Parallel polymorphisms for supernumerary heterochromatin in *Dichroplus elongatus* (Orthoptera):

Effects on recombination and fertility

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Abstract

Natural populations of *Dichroplus elongatus* often exhibit diverse forms of supernumerary heterochromatin. In Tafí Viejo, Tucumán Province (Argentina), parallel polymorphisms for extra segments on pairs M_6, S_9 (proximal) and S_{10} (distal) and a mitotically unstable B chromosome were detected. The segments produce intrachromosome effects excluding chiasmata from their neigbourhood and displacing them to regions distal to the extra heterochromatin. The individuals bearing simultaneously the B and the segment on M_6 show a decrease in the frequency of interstitial chismata (X_i) while those carrying only segments on M_6 or S_{10} have an increase of this variable when compared with standard males. The between cell variance of X_i is also higher in M_6 segment carriers. B individuals present an increase of abnormal spermatid frequency which may be explained by the simultaneous occurrence of two mechanisms: 1) lagging of supernumeraries and 2) physiological effects of B's affecting the meiosis even in cells lacking them. The intra and interchromosome effects may be important for the plasticity of populations allowing recombination to explore new chromosome regions. The B polymorphism seems to be stable which points to an advantage of B carriers and/or the occurrence of accumulation mechanisms counterbalancing their negative effects on fertility.

Introduction

The variation in the amount of the constitutive heterochromatin has played an important role in the chromosome changes which have ocurred in the phylogeny of the order Orthoptera (Hewitt, 1979). This variation involves the occurrence of extra or B chromosomes and supernumerary segments in regular members of the complement.

In Orthoptera, these kinds of chromosome mutations are frequently associated with characteristic effects on chiasma formation and, particularly B chromosomes may affect the production of normal sperm (Puertas, 1975; Jones & Rees, 1982). Since genetic variability, such as chiasma distribution and male fertility, affects the quantity of functional sperm

and is characteristic of adaptative significance, the maintenance of heterochromatin polymorphism should largely depend on such effects.

In *Dichroplus* diverse chromosome rearrangements (including heterochromatin variations), have been involved in the origin of new species (Sáez & Pérez Mosquera, 1971). Thus, this genus constitutes a particulary interesting material for evolutionary studies.

In a population of *Dichroplus elongatus* of Raco (San Javier Hill, Tucumán Province), a polymorphism for a mitotically unstable B chromosome has been detected (Vilardi, 1985; Remis & Vilardi, 1986). This chromosome produced an increase of between cell variance of total chiasma frequency without affecting the mean number of total chiasma per cell. In

a more detailed study, Remis and Vilardi (1986) observed that in B chromosomes bearing individuals the interstitial chiasma frequency increased with the number of B chromosomes per cell. This suggests that supernumerary chromosomes have the ability to alter the effective genetic recombination patterns.

In the present study, another population of this species situated near the foot of the San Javier Hill was analyzed. Parallel polymorphisms for B chromosomes and three supernumerary segments were found and their effects on recombination and fertility were analyzed.

Materials and methods

A sample of 42 males was collected at Tafi Viejo, Tucumán Province (Argentina) in January 1987.

Testes were dissected and fixed in 3:1 (ethanol: acetic acid). The cytological preparations for the analysis of the distribution of chiasmata were made by squashing in acetic orcein. The chiasma were classified in each chromosome pair as interstitial and terminal as described in Remis and Vilardi (1986). The slides for the analysis of abnormal spermatids were made by squashing of individual follicles in propionic haematoxilin. Spermatids were classified as normal, macro and micro according to their size and number of centriolar adjunts.

