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CHROMOSOMA

The Effect of C-Heterochromatin in Chiasma Terminalisation in *Chorthippus biguttulus* L. (*Acrididae, Orthoptera*)

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Abstract. Using the Giemsa C-banding procedure, a polymorphism in chromosome banding pattern has been found in a spanish population of *Chorthippus biguttulus*. The variation in C-banding pattern shown by bivalent M 6 allowed to study the effect of C-heterochromatin on chiasma terminalisation. The results indicate that interstitial heterochromatin acts as a barrier preventing chiasmata to pass. Anaphase separation seems to be normal but could be slightly delayed. A similar role for telomeric C-heterochromatin is suggested.

Introduction

Chiasma terminalisation was defined by Darlington (1929) as the movement of chiasmata towards the chromosome ends. This phenomenon was thought to begin at pachytene and finish at metaphase I overlaping with the onset of anaphase.

In the cases in which terminalisation has been analysed the conclusions appear to be contradictory. For instance, Hearne and Huskins (1935) in *Melanoplus femur-rubrum,* concluded that there is some movement of chiasmata from early diplotene to diakinesis. However, Fox (1973) in *Schistocerca gregaria* using changes in X-chromosome morphology to sequence cells in diplotene arrived at the conclusion that no movement of chiasmata was occuring during this stage, Hultén (1974) found no evidence of chiasma movement during diakinesis in human spermatocytes and Jones (1977) using autoradiographic methods extended his studies to pachytene in *Schistocerca gregaria* and came to the same conclusions. The existence of apparently terminal chiasmata at metaphase I of many organisms could be taken as a proof of terminalisation. In this phase when the spindle is organized centromeric forces could move chiasmata towards the telomeres. However, Jones (1978) using Giemsa C-banding in rye concludes that most terminalisation at metaphase I could be only apparent (pseudoterminalisation) and that chiasmata do not move but are stretched out.

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Using a C-banding technique, we have detected a heterochromatin polymorphism in some of the telocentric chromosomes of *Chorthippus biguttulus*.In the work reported here a comparison between chiasma positions at diplotene and at metaphase I has been made in bivalents with different heterochromatin patterns. The possible effect of C-heterochromatin segments on chiasma positions at metaphase I has been studied.

Material and Methods

Individuals of a population of *Chorthippus biguttulus* from The Campus of Complutense University, Madrid, Spain, have been employed in this study.

Arrested mitotic metaphases were obtained from gut caeca as follows: males and females were injected with 0.1% colchicine in insect saline. The gut caeca were fixed in acetic-alcohol 1:3 for aproximately 4 h after injection. Testes of non colchicine treated males were fixed in acetic-alcohol 1:3 for meiotic observations.

Preparations were made by squashing testes and gut caeca in 45% acetic acid. Coverslips were removed by the dry ice procedure. The slides were then dehydrated in absolute alcohol prior to air drying.

Air dried slides were immersed in saturated $(OH)_2Ba$ at room temperature for 10 min, washed in tap water and immersed in 2 × SSC at 60° C for 1 h. They were then stained in a 2% Giemsa solution (GURR's improved R66) in phosphate buffer pH 7. Stainig was checked until appropiate contrast was obtained. Then, slides were washed in tap water, rapidly air dried, immersed in xylene for 5 min and mounted in DEPEX.

Results

Chorthippus biguttulus has 17 chromosomes (16+X) in the male and 18 chromosomes (16+XX) in the female. As in other species of the same genus (e.g. *Chorthippus brunneus*, John and Hewitt, 1966) three pairs of autosomes (L1, L2 and L3) are long and submetacentric, whereas the remaining five pairs of autosomes and the X-chromosome are of medium to small size and seem to be telocentric.

At mitotic metaphase as well as at diplotene the telocentric chromosomes can be distinguished by their length. Arm length ratio can be used in addition to length for the identification of submetacentric chromosomes. At metaphase I the identification of bivalents presents some problems due to chromosome contraction. Chromosome identification in colchicine untreated males was carried out at diplotene.

