A test for equilocality of supernumerary heterochromatin distribution in grasshoppers

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The presence of extra segments in four or more nonhomologous chromosome pairs led us to test the applicability of the principle of equilocality of heterochromatin distribution to supernumerary heterochromatin. In *Chorthippus brunneus*, which carries extra segments on all chromosomes except the L_3, the 13 heterochromatic variants occupy all possible locations with 3 proximal, 5 interstitial, and 5 distal. This indicates that equilocality does not seem to apply to the supernumerary heterochromatin in this species. On the other hand, the four extra segments found in *Chorthippus parallelus* and five of the seven extra heterochromatic segments found in *Chorthippus binotatus* are all located distally on the M_5–S_8 chromosomes. Thus for these groups of chromosomes the extra heterochromatic segments do appear to show a tendency for an equilocal distribution.

Key words: *Chorthippus*, heterochromatin.

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La présence de segments surnuméraires chez quatre paires ou plus de chromosomes non homologues nous a conduit à vérifier l'applicabilité du principe de l'équilocalité de la distribution de l'hétérochromatine à l'hétérochromatine excédentaire. Chez le *Chorthippus brunneus*, qui est porteur de segments surnuméraires sur tous les chromosomes sauf le L_3, les 13 variants hétérochromatiques occupent toutes les localisations possibles, dont 3 proximales, 5 interstitielles et 5 distales. Ceci indique que équilocalité ne semble pas s'appliquer à l'hétérochromatine excédentaire chez cette espèce. D'autre part, les quatre segments surnuméraires observés chez *C. parallelus* et les cinq segments hétérochromatiques surnuméraires sur sept trouvés chez *C. binotatus* sont tous localisés de façon distale chez les chromosomes, M_5–S_8. Il s'ensuit donc que pour ces groupes de chromosomes, les segments hétérochromatiques surnuméraires semblent présenter une tendance vers une distribution équllocale.

Mots clés : *Chorthippus*, hétérochromatine.

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Introduction

The principle of equilocality of heterochromatin distribution (Heitz 1933, 1935) is generally applicable to the distribution of constitutive heterochromatin in grasshoppers, more especially with regard to the paracentromeric C-bands (King and John 1980; John *et al.* 1985). Whether this principle applies also to supernumerary heterochromatin has never been tested. The present report is based on the fact that Spanish populations of *Chorthippus brunneus* are polymorphic for supernumerary heterochromatic segments on almost all chromosomes. This provides an opportunity to examine whether the extra heterochromatin is distributed in an equilocal manner. Furthermore, the presence of extra segments on the four smallest chromosome pairs in both *C. parallelus* and *C. binotatus* can be used to determine whether equilocality of additional heterochromatin may sometimes apply to restricted chromosome groups (John *et al.* 1985).

Materials and methods

Natural populations of *Chorthippus parallelus*, *C. binotatus*, and *C. brunneus* were sampled in the South of the Iberian Peninsula (Table 1). The cytogenetic techniques employed are the same as in Camacho *et al.* (1984).

Results

Chorthippus parallelus

We have analysed male adults from two populations in the Sierra Nevada (Granada, Spain) and have found supernumerary segments distally located in the four smallest (M_5, M_6, M_7, and S_8) chromosomes. The response of the extra segments to C-banding was reported by Camacho *et al.* (1984); while those on the M_5, M_6, and M_7 C-band darkly, that on the S_8 does not C-band at all. Table 2 summarizes the chromosomal and karyomorphic frequencies in the two populations analysed. In Soportujar, where a large number of individuals was analysed, the frequencies of the different karyomorphs for the M_7 and S_8 chromosomes did not differ significantly from those expected from a Hardy–Weinberg equilibrium ($\chi^2_{(1)} = 0.45$, $P > 0.05$ for the M_7; $\chi^2_{(1)} = 0.446$; $P > 0.05$ for the S_8).

Chorthippus binotatus

All the populations analysed are highly polymorphic for the presence of eight different supernumerary chromosome segments on the four smallest chromosomes. Each of these may carry two extra segments differing from one another in chromosomal location, size, and C-banding reaction. A detailed examination of the response of these extra segments to C-banding and their frequencies in different populations was reported in a separated paper (Cabrero *et al.* 1986). A summary of the data is shown in Table 3 of the present report.

Chorthippus brunneus

Of the species of *Chorthippus* analysed to date, *C. brunneus* shows the most extensive variation for supernumerary segments and small C-bands, the latter being detectable only in embryonic neuroblasts where the chromosomes are much larger. Table 4 summarizes the frequencies of the polymorphisms found for each chromosome pair.