Results

Basic complement and chromosome polymorphism

The standard karyotype of *Dichroplus elongatus* is composed of 2n: 23 telocentric chromosomes in males with an XX/XO sex determining mechanism. The autosomes can be grouped in three large (L_1-L_3), five medium (M_4-M_8) and three small (S_9-S_{11}) pairs (Lafuente & Guerra, 1977; Vilardi, 1985; Remis & Vilardi; 1986).
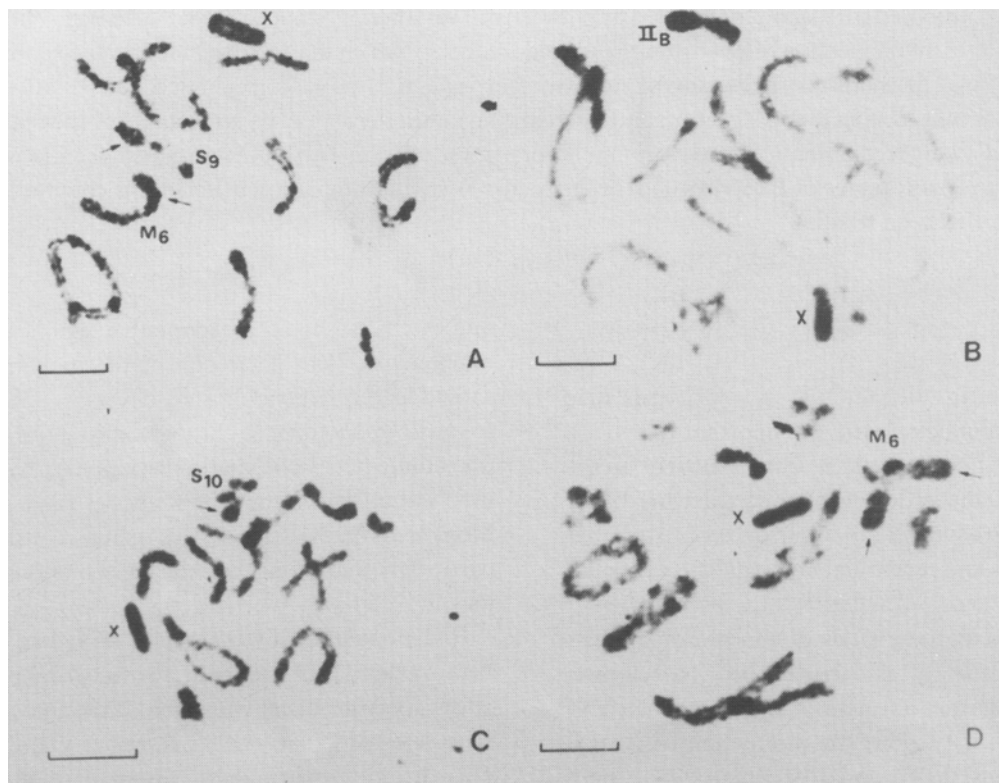

Fig. 1. Cells in diplotene showing the supernumerary segments (arrow) in heterozygous condition in the pairs M_6 and S_9 (A), S_{10} (C), in homozygous condition in M_6 (D) and a B bivalent (II_B) (B). Bar: 10 μm.
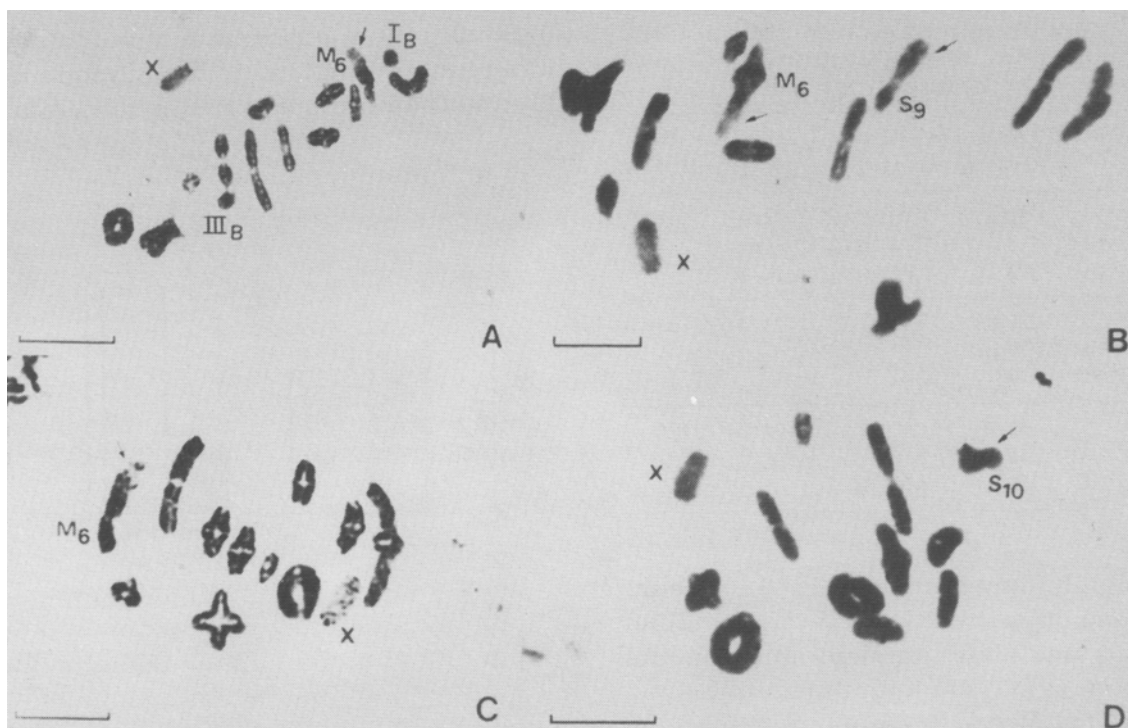
Fig. 2. Cells in metaphase I showing the supernumeraries segments in heterozygous condition (arrow) in pairs M_6 (A, C), M_6 and S_9 (B) and S_{10} (D) and B chromosomes forming a univalent (I_B) and a pseudotrivalent (III_B) (A). Bar: 10 μm.

