Chromosome C-banding patterns in Spanish Acridoidea

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Abstract

The karyotypes of 47 Spanish acridoid grasshoppers have been analyzed by means of C-banding in both mitotic and meiotic cells. The most frequent location of C-heterochromatin occurs in centromeric and telomeric regions whereas interstitial C-bands are very scarce. No clear relationship between similarities in C-banding patterns and taxonomic proximity has been found. Negatively heteropycnotic regions are described; their uniform location and distribution suggest that they could represent a functional chromosome structure as nucleolar organizers.

Introduction

Among the animal groups studied karyomorphologically, the Acridoidea are an example of apparent chromosome conservation. As John and Hewitt (1968) pointed out, this uniformity does not imply necessarily that few chromosome changes have occurred in the evolution of this group, but that these changes have not altered the number and morphology of the chromosomes. In an attempt to establish the patterns of change which have occurred during evolution through the analysis of the regularities and restrictions in the C-banded karyotypes of Australian grasshoppers, King and John (1980) found a remarkable degree of C-band variation between species. In addition, the number and location of C-heterochromatic bands in the Acrididae show a variation even within the same species (Shaw *et al.*, 1976; Webb, 1976; John & King, 1977).

In order to determine the possible evolutionary significance of the C-heterochromatin distribution we studied the variation in C-banding patterns between karyotypes with different degrees of relationship (within species, between species of the same genus and genera of the same subfamily).

Material and methods

Gangwere and Morales (1970) have described 324 species of orthopterans in the Iberian Peninsula; 121 of these belong to Acridoidea. Table 1 shows the species analyzed in this paper and the Spanish provinces where they were collected. The taxonomic classification used has been the one proposed by Harz (1975).

Cytological observations were made in mitosis and meiosis. Mitotic cells were obtained from embryos or gut caeca of adult individuals as follows: embryos were dissected from eggs at 20 to 30 days after laying, treated with 0.1% colchicine in insect saline for 3–4 h and fixed in acetic-alcohol 1:3. Adult males and females were injected with 0.15% colchicine in insect saline for 3–4 h, after gut caeca were removed and fixed in acetic-alcohol 1:3.

Testes of non-colchicine treated males were fixed in acetic-alcohol 1:3 for meiotic observations.

Squash preparations of the fixed material were stained following the Giemsa C-banding technique

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described previously (Santos & Giráldez, 1978).

The chromosomes of all species analyzed have been classified according to their relative length.

Results

Chromosome number and shape

Although apparent conservation of chromosome number and shape in Acridoidea has been described, some differences can be detected when karyotypes of several species are compared. In Table 1 the number and shape of the chromosomes in the species analyzed are shown. Due to the low number of species studied with 19–20 chromosomes, only two main groups were considered according to their karyotypical features: species with 23–24 chromosomes and species with 17–18 chromosomes.

In both groups, the same types of chromosome variation were found between species, namely the existence of short arms in some of the pairs of the complement, and differences in the relative size of the chromosomes giving rise to karyotypes with different numbers of small pairs.

The short arms can be euchromatic (*Oedaleus decorus, Paracinema tricolor, Chorthippus binotatus*) or heterochromatic (*Dociostaurus crassiusculus,* Fig. 4b). It is noteworthy that the occurrence of short arms usually affects several pairs of the complement.

Differences in the relative size of the chromosomes are sometimes evident, for instance two (*Calliptamus barbarus,* Fig. 1c, genus *Oedipoda,* Fig. 2) or three (*Aiolopus strepens,* Fig. 3d) pairs of small autosomes can appear. Within species of the same genus, the uniformity of the chromosome set seems to be greater, although in these cases, differences in the relative chromosome size can be revealed by measuring the chromosomes as demonstrated by Camacho (1980) in the genus *Chorthippus.*

Chromosome C-banding patterns

Another type of variation within the karyotypes of Acridoidea is related to the distribution of C-bands. When the C-banding technique is applied, two types of bands appear: positive C-bands (more stained than the euchromatic regions) and the ones we have called negative C-bands because they appear less stained than the remaining regions within the chromosome.

Positive C-bands

Positive C-bands can be classified according to their location within the chromosome as follows: paracentromeric bands (when they are located in or next to the centromere), terminal bands (when they occupy telomeric regions) and interstitial bands.