(*i*) L_3 chromosome. In two of the nine populations analysed,

TABLE 1. Material analysed

Species and Population	Province[a]	Year of sampling	No. of individuals analysed: Males	Females	Embryos
C. parallelus					
Las Sabinas (SN)	Gr	1980	7	6	—
Soportújar	Gr	1983	32	—	—
C. binotatus					
Alto del Chorrillo (SN)	Gr	1982	17	10	8
Loma de Piedrablanca (SN)	Gr	1982	6	6	—
Dornajo (SN)	Gr	1982	14	15	—
		1983	18	19	—
Collado Ruquino (SN)	Gr	1983	24	4	—
Valle del Lanjarón (SN)	Gr	1983	22	13	—
Santiago la Espada	J	1983	8	—	—
C. brunneus					
Carretera SN	Gr	1981	—	6	—
Río Aguas Blancas	Gr	1981	—	—	11
La Alhambra	Gr	1982	5	3	80
Prados del Pinar	Gr	1982	15	12	—
Alfaguara-1	Gr	1982	4	5	75
Alfaguara-2	Gr	1982	1	5	42
Padul	Gr	1982	5	1	—
Las Sabinas (SN)	Gr	1982	1	—	—
Alcalá la Real	J	1982	5	—	55
Totals			184	105	271

[a]SN, Sierra Nevada, Gr, Granada; J, Jaén.

TABLE 2. Chromosomal and karyomorphic frequencies in two populations of *Chorthippus parallelus*

Chromosome and population	Karyomorphic frequencies[a]	Segmented chromosome frequency (q_s)
M_5		
Las Sabinas	12 BB : 1 BS	0.038
M_6		
Las Sabinas	10 BB : 3 BS	0.115
M_7		
Las Sabinas	4 BB : 9 BS	0.346
Soportújar	3 BB : 11 BS : 18 SS	0.734
S_8		
Las Sabinas	11 BB : 1 BS : 1 SS	0.115
Soportújar	14 BB : 13 BS: 5 SS	0.359

[a]B, basic chromosome; S, segmented chromosome.

a total of 11 embryos were heterozygous for the size of the paracentromeric C-band (Fig. 1*a*).

(*ii*) L_2 chromosome. Variation for the size of the interstitial C-band in the short arm which, as was demonstrated by Cabrero and Camacho (1986), is associated with a nucleolus organizer region (NOR). One of both of the L_2 homologues carried a double sized interstitial C-band in 34 embryos from Alhambra and two from Alfaguara-1 (Fig. 1*b*), while in the latter population the abnormal L_2 chromosomes lacked the basic paracentromeric C-band (Fig. 1*c*).

(*iii*) X chromosome. Two adult females from Alhambra carried a small extra C-band located interstitially on one or both of the sex chromosomes (Fig. 1*d*).

(*iv*) M_4 chromosome. Three variants have been observed for this chromosome, one possessing only the paracentromeric C-band, a second carrying also two small interstitial C-bands (Fig. 1*e*), and a third carrying a large supernumerary segment located subdistally and lightly C-banded (Fig. 2*a*). This extra segment was previously reported by Camacho *et al.* (1984).

(*v*) M_5 chromosome. Two types of supernumerary segments that differ only in location. Both C-band darkly and are small, but while one is distally located (Fig. 2*a*) the other is interstitial (Fig. 2*b*). We have observed them in embryos from the same population but not in the same individual.

(*vi*) M_6 chromosome. Besides the basic M_6 chromosome type, which shows only a paracentromeric C-band, we have

FIG. 1. Supernumerary segments in embryos of *Chrothippus brunneus*. (*a*) Presence of a reduced paracentromeric C-band in one of the L_1 chromosomes (arrow) together with two extra C-band segments on one of the M_6 homologues. (*b* and *c*) The NOR- associated C-band interstitially located in the short arm f one L_2 chromosome (arrow) is double the size of that normally found. The M_6 pair is again heterozygous for two extra segments. Note in *c* the absence of the paracentromeric C-band in the L_2 chromosome which possesses the large interstitial C-band. (*d*) Small interstitial C-band in the X chromosome (arrow). (*e*) Two extra C-bands, interstitially located, in one of the M_4 chromosomes.

found three M_6 variants. One lacks the paracentromeric C-band (Figs. 2*c* and 2*d*), another carries a distal extra segment that is lightly C-banded (Fig. 2*d*), and a third possessing two large extra segments one distally and the other subdistally located, both of which C-band darkly (see Figs. 1*a*–1*c* and 2*e*). Both extra segments were described by Camacho *et al.* (1984). We have analysed the meiotic behaviour of both extra segments in heterozygous males. Anaphase I segregation of the heteromorphic bivalents in them is always equational, as is expected from the restriction of chiasma formation to the proximal regions. This, together with the fact that both extra segments have never been found separately in different chromosomes, demonstrates