B chromosomes and supernumerary segments in the pairs M_6 (SS_6), S_9 (SS_9) and S_{10} (SS_{10}) were found in several individuals (Figs. 1, 2). These variants may occur simultaneously in the same individual and the following combinations were observed: B (1 individual), B+SS6 heterozygotes (3 individuals), heterozygous SS_6 (3 individuals), Heterozygous SS_6+heterozygous SS_9 (1 individual), Heterozygous SS_9 (2 individuals), heterozygous SS_{10} (4 individuals), homozygous SS_6 (1 individual).

The incidence of the B chromosomes is 9.5% (4 out of 42). It is positive heteropycnotic in diplotene (Fig. 1B) and negative heteropycnotic to isopycnotic in metaphase I (MI) (Fig. 2) and its size is about a third of X. The B chromosome is mitotically unstable, and its number varying from 0 to 4 in the cells analysed of the carriers.

The SS_6 and SS_9 are proximal while SS_{10} is distal (Fig. 1A, C, D and 2). Their respective estimated frequencies were 0.107, 0.036 and 0.048. SS_6 size is about 2/3 of X while the SS_9 and SS_{10} are smaller than 1/3 of the sex chromosome. All supernumerary

segments are positive heteropycnotic from early prophase I to diplotene and slightly negative heteropycnotic to isopycnotic in MI (Figs. 1A, C, D and 2).

Effects of supernumerary heterochromatin on chiasma condition

Intrachromosome effects

In order to analyze the effect of supernumerary segments on chiasma frequency and distribution in the involved bivalents, heterozygotes and standard homozygotes were analyzed (Table 1).

The effect of supernumerary segments were determined through the comparison of the interstitial (Xi) and terminal chiasma frequency among karyomorphs.

The M_6, S_9 and S_{10} pairs always have one chiasma per bivalent in all analyzed cells. In homozygous condition the frequency of Xi varies from 0.56 to 0.60 (Table 1). The heterozygotes for supernumerary segments in proximal position (SS_6 and SS_9) present a highly significant decrease in the frequency of Xi with respect

Table 1. Percent of interstitial chiasmata (Xi), number of males (NM) and number of cells analyzed (NC) of standard homozygotes and heterozygotes for supernumerary segments.

		Standard homozygotes	Hetero-zygotes	X^2	P
	X_1	56.31	22.22	14.66	10^{-4}
SS_6	NM	8	3		
	NC	103	45		
	Xi	60.00	12.20	27.34	10^{-6}
SS_9	NM	10	3		
	NC	145	41		
	Xi	58.60	95.50	27.52	10^{-6}
SS_{10}	NM	10	4		
	NC	128	67		

to standard homozygotes (Table 1, Fig. 3 a, b). On the other hand, the presence of distal SS_{10} in heterozygous condition show a highly significant increase in Xi at the expense of Xt (Table 1, Fig. 3 c).

Interchromosome effects

Analysis of mean chiasma frequency. The effect of supernumerary heterochromatin on chiasma conditions in other chromosome pairs, *i.e.* interchromosome effects, were analysed by comparing chiasma frequencies among different karyotypes. The separate analysis of total (X_T) and interstitial (X_i) chiasma frequencies allowed a separate analysis of the effects of the heterochromatin on the total number and the distribution of chiasma per cell.

