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Equilocality of heterochromatin distribution and heterochromatin heterogeneity in acridid grasshoppers*

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Abstract. Comparative fluorescence studies on the chromosome of ten species of acridid grasshoppers, with varying amounts and locations of C-band positive heterochromatin, indicate that the only regions to fluoresce differentially are those that C-band. Within a given species there is a marked tendency for groups of chromosomes to accumulate heterochromatin with similar fluorescence behaviour at similar sites. This applies to all three major categories of heterochromatin – centric, interstitial and telomeric. Different sites within the same complement, however, tend to have different fluorescence properties. In particular, centric C-bands within a given species are regularly distinguishable in their behaviour from telomeric C-bands. Different species, on the other hand, may show distinct forms of differential fluorescence at equilocal sites. These varying patterns of heterochromatin heterogeneity, both within and between species, indicate that whatever determines the differential response to fluorochromes has tended to operate both on an equilocal basis and in a concerted fashion. This is reinforced by the fact that structural rearrangements that lead to the relocation of centric C-bands, either within or between species, may also be accompanied by a change in fluorescence behaviour.

Introduction

It was Heitz (1933, 1935) who first drew attention to the fact that the constitutive heterochromatin of non-homologous members of the same diploid set tends to be located at similar sites. More recently, this concept of equilocality has received additional support from studies that have used Giemsa C-banding as a means of defining the location of heterochromatic regions in condensed metaphase chromosomes (Schweizer and Ehrendorfer 1976). Using this same approach with the essentially uniarmed complements of acridoid grasshoppers it has been possible to demonstrate two rather different forms of equilocality. On the one hand a majority, or all, of the members of a chromosome set may carry C-bands at comparable locations, as is the case for the terminal C-bands of *Atractomorpha similis* (John and King 1983) or the interstitial bands of genus nov. 95 (King and John 1980). On the other hand, a particular subset of chromosomes may behave in a uniform manner. This is the case in the northern chromosome race of *Cryptobothrus chrysophorus* in which only the medium sized members of the complement carry fixed terminal C-blocks; here the large chromosomes have no such blocks while the small chromosomes are highly polymorphic for them (John and King 1977). Additionally, there are also clear exceptions to the principle of equilocality in acridoids. These are most evident in the *X* chromosome and, where present, the megameric pair, both of which show differential heteropycnosity at prophase of the first meiotic division in the male (King and John 1980). In plants with biarmed chromosomes Schweizer and Ehrendorfer (1983) conclude that the best fit to equilocality in respect of distal and terminal C-bands is to be found in species with chromosomes of uniform size and arm ratio, whereas species with chromosomes that differ markedly in size and arm ratio are more likely to carry interstitial C-bands. Clearly, it would be of considerable value to identify the precise causes that underlie the varying kinds of equilocal patterns.

While it is certainly possible to obtain differential C-banding (John and King 1983), the introduction of fluorescent DNA-binding dyes of different specificities has made it possible to characterise heterochromatic regions in a much more precise manner. Such dyes fall into two major categories depending on whether they exhibit AT-specific (Quinacrine, Hoechst 33258, and DAPI = 4′-6-diamidino-2-phenylindole) or GC-specific (chromomycin A_3 (CMA), and mithramycin (MM)) binding and/or fluorescence properties. On this basis at least three major categories of constitutive heterochromatin can be distinguished, all of which stain positively with Giemsa C-banding (Table 1). First there are those that are AT-rich and $DAPI^+$, CMA^-, where DAPI gives bright (+) fluorescence while CMA produces negative (−) fluorescence leading to the production of clear unstained region. Combinations of the two DNA dyes distamycin and DAPI, which have similar AT-specificity but non-identical binding modes, produce especially intense fluorescing DA-DAPI bands in some species (see Schweizer 1979, Schweizer et al. 1983). The combination of DAPI and actinomycin D (DAPI-AMD) usually gives a comparable result in grasshoppers, but with improved resolution. The

* We dedicate this paper, with affection, to Professor Hans Bauer on the occasion of his 80th birthday, and in appreciation of his singular contribution to the study of chromosomes

Table 1. Categories of heterochromatin defined by the fluorochromes DAPI and CMA

Category	Example		Results of in situ hybridisation	Reference
	Species	C-band site		
(1) Enhanced fluorescence with DAPI, or Hoechst 33258	*Mus musculus*	Centric	Contains AT rich satellite DNA	Hilwig and Gropp 1972
	Sus scrofa	Centric blocks of all acrocentrics		Schwarzacher et al. 1984
	Ornithogalum caudatum	Proximal and intercalary		Schweizer 1976
	Bufo americanus	Terminal		Schmid 1980
(2) Enhanced fluorescence with CMA	*Macropus robustus*	Centric	Contains GC rich satellite DNA	Dawson and Marshall-Graves 1984
	Sus scrofa	Centric blocks of all metacentrics		Schwarzacher et al. 1984
	Scilla siberica	Intercalary and terminal	Contains GC rich satellite DNA	Deumling and Greilhuber 1982
	Allium cupani	Terminal and subterminal		Loidl 1983
(3) No enhanced fluorescence with either DAPI or CMA	*Warramaba virgo*	Majority of centric sites		Schweizer et al. 1983
	Allium flavum	Some subterminal sites		Loidl 1983
	Secale cereale	Terminal		Schweizer 1979

Table 2. Details of ten species of Australian Acrididae analysed in this paper

Sub-family	Species	Locality collected
Catantopinae	*Apotropis tricarinata* (Stal.)	Bolivia Hill, NSW
	Rectitropis sp. 1 (Sjost)	Kurangi Swamp, NT
	Urnisa sp.	Bolivia Hill, NSW
	Stenocatantops angustifrons (Walk.)	Kurangi Swamp, NT
Acridinae	*Froggattina australis* (Walk).	Bolivia Hill, NSW
	Pseudailopus keyi (Hollis)	Kurangi Swamp, N.T.
	Cryptobothrus chrysophorus (Rehn)	
	Northern – race	Bolivia Hill, NSW
	Southern – race	Forbes Creek, NSW
	Heteropternis obscurella (Blanch.)	Kurangi Swamp, NT
	Gastrimargus musicus (Fabr.)	Uriarra, ACT
Oxyinae	*Oxya japonica*	Kurangi Swamp, NT

second category is GC-rich and CMA^+, $DAPI^-$, where CMA produces bright (+) fluorescence while DAPI gives negative (−) fluorescence. A third category fails to give positive or negative fluorescence with either class of fluorochrome, so that the material in question has a uniform dull fluorescence. Here, presumably, AT or GC bases are not clustered. In *Allium flavum*, however, sequential staining with the complementary fluorochromes CMA and DAPI reveals a class of heterochromatin that has the quite unexpected property of showing negative fluorescence with both dyes (Loidl 1983). The basis of this behaviour is unknown.

The above dye combinations thus provide an opportunity to examine the extent to which heterogeneity exists in C-band positive regions within a given chromosome set. This, in turn, allows a reconsideration of the concept of equilocal heterochromatin distribution in more refined terms. In this paper we have used CMA, DA-DAPI and DAPI-AMD fluorescence patterns to examine heterochromatin heterogeneity in relation to equilocality of C-bands in acridid grasshoppers.

Materials and methods

The ten species used in this study are all Australian members of the family Acrididae. Their subfamilial affinities are summarised in Table 2. In each case wild-caught specimens were established as breeding colonies at Can-

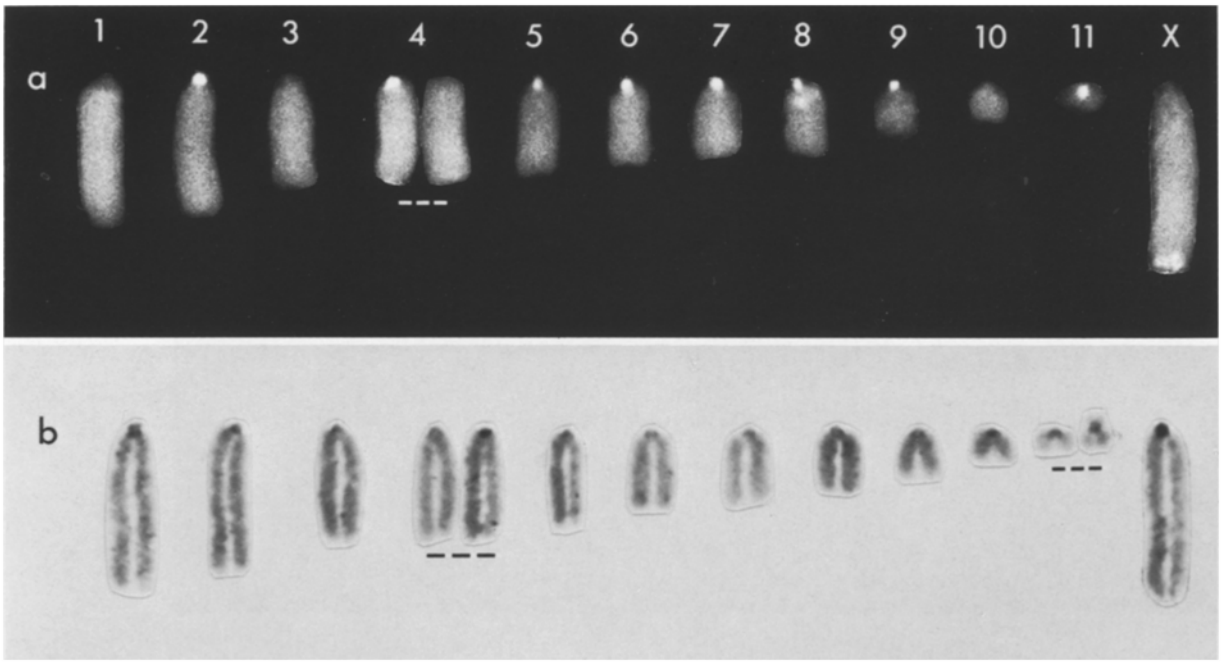


Fig. 1. *Apotropis tricarinata* Haploid complement **a** with CMA-fluorescence and **b** after C-banding. Note heteromorphism for centric heterochromatin and fluorescence pattern in autosome *4* (– – –) in both (**a**) and (**b**) as well as for pericentric inversion in pair *11* of (**b**)

berra so that egg pods could be obtained from laying females. Embryos were extracted from these pods at appropriate time intervals after laying and air-dried preparations of these were made following treatment with colchicine and subsequent fixation in Carnoy 3:1. Some of these preparations were processed for C-banding following the technique of Webb (1976). Other were analysed for fluorescence behaviour in Vienna using the procedures developed by Schweizer (1976, 1979). Photographs of the CMA and DA-DAPI patterns were usually taken from the same cell. The DAPI-AMD patterns were obtained following destaining and restaining and the karyotypes shown are, with few exceptions, from the same cell. The C-band patterns shown are from different metaphases and may stem from a different animal.