Giemsa C-banding applied to mitotic and meiotic cells revealed centromeric heterochromatin in all chromosomes. Telomeric heterochromatin was found only in chromosomes M5, M6, M7 and S8. Although variable in size, telomeric heterochromatin was always present in chromosome M7. In chromosomes M5, M6 and S8 telomeric heterochromatin was variable in size and could even be absent. This variation is especially apparent in diplotene cells in which the telomeric heterochromatin amount of two homologous chromosomes forming a bivalent can be compared (Fig. 1). Two individuals (male and female) were heterozygotes for an interstitial band in chromosome M6 (Fig. 1a–b). No homozygotes were found and all other individuals lacked bands at this locus.

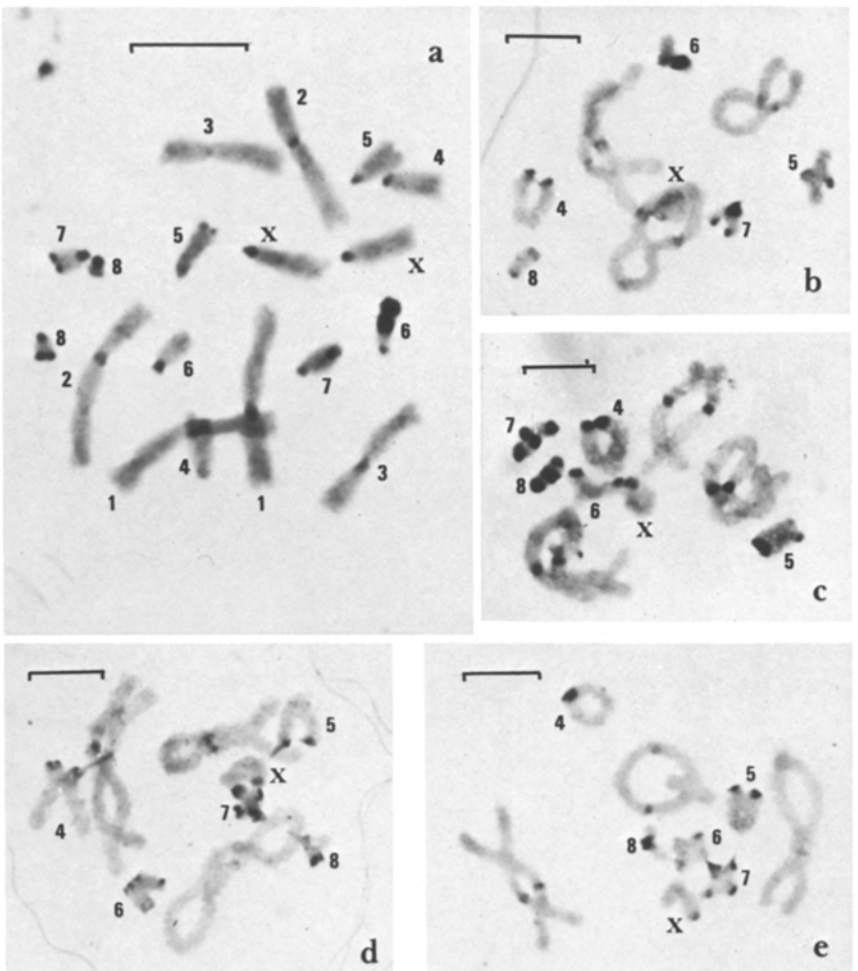

Fig. 1. a Mitotic metaphase cell obtained from gut caeca of the female heterozygous for both an interstitial and a distal C-heterochromatin band in chromosome M6. **b** C-banded diplotene cell of the male heterozygous for the interstitial band showing a proximal chiasma in bivalent M6. **c–e** C-banded diplotene cells of different individuals showing distal (**c**), proximal (**d**) and interstitial (**e**) chiasma in bivalent M6. Bar represent 10 μm

The frequency of telomeric heterochromatin in each chromosome could be measured. Then, for chromosome pairs M5, M6 and S8 three types of individuals were considered: homozygotes for telomeric heterochromatin presence (PP); homozygotes for absence (AA); and heterozygotes (PA). Table 1 shows the number of individuals of each type for these three chromosomes found in the population studied.