Only four karyomorphs, standard homozygotes, heterozygous SS_6 with or without B's and heterozygous SS_{10} individuals, were considered because the rest of karyomorphs were represented by only one or two individuals.

X_T frequencies (Table 2) apparently do not differ among karyotypes. This was confirmed since no significant differences were detected through an analysis of variance $(F_{3, 17}= 0.34; p= 0.8)$.

On the contrary, X_i frequencies (Table 2) seems to vary. B bearing individuals show lower values while those free of B's with supernumerary segments have higher number of X_i per cell than standard ones. The analysis of variance revealed that the differences among karyotypes are significant $(F_{3, 17}=4.19; p= 0.028)$. The comparisons of mean chiasma frequencies

by Scheffé's (1959) method indicated that the significance of the analysis of variance is mostly attributable to a reduction of X_i frequency in B bearing individuals.
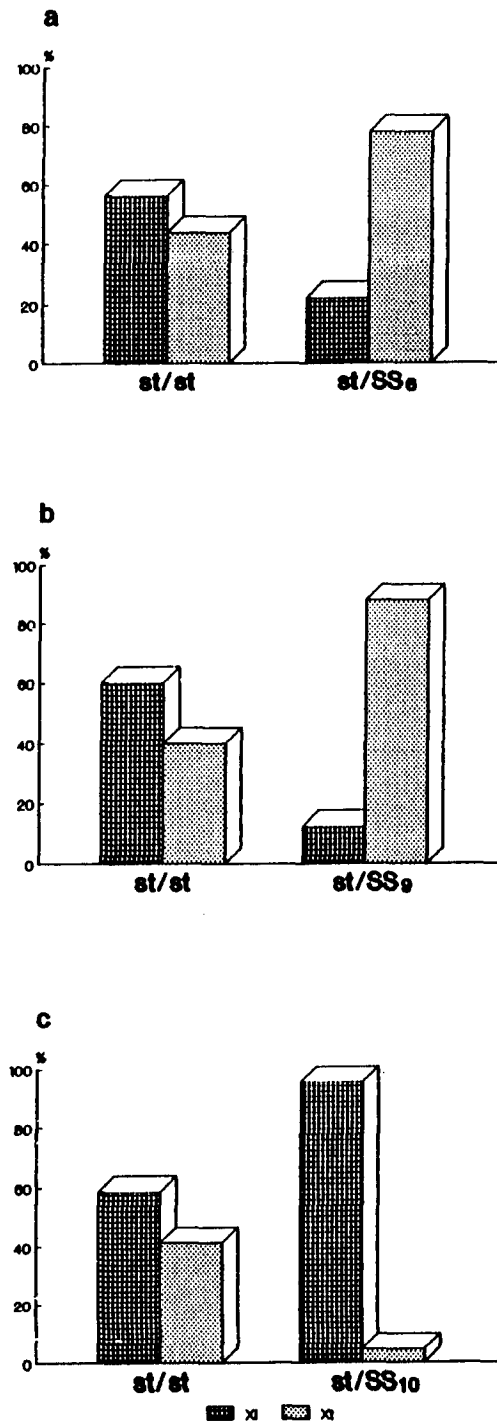
Fig. 3. Percent of interstitical (X_i) and terminal (X_t) chiasmata of standard homozygotes (st/st) and heterozygotes (st/SS) for supernumerary segments. a) pair M_6, b) pair S_9, c) pair S_{10}.

Table 2. Mean frequency sample variance (S^2) of Total (X_T) and interstitial (X_i) chiasmata per cell in the different karyomorphs analyzed. (N= number of males analyzed).

| | Standard homozygotes | | SS_6+B | | SS_6 | | SS_{10} | |
	Mean	S^2	Mean	S^2	Mean	S^2	Mean	S^2
X_T	12.77	1.06	12.96	4.40	12.70	5.74	12.97	2.75
X_i	5.45	2.88	4.18	0.36	6.35	1.00	6.54	0.67
N	10		3		3		4	

In fact, B carriers showed significant differences when compared a) with all the remaining karyomorphs (F= 3.26; p= 0.047); b) with the average between SS_6 and SS_{10} (F= 3.91; p= 0.027) and c) with SS_{10} (F= 3.32; p= 0.045). The difference with respect to SS_6 is on the borderline significance (F= 2.82; p= 0.07). On the other hand, the other three karyomorphs do not differ significantly from each other.