Results

Before describing the individual behaviour of the ten species examined there are four general points that need to be emphasised:

1) With the exception of *Stenocatantops angustifrons*, which has $2n=22$ ♂ (*XY*), 22 ♀ (*XX*), all the other species have $2n=23$ ♂ (*XO*), 24 ♀ (*XX*). Six of the species include a graded series of uniarmed chromosomes. This arrangement is complicated in *Stenocatantops angustifrons* by the presence of a metacentric *X*, the product of an *XA* fusion, while in *Froggattina australis* chromosome *7* has a minute short arm. In *Pseudailopus keyi*, by contrast, there are short arms on all but the three shortest members of the complement, while in *Gastrimargus musicus* all but the two shortest autosomes carry them.

2) In each case, chromosomes within a complement have been arranged and numbered in decreasing size order according to their content of euchromatin. As we have pointed out earlier (King and John 1980) this should not be taken to imply that chromosomes in different species that are assigned the same number are necessarily homologous.

3) Paracentromeric C-bands, which occur in the vicinity of the centromere regions, can be subdivided into three categories. Where only minute terminal bands are present they appear to represent the substance of the centromere region proper and we have referred to these as centric. In uniarmed chromosomes, however, the proximal region of the arm itself may also give a positive C-band reaction so that what, at first sight, appears as a single band in reality consists of both a centric and a proximal arm component. Finally, in the two cases in which biarmed chromosomes with C-band positive short arms are present these too have been considered as part of the paracentromeric system.

4) In describing the fluorescence behaviour of individual C-bands, we comment only on those that give a differential response. Those that give a dull fluorescence, equivalent to that shown by the euchromatin, are not referred to individually.

With these general points in mind, we can define the behaviour patterns of the ten species analysed in the following terms:

Apotropis tricarinata (Fig. 1)

This species has the simplest pattern of heterochromatin distribution that we have encountered in any acridid. There are no terminal or interstitial C-bands and while centric bands are present on all the chromosomes only in the *X* do they reach even a modest size. In terms of fluorescence behaviour, the centric bands on *1, 3* and *10* are CMA^- while those of *2, 5–9* and *11* are CMA^+. Those of *4* are

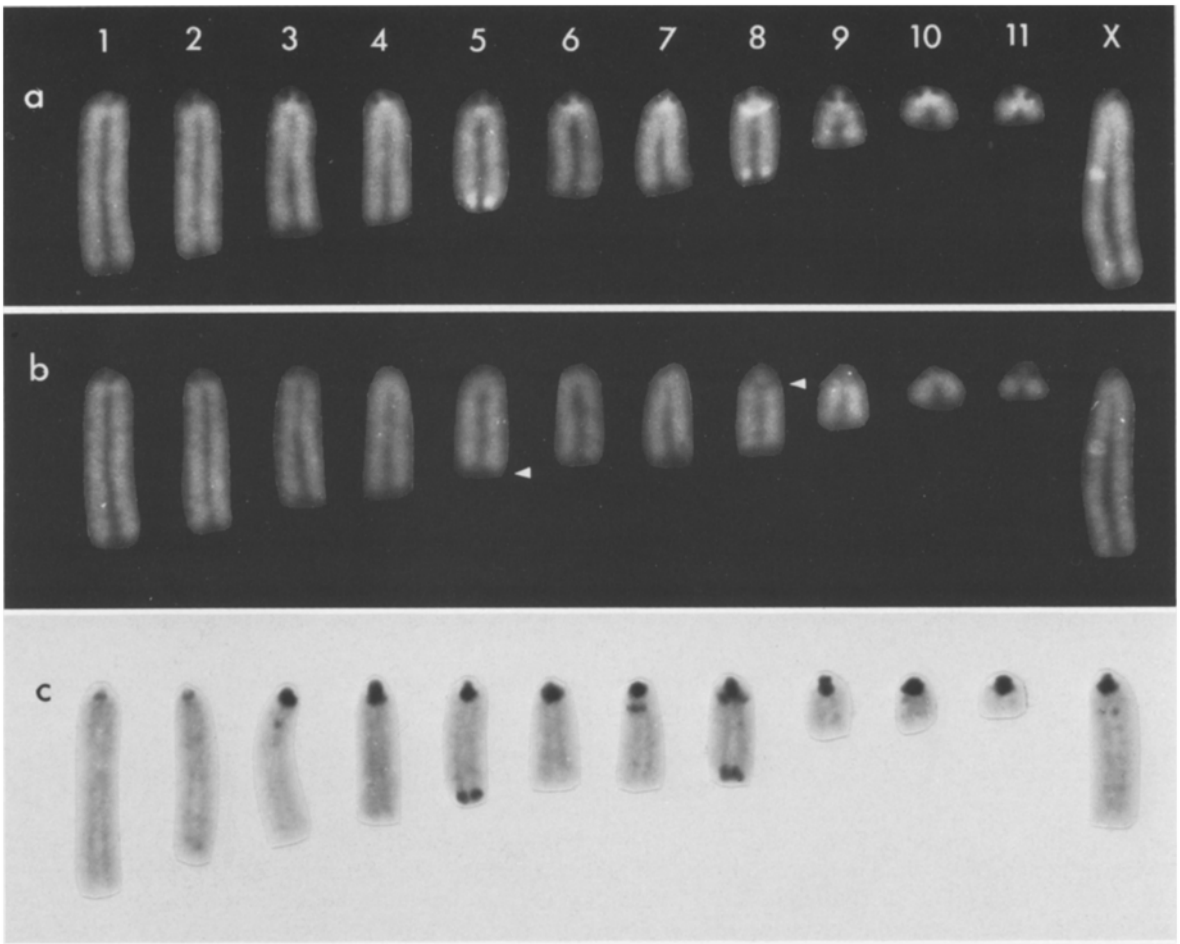


Fig. 2. *Rectitropis sp. 1.* Haploid complement **a** with CMA-fluorescence, **b** with DAPI-AMD fluorescence and **c** after C-banding. The *arrowheads* demarcate regions of negative fluorescence

polymorphic and may be either CMA^- or CMA^+, a feature that corresponds to an obvious C-band heteromorphism. The centric band of the *X* shows no differential fluorescence, but a terminal CMA^+ band is sometimes found on the *X*. Both *X* bands give a uniform dull fluorescence pattern with DA-DAPI and with DAPI-AMD.

Rectitropis sp. 1 (Fig. 2)

Here the heterochromatin pattern defined by C-banding is only slightly more complex than that of *Apotropis*. With the exception of autosomes *1* and *2*, however, the paracentromeric C-bands are noticeably larger in *Rectitropis*. Additionally, there are terminal segments on *5* and *8* and faint interstitial C-bands on *7* and the *X*. The fluorescence pattern also shows some differences compared with *Apotropis*. The small centric C-bands on *1* and *2* show no differential response but all the remaining centric bands are CMA^+. Additionally, the blocks on *7–11* include a proximal component which fluoresces positively with CMA. In chromosome *8* the positive fluorescence with CMA is intense and is distinctive in giving a negative response to DAPI-AMD. The terminal blocks on *5* and *8* also fluoresce intensely with CMA and are negative with DA-DAPI. The interstitial bands on *7* and the *X* fail to fluoresce differentially with any dye combination.

Urnisa sp. (Fig. 3)

In this case there are paracentromeric C-bands on all the chromosomes and these are fairly even in size. Otherwise there are only small interstitial C-bands on *9* and *10*. The parencentromeric bands are characterised by the general absence of differential fluorescence with CMA, but four of them, those on *5–7*, give a positive fluorescence with DA-DAPI. There is intense fluorescence with CMA for the proximal bands on *8* and *11* and the interstitial C-bands on *9* and *10*, coupled with a negative fluorescence with both DA-DAPI and DAPI-AMD of these same regions on *8, 9* and *10*.

