indicates the secondary constriction on the X chromosome. C, metaphase I of an individual heterozygous for centric fusion between chromosomes of pairs L2 and L3; arrow: trivalent formed by the two not fused telocentric and the fused submetacentric chromosomes.
even more evident because of the presence of an elastic constriction on the chromosome carrying the segment, which separates it from the rest of the chromosome (Fig. 3B). This constriction was observed in all the individuals carrying the supernumerary segment and, therefore, it might be supposed that the constriction is, in some way, associated with the segment. At diakinesis the constriction, though yet visible,
is notably less stretched than in previous stages and at MI, because of the high chromosome condensation, it is hardly distinguishable. Many cells were studied in the heterozygous individuals and no evidence of translocation was found that could

Fig. 3. A, pachytene of an individual heterozygous for the supernumerary segment on pair L2; arrow: non paired procentric segment. B, diplotene of another individual carrying the same rearrangement. The supernumerary heterochromatic block is separated from the rest of the chromosome by an elastic constriction (arrow). C, karyogram of a heterozygote for the supernumerary segment; arrow: constriction.
have originated the segment. The proximal localization of the segment determined that chiasmata were always formed distally and consequently, the first division was invariably reductional for this heteromorphism.

Fig. 4. A, diplotene of a heterozygote for an inversion in pair M8; arrow: heteromorphic bivalent. B, pachy-diplotene showing a distended constriction on the chromosome X (arrow). C, pachytene of an individual having two elastic constrictions on the chromosome X. D, pachytene of a heterozygote for an elastic constriction in pair L3 (arrow).
The study of gonial dividing cells allowed the karyotype analysis in the heterozygotes. The corresponding karyogram shows both the segment and the constriction (Fig. 3C).

**Pericentric inversion**

One individual from AR exhibited a heteromorphic bivalent corresponding to pair M8 (Fig. 4A), in which one member was more condensed and formed a right angle with its homologue. This feature was interpreted as the occurrence of a pericentric inversion, the consequence of it being the displacement of the centromere from one chromosome end to the opposite one, while the nF remains the same. The chiasmata would occur only within the terminal segment of the non inverted chromosome long arm and the inverted chromosomes short arm (not visible at L.M.). The fact that ditactic bivalents have been observed in this organism with a certain fre-
Elastic constrictions

In the present study, elastic constrictions were demonstrated in relation with the supernumerary segment in pair L2. The same type of phenomenon was also found in chromosome X and in pair L3.

In all the individuals analyzed, chromosome X showed a constriction near the centromeric region, as previously described. Nevertheless, one individual from AR showed this constriction extremely stretched (Fig. 4B), which was specially evident in diplotene. Another individual from the same population had a second constriction in the X', both of them being very stretched, giving the chromosome a tripartite appearance (Fig. 4C). At MI the constrictions were not stretched, but looked like ordinary ones.

One individual from AR exhibited an elastic constriction in one chromosome of pair L3. This was very evident in every cell analyzed at pachytene (Fig. 4D), and progressively less visible in more condensed stages.

Chiasma frequencies

Total chiasma frequency and the ratio interstitial/total chiasmata at diplotene, diakinesis and MI in both AR and PQ populations were calculated, trying to de-
termine the differences if any, between stages and populations, and the effects of the observed rearrangements on chiasma conditions.

Firstly, the total chiasma frequency at diplotene, diakinesis and MI were compared by an analysis of variance (Table 1). For this study, 13 individuals from both populations were analyzed by studying 5 cells from each one at each stage. The means for each stage were as follows: diplotene, $12.877 \pm 0.307$; diakinesis, $12.477 \pm 0.165$; MI, $12.246 \pm 0.237$.

The statistical analysis demonstrated significant differences between individuals and between stages (Table 1). Contrasts were made by Scheffe's method (Scheffe 1959) and they indicated that there are significant differences between diplotene and the other two stages at the 5% level, but diakinesis and MI do not differ significantly (Table 1).

The same type of comparison for the ratio interstitial/total chiasmata per cell shows significant differences between diplotene ($\bar{x}=0.522 \pm 0.003$) and the other two stages (Table 2), but there were also significant differences between diakinesis ($\bar{x}=0.420 \pm 0.003$) and MI ($\bar{x}=0.338 \pm 0.003$). From these results it may be concluded that even though the total number of chiasmata per cell is the same in diakinesis and in MI, in the first stage the proportion of interstitial chiasmata is higher than in the second one.

The two populations were then compared to each other with respect to total and interstitial/total chiasmata per cell at diplotene, studying 19 individuals from AR and 16 from PQ by the analysis of approximately 10 cells per individual. The
mean of total chiasmata per cell for AR was 12.584 and for PQ the value was 13.125. These two means were compared by an analysis of variance (Table 3) and no significant differences were found between populations. The mean of interstitial/total chiasmata per cell at diplotene was calculated in both populations, and the values (0.466 for AR and 0.519 for PQ) were compared using the same method. The F value indicated no significant differences between populations (Table 4).