Of especial interest is the male heterozygous for the interstitial band. At diplotene, bivalent M6 showed invariably only one chiasma. Two types of chiasma position could be unequivocally distinguishted: proximal (between the interstitial band and the centromere) (Fig. 1 b) and distal (between the interstitial

Table 1. Number of individuals homozygotes for telomeric heterochromatin presence (PP); homozygotes for absence (AA); and heterozygotes (PA); in chromosome pairs M5, M6 and S8

Telomeric heterochromatin constitution	Chromosome pair		
	M5	M6	S8
PP	7	4	29
PA	27	22[a]	13
AA	15	23[b]	7

[a] One female was in addition heterozygous for an interstitial band

[b] One male was heterozygous for an interstitial band

Table 2. Positions of chiasma in bivalent M6 at diplotene and at metaphase I in the male heterozygous for the interstitial C-heterochromatin

Diplotene		Metaphase I		Contingency χ^2
Prox.	Dist.	Prox.	Dist.	
7	15	95	180	0.0683*

* not significant

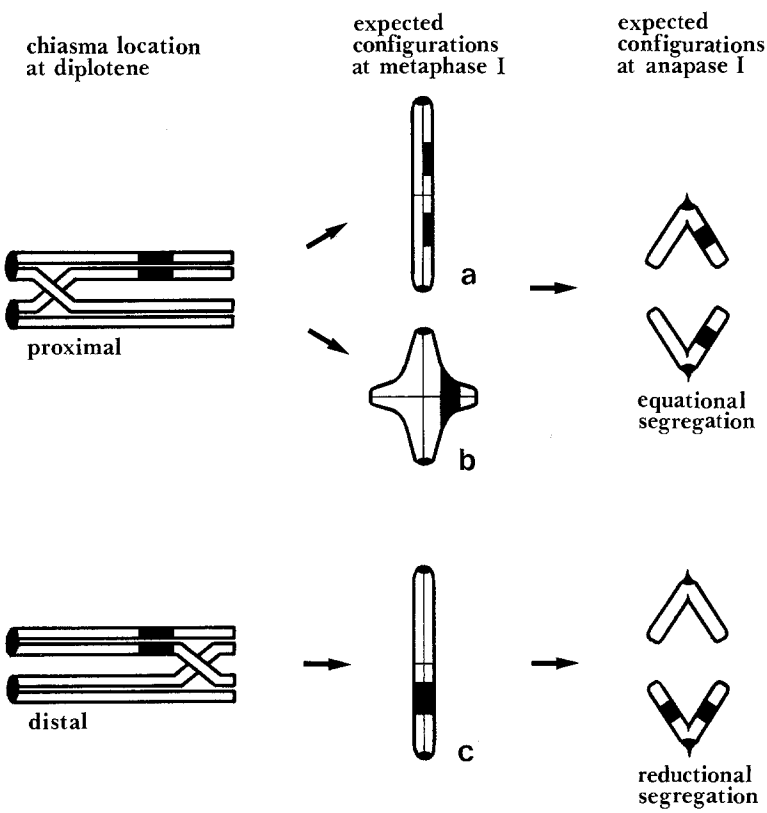

Fig. 2. Diagrams illustrating the expected configurations at metaphase I and at anaphase I of bivalent M6 heterozygous for the interstitial heterochromatin band arising from the two possible locations of the chiasma at diplotene (see text)

band and the telomere). The results concerning the position of the chiasma at this stage are given in Table 2.

Figure 2 shows the three types of bivalents which could be theoretically found at metaphase I, namely: bivalents resulting from a terminalised proximal chiasma (a), bivalents resulting from a non-terminalised proximal chiasma (b); and bivalents resulting from a distal chiasma (c). Only two types of these metaphase I bivalents were observed: those resulting from a distal chiasma (c) and those resulting from a non terminalised chiasma (b) (Fig. 3c–d).

The frequencies of these two types of bivalents are given in Table 2. Chiasma positions in bivalent M6 were also studied in seven individuals which did not have the interstitial band: two homozygotes for telomeric heterochromatin presence (PP), two homozygotes for absence (AA) and three heterozygotes (PA). In order to identify bivalent M6 at metaphase I individuals showing differences in C-banding pattern between bivalents M5, M6 and M7 were chosen. For instance, Fig. 1c, 3a and b show different cells of the same individual. From