Froggattina australis (Fig. 4)

Paracentromeric C-bands are again present on all the chromosomes. These are small on *1–7* and the *X* but large on *8–11*. Additionally, there are small terminal C-bands on *1* and *X* and small interstitial C-bands on *4* and the *X*. Chromosome *9* is very distinctive in its C-band pattern since it is not only multi-banded but additionally gives an overall, dull C-band response in its non-banded areas. Its unique behaviour can be related to the fact that it forms the megameric element in this species at male meiosis. Chromosomes *1–6* generally lack any differential centromeric fluorescence. The centric bands on *8–11*, by contrast, give intense

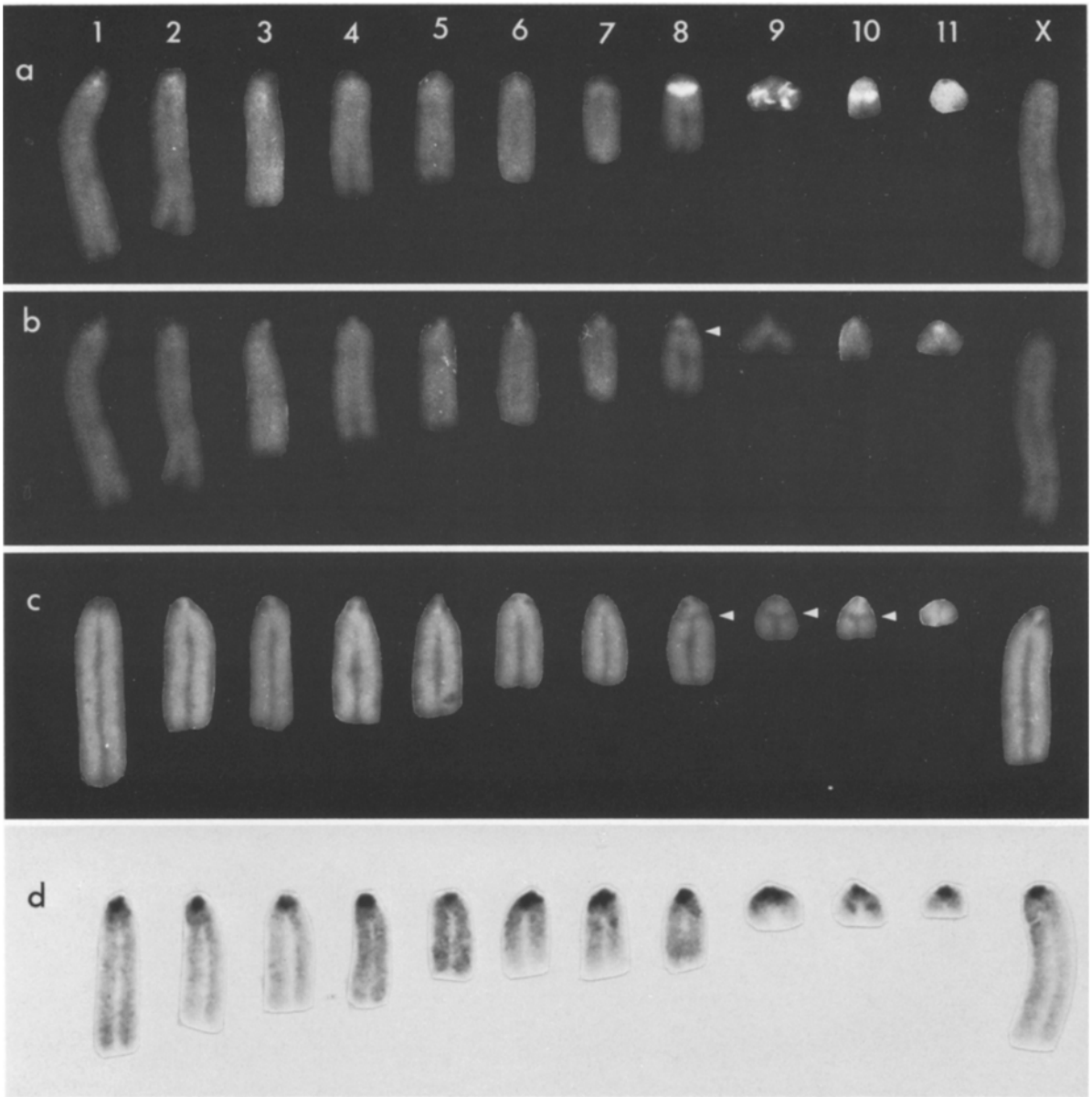


Fig. 3. *Urnisa sp.* Haploid complement **a** with CMA-fluorescence, **b** with DA-DAPI fluorescence, **c** with DAPI-AMD fluorescence, and **d** after C-banding. The *arrowheads* demarcate regions of negative fluorescence

fluorescence with CMA and are also DA-DAPI and DAPI-AMD negative. The centric band on the *X* fluouresces negatively with both CMA and DA-DAPI, while that of autosome *7* is DA-DAPI negative. This chromosome also includes a proximal C-band region in the form of a minute short arm which is both CMA and DAPI-AMD positive. The proximal region of the long arm, however, gives no differential fluorescence whereas the proximal regions of *8–11* all show intense fluorescence with CMA and give a positive, though reduced, fluorescence with DA-DAPI and a negative reaction with DAPI-AMD. The megameric *9* has a brightly CMA fluorescing and DA-DAPI negative interstitital band in addition to its proximal band. A similar small interstitial C-band on the *X* is also DAPI-AMD$^-$. Finally, the terminal band on *1* gives an intense positive fluorescence with CMA but responds negatively with DAPI-AMD.

Stenocatantops angustifrons (Fig. 5)

The neo *XY* ♂, neo *XX* ♀ sex chromosome system which characterises this species has evidently arisen by an *X5* fusion from a progenitor *XO* ♂ *XX* ♀ system of the kind still found in its sister species *Stenocantops vitripennis*. In *angustifrons*, all the chromosomes show a paracentromeric C-band but this is much smaller in the neo *Y* than in any other member of the complement. The fact that all the chromosomes of *vitripennis* carry a substantial paracentromeric C-band reinforces our earlier suggestion that the neo *Y* has lost heterochromatin since its partner fused with the progenitor uniarmed *X*. As a result of this it is now slightly reduced in size compared to its homologous arm in the metacentric *X*. All the chromosomes other than the neo *Y* have large centromeric C-bands, which morphologically look composite in character. This is confirmed by fluores-

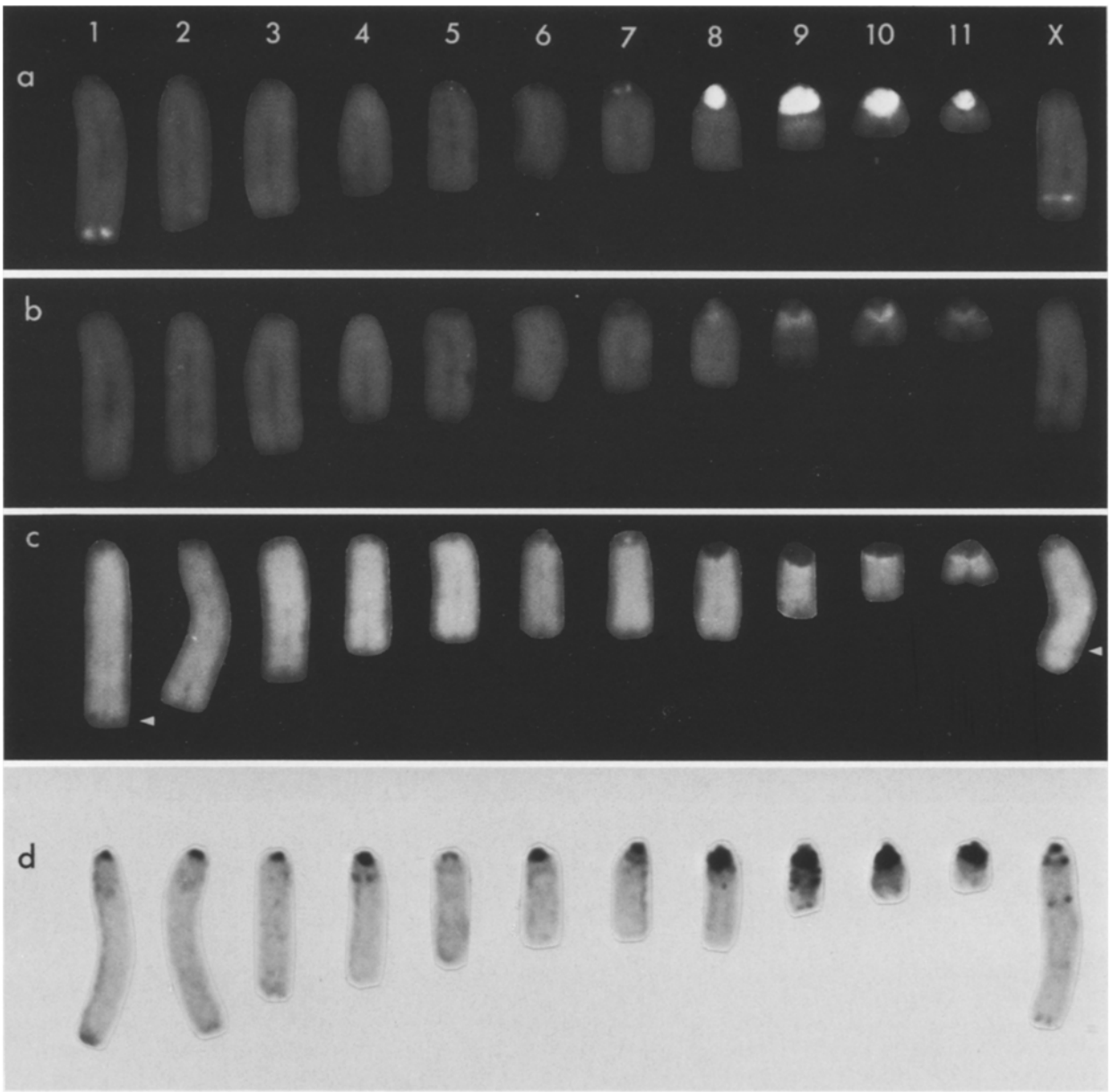


Fig. 4. *Froggatina australis*. Haploid complement **a** with CMA-fluorescence, **b** with DA-DAPI fluorescence, **c** with DAPI-AMD fluorescence, and **d** after C-banding. The *arrowheads* refer to regions of less obvious negative fluorescence

cence. In each case there is a very small CMA^-, DA-$DAPI^+$, DAPI-AMD^+ centric band which is double in the *X*, reflecting its fusion origin. In all cases, however, these regions are very much smaller in size than that of the C-bands themselves. The interstitial band on *8* is intensely CMA^+, but both DA-$DAPI^-$ and DAPI-AMD^-. Autosome *9* is again distinctive, reflecting its megameric behaviour at male meiosis, and is multi C-banded. The only band which fluoresces intensely in this chromosome, however, is the most distal which is CMA^+, DA-$DAPI^-$, DAPI-AMD^-. This is also the case with the interstitial band on *2*.