In Table 2 a–c the distribution of positive C-bands in different karyotypes of the species analyzed is explained in detail. From these tables some regularities can be derived:

(1) The paracentromeric C-bands appear in all chromosomes of every species analyzed. In some cases, these bands are restricted to the centromeric region (thin C-bands), e.g. in the whole complement of *C. barbarus* (Fig. 1c) and *Oedipoda coerulescens* (Fig. 2b). In other cases, they also occupy the region next to the centromere (thick C-bands) as in *Pyrgomorpha conica* (Fig. 1a) and *Oedipoda germanica* (Fig. 2c). In submetacentric chromosomes when these thick C-bands appear, they are

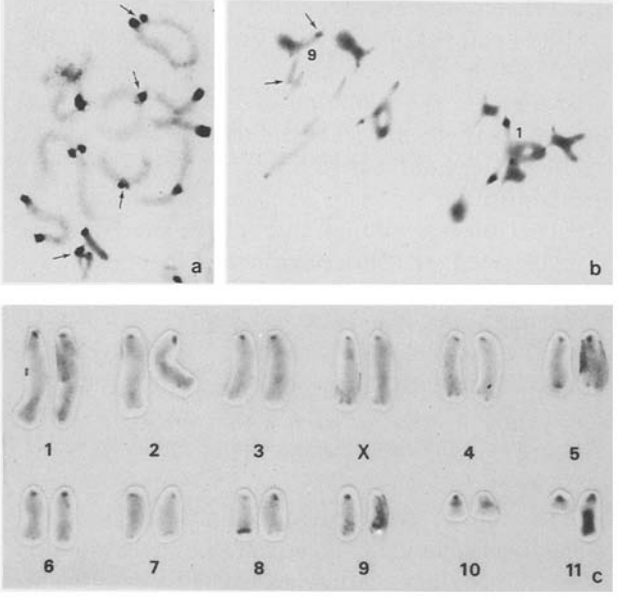

Fig. 1a–c. C-banding in Pyrgomorphidae (a) and Catantopidae (b–c): (a) Diplotene of *Pyrgomorpha conica;* – (b) Metaphase I of *Calliptamus wattenwylianus;* – (c) Female karyotype of *C. barbarus,* note heteromorphic pair S11. (Arrows indicate quantitative variation in paracentromeric C-heterochromatin).

Table 1. Taxonomic classification and general karyotypical features of the 47 species of Acridoidea analyzed and provinces from which they were collected.

