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C-Heterochromatin Polymorphism and Variation in Chiasma Localization in *Euchorthippus pulvinatus gallicus* (Acrididae, Orthoptera)

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Abstract. Eight populations of the grasshopper *Euchorthippus pulvinatus gallicus* have been analyzed by means of C-banding. Chromosome pairs M6, M7 and S8 show both quantitative and qualitative variation in their C-heterochromatin. There are at least four different types of M6, three of M7 and two of S8. Differences in the frequencies of these chromosome types have been found between populations. Within a given population the frequencies of the different M7 and S8 chromosomes fit a Hardy-Weinberg distribution and they remain constant within and between generations. The possible adaptative role of supernumerary heterochromatin as leading to a redistribution of chiasmata in the heterochromatin carrier chromosomes is discussed.

Introduction

C-heterochromatin variability is a general feature among eukaryotes. Within a species homologous chromosomes can differ not only in the number and location of C-bands (qualitative differences) but also in the amount of heterochromatin in a specific C-band (quantitative differences). The detection of these two types of differences in C-banding pattern depends on several factors such as the magnitude of the variation and the degree of chromosome contraction. As the measurement of the C-heterochromatin amount in a specific band is difficult and chromosome contraction can lead to the apparent fusion of nearly located C-bands, only large differences are usually detected, resulting in an underestimation of the actual variability in C-banding pattern.

There are several cases in which a relationship between presence of heterochromatin and chiasma location (Fox et al., 1973; Klášterská et al., 1974; Morgan, 1978) has been described. Polymorphism for C-heterochromatin can apparently lead to variation in chiasma localization.

In *Euchorthippus pulvinatus* there is variation in the C-heterochromatin banding pattern of chromosomes M6, M7 and S8. When present, C-heterochromatin is interstitially located in chromosomes M6 and S8 and its position is apparently

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distal in chromosome M7 (Arana et al., 1980). At meiosis each of these three chromosomes forms one chiasma that can be located at either side of the C-heterochromatin, the frequency of proximal and distal chiasmata varying among individuals.

In this paper, the variation in C-banding pattern between and within different Spanish populations of *E. pulvinatus* and its relation with variation in chiasma localization are analyzed.

Material and Methods

Euchorthippus pulvinatus gallicus is a grasshopper species widely distributed in the Iberian Peninsula. Adult individuals are found from the end of June to the end of September. Since the eggs must pass through a diapause period, only one generation per year is produced.

Adult males of *E. pulvinatus* were collected during 1978, 1979 and 1980 at eight localities, three of them near Madrid (Valdemoro, Ciudad Universitaria and Majadahonda), three near the Sierrra of Guadarrama (Montegolf, Miraflores and Desviación) and the remaining two near Calatayud, Zaragoza (Calatayud and Galápago) (Fig. 1). Females were also collected at Valdemoro, Majadahonda, Ciudad Universitaria, Montegolf and Calatayud.

Mitotic metaphases can be obtained either from gut caeca of adult individuals or from embryos. In the first case a treatment with colchicine is necessary in order to accumulate an appreciable number of metaphase cells, whereas in embryos the high frequency of dividing cells makes this treatment unnecessary. Adult males and females were injected with 0.25% colchicine in insect saline. The gut caeca were fixed in acetic alcohol 1:3 aproximately 4 h after injection. Egg pods obtained from females (fertilized in the locality of origin) were incubated at 28–30° C and embryos were dissected from the eggs 20 days after laying and fixed without colchicine treatment in acetic alcohol 1:3.