Pseudailopus keyi (Fig. 6)

As we have already mentioned, this species is distinctive by virtue of the fact that ten of the autosomes and the *X* carry short arms. These short arms have a variable C-band appearance. Those on *1, 4, 6, 8* and *10* are totally C-band positive. Chromosomes *2, 3, 5, 7, 11* and the *X* have short arms in which the distal portion is C-band positive but the proximal portion is not. Additionally, there is a subterminal C-band on *2*, faint interstitial C-bands on *6* and *7*, and a prominent interstitial band on *8*. Chromosome *9*, the megameric, is again multibanded.

The short arms of *1, 3, 4, 5* and *8* are DA-$DAPI^+$. That on *6* is polymorphic, including one variant which is CMA^+ and another which is not. The centric regions, which invariably show up as C-band positive and which are especially prominent in the biarmed members, where they often take the form of extended 'necks', all give an undifferentiated response to fluorochromes. Chromosome *9* has a very bright CMA^+ interstitial band which is DA-$DAPI^-$ and an equivalent band is also present in *10*.

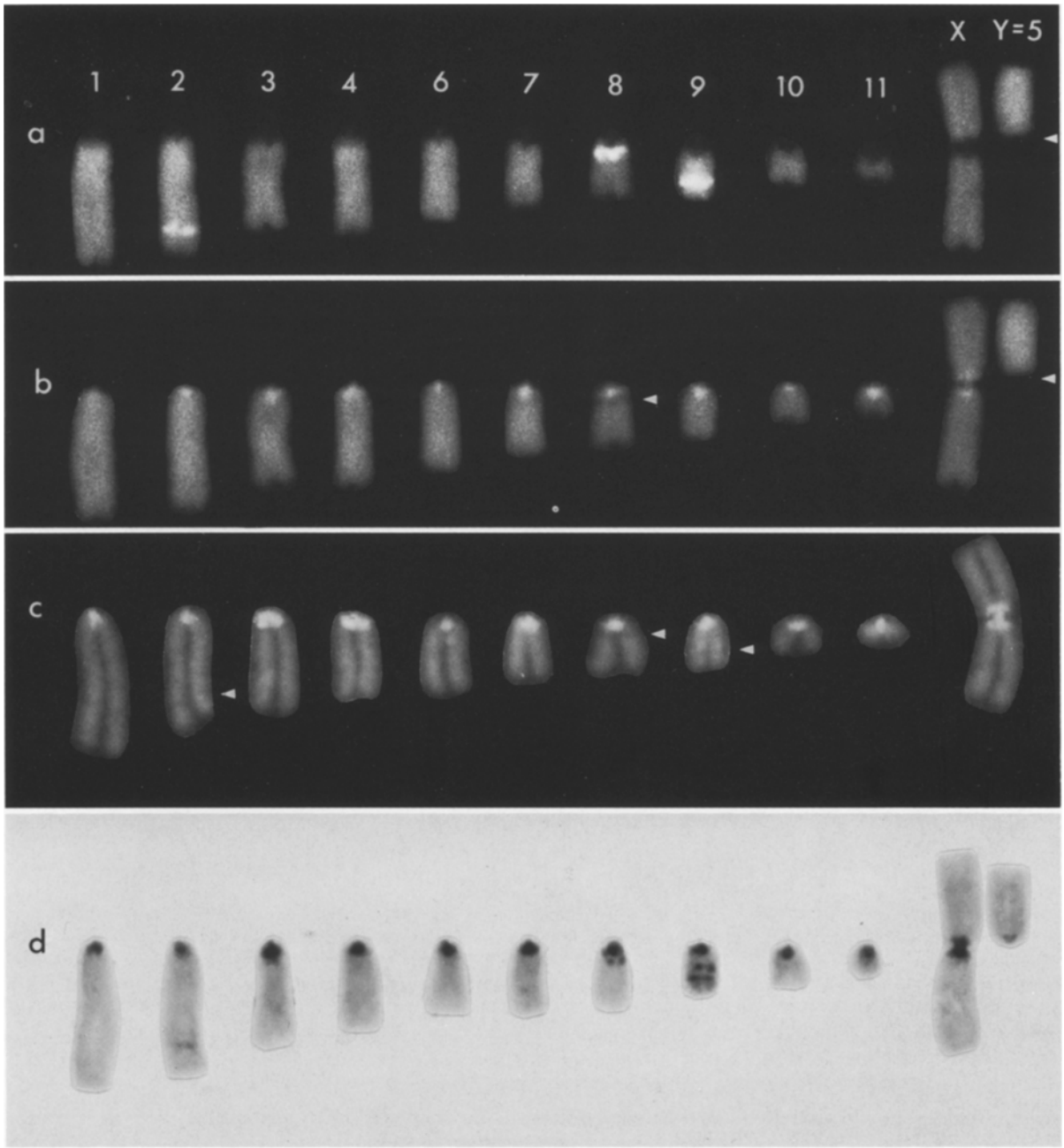


Fig. 5. *Stenocatantops angustifrons*. Haploid complement **a** with CMA fluorescence, male cell, **b** with DA-DAPI fluorescence, male cell, **c** with DAPI-AMD fluorescence, female cell, and **d** after C-banding, male cell. The *arrowheads* demarcate regions of less obvious negative fluorescence

Oxya japonica (Fig. 7)

The C-band arrangement of the standard members of the karyotype in this species shows a complex pattern of heterochromatin distribution. In addition to the paracentromeric bands present on all the chromosomes, which vary both in size and complexity, there are terminal bands on *4–10*, interstitial bands on both *2* and *8*, while *10*, the megameric, is again multibanded. The range of fluorescence response is even more striking. Of the paracentromeric C-bands, that on *1* shows no differential behaviour; those on *2, 3, 6* and *11* include CMA^+ centric regions, which are also DAPI-AMD^- in *2, 3,* and *6* and DAPI-AMD^+ in *11*. The remaining centric C-bands are all CMA^-, DAPI-AMD^-. The proximal regions of the paracentromeric C-bands on *3, 6–8, 10, 11* and the *X* are all CMA^+, intensely so in the case of *3, 8, 10* and the *X*. Negative DA-DAPI regions coincide with these in *3, 8, 10, 11* and the *X*.

Chromosome *10* is not particularly distinctive in terms of fluorescence, though it is certainly distinctive in terms of its C-banding. The two interstitial bands on *2* are both CMA^-, DA-$DAPI^+$, DAPI-AMD^+ while the intense interstitial band on *8* is CMA^+, DA-$DAPI^-$, DAPI-AMD^- giving the same reaction as the proximal band in this chromosome. Finally, the terminal C-bands, on *4–10* are all CMA^-, DA-$DAPI^+$, DAPI-AMD^+ though those on *6, 8*

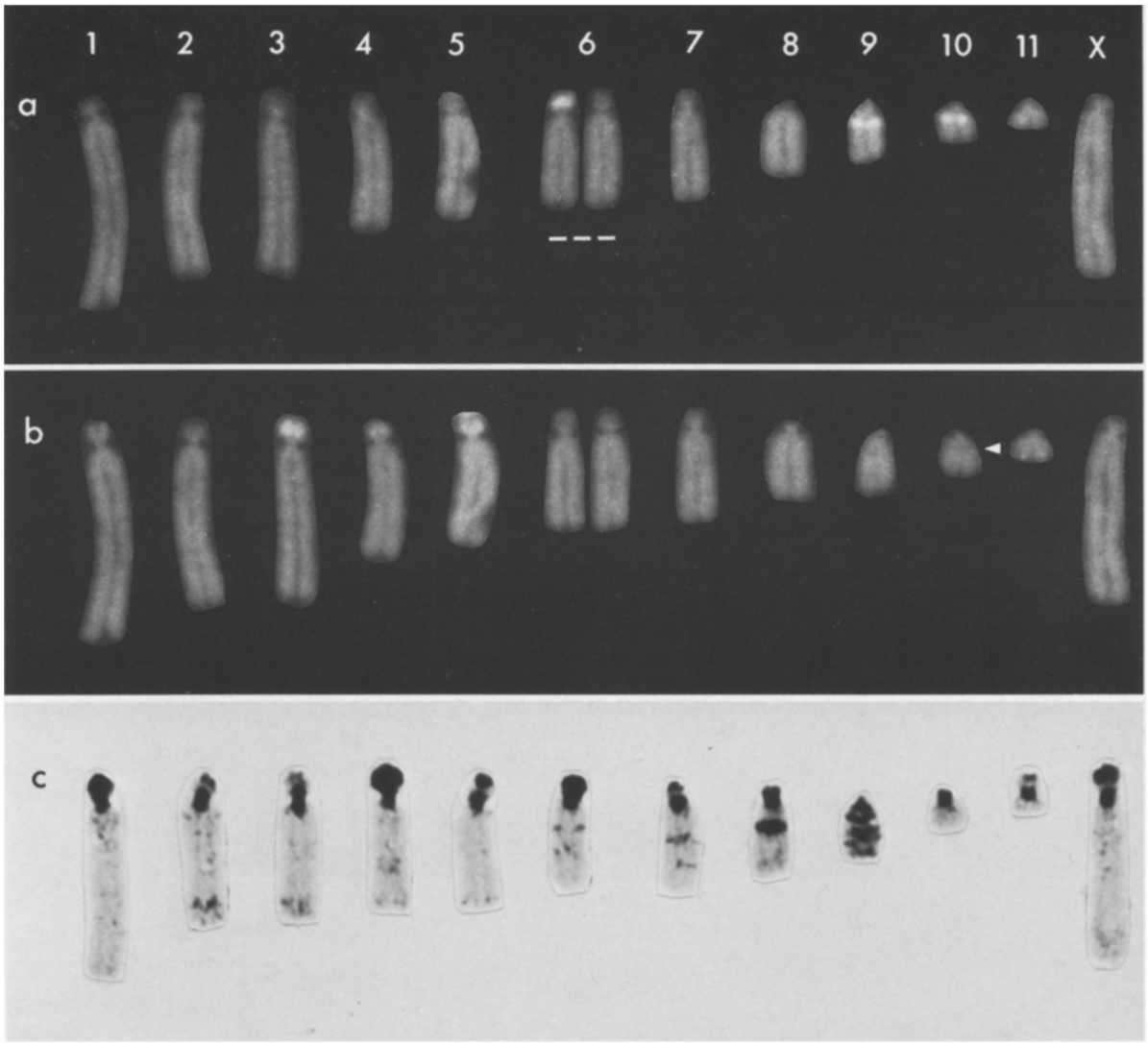


Fig. 6. *Pseudailopus keyi.* Haploid complement **a** with CMA-fluorescence, **b** with DA-DAPI fluorescence, and **c** after C-banding. Note the heteromorphism for the fluorescence behaviour of the short arm of autosome *6* (– – –) in **a**. The *arrowhead* demarcates a region of less obvious negative fluorescence

and *9* fluoresce more intensely with both DA-DAPI and DAPI-AMD.