Analysis of among cell variances. Another effect of the supernumerary heterochromatin polymorphisms here observed was their influence on the between cell variance of chiasma frequencies. The same karyomorphs indicated above were analyzed and mean variance values for each karyomorph are indicated in Table 2.

A frequency histogram was made both for X_T and X_i between cell variance (Fig. 4a, b) in which the individuals with each karyomorph are represented. In the case of X_T, the distribution is unimodal, the individuals carrying chromosome mutations (B or supernumerary segments) are more or less evenly distributed along the overall range, and no defined tendencies may be detected (Fig. 4a).

For X_i variances the distribution is bimodal (Fig. 4b). The heterozygotes for SS_6, with or without B's, tend to be on the right side of the distribution range and three of them are included in the interval of the highest variance mode. If these individuals were excluded from the histogram, this would turn to unimodal, coinciding with the distribution of standard individuals. The rest of karyomorphs are distributed around the lowest variance mode. Therefore, the only condition that seems to affect X_i variance is the presence of SS_6. In order to demonstrate this hypothesis, statistically, among cell variance of X_i was first

compared between SS_6 and SS_6+B karyomorphs. Since the t-test indicated that such differences were not significant (t_4= 0.74; p= 0.25), these individuals were pooled and compared with standards. The test indicated that SS_6 heterozygotes have among cell variance statistically higher than standard individuals (t_{14}= 2.45; p= 0.014).

Effects on sperm formation

The influence of chromosome mutations on the processes of spermiogenesis and spermatogenesis was assessed by an analysis of abnormal spermatid frequencies. In order to analyze these effects, the haploid (normal), macro and microspermatids (abnormal)
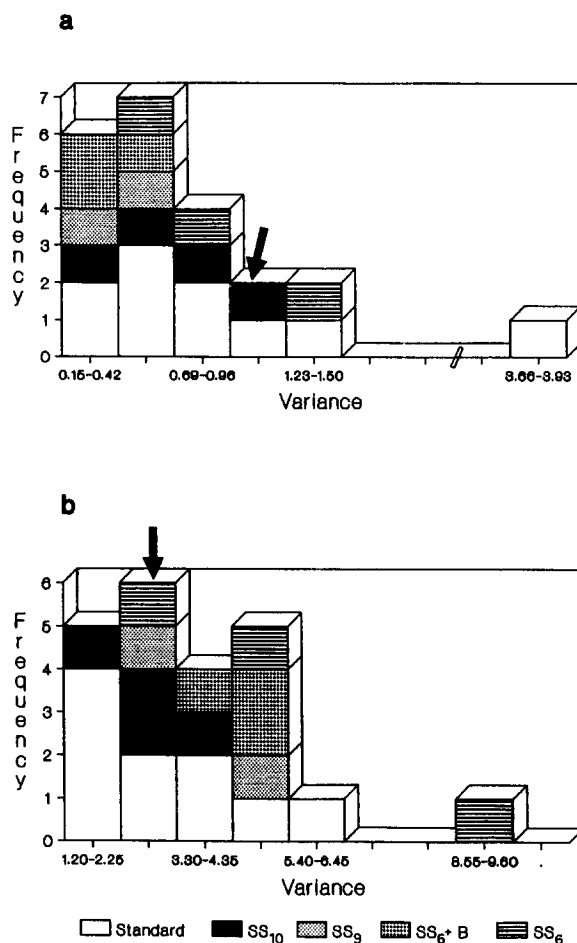
Fig. 4. Frequency histogram for between cell variance of a) total chiasmata per cell and b) intersticial chiasmata per cell. The different karyomorphs are represented as indicated on the foot.

were scored in individual follicles in B and non B carrying individuals (Table 3).

Macro and microspermatid occur in both B and non B individuals, but seem to be higher in the former. In order to test the statistical significance of this trend, chi-square tests were made between the following classes:

i)-follicles of non B carriers vs. OB follicles of B carrying individuals.

ii)-follicles with different number of B's in B males.

The first comparison indicated that macrospermatids are more frequent in follicles without B's or B bearing individuals than in the standard (non B) males, the difference being highly significant (Table 4a). On the contrary, the frequency of microsperma-

Table 3. Percent of normal and abnormal (Macro and Micro) spermatids in individuals with (b, c and d) and without B chromosomes (a).