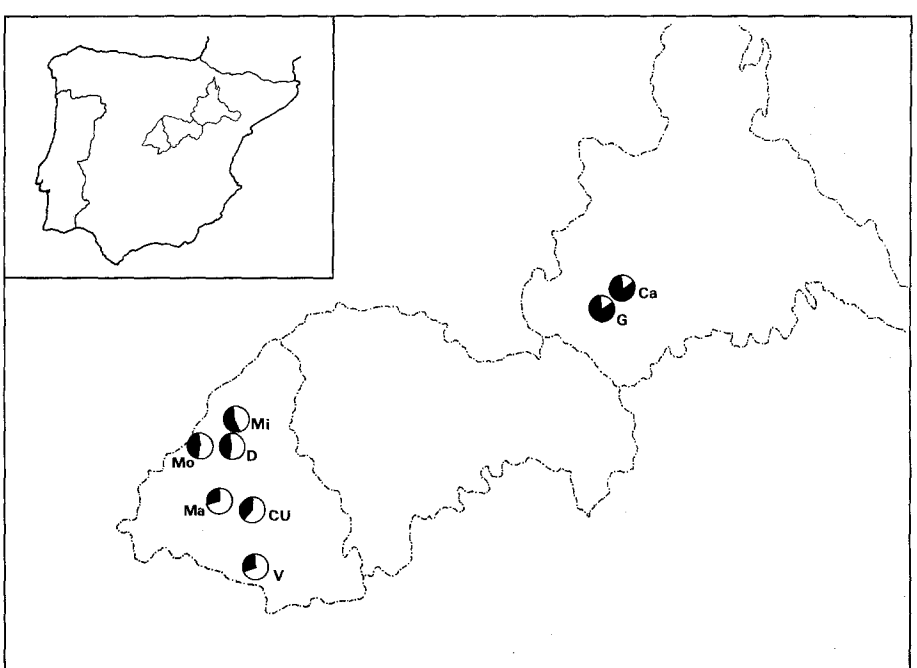

Fig. 1. Eight localities of collection: *Ca* Calatayud; *G* Galápago; *Mi* Miraflores; *D* Desviación; *Mo* Montegolf; *Ma* Majadahonda; *CU* Ciudad Universitaria; *V* Valdemoro. White sectors of circles represent frequency of chromosome S8 without supernumerary C-heterochromatin

Testes of non-colchicine treated males were fixed in acetic alcohol for meiotic observations. The fixed material was squashed in 45% acetic acid and stained following the Giemsa C-banding technique described previously (Santos and Giráldez, 1978).

Results

C-banding Pattern Variability

E. pulvinatus has 17 chromosomes in the male (16+X) and 18 in the female (16+XX). Three pairs of autosomes are long and submetacentric, whereas the remaining five pairs and the X are of medium to small size and telo or subtelocentric (Fig. 2b). All members of the complement show centromeric C-heterochromatin.