Cryptobothrus chrysophorus, northern race (Fig. 8a, b)

The C-band pattern of this species has been described in detail in an earlier publication (John and King, 1977). It is complicated by the occurrence of two distinct chromosome races, North and South, which differ strikingly in heterochromatin content. Both races have centric blocks on all the chromosomes. These are C-band positive and are noticeably larger in the southern form. By contrast, chromosomes *4, 5, 6, 8* and *9* are consistently larger in individuals of the northern race, where they carry fixed terminal blocks of heterochromatin which, with the exception of those on *5*, are also C-band positive. Equivalent terminal blocks are not present on their southern homologues though polymorphisms for small terminal C-band regions are present on chromosomes *5, 6* and *8* in some of the southern populations. There are also differences in the banding character and sometimes the size of chromosome *7*, the megameric. Finally, both races carry polymorphisms for a series of supernumerary heterochromatic segments on one or both of the two smallest chromosomes, *10* and *11*. These are more variable, more often larger and more commonly C-band positive in the northern race. Additionally, the Forbes Creek population, which we have used as a source of southern individuals, is distinctive in carrying up to ten pericentric inversions and these are especially common in *3, 7* and *8*.

The two races also show some striking differences in their fluorescence patterns. The centric C-bands of the northern race while small are all CMA^+, $DA\text{-}DAPI^-$, $DAPI\text{-}AMD^-$. The larger centric C-bands of the southern race give a variable response. In the telocentric state only those of *8, 9, 10* and *11* are CMA^+, but whereas *8* and *10* are also $DA\text{-}DAPI^-$, $DAPI\text{-}AMD^-$ the centric C-bands of *9* and *11* are $DA\text{-}DAPI^+$, $DAPI\text{-}AMD^+$. Finally, while the centric C-bands of *3* and *7* do not fluoresce differentially when telocentric they, and the inverted centromere of *8*,

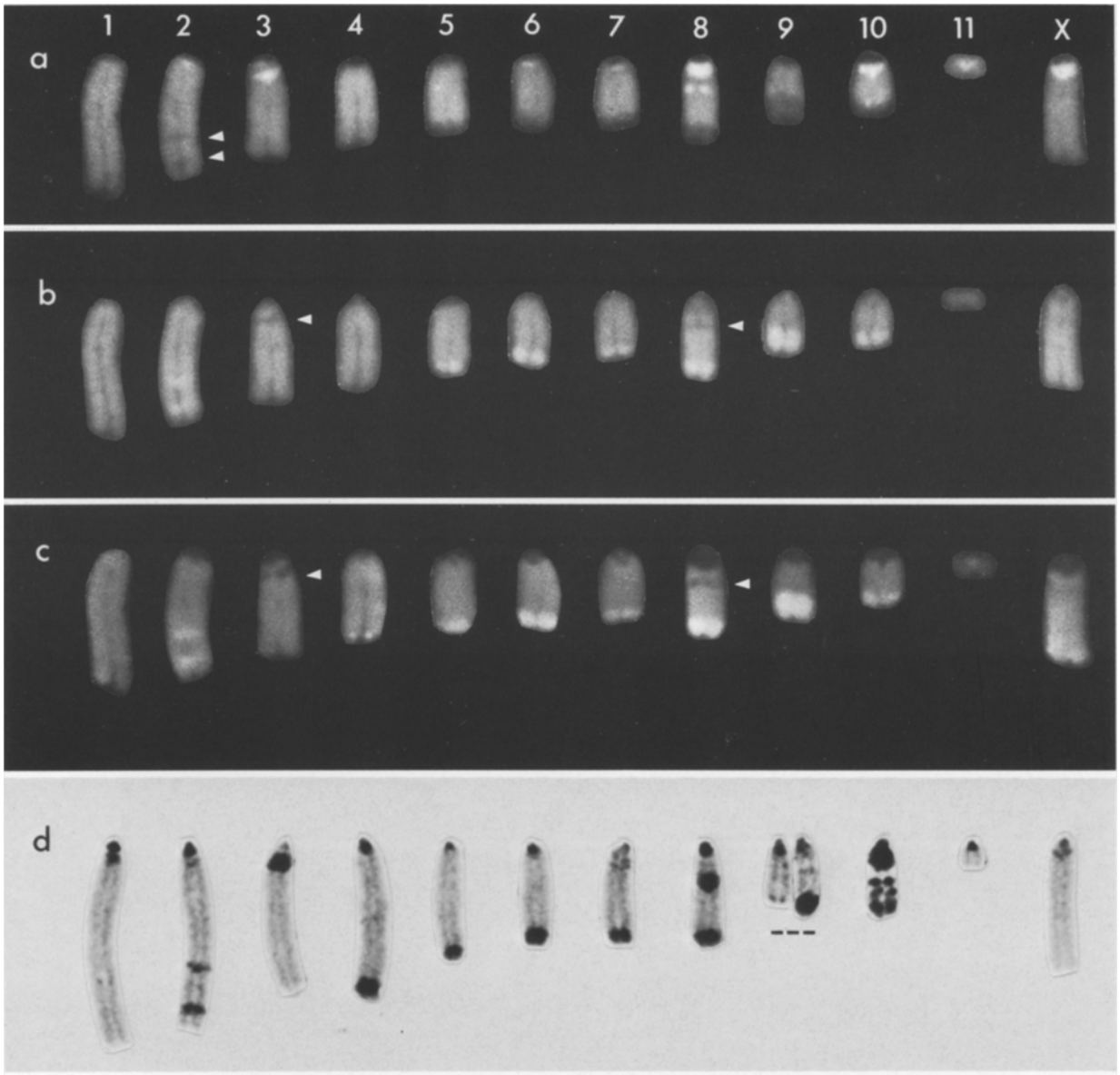


Fig. 7. *Oxya japonica.* Haploid complement **a** with CMA-fluorescence, **b** with DA-DAPI fluorescence, **c** with DAPI-AMD fluorescence, and **d** after C-banding. The *arrowheads* demarcate regions of less obvious negative fluorescence

show intense CMA^+ fluorescence, coupled with a $DA\text{-}DAPI^-$, $DAPI\text{-}AMD^-$ behaviour, when they occupy a submetacentric position.

In the northern race the terminal C-bands on band *8* are CMA^+, but those on *4, 5* and *9* do not exhibit any differential fluorescence. By comparison, the smaller polymorphic terminal C-bands on *6, 7* and *8* of the southern race are $DA\text{-}DAPI^+$ and $DAPI\text{-}AMD^+$. None of the supernumerary heterochromatic blocks present on either 10 or 11 of either race fluoresce differentially. As far as the interstitial C-bands are concerned those of *7* and *8* are CMA^+, $DA\text{-}DAPI^-$, $DAPI\text{-}AMD^-$ in both races, as too is the interstitial C-band found on chromosome *11* of the southern race.

Heteropternis obscurella eastern form (Fig. 9).

A detailed description of the C-band pattern of this species can be found in John and King (1982). There are substantial paracentromeric C-blocks on all the autosomes other than *9*, the megameric chromosome, which is however multibanded. There are terminal bands on *3–8*, any or all of which may be polymorphic either for size, for presence or absence, or for both features. There is also a distinctive interstitial band on *8*. Intensely CMA^+ material is present in the proximal C-bands on *5, 8* and *10*. the interstitial bands on *8, 9* and *10*, and the terminal bands on *11* and the *X*. Those on *8* and *9* are also $DA\text{-}DAPI^-$. The centric regions of *2–11* and the *X* are all $DA\text{-}DAPI^-$, $DAPI\text{-}AMD^-$.