Family Subfamily	Species	Chromosome set 2n ♂	Submet.	Acrocentric	Province
Pamphagidae					
Pamphaginae	*Ocnerodes brunneri* (Oliv.)	19XO			Madrid
	Eumigus cucullatus (Bol.)	19XO			Alicante
	E. sp.	19XO			Granada
Akicerinae	*Prionotropis flexuosa* (Serv.)	19XO			Madrid
Pyrgomorphidae	*Pyrgomorpha conica* (Oliv.)	19XO			Madrid, Zaragoza
Catantopidae					
Catantopinae	*Pezotettix giornae* (Rossi)	23XO			Madrid, Granada
Calliptaminae	*Calliptamus wattenwylianus* (Pant.)	23XO			Madrid
	C. barbarus (Costa)	23XO			Madrid, Zaragoza
Eyprepocnemidinae	*Eyprepocnemis plorans* (Charp.)	23XO			Valencia, Alicante
	Heteracris littoralis (Ramb.)	23XO			Valencia
Cyrtacanthacridinae	*Anacridium aegyptium* (L.)	23XO			Madrid
Acrididae					
Acridinae	*Acrida* sp.	23XO			Alicante
Locustinae	*Oedipoda fuscocincta* (Luc.)	23XO		1-11, X	Madrid, Avila
	O. charpentieri (Fieb.)	23XO			Madrid
	O. coerulescens (L.)	23XO			Madrid, Avila
	O. germanica (Latr.)	23XO			Baleares
	Locusta migratoria (L.)	23XO		1-3, several M	Valencia
	Acrotylus insubricus (Scop.)	23XO			Madrid, Zaragoza
	A. fischeri (Azam)	23XO			Madrid, Zaragoza
	Sphingonotus coerulans (L.)	23XO		1-11, X	Madrid, Zaragoza
	S. azurescens (Ramb.)	23XO			Madrid, Zaragoza
	Oedaleus decorus (Germ.)	23XO		1-11, X	Madrid, Zaragoza
	Aiolopus strepens (Latr.)	23XO			Madrid, Zaragoza
	A. thalassinus (Fabr.)	23XO			Madrid, Valencia
	Paracinema tricolor (Thunb.)	23XO		several M, 9, 10 and 11	Madrid
	Callephorus compressicornis (Latr.)	23XO			Zaragoza
Gomphocerinae	*Arcyptera tornosi* (Bol.)	23XO			Madrid
	Brachycrotaphus tryxalicerus (Fisch.)	23XO			Baleares
	Dociostaurus crassiusculus (Pant.)	23XO		2, 3, 10, 11	Madrid
	D. genei (Ocsk.)	23XO		most chromosomes	Madrid
	D. hispanicus (Bol.)	23XO		most chromosomes	Madrid
	D. maroccanus (Thunb.)	23XO		most chromosomes	Madrid
	Ramburiella hispanica (Ramb.)	23XO			Zaragoza
	Myrmeleotettix maculatus (Thunb.)	17 XO	1-3		Madrid
	Omocestus panteli (Bol.)	17XO	1-3		Madrid, Avila, Zaragoza
	O. bolivari (Chopard)	17XO	1-3		Granada
	O. minutissimus (Bol.)	17XO	1-3	X	Madrid, Avila
	Stenobothrus festivus (Bol.)	17XO	1-3		Madrid
	S. stigmaticus (Ramb.)	17XO	1-3	5, 6, 7	Madrid
	Chorthippus apicalis (H.-S.)	17XO	1-3		Madrid, Avila
	C. binotatus (Charp.)	17XO	1-3	4-8, X	Madrid, Granada
	C. biguttulus (L.)	17XO	1-3	4-8, X	Madrid, Segovia
	C. parallelus (Zett.)	17XO	1-3		Madrid, Zaragoza
	C. jucundus (Fisch.)	17XO	1-3	4-8, X	Madrid, Zaragoza
	C. vagans (Everms)	17XO	1-3	4-8, X	Madrid, Zaragoza, Coruña
	Euchorthippus pulvinatus (F.-V.)	17XO	1-3		Madrid, Zaragoza
	E. albolineatus (Luc.)	17XO	1-3		Madrid

Table 2. a–c. C-heterochromatin location in the species of Acridoidea analyzed. Chromosomes showing quantitative variation for C-banding pattern are designed by (+) and those showing qualitative variation by asterisks: (*) indicates presence of C-heterochromatin in 95% of the chromosomes studied. (**) indicates presence of C-heterochromatin in frequencies between 5 and 95%. (***) indicates presence of C-heterochromatin in less than 5% of the cases.

(a) Distribution of C-heterochromatin in species $2n♂ = 19$, $2n♀ = 20$.

Species	Fig.	C-heterochromatin location			Number of individuals
		Centromeric region	Interstitial region	Telomeric region	
Prionotropis flexuosa		1-9, X	8	–	1
Eumigus sp.		1-9, X, 8+	–	–	1
E. cucullatus		1-9, X	8+	–	1
Ocnerodes brunneri		1-9, X, 8+	–	–	5
Pyrgomorpha conica	1a	(1-9) +, X	–	–	30

(b) Distribution of C-heterochromatin in species $2n♂ = 23$, $2n♀ = 24$.