	Number of male	No of esper-mat.	Normal	Macro	Micro	Total abnor-mal
a)						
OB	37	747	99,866	0,134	0	0,134
	43	1031	99,224	0,405	0,291	0,776
	64	895	100	0	0	0
	74	99	96,970	0,030	0	3,030
	79	784	99,745	0,255	0	0,255
TOTAL		3556	99,606	0,309	0,084	0,393
b)						
OB	31	1483	95,482	4,450	0,067	4,517
	32	3634	99,505	0,440	0,055	0,495
	86	664	99,699	0,151	0,151	0,302
	87	315	95,873	3,175	0,952	4,127
TOTAL		6096	98,360	1,526	0,115	1,641
c)						
2B	31	1312	94,969	4,802	0,229	5,031
	32	730	98,493	1,507	0	1,507
	86	1238	99,273	0,727	0	0,727
	87	1291	98,606	1,394	0	1,394
TOTAL		4571	97,725	2,209	0,066	2,275
d)						
4B	31	214	92,523	2,804	4,673	7,477
	87	1143	90,726	9,099	0,175	9,274
TOTAL		1357	91,009	8,106	0,884	8,99

Table 4. Values of X^2 for the comparison of macro and microspermatids between OB follicles of B and non B males (a) and among follicles with different number of B's of B bearing individuals (b, c, d, e).

	Macro			Micro		
	X^2	D.F.	p	X^2	D.F.	p
a) (OB males)- (B males)	31.18	1	$2.4 \ 10^{-8}$	1.16	1	0.280
b) OB-2B-4B	200.50	1	$3 \ 10^{-10}$	5.17	1	0.023
c) OB-2B	6.82	1	0.009	0.66	1	0.42
d) OB-4B	183.48	1	$2 \ 10^{-10}$	28.25	1	$1 \ 10^{-7}$
e) 2B-4B	107.53	1	$1 \ 10^{-10}$	29.86	1	$5 \ 10^{-9}$

tids does not differ statistically between these classes (Table 4a).

The second analysis revealed that there are highly significant differences in the production of macro and microspermatid among follicles with different numbers of B's (Table 4b). These chi-square tests were partitioned (Table 4c, d, e) determining that the frequency of macrospermatids increases with the number of B per cell and the differences are highly significant for all paired comparisons. For microspermatids the only significant results were those involving 4B follicles, while OB and 2B classes do not differ statistically from each other.

Discussion

Among the most frequent polymorphisms in Orthoptera are those involving heterochromatic variations (Hewitt, 1979; Jones & Rees, 1982; John, 1983). The absence of such variants in some individuals of the populations suggests that these morphs are not necessary for the normal individual development, but in several cases, their presence seems to be stable. With this in mind, it is interesting to analyze whether some fitness components may be involved in their maintenance.

As John (1981b) suggested, there are clear grounds for arguing that chromosome change has been restricted and promoted by its effects on meiosis. Indeed, the heterochromatic variants in grasshoppers are associated, in many cases, with effects on chiasma formation.

In *Dichroplus elongatus* the supernumerary segments SS_6, SS_9 and SS_{10} produce in heterozygous condition an intrachromosomic repatterning of chiasmata. Such situation was very often observed in other grasshopper species (Shaw, 1970, 1971; John, 1981a; John & King, 1982; Navas Castillo *et al.*, 1985; De la Torre *et al.*, 1986; Rufas *et al.*, 1986; Remis, 1989). These readjustment has been related with the fact that occurrence of crossing over is low or null near heterocromatin (John, 1981a). However more recently, the redistribution of chiasmata was explained by the asymetry of the bivalents (Camacho *et al.*, 1984; Navas Castillo *et al.*, 1985; Rufas *et al.*, 1986). Indeed, De la Torre *et al.* (1986) suggested that this asymetry in the involved pair might lead to modifications in the structure of the synaptonemal complex.

Whatever is the cause of these redistribution of chiasmata, the presence of supernumerary segments determines that crossing-over tends to be excluded from neighbourhood of heterochromatin. These effect may lead, in some cases, to the ocurrence of recombination in unexplored regions for the standard homozygotes. The consequence is that linkage distances would be drastically altered in polymorphic populations for such segments in respect to monomorphic ones.

With reference to interchromosome effects, the influence of supernumerary heterochromatin on chiasma frequency may vary among species and populations of the same species (Puertas, 1975; Hewitt, 1979; Jones & Rees, 1982; John, 1983; Confalonieri, 1990).