Gastrimargus musicus (Fig. 10)

Paracentromeric C-bands are again present on all the chromosomes and are especially prominent on the short arms of *1, 2, 3, 4, 8, 9* and the *X*. Those of *5, 6* and *7*, on the other hand, are C-band negative while *10* and *11* both

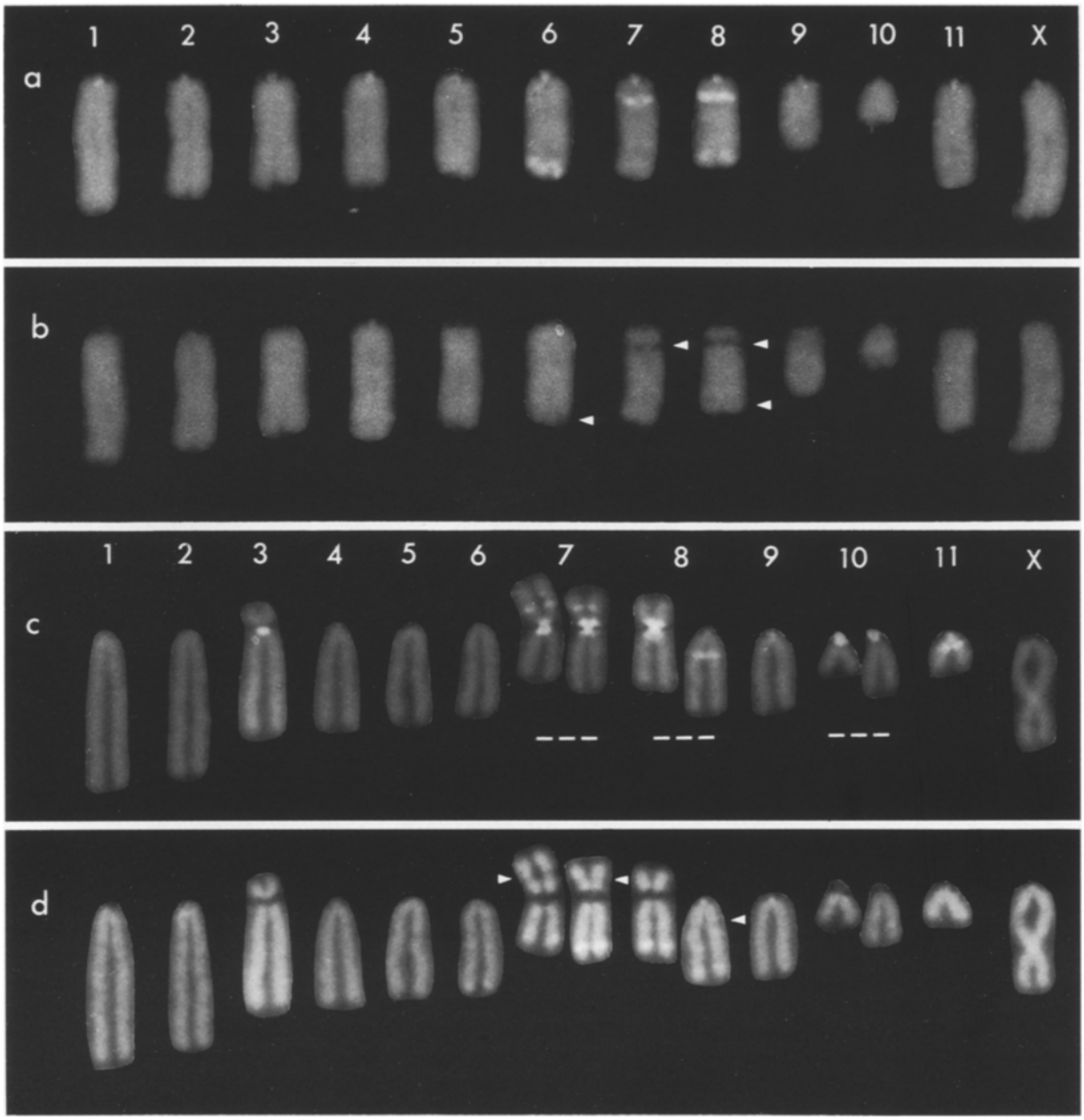


Fig. 8. *Cryptobothrus chrysophorus*. Haploid complements of northern (**a** and **b**) and southern (**c** and **d**) chromosome races; **a** and **c** with CMA-fluorescence, **b** and **d** with DAPI-AMD fluorescence. Note the heteromorphisms (– – –) for pericentric rearrangements in autosomes *7* and *8* in **c** and **d** as well as for supernumerary segments in 10 **c** and **d** and 11 **a** and **b**. The *arrowheads* demarcate regions of less obvious negative fluorescence

lack short arms (King and John 1980). The only other C-band regions are those found interstitially on *2, 8* and *9*, the megameric, where there is also a very small terminal C-band. With the exception of the *X*, which has a CMA^+, $DA\text{-}DAPI^+$, $DAPI\text{-}AMD^+$ centric region, neither the centromeres nor the short arms of any of the chromosomes show differential fluorescence. The proximal regions of *2, 8, 9, 10, 11* and the *X*, however, are $DA\text{-}DAPI^+$, $DAPI\text{-}AMD^+$, while the interstitial C-bands of *2* and *9* are intensely CMA^+ but $DA\text{-}DAPI^-$ and $DAPI\text{-}AMD^-$.

Discussion

The concept of equilocality, as originally defined by Heitz, was based solely on the location of heterochromatin within the chromosome complement. It said nothing about either the size or the structure of the heterochromatic segments involved. A summary of our fluorescence data (Table 3) indicates that even when heterochromatic segments occupy similar positions in non-homologous chromosomes within a species, they do not necessarily agree in size or in composition. This heterogeneity of heterochromatin, as inferred from differential fluorescence, implies that equilocality, as defined by position, does not invariably apply to structure. This finding has implications for any mechanism that attempts to explain equilocality.

From studies on C-band distribution in acridoid grasshoppers King and John (1980) were able to show that not only were there clear indications of an equilocal distribution of heterochromatin in these organisms but, additionally,

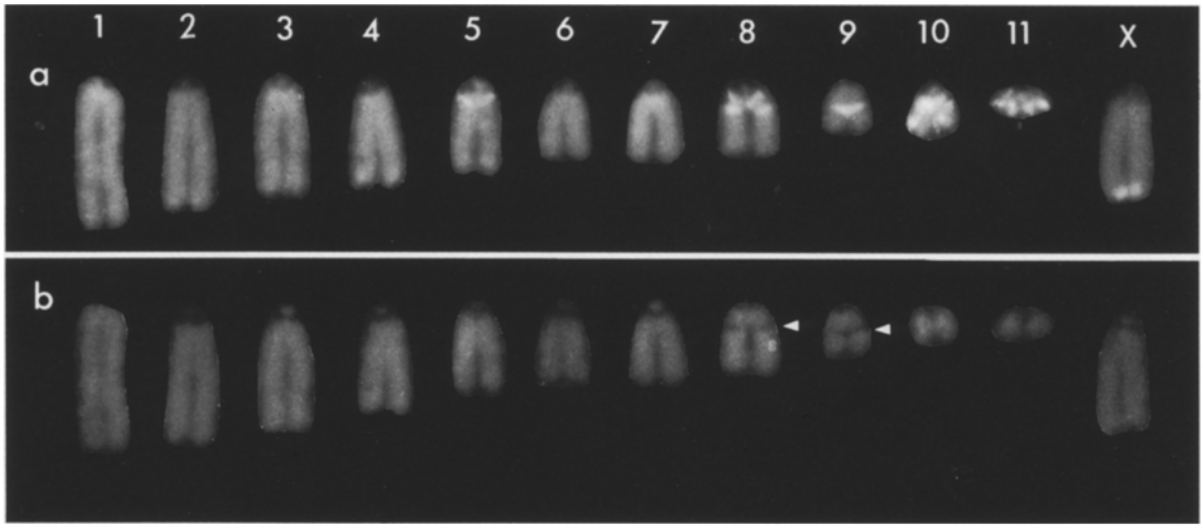


Fig. 9. *Heteropternis obscurella.* Haploid complement **a** with CMA-fluorescence, and **b** with DAPI-AMD fluorescence. The *arrowheads* demarcate regions of less obvious negative fluorescence

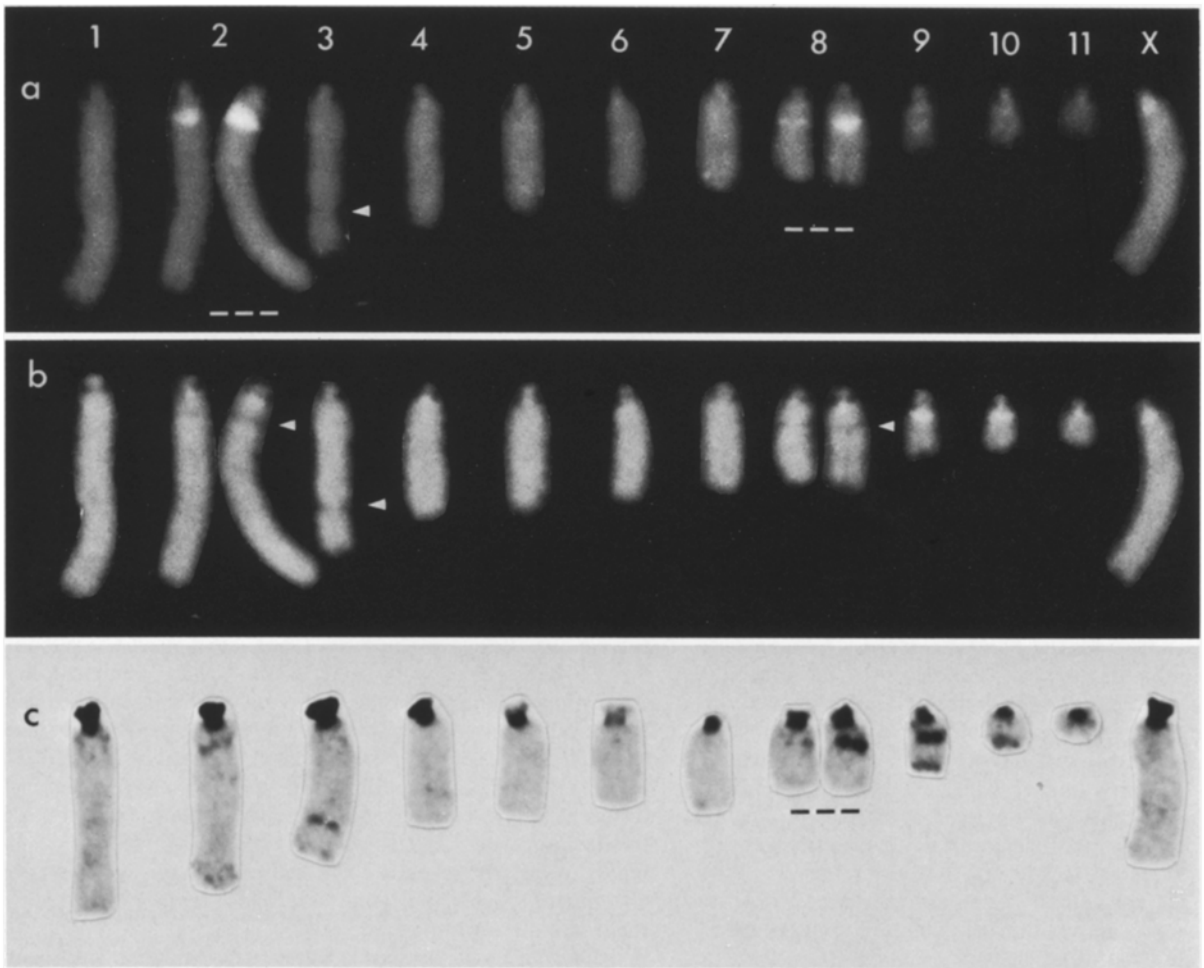


Fig. 10. *Gastrimargus musicus.* Haploid complement **a** with CMA-fluorescence, **b** with DAPI-AMD fluorescence, and **c** after C-banding. Note the heteromorphism for the intersitital bands in autosomes 2 and 8 (– – –) in **a** and **b**. The *arrowheads* demarcate regions of negative fluorescence