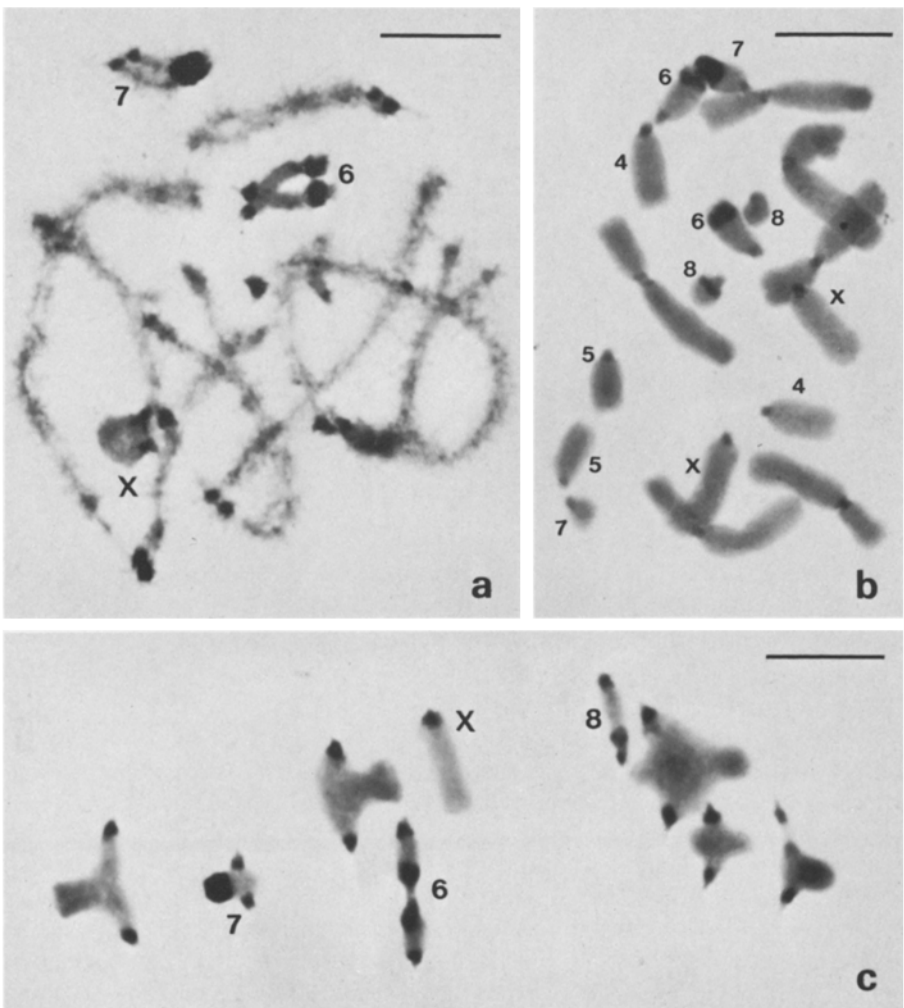

Fig. 2. a Pachytene cell showing megameric nature of bivalent M6. **b** Mitotic metaphase cell obtained from gut caeca of a female double heterozygous M7 BS, S8 BS. **c** Metaphase I cell of a male double heterozygous for chromosomes M7 and S8. Bar represents 10 μm

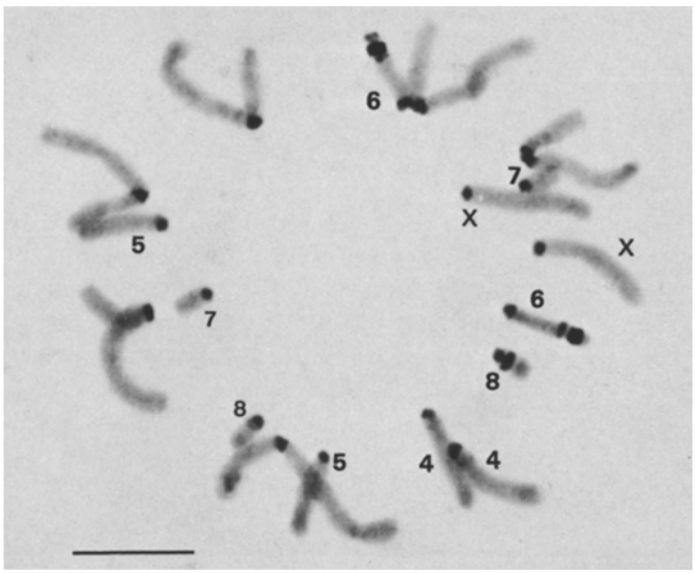

Fig. 3. Mitotic metaphase from female embryo. Note different C-banding pattern of two members of pair M6. Bar represents 10 μm

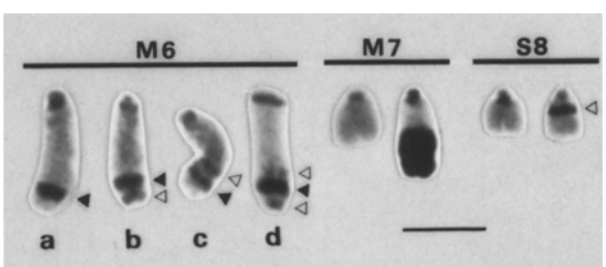

Fig. 4. Different C-banding patterns found in chromosomes M6, M7 and S8. Quantitative variation of C-bands was also observed. Bar represents 4 μm

The three smallest chromosomes (M6, M7 and S8) show variation in their C-banding pattern.