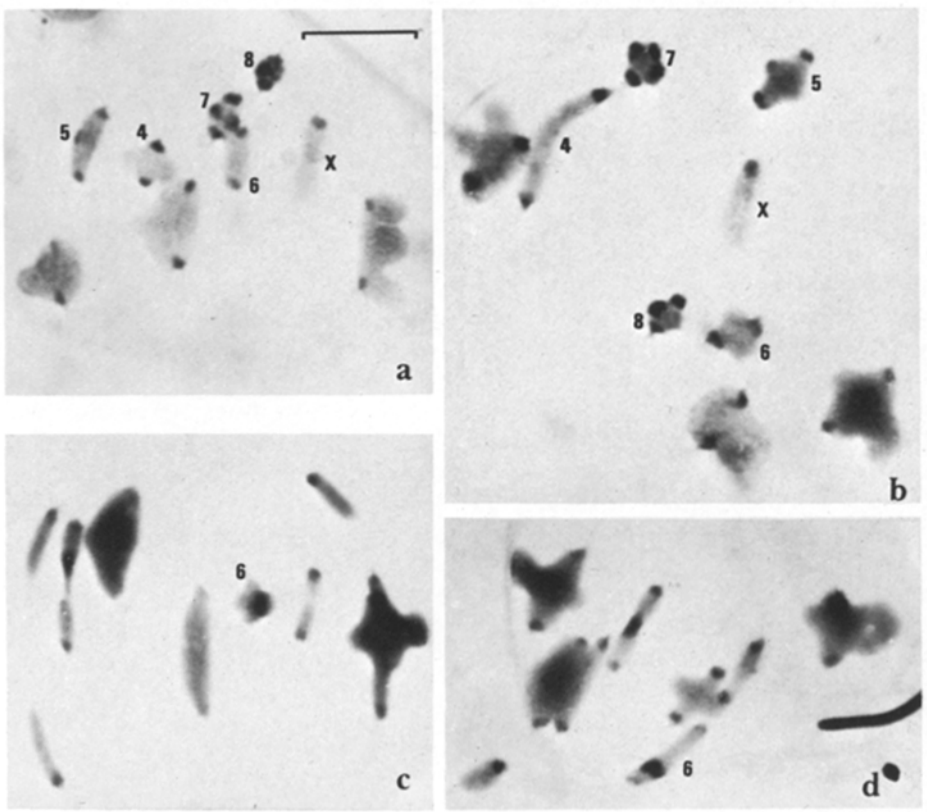

Fig. 3 a and b C-banded metaphase I cells of the same individual of figure 1c showing terminalised (**a**) and non terminalised (**b**) chiasma in bivalent M6. **c and d** C-banded metaphase I cells of the male heterozygous for the interstitial heterochromatin band showing proximal (**c**) and distal (**d**) chiasma in bivalent M6. Bar represent 10 μm

Table 3. Positions of the chiasma in bivalent M6 at diplotene and at metaphase I of individuals without interstitial heterochromatin

Telomeric hetero-chromatin constitution	Diplotene			Metaphase I		Contingency[a]	
	Prox.	Interst.	Dist.	Non-termi-nalised	Termi-nalised	χ^2	χ^2
PP[b]	20	10	37	41	133	1.0105*	10.4714**
PA[b]	19	13	40	48	199	1.6277*	18.5498**
AA[b]	12	7	22	44	176	1.7770*	13.0830**
Total	51	30	99	133	508	4.6501**	42.8834**
				Heterogeneity		0.2349*	0.7792*

[a] Interstitial and distal chiasmata at diplotene have been added.

[b] PP: homozygotes for telomeric C-heterochromatin; AA: homozygotes for absence of telomeric C-heterochromatin; PA: heterozygotes.

* not significant, ** significant at the level of 5%

diplotene cells (Fig. 1c) it can be concluded that in this individual bivalents M5 and S8 are heterozygous for telomeric heterochromatin (PA), bivalent M6 is homozygous for absence (AA) and bivalent M7 is homozygous for presence (PP). Then, in this individual such bivalents can be unequivocally identified at metaphase I according to their size and band pattern (Fig. 3a–b). At diplotene, bivalent M6 showed in all cases only one chiasma which could be proximally (Fig. 1d), interstitially (Fig. 1e) or distally (Fig. 1c) located. The frequencies of such locations are given in Table 3. At metaphase I two types of chiasma position (terminalised and non terminalised) were considered (Fig. 3a–b). The frequencies of both positions for the different types of individuals are given in Table 3. In these individuals comparison between the positions of the chiasma at diplotene and at metaphase I can be made in two ways: (i) adding the frequencies of interstitial and distal chiasmata at diplotene, and (ii) adding the frequencies of interstitial and proximal chiasmata at diplotene. The results of such tests are shown in Table 3. In the male heterozygous for interstitial heterochromatin both equational and reductional segregation for the heterochromatic segment was observed at anaphase I. Although no valid statistical test could be made due to the small number of anaphase I cells observed (2 equational and 10 reductional) it can be concluded that there is a good correspondence between anaphase I results and those obtained from earlier phases. In individuals heterozygous for telomeric heterochromatin (PA) only equational segregation for telomeric heterochromatin was found.