Table 3. Differential fluorescence of C-band positive regions in ten species of acridids

Species	Special characteristics	Dye	(1) centric C-bands										
			1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*X*
(1) *Apotropis tricarinata*		CMA	−	+	−	±	+	+	+	+	+	−	+	
		DA-DAPI												
		DAPI-AMD												
(2) *Rectitropis* sp. 1		CMA			+	+	+	+	+	+	+	+	+	+
		DA-DAPI												
		DAPI-AMD												
(3) *Urnisa* sp.		CMA												
		DA-DAPI					+	+	+	+				
		DAPI-AMD												
(4) *Froggattina australis*	*9* = megameric	CMA								⊕	⊕	⊕	⊕	−
		DA-DAPI							−	−	−	−	−	−
		DAPI-AMD								−	−	−	−	
(5) *Stenocatantops angustifrons*	*9* = megameric	CMA	−	−	−	−	−	−	−	−	−	−	−	−
	5 = neo *Y*	DA-DAPI	+	+	+	+	−	+	+	+	+	+	+	+
		DAPI-AMD	+	+	+	+	−	+	+	+	+	+	+	+
(6) *Pseudailopus keyi*	*9* = megameric	CMA												
	Proximal C-bands	DA-DAPI												
	= short arms	DAPI-AMD												
(7) *Oxya japonica*	*10* = megameric	CMA		+	+	−	−	+	−	−	−	−	+	−
		DA-DAPI												
		DAPI-AMD		−	−	−	−	−	−	−	−	−	+	−
(8) *Cryptobothrus chrysophorus*	*7* = megameric	CMA	+	+	+	+	+	+	+	+	+	+		+
N-race		DA-DAPI	−	−	−	−	−	−	−	−	−	−	−	−
		DAPI-AMD	−	−	−	−	−	−	−	−	−	−	−	−
S-race	I = inverted form	CMA			I				I	I	+	⊕	+	
		DA-DAPI			−				−	−	+	−	+	
		DAPI-AMD			−				−	−	+	−	+	
(9) *Heteropternis obscurella*	*9* = megameric	CMA												
		DA-DAPI	−	−	−	−	−	−	−	−	−	−	−	−
		DAPI-AMD	−	−	−	−	−	−	−	−	−	−	−	−
(10) *Gastrimargus muscus*	*9* = megameric	CMA												+
		DA-DAPI												+
		DAPI-AMD												+

N.B. Key: − = negative fluorescence, + = positive fluorescence and ⊕ = intense fluorescence. The dull background fluorescence seen in all chromosomes is not indicated in this table

that this equilocality was commonly reflected in the uniform behaviour of specific subsets of C-bands within a given complement. The fluorescence data allow us to take these observations a stage further in the case of the acridids. We would emphasise three points:

1) The only regions that fluoresce differentially in acridids are those that appear C-band positive with Giemsa.

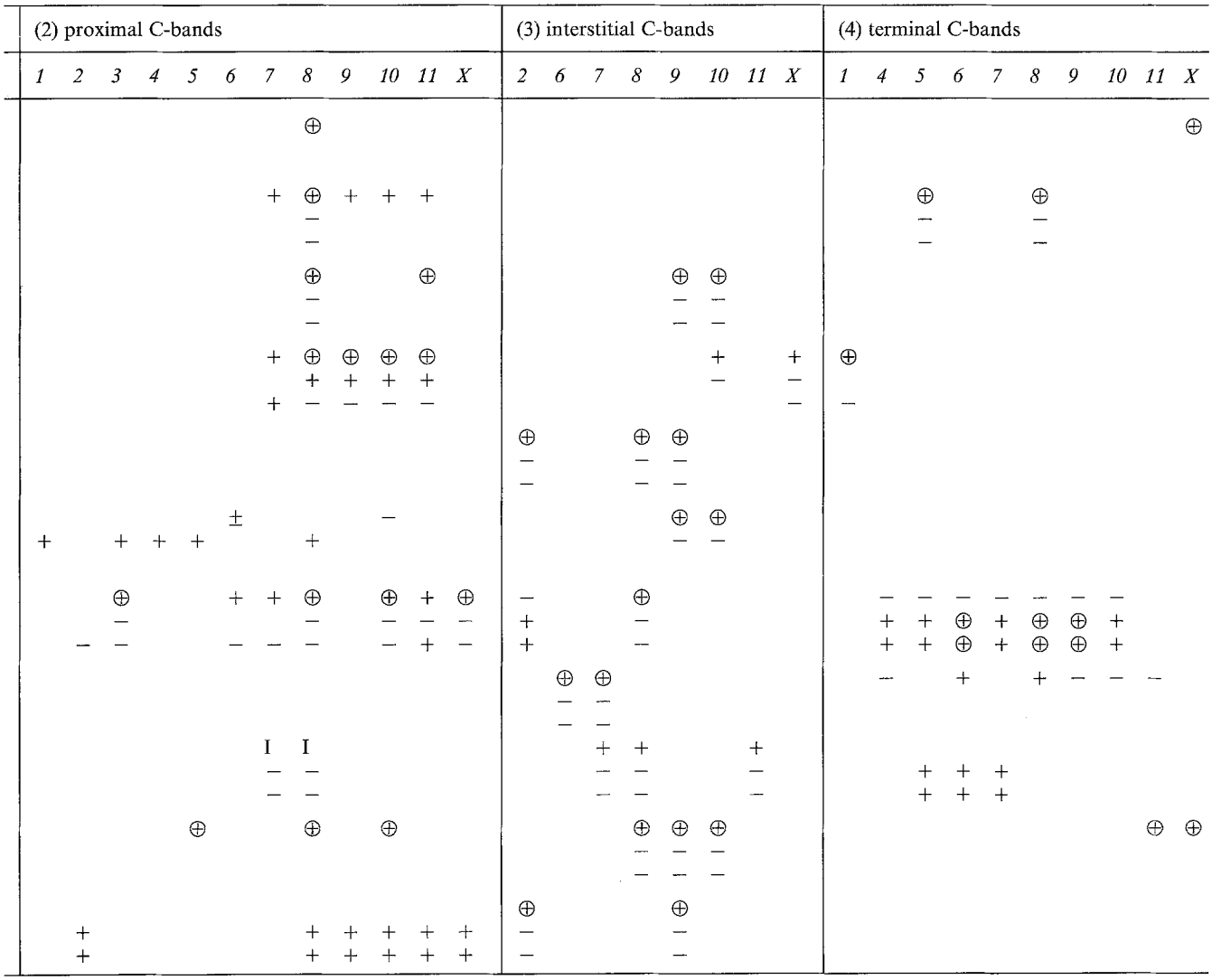
2) Not all C-bands, however, do fluoresce differentially. While DNA composition is the primary determinant of differential fluorescence, the possible influence of chromosomal proteins associated with the DNA remains to be clarified. Consequently, we are unsure whether the dull and non-differential fluorescence shown by a number of C-bands is a reflection simply of their similarity with euchromatin in terms of average GC-content. There is also a possibility that some of the very small bands seen following Giemsa staining may be below the limits of detection by the fluorescence technique.

3) There are some quite striking indications in our data that entire groups of chromosomes within a given species commonly accumulate heterochromatin with similar fluorescence behaviour at similar sites. This provides even more compelling evidence that the changes that go on in the heterochromatin content of a genome are not only equilocal but are also markedly non-random.

The most consistent form of equilocality shown within a species relates to the heterochromatic material found in the vicinity of the centromeres. Considering first the category of C-bands that we have defined as strictly centric, there is, in general, an impressive uniformity in their fluorescence behaviour with a given species. Thus in *Cryptobothrus* (N-race), *Stenocatantops, Heteropternis, Apotropis, Rectitropis* and *Oxya* a major fraction of the centric C-bands respond differentially to the same fluorochrome, or fluorochromes, in the same way. In *Urnisa* and *Froggattina,* on the other hand, a specific subset of C-bands, involving a cluster of chromosomes in a particular size class, respond in a uniform manner within each species.