Species	Fig.	C-heterochromatin location				Number of individuals
		Centromeric region		Interstitial region	Telomeric region	
		Thin-C band	Thick C-band			
Calliptamus wattenwylianus	1b	3-11, X, 9+	1, 2	–	–	21
Calliptamus barbarus	1c	1-11, X	–	9, 10	3-6, X, 8, 11**	17
Heteracis littoralis		1-7, 10, 11	8, 9, X	–	–	28
Eyprepocnemis plorans		1-11, X, 2+, 6+, 11+	–	7, 9, 10	–	42
Pezotettix giornae		1-4, 6-11, X, 8+	5	–	7***	11
Anacridium aegyptium		1-11, X	–	–	9, 10, 11	6
Acrida sp.		1-8, 10, 11, X	9	–	–	8
Oedipoda charpentieri	2a	2-6, X	1, 7+, 8+, 10+, 11+	6, 9	1, 2, 4, 5, 7-9	12
O. coerulescens	2b	1-11, X	–	–	–	12
O. fuscocincta		1-8, 10, X	9, 11	–	11**	8
O. germanica	2c	2	1, 3-11, X	8	–	6
Oedaleus decorus		1-10, X	11	–	–	7
Callephorus compressicornis		2-11, X	1	9	9, 10, 11	12
Locusta migratoria		1-11, X	–	–	–	3
Acrotylus insubricus	3a	1, 2, 3+, 4+, 7	5, 6, 8-11, X	–	9***	17
A. fischeri		1-11, X	–	–	5, 7-9, 10+, 11	15
Sphingonotus azurescens		1-11, X	–	9***	–	13
S. coerulans	3b	1-11, X	–	1-11, X, 6*, 9+	–	14
Paracinema tricolor		5-11, X	1-3, 4+	–	–	6
Ramburiella hispanica		7-11	1-6, X	3	–	3
Aiolopus thalassinus	3c	1-7, 9, X	8, 10, 11	4**	–	19
A. strepens	3d	1-11, X	–	9	10	12
Arcyptera tornosi		1, 2, 4-10, X	3, 11	3	1-11, X, 9+, 11**	23
Brachycrotaphus tryxalicerus	4a	1-11, X	–	1, X	7, 8, 9+	5
Dociostaurus crassiusculus	4b	1, 4-9, 8+, X	2, 3, 10, 11	9	–	2
D. genei		1, 3+, 4+, 5-7, 9-11, X	2, 8	–	–	11
D. hispanicus		1-11, X	–	–	–	8
D. maroccanus		3-10, X	1, 2, 11	–	–	16

Table 2. (continued).

(c) Distribution of C-heterochromatin in species 2n♂ = 17, 2n♀ = 18.

Species	Fig.	C-heterochromatin location				Number of individuals
		Centromeric region		Interstitial region	Telomeric region	
		Thin C-band	Thick C-band			
Stenobothrus festivus	4c	1-8, X	-	7, 8	4-6, 8	10
S. stigmaticus		1-8, X	-	-	-	8
Omocestus panteli		1-5, 7, 8, X	6	-	-	18
O. minutissimus		1-8, X	-	-	-	10
O. bolivari		1-8, X	-	-	-	6
Myrmeleotettix maculatus		1-5, 7, 8, X	6	-	-	28
Chorthippus apicalis	4d	4, 5, X	1, (2-3)+, 6-8	-	6***	32
C. binotatus		1-8, X	-	-	6, 7, 8***	31
C. jucundus		1-8, X	-	-	4-6, 7+	22
C. biguttulus		1-8, X	-	6***	5**, 6**, 7+, 8**	67
C. parallelus		1-8, X	-	6	4-8	31
C. vagans		1-3, 5-8, X	4	-	-	31
Euchorthippus pulvinatus	5a	1-8, X	-	6+	6*, **, 7**, *** 8*, **, ***	1734
E. albolineatus	5b	1-3, 8	4-7, X	3	1, 2	35

only present in the short arms (*Chorthippus apicalis*, Fig. 4d).

(2) When terminal C-bands are present, they are usually located in medium or small chromosomes (*Anacridium aegyptium, Chorthippus biguttulus, Stenobothrus festivus,* Fig. 4c) and sometimes in most of the members of the complement (*C. barbarus, Oedipoda charpentieri,* Fig. 2a). In submetacentric chromosomes they only appear in the short arms of pairs L1 and L2 of *Euchorthippus albolineatus* (Fig. 5b).

(3) Interstitial C-bands are very scarce. When they appear they are usually located near the paracentromeric region (pair L1 of *Brachycrotaphus tryxalicerus,* Fig. 4a; pair L3 of *Ramburiella hispanica*) or the telomeric region (pair M6 of *Euchorthippus pulvinatus,* Fig. 5a; M7 and S8 of *S. festivus,* Fig. 4c). Only in the species *E. albolineatus* does a submetacentric pair (L3) show an interstitial C-band.

The most extreme case of this type of C-heterochromatin location is *Sphingonotus coerulans* (Fig. 3d) since all chromosomes have small subterminal C-bands.