Chromosome M6. This is the megameric chromosome (Fig. 2a) and its banding pattern is rather complex. In mitotic cells obtained from gut caeca, as well as in metaphase I cells (Fig. 2b–c), chromosome contraction is very high and only a subterminal band can be seen.

However, in neuroblasts, not treated with colchicine (Fig. 3) chromosomes appear less condensed than in colchicine-arrested mitotic metaphases and the C-banding pattern of chromosome M6 shows more details. Figure 4 shows the four types of chromosome M6 found in such cells. In all types a subterminal C-band is present which may be accompanied at either or both sides by thin C-bands (interstitially or distally located). All these bands are variable in size.

Table 1. Frequencies of the different configurations of the four M6 types found in embryos obtained from females of Desviación and C. Universitaria populations (compare Fig. 4)

Population	Number of females analyzed	Embryos per female	Chromosome configuration										Numbers of embryos
			aa	ab	ac	ad	bb	bc	bd	cc	cd	dd	
Desviación	17	1–7	2	14	1	3	6	11	9	0	2	3	51
C. Universitaria	6	1–8	0	1	0	3	2	1	10	0	3	5	25

The resolution of Giemsa C-banding in other cells than untreated neuroblast is insuficient to permit analyses to be carried out such as were possible with M7 and S8.

Table 1 shows the frequency of each M6 type embryos obtained from females collected in two of the localities studied (Desviación and Ciudad Universitaria). Although the contingency test between the chromosome frequencies in the two localities is significant ($\chi^2 = 17.58$; df$=3$; $p < 0.001$), as a different number of embryos per female has been analyzed, the frequency of each chromosome type is not representative of the actual frequency in the adult individuals in the populations. The reason why this table is included, is to show the great variability of this chromosome. In most cases, a segregation for the C-banding pattern was observed among the offspring of a single female.

Chromosomes M7 and S8. The C-banding pattern variation is due to the presence or absence of a specific C-band. Chromosome M7 can have a thick C-band at or near the telomere, and chromosome S8 can have a thin interstitial C-band near the centromere (Figs. 2b–c and 4). Among individuals, some variation in the amount of C-heterochromatin in these bands can be observed. There are also quantitative differences between populations. The C-band present in chromosome M7 in the localities of Valdemoro, Ciudad Universitaria and Majadahonda had, as a rule, a smaller amount of C-heterochromatin than those present in other localities (compare Figs. 2c, 4 and 5).

Following the nomenclature proposed by Hewitt and John (1968), for both, M7 and S8, two types are distinguished: B (basic, absence of supernumerary heterochromatin) and S (structural, presence of supernumerary heterochromatin). Consequently, for both chromosomes three types of individuals can exist: homozygous for the chromosome without the C-band (BB), heterozygous (BS) and homozygous for the chromosome with the C-band (SS). Table 2 shows the frequencies of the different karyotypes in males and females considering these two chromosomes, in five populations in which females were collected. Within the same population the frequencies of the different karyotype in both sexes did not differ significantly.

The comparison between the observed frequencies of the different chromosome types within populations, with the expectations based on a Hardy-Weinberg distribution is shown in Table 3. It can be concluded that there is a good correspondence between the observed and expected values.