When different species are compared they fall into four groups with respect to their response to CMA. First, there are those in which a majority of the centric C-bands are CMA^+. This includes *Apotropis, Rectitropis* and *Crypto-*

Table 3 (continued)



bothrus (N-race). In a second class, a majority of the centric C-bands are CMA^-, as is the case in *Stenocatantops* and *Oxya*. A third class, which includes *Pseudailopus* and *Gastrimargus*, have centric C-bands that do not respond differentially to CMA, while in a fourth group there is a heterogeneous response within any one species. The point of particular interest is that whatever causal events determined this differential response, they have tended to operate not only on an equilocal basis but also in a concerted fashion. A particularly striking instance of this is seen in the pericentric inversion chromosomes that characterise the southern population of *Cryptobothrus* from Forbes Creek. Here, the relocation of the centromere is invariably accompanied by a change in the fluorescence behaviour of the centric heterochromatin. This leads to an especially intense and pronounced CMA^+, DA-$DAPI^-$, DAPI-AMD^- response, accompanied by an enlargement of the proximal block. This raises the more general issue of the extent to which structural rearrangement provides an opportunity for changing the character of heterochromatin, and especially paracentromeric heterochromatin, within a genome. The neo *XY* mechanism, which characterises *Stenocatantops angustifrons*, results from a fusion of two telocentric chromosomes, autosome *5* and the *X*. While the fused *5* present in the neo *X* gives a CMA^-, DA-$DAPI^+$, DAPI-AMD^+ centric response, equivalent to that found in all ten telocentric autosomes, the free *5*, which now constitutes the neo *Y*, shows no differential fluorescence at the centromere region. Here, too, structural rearrangement has been accompanied by a modification in the character of centric heterochromatin. Schwarzacher et al. (1984) have drawn attention to what may prove to be a comparable phenomenon in the domestic pig. In this species the haploid complement includes 12 biarmed autosomes and 6 uniarmed autosomes, all of which carry substantial blocks of paracentromeric heterochromatin. That of the metacentrics is CMA^+ but that of the acrocentrics is DA-$DAPI^+$. If, as Schwarzacher et al. believe, this bimodal karyotype has arisen from an ancestral unimodal karyotype by a series of Robertsonian changes, then we have a further case in which structural rearrangement within the genome has been accompanied by a concerted change in the fluorescence pattern of centric heterochromatin.

An additional point from the fluorescence data is that, where paracentromeric C-blocks include both a centric and a proximal component, the latter, which is invariably larger than the former, does not necessarily fluoresce in the same way. This is particularly evident in the case of *Oxya, Pseudailopus, Stenocatantops* and *Heteropternis*. In all these cases the paracentromeric C-bands are evidently composite structures. Thus those proximal blocks of *Oxya* that are CMA^+ have centric bands that are CMA^- and vice versa.

The proximal blocks are also of interest because they, and the interstitial C-bands, more commonly show intense positive fluorescence with CMA. Even so, despite their uniformity within a species, they too show the same heterogeneity between species as the centric C-bands. They thus provide additional evidence for concerted patterns of change, which represent an alternative form of expression of the original concept of equilocality. One class of chromosome which, when present, often shows a particularly consistent form of differential fluorescence behaviour in respect of either the proximal, the interstitial, or both of these categories of C-band is the megameric chromosome. Within a species, however, it commonly behaves like other interstitially sited C-bands in respect of its response to fluorochromes, despite the fact that the segments in question are certainly not equilocally distributed.

Finally, if we turn to the terminal C-bands, these exhibit what is undoubtedly the most uniform pattern of behaviour in respect of fluorescence. This applies to all four species belonging to the genera (*Cryptobothrus, Heteropternis, Oxya* and *Rectitropis*) that show two or more such bands. Like the centric blocks, the terminal bands tend to fall into distinct intra-specific patterns with respect to their behaviour with fluorochromes. Those of *Oxya*, for example, are uniformly CMA^-, whereas those of *Heteropternis* uniformly fail to give any differential response to any fluorochrome.

Where C-bands on different chromosomes have the same fluorescence properties, it has been argued that there has been a co-evolution of highly repeated DNA on non-homologous chromosomes (Schweizer and Ehrendorfer 1983, Schmid 1980). This, however, is an over-statement, since in most cases we do not know the precise sequence organisation of the heterochromatic regions involved. Despite this, the equilocal arrangement of supposedly similar C-bands within a complement has led to the assumption that there must be a mechanism, or mechanisms, which either induce the formation of C-bands at, or else distribute C-band material to, specific and often equilocal sites within a genome. This it is argued, regulates the co-evolution of such material.

In biarmed complements, the tendency for equilocality of heterochromatin has been explained by Schweizer and Ehrendorfer (1983) on the assumption that the DNA sequences within a given C-band can be transferred to other equilocal sites as a consequence of the specific disposition of telomeres, centromeres and chromosome arms in a spatially ordered interphase nucleus. Following Bennett (1982, 1983), they accept a model of the interphase nucleus in which there is a separation of haploid genomes, accompanied by a unique ordering of the individual chromosomes within each haploid set. This ordering, in turn, is determined by the association of pairs of short and long arms of most similar length, so that non-homologous arms of most comparable length lie adjacent to one another.

Such an ordered arrangement has, for example, been held to explain the co-evolution of similar types and amounts of telomeric C-bands in similarly sized arms of non-homologous chromosomes within the same haploid set. By extension, it has also been held to explain the equilocal siting of interstitial C-bands by coupling it with the additional assumptions that: (1) the shortest arm in the complement that is devoid of interstitial C-bands roughly determines a pericentromeric region in the long arms with little or no banding (Greilhuber and Loidl 1983), and (2) the shortest distance between the centromere and the most proximal of the interstitial C-bands in a long arm corresponds to the length of the shortest, terminally banded, short arm (Loidl 1983).

The final assumption implicit in such speculations is that either sequence transposition or else sequence conversion, both of which are known to operate in repeated sequence DNA of the kind commonly represented in heterochromatic segments, is more likely to take place between arm regions that lie in proximity. This, of course, leaves unexplained the initial event that led to the production of the progenitor C-band, which forms the starting point for the whole operation, and which may well have depended on some form of sequence amplification. Amplification has also, presumably, been involved in the production of those heterochromatin polymorphisms in which the size of a given C-band can be shown to vary both between different individuals of the same population and between different homologues in the same individual. An alternative to sequence transfer as a basis for equilocal distribution, therefore, is that concerted amplification occurs at similar sites in non-homologues.

The Bennett model, which forms the basis of the explanations for equilocality adopted by both Schweizer and Ehrendorfer and by Loidl, has recently been criticised by Callow (1984). He points out that both the ordination technique used in the construction of the model, and the statistical validation of the model are lacking in rigour. However, even if the assumptions involved in the Bennett model are accepted as valid for biarmed complements, and there are indications that at least one of the predictions of this model cannot be sustained when chromosomes are structurally reorganised (Maguire 1984), they run into a major difficulty in complements which contain mixtures of uni- and biarmed chromosomes. Coates and Smith (1984) have used F_1 hybrids between the Moreton and Torresian taxa of the grasshopper *Caledia captiva* to examine an approximation to such a situation. The Moreton genome consists of distinctly biarmed chromosomes, all of which can be individually identified by centromere position and C-band pattern. The Torresian genome, on the other hand, has acrocentric chromosomes in which the small short arms are C-band positive and where individual chromosomes can be identified by length. Untreated, air-dried and C-banded neuroblast preparations from F_1 embryos obtained by crossing these two chromosomal taxa have a convenient, hollow and radial, metaphase configuration. From an analysis of such radial metaphase cells Coates and Smith find that, while some non-homologous chromosomes in the Moreton genome are organised non-randomly, there is no evidence that this non-randomness reflects a unique order of the kind predicted by the Bennett model, which is an order based on arm length similarity.

Completely uniarmed complements, of the kind commonly found in acridids, pose even more formidable prob-

lems. A logical extension of Bennett's model would imply that, in such cases, interphase order would involve progressively reducing size classes. This would leave a major discontinuity between the largest and smallest members of the complement so that they would be expected to lie adjacent to one another in a radial arrangement. Two earlier studies have examined the distribution of chromosomes in radial metaphase plates of this kind. Fox et al. (1975) analysed diploid neuroblast cells of *Locusta migratoria* and found no evidence to indicate that any combination of non-homologous chromosomes was associated in a consistent order. Nur (1973) carried out an equivalent examination of haploid second metaphase cells of *Melanoplus femur rubrum*, which also show a radial arrangement, and also reported a random distribution of chromosomes.

Bennett (1982) has argued that squashed cells of the kind used by Fox and Nur introduce a large amount of random chromosome distortion and cannot therefore be used to infer anything about the interphase order. Paradoxically, the initial observations that led to the inference that genome separation occurred at interphase were in fact made on squashed metaphase cells (Finch et al. 1981). It is, of course, appreciated that for the metaphase arrangement of centromeres and arms to reflect accurately their interphase distribution, requires that the formation of the spindle and the orientation of the centromeres on it leave centromere arrangement essentially undisturbed. In the giant neuroblast cells of acridids, with their hollow spindles, if the radial arrangement of chromosomes remains intact following squashing or air drying then it must follow that the treatment process can hardly have disrupted centromere arrangement. The key question, therefore, is to what extent the observed metaphase arrangement is indicative of interphase order, or the lack of it.

Neuroblasts are unique for two other reasons. First, because the spindle is hollow, the interphase nucleus frequently has a doughnut organisation with the centromeres arranged around the central hollow and the telomeres towards the outer periphery. Second, the interphase period of the cell cycle is very short so that the nucleus re-enters mitosis once replication is complete. This means that there is minimal time for any interphase movement of chromosomes. Under these circumstances, and given a fixed order within the interphase nucleus, it would be anticipated, that the metaphase arrangement ought to reflect this order. The fact that in *Caledia* and *Locusta* there is no consistent metaphase order, of the kind proposed by Bennett, implies that no such order exists at interphase either.

This does not deny the possibility that interactions, resulting from proximity, can and do occur between individual centromeres or groups of centromeres, or between individual telomeres or groups of telomeres, during interphase. Such regions are known to associate in various ways during the interphase period, especially in cases where they are heterochromatic (Comings 1980). Interactions of this kind, however, would not be those predicted from an ordered arrangement of the type proposed by Bennett. While we accept that the rules governing equilocality may differ in organisms with distinct patterns of karyotype organisation, we conclude that in acridids, at least, there is no evidence that a consistent ordering of chromosomes in the interphase nucleus, based on arm length, determines the patterns of equilocality that are apparent in the distribution of heterochromatic segments.

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