(4) The submetacentric chromosomes of species with karyotypes 2n = 17 or 18 show a great uniformity in their C-heterochromatin distribution since in all species (except *E. albolineatus*) only centromeric bands appear.

It is also worth mentioning that the C-hetero-

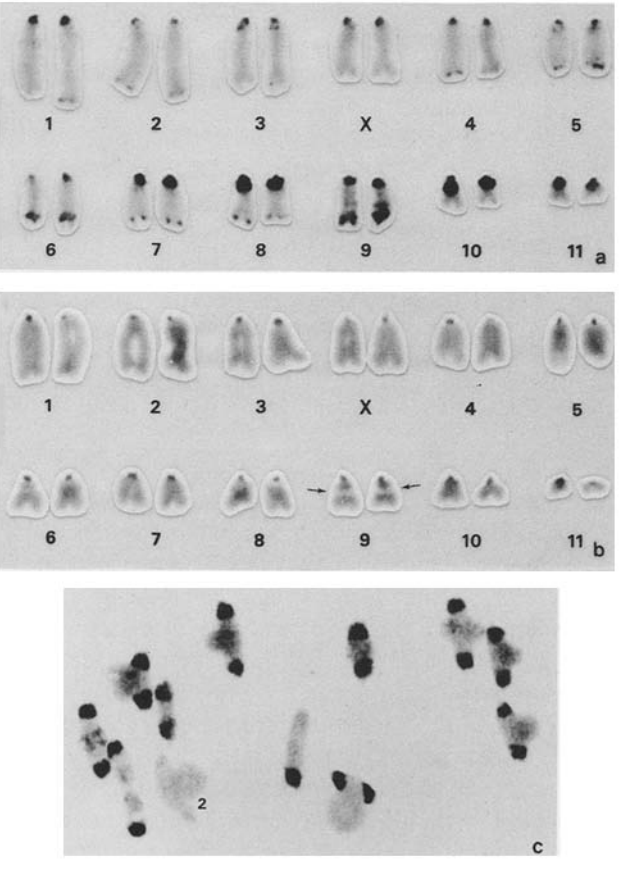

Fig. 2a-c. C-banding in *Oedipoda:* (a-b) Female karyotypes of *O. charpentieri* (a) and *O. coerulescens* (b, arrows: negative C-bands); - (c) Metaphase I of *O. germanica.*

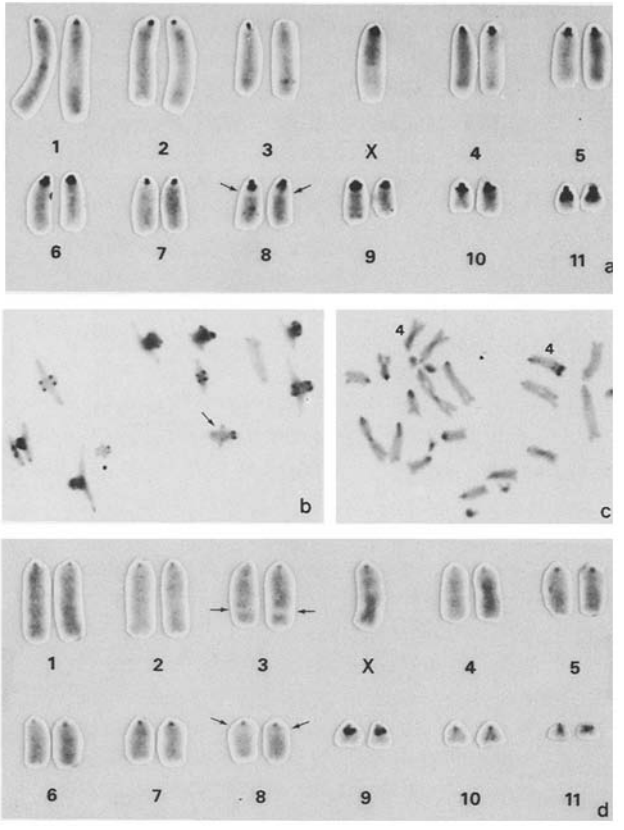

Fig. 3a–d. C-banding in Locustinae: (a) Male karyotype of *Acrotylus insubricus,* arrows indicate negative C-bands in pair M8; – (b) Metaphase I of *Sphingonotus coerulans,* arrow indicates heteromorphic bivalent; – (c) Female mitotic metaphase of *Aiolopus thalassinus,* pair M4 is heterozygous for interstitial heterochromatic bands; – (d) Male karyotype of *A. strepens,* arrows indicate negative C-bands.

chromatin content of two homologous chromosomes is subjected to both qualitative (presence or absence) and quantitative (more or less) variation. The observations under a light microscope allow the detection of the last type of variation only when large differences exist, resulting in an underestimation of the actual variability.