Discussion

The results shown in tables 2 and 3 indicate that the location of the chiasma at diplotene in bivalent M6 is similar in all individuals studied except the

one heterozygous for the interstitial band. In this individual the lack of interstitial chiasmata and the presence of heterochromatin in the middle of the chromosome are probably causally related. The absence of chiasmata in C-heterochromatic zones has been demonstrated by other authors (Fox et al., 1973; Hultén, 1974; Marks, 1974; Jones, 1978).

In the individual heterozygous for the interstitial band, proximal chiasmata in bivalent M6 do not move towards the telomere. This fact is clearly demonstrated both by the lack of bivalent type (a) (see Fig. 2) and by the fit between frequencies of proximal and distal chiasmata at diplotene and at metaphase I (Table 2).

The differences in chiasma location at diplotene and at metaphase I in the bivalents without interstitial heterochromatin are significant in all cases when proximal and interstitial chiasmata at diplotene are added (Table 3). Although this could indicate the existence of terminalisation in this bivalent, probably this terminalisation is overestimated since due to the lack of a marker in the bivalents without interstitial heterochromatin, the distinction between non terminalised and terminalised chiasmata at metaphase I could be subject to some error. The diplotene interstitial chiasmata could terminalise enough to appear as terminal chiasmata at metaphase I if pseudoterminalisation (Jones, 1978) is occuring in this material. This problem can be obviated by adding the frequencies of interstitial and distal chiasmata at diplotene for the comparison with chiasma locations at metaphase I. A significant trend towards an increase in the frequency of terminal chiasmata at metaphase I over that observed at diplotene is suggested by the result of the overall χ^2 test, although individual tests carried out in the three groups of individuals (PP, PA and AA) resulted in non significant differences.

When terminalisation as well as anaphase separation take place the two sister chromatids from each chromosome separate between the telomere and the most distal chiasma. Our results indicate that interstitial C-heterochromatin prevents terminalisation. This may be explained by the association between chromatids in the heterochromatic region being tighter than association in the euchromatic region. If this is so, some delay in the anaphase separation of chromosomes with interstitial heterochromatin will be expected when a proximal chiasma occurs.

From the results shown in Table 3 on comparing the proportion of terminalised versus non terminalised chiasmata at metaphase I in individuals PP, PA and AA, one can infer a certain effect of telomeric heterochromatin on terminalisation (or stretching), suggesting that telomeric heterochromatin could hinder chromosome separation until anaphase I. In the population of *Chorthippus biguttulus* analysed in this report only medium and short chromosomes (M5, M6, M7 and S8) have been found to show telomeric heterochromatin, the shortest chromosomes (M7 and S8) being the ones in which telomeric heterochromatin appears with the highest frequency (Table 1).

Our preliminary results obtained in a study concerning the distribution of C-heterochromatin in the karyotypes of spanish Acrididae reveal that in those species in which C-heterochromatin has been found at or near telomeres (*Acrotylus insubricus, Calliptamus sp., Chorthippus apicalis, C. jucundus, C. paralellus,*

C. binotatus, Euchorthippus pulvinatus gallicus, Stenobothrus festivus, Sphingonotus coerulans carsicus) it is restricted to medium and short chromosomes. A similar phenomenon can be observed in *Schistocerca gregaria* in which all chromosomes show centromeric heterochromatin, but only the shortest ones show in addition C-heterochromatin near telomeres (Fox et al., 1973).

The reason for this apparent tendency of this kind of heterochromatin to be located in medium and short chromosomes is an open question. We would like to propose that it has some relation with delay of anaphase separation which may improve synchronisation of these short chromosomes with a single often distal chiasma, with the larger chromosomes which usually have more chiasmata.

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