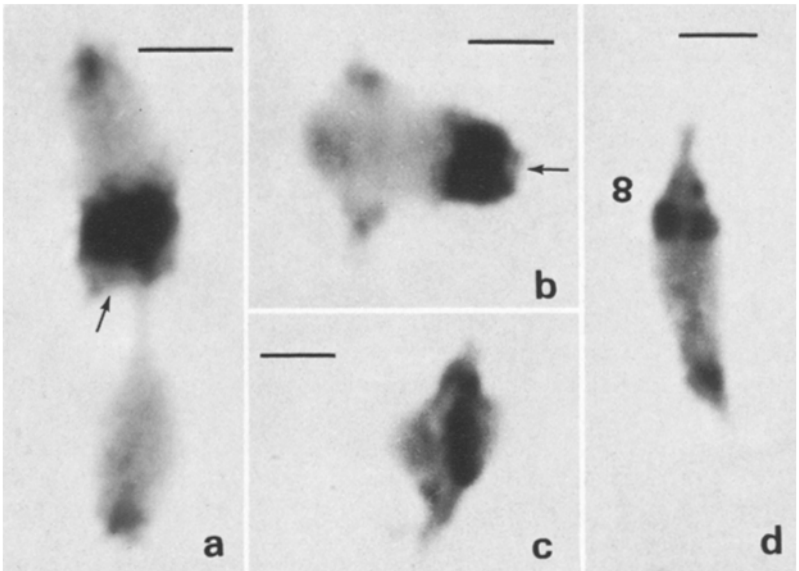

Fig. 5a–d. The two types of chiasma localization found in bivalents M7 (**a, b**) and S8 (**c, d**) heterozygous for supernumerary C-heterochromatin. *Arrows* show telomeric euchromatin in chromosome M7 S. Bar represents 2 μm

Table 2. Distribution of karyotypes (females and males) in five populations of *Euchorthippus pulvinatus*. (BB: homozygotes without heterochromatin, BS: heterozygotes for supernumerary heterochromatin, SS: homozygotes for supernumerary heterochromatin). Chromosomes M7 and S8

Population	Chromosome	Females			q_B	Total	Males			q'_B	Total	χ^2
		BB	BS	SS			BB	BS	SS			
Calatayud	M7	51	7	0	0.94	58	375	43	1	0.95	419	0.172
	S8	1	11	46	0.11		13	99	307	0.15		0.714
C. Universitaria	M7	13	2	0	0.93	15	43	5	0	0.95	48	–
	S8	5	8	2	0.63		18	24	6	0.65		0.079
Majadahonda	M7	17	3	0	0.93	20	47	3	0	0.97	50	–
	S8	8	10	2	0.65		26	21	3	0.73		0.617
Montegolf	M7	19	0	0	1.00	19	29	1	0	0.98	30	–
	S8	5	11	3	0.55		9	15	6	0.55		0.165
Valdemoro	M7	37	6	1	0.91	44	264	27	0	0.95	291	0.920
	S8	25	18	1	0.77		132	132	27	0.68		0.980

All χ^2's not significant at 5% level. (classes < 5 excluded)

The analysis of the different karyotype combination frequencies indicates that the heterochromatin distributions for chromosome M7 and S8 fit the assumption of independence.

In the eigth populations of *E. pulvinatus* the frequencies of the two types of chromosomes M7 (B and S) are rather similar, however, for chromosome

Table 3. A comparison of the observed frequencies of karyotypes found in eight populations of *E. pulvinatus* with expected frequencies based in a Hardy-Weinberg distribution

Population	Total sample	Chromosome	Distribution of karyotypes							χ^2
			Observed			q_B	Expected			
			BB	BS	SS		BB	BS	SS	
Calatayud	477	M7	426	50	1	0.94	425.97	49.58	1.45	0.004
		S8	14	110	353	0.15	10.03	118.27	348.70	2.32
Galápago	47	M7	37	9	1	0.88	36.66	9.73	0.61	0.058
		S8	0	14	33	0.15	1.06	11.98	33.96	0.368
Montegolf	49	M7	48	1	0	0.99	48.02	0.97	0.01	–
		S8	14	26	9	0.55	14.88	24.25	9.87	0.25
Desv. Manzanares	611	M7	567	41	3	0.96	563.09	46.93	0.98	0.776
		S8	175	318	118	0.55	184.83	302.44	123.73	1.59
Miraflores	79	M7	76	3	0	0.98	75.87	3.09	0.03	–
		S8	17	40	22	0.47	17.45	39.36	22.19	0.02
Majadahonda	70	M7	64	6	0	0.96	64.10	5.76	0.14	0.01
		S8	34	31	5	0.71	34.98	29.00	6.02	0.33
C. Universitaria	63	M7	56	7	0	0.94	56.13	6.66	0.21	0.018
		S8	23	32	8	0.62	24.22	29.69	9.09	0.37
Valdemoro	335	M7	301	33	1	0.95	301.07	33.02	0.91	0.0001
		S8	157	149	29	0.69	159.49	143.32	32.19	0.59