Quantitative variation occurs, for instance, in several pairs of *P. conica,* S11 of *Eyprepocnemis plorans,* M9 of *S. coerulans* and L2 and L3 of *Ch. apicalis* (see Table 2 a–c). These observations suggest that this type of variation appears in large as well as in medium or small chromosomes.

Qualitative variation in interstitial regions as well as in telomeric ones, has only been found in medium or small chromosomes (see Fig. 3c, M4 pair; Fig. 5a, S8 pair; and Fig. 1c, S11 pair).

Negative C-bands

These bands usually appear in C-banded preparations which have been subjected to prolonged stain. When these bands are observed they are present in one or more chromosome pairs of the complement, in both mitotic and meiotic cells.

The distribution and location of negative C-bands in different karyotypes of the species analyzed is shown in Table 3. These bands are always subterminally located in large telocentric chromosomes (Fig. 3d) and near the centromere in most of the medium or small chromosomes (Figs. 2b, 3a and 5a). When they appear in submetacentric

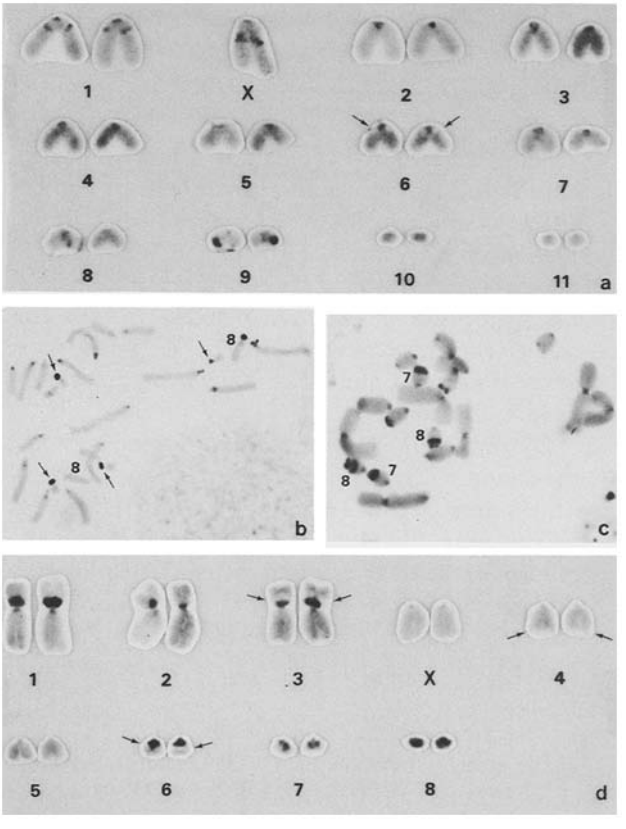

Fig. 4a–d. C-banding in Gomphocerinae: (a) Male karyotype of *Brachycrotaphus tryxalicerus* from anaphase-I cell of heterozygote for terminal C-heterochromatin in pair M9; – (b) Female mitotic metaphase of *Dociostaurus crassiusculus,* arrows indicate hetrochromatic short arms; – (c) Female mitotic metaphase of *Stenobothrus festivus;* – (d) Male karyotype of *Chorthippus apicalis.* Arrows in (a) and (d) indicate negative C-bands.

Table 3. Localization of the negative C-bands in the species analyzed. The megameric pair is also indicated.