Since there were no differences, the data of both populations were pooled together and a histogram was made to analyze the distribution of the individuals according to its total chiasma frequency per cell at diplotene (Fig. 5A). The mean was 12.817 and the dispersion was high, since the differences between the individuals are significant (Tables 1 and 3). The type of structural alteration found in individuals belonging to each frequency interval is indicated in the graphic, and it is possible to observe that individuals carrying rearrangements are distributed more or less all over the distribution range, not being possible to group them in relation to its corresponding chiasma frequency.

Making a histogram for interstitial/total chiasma frequency distribution (Fig. 5B), the mean was 0.490 and similar conclusions were obtained, i.e., the individuals carrying rearrangements are distributed at both sides of the mean.

**C-banding**

The C-banding technique allowed the characterization of diplotene bivalents according to their banding patterns. Figure 6 shows the C-banded meiotic karyogram at diplotene. Interstitial and telomeric positive regions can be observed in several bivalents. The pair M7 is the megameric one in this species, and like X', seems to be totally heterochromatic. The heterochromatic bands are also visible at
pachytene. From diakinesis to later stages they become less distinguishable as the chromosome condensation progresses.

Discussion

The species of *S. longicornis* presents a primitive karyotype since it corresponds to the basic scheme for the family *Acrididae*, *i.e.*, 23 uniarmed chromosomes in males, and sexual mechanism X0♂/XX♀ (White 1973a, Hewitt 1979). The procentric constriction on the X is also a relatively frequent characteristic in the group. Nevertheless, a number of features were observed in the present populations which would imply that karyotype and meiotic behaviour are not, in this species, so steady as it could be expected.

White (1957b), described in *Keyacris* (formerly *Moraba*) *scurra* heteromorphic bivalents with chromosome segments highly stretched during the first meiotic division, and named these segments elastic constrictions. Such constrictions were described in many other orthopterans. They occur invariably in megameric chromosomes that bear the nucleolar organiser and, in other chromosomes, this extension only develops in cases where the organiser lies between the centromere and a distally located chiasma (John 1976). In *S. longicornis* the X chromosome showed elastic constrictions even though it can not have chiasmata. The constrictions observed in the autosomes were in all of the cases situated immediately close to the pericentric region, and in one of the cases, a certain relation between the constriction and a heterozygous supernumerary heterochromatic segment could be demonstrated. In the cases presented here the assumption that all the elastic constrictions observed are nucleolar organisers seems to be unlikely, and therefore it is possible to agree with John (1976) who considers improbable that all the elastic constrictions represent stretched nucleolar organisers.

The supernumerary segment found in this work has similar characteristics to
that observed by Shaw (1971a) in a mutant of *Schistocerca gregaria*. In this mutant, the heterozygosity was observed in 14 out of 24 follicles analyzed, and there was no evidence of material movement between chromosomes. This suggests that the segment arose "de novo" through duplication and heterochromatinization. In the case herewith presented, the follicles were not analyzed individually because most of the slides were made by dispersion, but the segment was clearly visible in all of the cells studied (more than 60) at pachytene and diplotene. Though there is no conclusive evidence about the origin of the supernumerary segment, one might think that analogically to the case described by Shaw, it arose through duplication. Surprisingly, the segment described in this work occurs in a large chromosome, whilst in most of the cases previously described this type of rearrangement occurred in small ones (John 1973).

An inversion similar to the one found in this species was described by Sáez (1963), fixed in the Neo-Y of *Dichroplus bergii*. In the case herewith presented it is assumed that as a consequence of the inversion, the entire but the terminal region has its sequence inverted respect to the original one (Fig. 7). Since no loops were seen at pachytene, the pairing zone would be reduced to the terminal regions. This phenomenon of pseudosynapsis has been observed in orthopterans (Nur 1968, Fletcher and Hewitt 1978) and other insects and also in rodents, polymorphic for pericentric inversions (White 1973b), and would avoid the diminution in fertility caused by crossing over within the inverted region in the heterozygotes.

C-banding demonstrated centromeric, telomeric and interstitial bands. Interstitial bands have been described in orthopterans occurring in B and megamergeric chromosomes (Fontana and Vickery 1974, Klásterská et al. 1974, John and Freeman 1976, Webb and Westerman 1978). In *Zoniopoda tarsata*, bands of this type were described in the larger chromosome pairs (Cardoso and Di Tomaso 1980). The case presented here would be comparable to the latter, since the bivalents with interstitial bands have regular behaviour during the meiosis.