All χ^2's not significant at 5% level (classes < 5 excluded)

S8 remarkable differences are apparent (Table 3 and Fig. 1). These differences are related to the situation of the localities analyzed. Three geographical areas can be considered according to the frequencies of chromosome S8 without heterochromatin (q_B): 1) Calatayud and Galápago $q_B = 0.15$ (Zaragoza province). 2) Miraflores, Montegolf and Desviación $q_B = 0.47$–0.55 (Sierra of Guadarrama). 3) Majadahonda, Ciudad Universitaria and Valdemoro $q_B = 0.62$–0.71 (Middle and South of Madrid province).

In these geographical areas the frequencies of the different types of chromosomes M7 and S8 have been studied at different times: along a generation (July-September) in the populations of Calatayud (1978) and Desviación (1979) and along consecutive generations (1978–1980) in the populations of Calatayud, Desviación and Valdemoro. In no case the differences (as measured by contingency test) within and between generations were significant.

Chiasma Localization Variability

Bivalents M6, M7 and S8 carrying C-heterochromatin form invariably one chiasma that can be located between the C-band and the centromere (proximal) or between the C-band and the telomere (distal) (Figs. 2c and 5). In these bivalents the frequencies of distal and proximal chiasmata at diplotene were similar to those found in metaphase I. Evidence of terminalization across the

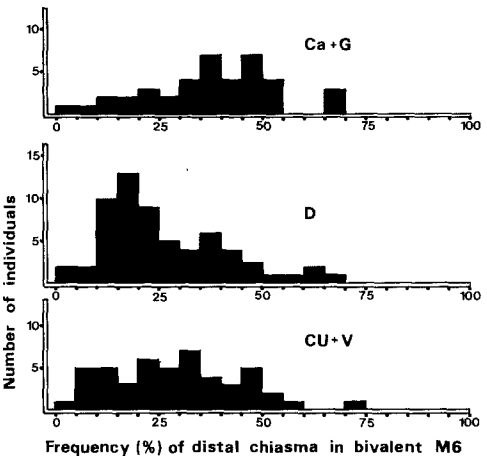

Fig. 6. Frequency (%) of distal chiasma in bivalent M6 in individuals of three geographical areas analyzed

C-band was not found. It could not be decided whether this was due to an effect of the C-band or to a general absence of terminalization in these chromosomes.

Figure 6 shows the frequency (%) of distal chiasmata in bivalent M6 in individuals of the three geographical areas analyzed (no significant differences between the localities belonging to the same area were found). An average of 45 diplotene and metaphase I cells per individual were analyzed. The mean frequency of a distal chiasma in the Zaragoza province area (CA+G) is significantly different from that of the other two areas (the Student t test for the percentages transformed to angles were $t=3.021$, $df=91$; $p<0.01$ when compared with Sierra of Guadarrama area (D) and $t=2.477$; $df=86$; $0.05>p>0.02$ when compared with Middle and South of Madrid (CU+V) area). There was no significant difference between the other two areas, D and CU+V ($t=0.415$; $df=99$; $0.7>p>0.6$).