Species	Negative C-bands location		Megameric pair
	Chromosome pair	Chromosomic region	
Eumigus sp.	8	paracentromeric	-
Calliptamus wattenwylianus	-	-	9
Calliptamus barbarus	-	-	9
Eyprepocnemis plorans	-	-	9
Oedipoda fuscocincta	-	-	9
O. charpentieri	-	-	9
O. coerulescens	9	paracentromeric	9
O. germanica	9	paracentromeric	9
Acrotylus insubricus	8	paracentromeric	9
A. fischeri	8	paracentromeric	9
Sphingonotus azurescens	2	subterminal	9
	9	paracentromeric	
Oedaleus decorus	-	-	9
Aiolopus strepens	3	subterminal	9
	8	paracentromeric	
Callephorus compressicornis	-	-	9
Arcyptera tornosi	-	-	9
Brachycrotaphus tryxalicerus	6	paracentromeric	-
Ramburiella hispanica	2	subterminal	-
	8 or 9	paracentromeric	
Myrmeleotettix maculatus	-	-	6
Omocestus panteli	3 short arm	paracentromeric	6
O. bolivari	-	-	6
Chorthippus apicalis	3 short arm	paracentromeric	6 or 7
	4	telomeric	
	6	paracentromeric	
C. binotatus	-	-	6
C. biguttulus	3 short arm	paracentromeric	6
C. parallelus	-	-	6
Euchorthippus pulvinatus	3 short arm	paracentromeric	6
	8	paracentromeric	
Euchorthippus albolineatus	-	-	6

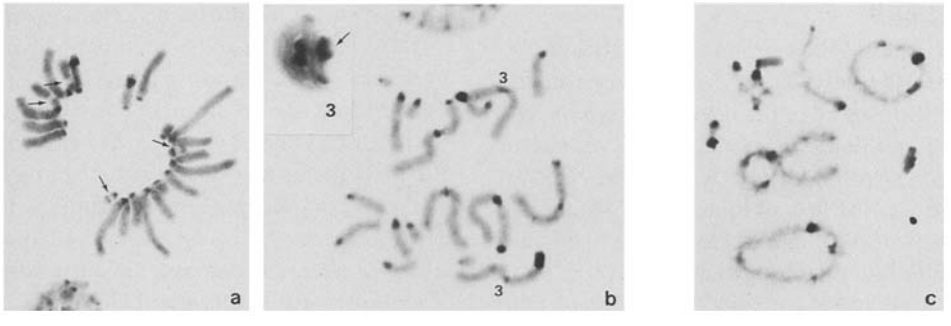

Fig. 5a–c. C-banding in *Euchorthippus:* (a–b) Mitotic metaphases from neuroblasts of *E. pulvinatus* (a, arrows indicate negative C-bands) and *E. albolineatus* (b, inset shows L3 metaphase-1 bivalent, arrow indicates interstitial C-heterochromatin in short arms); – (c) Diplotene of artificial hybrid *E. pulvinatus* × *E. albolineatus.* (Micrography 5a has been kindly supplied by Dr. E. Ferrer).

chromosomes they are always located in the short arm of the L3 pair (see Figs. 4d and 5a).

In contrast with the positive C-bands, the chromosomal position of the negative C-bands did not vary between individuals of the same species.

Megameric chromosomes

The name 'megameric pair' is given to the two homologous chromosomes precociously condensed during the first meiotic prophase (Corey, 1938). This bivalent, which appears in many acridoids, is characterized by its deeply positive heteropycnosity. In some of the species analyzed such as *Eumigus cucullatus, B. tryxalicerus* and the genus *Dociostaurus,* this bivalent can not be detected and in some others its identification is difficult (*Ch. apicalis* and *S. festivus*). As shown in Table 3, in species with 23–24 chromosomes, the megameric bivalent is always the M9 pair and in species with 16–17 chromosomes, the megameric bivalent is the M6 pair.

In the species analyzed, a trend towards accumulation of C-heterochromatin in the megameric pair has been observed. Nevertheless, there are some species in which its amount of C-heterochromatin is not higher than that of the rest of the complement. This fact indicates that there is no functional relationship between C-heterochromatic content and particular meiotic chromosome behaviour.

Discussion

Chromosome C-banding patterns

Positive C-bands

From the regularities observed in the distribution of these bands (Tables 2 a–c), it can be concluded that the distribution of paracentromeric and telomeric C-bands in Spanish acridoid species is similar to that in the Australian ones (King & John, 1980). However, the distribution of interstitial C-bands is rather different since they are uncommon in Spanish species. This fact could suggest that in these two geographic areas the possible mechanisms of change affecting C-band distribution have behaved in a different way. This idea is also supported by the differences found in the C-heterochromatic patterns of Spanish and Australian individuals of the same species (*Locusta migratoria* and *Aiolopus thalassinus*).