FIG. 2. Supernumerary segments in embryos of *Chorthippus brunneus*. (*a*) Lightly C-banded extra segment subdistally located on one of the M_4 chromosomes (arrow) together with an extra distal C-band in one of the M_5 chromosomes. (*b*) Interstitial C-band in the M_5 chromosomes (arrow). (*c*) One of the M_6 homologues lacks the paracentromeric C-band (arrow). (*d*) Lightly C-banded extra segment distally located on one of the M_6 chromosomes (arrow). Note the other M_6 lacks the paracentromeric C-band while one S_8 possesses a large and darkly C-banded extra segment (arrow). (*e*) Extra segments in both M_6 chromosomes, basic M_7 chromosome showing a small distal C-band, and both S_8 chromosomes showing a distal and darkly C-banded small extra segment. (*f*) Heterozygosity in the M_7 and S_8 pairs for the presence of small distally located and darkly C-banded extra segments (arrow).

the total absence of crossing-over in the euchromatic zone between them.

(*vii*) M_7 chromosome. We have found two types of M_7 chromosomes. The most frequent type shows a small C-band distally located (Fig. 2*e*) and the other carries a large distally located supernumerary segment that C-bands darkly (Fig. 2*f*). All the populations analysed were polymorphic for the M_7 chromosome pair.

TABLE 3. Chromosome location, C-banding response, and frequency of supernumerary segments in *Chorthippus binotatus*. In M_5 and M_7 chromosomes, we have designated S as the larger and s as the smaller extra segment. The data of frequency have been summarized from those shown in Cabrero *et al.* (1986)

Chromosome and supernumerary segment	Chromosome location	C-banding response	Frequency
M_5			
s	Distal	Darkly C-banded	0.028–0.167
S	Distal	Partly darkly C-banded	0.018–0.167
M_6			
E[a]	Distal	Does not C-band	0.105
I	Interstitial	Darkly C-banded	0.068
M_7			
s	Distal	Darkly C-banded	0.034–0.200
S	Distal	Lightly C-banded	0.086
S_8			
P	Proximal	Does not C-band	0.014–0.067
D	Distal	Darkly C-banded	0.018–0.042

[a]This extra segment is euchromatic.

TABLE 4. C-banding variation and supernumerary segments in *Chorthippus brunneus* (B, basic). The embryos of Alhambra population were obtained from eight egg pods, those of Alfaguara-1 from seven, those of Alfaguara-2 from four, and those of Alcalá la Real from seven

Chromosome and population	Stage	Chromosome variant	Karyomorphic frequencies	Chromosome frequency
L_1				
Alfaguara-1	Embryos	s, reduced paracentromeric C-band	68 BB : 7 Bs	$q_s = 0.046$
Alfaguara-2	Embryos	s, reduced paracentromeric C-band	38 BB : 4 Bs	$q_s = 0.047$
L_2				
Alhambra	Embryos	s, interstitial basic C-band double sized	46 BB : 34 Bs	$q_s = 0.212$
Alfaguara-1	Embryos	s, interstitial basic C-band double sized and absence of the paracentromeric C-band	73 BB : 2 Bs	$q_s = 0.013$
X				
Alhambra	Embryos	s, with an interstitial C-band	78 BB : 1 Bs : 1 ss	$q_s = 0.019$
M_4				
Alfaguara-1	Embryos	S, with a subdistal extra segment lightly C-banded; s, with two interstitial C-bands	54 BB : 17 BS : 4 Bs	$q_S = 0.113$, $r_s = 0.027$
Alhambra	Adults	S, as in Alfaguara-1	7 BB : 1 BS	
Alhambra	Embryos	S, as in Alfaguara-1	76 BB : 4 BS	$q_s = 0.025$
Alcalá la Real	Embryos	S, as in Alfaguara-1	58 BB : 17 BS	$q_s = 0.113$
M_5				
Alfaguara-1	Embryos	s, with a distal extra segment darkly C banded; I, with an interstitial extra segment	68 BB : 4 Bs : 2 BI : 1I	$q_s = 0.027$, $r_I = 0.027$
M_6				
Alhambra	Embryos	d, lacking of paracentromeric C-band; S, with large distal and subdistal extra segments darkly C-banded; s, with a small distal extra segment lightly C-banded	34 BB : 15 BB^d : 28 B^dB^d : 1 B^dS^d : 1 Bs^d : 1 B^ds^d	$q_B^d = 0.456$, $r_S^d = 0.006$, $s_s^d = 0.013$
Alfaguara-1	Embryos	d, as in Alhambra; S, as in Alhambra; s, as in Alhambra	27 BB : 12 BB^d : 13 B^dB^d : 7 BS^d 3 SS^d : 2 B^dS^d : 1 S^dS^d : 4 B^ds 2 Bs^d : 2 Bs : 2 ss	$q_B^d = 0.293$, $r_S = 0.02$, $s_S^d = 0.093$, $t_s = 0.067$, $u_s^d = 0.013$
Alfaguara-2	Embryos	d, as in Alhambra; S, as in Alhambra	18 BB : 10 BB^d : 11 B^dB^d : 3 B^dS^d	$q_B^d = 0.417$, $r_S^d = 0.036$
Alfaguara-1	Adults	S, as in Alhambra	7 BB : 2 BS	
Alfaguara-2	Adults	S, as in Alhambra	5 BB : 1 BS	
Padul	Adults	s, as in Alhambra	5 BB : 1 Bs	