Figure 7A shows the frequency (%) of a distal chiasma in bivalent M7 heterozygous for the C-band in individuals of the three geographical areas (no significant differences between the localities belonging to a same area were found). An average of 68 diplotene and metaphase I cells per individual were analyzed. The frequency of distal localization is significantly higher in CU+V when compared with that of the other two areas (CA+G and D) ($t=6.46$; $df=64$; $p<0.001$), in which in most individuals bivalents with distal chiasma were not observed.

Fig. 7B shows the frequency (%) of distal chiasma in bivalent M7 homozygous without C-heterochromatin in individuals of the three geographical areas. An average of 19 diplotene cells per individual were observed. Since M7 and S8 are very similar in size, in order to identify bivalent M7 (BB) at diplotene, individuals heterozygous for the C-band in chromosome S8 had to be used. No significant differences between localities belonging to the same area nor between different areas were found.

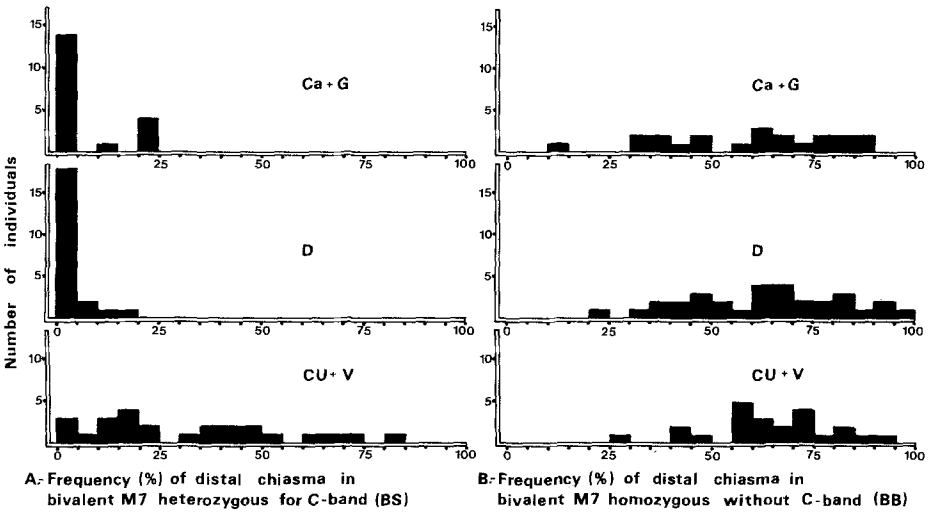

Fig. 7A–B. Frequency of distal chiasma in bivalent M7 in individuals of three geographical areas analyzed. **A** M7 BS. **B** M7 BB

In bivalents M7 carrying C-heterochromatin most proximal chiasmata were located close to the centromere, whereas in bivalents M7 without C-heterochromatin (M7 BB) chiasmata could be located all along the length of the chromosome. In these bivalents only telomeric associations were considered as distal chiasmata. In bivalents M7 homozygous for C-heterochromatin (SS) no distal chiasmata appeared at all.

The frequency of a proximal chiasma in bivalent S8 (BS) was similar in all localities analyzed (about 4%). This low frequency did not allow the realisation of valid comparison concerning chiasma localization for this bivalent.

In each geographic area the possible relationships between chiasma localization frequencies of bivalents M6 and M7 was investigated. The between and the within-individual correlations indicated that chiasma localization in bivalents M6 and M7 was independent in all cases. This can support the assumption that chiasma localization frequencies of chromosomes M7 and S8 are also not correlated.

Discussion

The results shown in Figs. 6 and 7A indicate that there is a significant difference between populations in the localization of chiasmata in bivalents M6 as well as M7 BS. The absence of a difference in localization pattern for bivalent M7 BB (Fig. 7B) suggests that the variation in M7 BS is due to the structural difference between the members of this bivalent. This variation can be related with the between-population differences in amount of C-heterochromatin found for chromosome M7 S (Figs. 2, 4 and 5). The smaller amount of C-heterochromatin present in the population of the Middle and South of Madrid area would allow chiasmata to form distally.