A population of *D. elongatus* situated at Raco upper San Javier Hill (Tucumán) (Vilardi, 1985; Remis & Vilardi, 1986) presented a B chromosome which, according to its morphological and behavioural characteristics, would be the same as the one found here. In that population it affected the among cell variance of the number of total chiasmata per cell. Moreover, in B chromosome bearing males the interstitial chiasma frequency increases with the number of B chromosomes per cell. In the present population, only one

individual with standard A complement was found bearing a B chromosome, while the rest of B carriers were heterozygotes for SS_6 segment. These individuals show a significant decrease in interstitial chiasma frequency. This decrease is conterbalanced by an increase of terminal chiasmata since the mean total chiasma frequency remains the same. The fact that such effect was not observed in heterozygotes for SS_6 without B indicates that the alteration in chiasma distribution depends on the presence of the extra chromosomes. Consequently, the effect of B chromosomes in the present population seems to be the opposite from those observed in Raco where they increase the number of interstitial chiasmata per cell.

Finally, the effects of heterochromatin on the variance of chiasma frequency were also analyzed in this population. The variance of X_T is not affected by the karyomorph but the presence of SS_6 increases the variance of X_i. In this case, a difference is again observed between the present results and those previously obtained in the Raco population (Vilardi, 1985). There, the B chromosome produced an increase in the variance of X_T. These observations indicate that B chromosomes might produce varying effects in different populations. A similar situation was found in natural populations of *Trimerotropis pallidipennis* (Confalonieri, 1990). In this case, B chromosomes produce a decrease in mean total chiasma frequency in only 2 out of 15 populations studied. Consequently, the influence of supernumerary chromosomes on chiasma control would be affected by the environment and/or the genetic background.

Another effect of the supernumerary chromosomes, studied here, was related to abnormal spermatid production. The presence of B chromosomes is associated with an increase in macro and microspermatid frequencies.

It is often argued that the blocking of cytokinesis at first or second meiotic division is responsible for macrospermatid formation (Nur, 1969; Bidau, 1988; Bidau & Confalonieri, 1988). Suja *et al.* (1986b) and Bidau (1987) have proposed an alternative mechanism involving cellular fusion to account the occurrence of macrospermatids with odd levels of ploidy.

In *Dichroplus elongatus* there is evidence which supports both mechanisms operating simultaneously. The proportion of macrospermatids increases with the

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number of B chromosomes per cell. This is expected according to the first mechanism from the fact that the higher the number of B chromosome is, the higher is the opportunity to lagg. The lag of B chromosomes may also explain the increase of microspermatid frequencies in the follicles 4B with respect to 0 and 2B ones. In cells with 4B's, there is an increase in the probability of univalent formation which may be excluded in the micronucleus.

On the other hand, lagging is not the only mechanism related to the non-functional sperm formation. In order to explain the higher frequency of macrospermatids in follicles OB of B carriers compared to individuals free of B chromosomes, another mechanism mediated by physiological effects of B's must be postulated. Though, no direct evidences for the occurrence of cellular fusion in *D. elongatus* has thus far been obtained, this is a plausible explanation for the differences between follicles without B's of standard and B individuals.

According to the results here obtained, in *D. elongatus* the supernumerary heterochromatin may affect the chiasma formation in different ways. Since the release of genetic variability is affected directly by chiasma conditions, the occurrence of polymorphisms which affect this character, may be important in the adaptation of populations. Supernumerary segments were observed in several populations of this species. In Raco, Vilardi (1985) detected two segments in the pairs 8 and 9 respectively. In other population of Buenos Aires Province, Remis (1989) described a polymorphism for a distal segment in the S_{10} pair, similar to that reported here. Since most segments displayed intrachromosomic effects and some of them show interchromosome ones (among cell variances of X_i), the presence of these effects in the population might be important for genetic plasticity to allow the production of new genetic combinations.

The polymorphism for B chromosomes, in Tucumán Province, seems to be stable since it was detected in samplings obtained at different years in two populations (Vilardi, 1985; Remis & Vilardi, 1986; unpublished data). Therefore, the effects of B's on the fertility of its carriers (higher proportion of abnormal sperm) must be counterbalanced by mitotic unstability, which may lead to accumulation mechanisms (Nur, 1963, 1969) and/or by its adaptive effects. On

the contrary, in populations from other geographical regions (Bs. As. Province), they were not found (Remis, 1989). The restriction of B to particular regions might be the reflection of the adaptive importance of such chromosomes.

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