TABLE 4. (*concluded*)

Chromosome and population	Stage	Chromosome variant	Karyomorphic frequencies	Chromosome frequency
M_7				
Alhambra	Embryos	s, with a darkly C-banded distal extra segment	39 BB : 32 Bs : 9 ss	$q_s = 0.313$
Alfaguara-1	Embryos	s, as in Alhambra	46 BB : 21 Bs : 8 ss	$q_s = 0.247$
Alfaguara-2	Embryos	s, as in Alhambra	11 BB : 12 Bs : 19 ss	$q_s = 0.595$
Alcalá la Real	Embryos	s, as in Alhambra	36 BB : 17 Bs : 2 ss	$q_s = 0.191$
Alhambra	Adults	s, as in Alhambra	7 BB : 1 Bs	
Alfaguara-2	Adults	s, as in Alhambra	3 BB : 3 Bs	
Alfaguara-1	Adults	s, as in Alhambra	4 BB : 5 Bs	
Prados del Pinar	Adults	s, as in Alhambra	19 BB : 5 Bs : 3 ss	$q_s = 0.204$
Padul	Adults	s, as in Alhambra	5 BB : 1 Bs	
Carretera Sierra Nevada	Adults	s, as in Alhambra	5 BB : 1 Bs	
S_8				
Alhambra	Embryos	s, with a small and darkly C-banded distal extra segment; S, with a large and darkly C-banded distal extra segment	10 BB : 6 Bs : 52 BS : 12 ss	$q_s = 0.188$, $r_s = 0.325$
Alfaguara-1	Embryos	s, as in Alhambra	4 BB : 19 Bs : 52 ss	$q_s = 0.820$
Alfaguara-2	Embryos	s, as in Alhambra	6 BB : 21 Bs : 15 ss	$q_s = 0.607$
Alcalá la Real	Embryos	s, as in Alhambra	23 BB : 14 Bs : 18 ss	$q_s = 0.455$
Alhambra	Adults	s, as in Alhambra	6 BB : 1 Bs : 1 ss	
Prados del Pinar	Adults	s, as in Alhambra	25 BB : 2 Bs	$q_s = 0.037$
Alfaguara-1	Adults	s, as in Alhambra	3 BB : 6 Bs	
Alfaguara-2	Adults	s, as in Alhambra	3 BB : 3 Bs	
Padul	Adults	s, as in Alhambra	2 BB : 2 Bs : 2 ss	
Carretera Sierra Nevada	Adults	s, as in Alhambra	1 BB : 4 Bs : 1 ss	
Las Sabinas	Adults	S, as in Alhambra	1 BS	

(*viii*) S_8 chromosome. In addition to the paracentromeric C-band that is the only one present in the basic S_8 chromosomes, we have seen two types of supernumerary segments. Both are distal and C-band darkly but one (S, Fig. 2*d*) is twice the size of the other (s, Figs. 2*e*–2*f*). The smaller segment was present in all populations analysed, but the larger segment was found in only two.

Discussion

Variations for extra heterochromatin occurred in four or more nonhomologous chromosomes in all three species examined. While in *C. brunneus* all the chromosome pairs, except the L_3, are polymorphic for extra heterochromatic segments, in the two other species polymorphisms were confined to the four smallest chromosome pairs (M_5–S_8). The 13 heterochromatin variants present in *C. brunneus* included 3 proximal, 5 interstitial, and 5 distal types (Table 4), which demonstrates that equilocality, while is commonly found in most of the fixed heterochromatic regions within a species, does not seem to apply to the supernumerary heterochromatin in this species. On the other hand, *C. binotatus* and *C. parallelus* provide evidence for the occurrence of a second type of equilocality which is restricted to a particular group of chromosomes (cf. John *et al.* 1985). In both species there is a tendency for a distal location of extra heterochromatic segments on the M_5–S_8 chromosomes. This applies to the four extra segments found in *C. parallelus* and to five of the seven extra heterochromatic segments in *C. binotatus* (Tables 2 and 3). Thus for this group of chromosomes the extra segments do indeed show a tendency for an equilocal distribution. On the other hand, the four smallest pairs in *C. brunneus* include proximal, interstitial, and distal variants and why these should differ from the equilocal patterns present in the same chromosome group in the two other species is not clear.

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