In bivalents M7 BS with a distal chiasma, a telomeric euchromatic segment could be observed at late metaphase I (Fig. 5a). However, this euchromatic segment was not observed in mitotic chromosomes and could only exceptionally be observed in bivalents having a proximal chiasma (Fig. 5b) in individuals of the Middle and South of Madrid area. This euchromatic segment is probably masked by the C-heterochromatic band close to it, appearing mainly when metaphase I tension of bivalents pulls it out.

Occurrence of distal chiasmata in bivalents heterozygous for C-heterochromatic segments located at or near the telomere is a point of controversy. The observations of reductional anaphase segregations for these segments led to the conclusion that they were actually subdistally located (Nur, 1961; Shaw, 1970), chiasmata being formed in the euchromatic subtelomeric region. However, in other instances, it has been claimed that these distal chromosome bonds were associations between two heterochromatic or a heterochromatic and a euchromatic segment (John, 1973; John and Freeman, 1976; John and King, 1977a; King and John, 1980).

The data reported here suggest that in *E. pulvinatus* these distal chromosome bonds, either chiasmatic or simple associations have been formed in euchromatic segments. Moreover, C-heterochromatin located close to the telomeres decreases the frequency of such associations.

The differences between populations in chiasma localization for chromosome M6 can also be due to differences in C-heterochromatin banding pattern. In this species, as in *Cryptobothrus chrysophorus* (John and King, 1977b) the megameric chromosome is the one presenting the highest variability in C-banding pattern. The existence of at least four different types of this chromosome (Fig. 4) can only be appreciated in mitotic cells of embryos. This makes it difficult to establish possible relationships between chiasma localization and C-band variability.

The results shown in Figs. 6 and 7 suggest a within population variation for chiasma localization in M6 and M7 BS, which can be due to differences in C-banding pattern of the chromosomes involved. The independent distal chiasma localization frequencies in bivalents M6 and M7 would agree with this conclusion. However, this is probably not the reason for the variation found in bivalent M7 BB. The existence of genetic or environmental factors influencing chiasma localization pattern cannot be excluded.

The results shown in Table 3 indicate that the polymorphisms for chromosomes M7 and S8 fit a Hardy-Weinberg distribution, also, the frequencies of different karyotypes did not vary within nor between generations. Studies of similar polymorphisms have revealed different results. Thus, in *Chorthippus parallelus* (Hewitt and John, 1968, 1970; John and Hewitt, 1969; Westerman, 1969, 1970) populations fit a Hardy-Weinberg distribution for chromosome S8 but not for M7, in which there is an excess of homokaryotypes (BB and SS). Southern (1970) reported an analogous situation for pair L4 of *Metrioptera brachyptera*. The important role played by inbreeding in these populations is the explanation proposed by these authors.

The importance of supernumerary heterochromatic segments in the redistri-

bution of chiasmata within the bivalents carrying them as a source of variability in natural populations has been pointed out on several occasions (John and Miklos, 1979). In the three geographical areas studied, at least two types of bivalents M7 carrying C-heterochromatin have been found. Chiasma redistribution was different in each case (Fig. 7). However, the frequency of chromosome M7 B was similar in all populations and did not change along generations.

The reverse situation is found in bivalent S8. Although the frequency of proximal chiasmata in bivalents S8 BS and S8 SS was similar in all cases, the frequency of chromosome S8 carrying C-heterochromatin was different in the three geographical areas (Fig. 1, Table 3).

The quantitative and qualitative variation in supernumerary C-heterochromatin both between and within populations of *E. pulvinatus* reveals the dynamic nature of this chromosomal complement. However the possible role of chiasma redistributions produced by such C-heterochromatin remains obscure as one does not know if the patterns of redistribution of chiasmata described above represent different optima in different populations or, alternatively, populations differ as a consequence of the neutral nature of the pattern.

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