Chiasma frequency generally shows a non-random distribution (Haldane 1931), which would indicate that chiasma number and position must be controlled in some way. Shaw (1971b) demonstrated the existence of an environmental component in chiasma regulation. Likewise, cases have been presented in which Mendelian genes (Beadle 1930, Jones 1967), gene interaction (Wagenaar 1960) and polygenic systems (Gale and Rees 1970) could affect chiasma frequency. Furthermore, there are many works that indicate that supernumerary chromatin would have some effect on chiasma conditions, this being its only effect identified so far (John and Hewitt 1965, 1966, Jones and Rees 1967, Shaw 1971a). Finally, there is an information about the influence of inversions on chiasma frequency (De Vaio et al. 1979). In the present case chiasma frequency and terminalization were analyzed in the two populations studied. Chiasma movement was not studied along diplotene as in the work by Fox (1973) on *S. gregaria*, but it is based on the comparison of chiasma frequency at different stages. The results obtained would indicate a continuous movement of chiasmata from diplotene to MI, since there are differences between diplotene and diakinesis respect to total chiasmata per cell and between diakinesis and MI in the ratio interstitial/total chiasmata per cell. This implies that some
chiasmata are released from diplotene to diakinesis (diminishing the total amount of chiasmata), while the rest would continue terminalizing till MI (the chiasmata become more terminal). The differences can not be explained on the basis of cyst differences as it was supposed in other cases (Shaw 1971b, Ehnis 1972), since the slides were made by dispersion and each of them had cells originating from several cysts. Furthermore, the non-significant interaction in the analysis of Tables 1 and 2 indicates that all of the individuals showed the same type of variation between stages.

If chiasma frequency were randomly distributed, this distribution would have to be poissonian (Haldane 1931). One property of this distribution is that the mean have to be equal to the variance. Nevertheless, in every individual the variance between cells was much lower than the mean. The events taking place before and during the chiasma formation are regulated both, genetically and environmentally (Rees 1961, Lindsley et al. 1968), and therefore, since the variance of frequencies is much lower than the mean, it is possible that genic or environmental determinants are acting canalizing the frequencies and reducing the intraindividual variance (between cells).

Comparing individuals from the same and different populations instead of different stages, one of the first remarkable facts was the high interindividual variability in both total and interstitial/total chiasma frequencies (significant differences between individuals in Tables 3 and 4). The populations studied were separated approximately 30 km from each other, but between them there is a barrier constituted by La Rioja City. Furthermore, the ecologic conditions were different in them. AR was situated in a plain and sandy zone, where Larrea cuneifolia was the dominant vegetation, very modified by human activity. PQ was located at the foot of Velazco Hill, on a stony ground, where Bulnesia foliosa was abundant besides some Gramineae. Taking account of the fact that the populations do not differ in chiasma frequencies notwithstanding the environmental differences, one might assume that the environmental effect on them is not too important or, at least, is not the only one. This does not agree with Shaw’s findings studying S. gregaria and Stethophyma grossum (Shaw 1971a), although in that study the working conditions were controlled because he dealt with an experimental population. Nevertheless, in S. longicornis the internal variation between cysts, the influence of which was observed by Shaw (1971a) and Ennis (1972), could be of greater importance. In any case, the high variance observed would have to be also closely associated with a high genetic variation. Some authors claim that chromosome structural alterations may influence chiasma conditions (White 1973b, Hewitt 1979). In the case presented here no relation can be demonstrated between a certain chromosome rearrangement and variations in chiasma frequencies. Rather, the interindividual variation was much higher than what it could be explained on the basis of the rearrangements, since the individuals carrying chromosome alterations are distributed more or less all over the frequency variation range. This is something to take into account when studying the influence of certain rearrangements on chiasma conditions. There is a series of works (Kayano and Nakamura 1960, Hewitt and John 1965, Sannomiya 1968, Arana et al. 1980) in which phenomena such as chiasma interference and centromere co-orientation are studied in individuals heterozygous for
translocations and fusions comparing the data obtained from one (or a few) spontaneous mutant(s) with normal individual values. On the basis of the present study it is evident that this type of comparison is not valid unless it can be demonstrated that interindividual variation within the same karyotype is negligible. This is not the case of the population under consideration in this study, and so it would be erroneous the use of data from one individual carrying a fusion or an inversion for studying the influence of such rearrangement on chiasma conditions. The individuals having the supernumerary segment are very dispersed in the frequency distribution histogram (Fig. 5), and so they would have no evident effect on chiasma frequencies.

Possibly, in the cases previously mentioned, the populations studied exhibited a lesser individual variation, since they were experimental ones, or the chromosomes involved in the rearrangements contained recombination controlling genes. In the case described here, on the contrary, the chromosomes with structural alterations (4 out of 12) would not have genes involved in the control of recombination or, if they do have, the rearrangement would not affect its genic expression.

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Summary

Karyotype and meiotic behaviour were analyzed in two Argentine populations of *S. longicornis* (G. Tos). It was found that the basic karyotype in this species is composed by 23 telocentric chromosomes in males (22 autosomes+X) with a sexual mechanism X0♂♀/XX♀♀. Chromosome variations involving a centric fusion, a pericentric inversion, elastic constrictions and a supernumerary segment were detected. The study of chiasma frequency and movement allowed to establish that: 1) there is a continuous chiasma movement and terminalization from diplotene to MI; 2) the differences between populations are non significant though there is a high within population variability; 3) the rearrangements observed have no obvious influence on chiasma conditions. C-banded meiotic karyogram at diplotene, made with Ba (OH)₂ and 2×SSC, is described. It demonstrates centromeric, interstitial and telomeric bands in meiotic autosomes. The X and megameric pair were totally heterochromatic.
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