Qualitative variation in positive C-bands have been described by other authors (for review see Hewitt, 1979). This type of variation can form true polymorphisms in natural populations of some species: *Chorthippus parallelus* (Hewitt & John, 1968, 1970; John & Hewitt, 1969), *Stetophyma* (Shaw, 1970, 1971) and *Euchorthippus pulvinatus* (Santos & Giráldez, 1982).

Negative C-bands

The uniformity of the distribution and location of negative C-bands (Table 3) suggests that they could represent a functional chromosome structure as nucleolar organizers. Trying to elucidate the nature of these heterpycnotic bands, a specific NOR staining technique (Rufas *et al.,* 1982) has been applied in some of the species analyzed: *E. pulvinatus* and *Chorthippus biguttulus,* obtaining a good correspondence between C-bands and NOR's.

A similar trend towards uniformity in location and distribution of special chromosome regions has been described by Schmid (1978 a, b) in nucleolar organizers of related species of Amphibia; in these cases, NOR's appear as negative heteropycnotic regions when stained by fluorescence and, in some cases, C-banding procedures.

C-banding within a species

Some regularities can be detected in C-banding patterns within species. The most striking feature observed is the one named 'equilocal distribution' (Heitz, 1933, 1935). This concept refers to the fact that in certain species the heterochromatin of non-homologous chromosomes tends to be located in similar regions in most members of the chromosome set. The C-banding patterns of several Spanish acridoid species support this form of regularity. Thus, thick C-bands next to the centromere regions are present in many chromosome pairs of *P. conica* and *O. germanica;* telomeric bands are frequent in chromosomes of *C. barbarus, Arcyptera tornosi* and *O. charpentieri* and all chromosomes of *S. coerulans* have subterminal C-bands.

As King and John (1980) pointed out, this uniformity in C-banding patterns could suggest either the existence of a mechanism responsible for simultaneous changes in the heterochromatin content of

many members of a complement, or else that there is one mechanism to change the form of heterochromatin and another to distribute that changed form elsewhere in the complement. However, in some of these examples of equilocal distribution within the karyotype there is additional evidence which overlies the general pattern, for instance, the lack of a thick paracentromeric C-band in the L2 pair of *O. germanica* and in X, M4 and M5 of *Ch. apicalis* and the presence of an interstitial band in X and L1 of *B. tryxalicerus* or in the M9 pair of *A. strepens*. Nevertheless, there are chromosome regions which retain some independence with regard to the mechanism which regulates the pattern of distribution of C-heterochromatin.

C-banding between species

The same types of C-heterochromatin variation found within a species have been observed in the study of variability between species. However in the latter case, either qualitative or quantitative differences affect most of the members of the chromosome set.

The analysis of C-banding patterns of different species of the same genus and different genera of the same subfamily reveals no clear correlation between these patterns and the degree of relationship. Identical C-banding patterns appear in species of different genera: for instance, *O. coerulescens* and *Sphingonotus azurescens* have thin C-bands in paracentromeric regions of all chromosomes and thick C-bands are present only in the M6 pair of *Myrmeleotettix maculatus* and *Omocestus panteli* (see Table 2 b, c).

On the other hand, species of the same genus are not uniform in their C-banding pattern (see genera *Calliptamus*, Figs. 1 b, c; *Oedipoda*, Fig. 2; and *Euchorthippus*, Fig. 5). Analogous results have been reported in other genera of animals and plants (*Peromyscus*, Pathak *et al.*, 1973; *Mus*, Markwong *et al.*, 1973; *Anemone*, Marks & Schweizer, 1974; *Neotoma*, Mascarello & Hsu, 1976; *Allium* and *Hordeum*, Vosa, 1976 a, b).

Differences between species of the same genus and between genera of the same subfamily can be due to the mechanism responsible for the equilocal distribution of C-heterochromatin. Whatever may be the explanation, the dynamic nature of this class of heterochromatin is evident; mechanisms of change affecting the C-heterochromatin distribution could then act independently of the chromosome changes implied in the evolution of these species. The analysis of meiotic pairing in the artificial hybrid *E. pulvinatus* × *E. albolineatus* (unpublished data) supports this idea since it shows a high frequency of bivalents (Fig. 5c), indicating a close relationship between the two parental species although they present very different C-banding patterns.

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