

New hypotheses about the origin of supernumerary chromosome segments in grasshoppers

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The existence of euchromatic supernumerary chromosome segments which do not seem to represent repeated material cannot be explained by the current hypotheses (duplication or translocation) concerning the origin of extra segments. An hypothesis is proposed to explain the origin of the euchromatic extra segments distally located on the megameric M_6 chromosome of the species *Omocestus bolivari* and *Chorthippus binotatus*, namely that these segments represent a relic of an euchromatic region which formed part of the ancestral M_6 chromosome of the gomphocerine grasshoppers. On the other hand, the absence of paracentromeric C-bands in some chromosomes of *Chorthippus vagans* and *Ch. brunneus* could have been achieved by means of transformation of heterochromatin into euchromatin.

INTRODUCTION

Two mechanisms have been proposed for the origin of the supernumerary heterochromatic segments (White, 1954, 1973), both involving the repetition of chromosome segments. Firstly, by translocation from a chromosome of the standard complement or a B chromosome. Secondly, by amplification of some part of the genome. Although the second hypothesis is accepted by the majority of authors (Shaw, 1971; John, 1973; Hewitt, 1979; Camacho *et al.*, 1984), we have recently obtained some evidence for the origin of an extra segment in the grasshopper *Oedipoda fuscocincta* by translocation from the megameric M_9 chromosome (Camacho *et al.*, 1986).

In this paper we analyse a series of supernumerary segments including two new forms of chromatin variation, one involving euchromatin and the other heterochromatin, whose origin needs to be explained by means of new hypotheses.

MATERIALS AND METHODS

Several natural populations of the grasshopper species *Euchorthippus pulvinatus*, *Stenobothrus festivus*, *Omocestus raymondi*, *Chorthippus apicalis*, *Ch. binotatus*, *Ch. vagans* and *Ch. brunneus* were sampled in the South of the Iberian Peninsula. The

cytogenetic techniques employed for analysing these materials were the same as in Camacho *et al.* (1984).

RESULTS

Euchorthippus pulvinatus (fig. 1A)

While the M_6 chromosome carries a subdistally located C-band in the majority of Spanish populations analysed (Santos and Giráldez, 1982), one male from the Sierra de Alfacar (Granada) was heterozygous for the presence/absence of such C-band. On the other hand, the S_8 is frequently polymorphic for a supernumerary segment located proximally and darkly C-banded (Santos, 1980; Riva *et al.*, 1984). We have found it in one out of 13 females analysed from Maitena (Granada).

Stenobothrus festivus (fig. 1B)

The M_7 and S_8 chromosomes may carry distal supernumerary segments which C-band darkly, although that on the M_7 is much larger than that on the S_8 .

Omocestus raymondi (fig. 1C)

This species shows variation for the size of the interstitial C-band in the X chromosome which

coincides with the location of the nucleolar organiser region in this species (Cabrero and Camacho, 1986b). Furthermore, there was variation for the presence of two types of darkly C-banded extra segments located distally on the M_7 chromosome.

Chorthippus apicalis (fig. 1D)

A darkly C-banded supernumerary segment located distally on the M_7 chromosome was found in Alcalá la Real population with the following karyomorph frequencies: 4BB, 8Bs, 1ss.

Chorthippus binotatus (fig. 1E)

We have found eight different supernumerary segments in Spanish populations of this species (Cabrero *et al.*, 1986). Here we will mention only the euchromatic segment located distally on the M_6 chromosome.

Chorthippus vagans (fig. 1F)

In this species, we have found C-band variation in three chromosome pairs: L_3 , M_4 and M_5 . Some L_3 chromosomes possess an interstitial extra C-band in their short arm while some M_4 and M_5 chromosomes lack the paracentromeric C-band.

Chorthippus brunneus (fig. 1G)

The majority of populations analysed were polymorphic for C-band variations and supernumerary chromosome segments. With the single exception of the L_3 , all remaining chromosomes show some type of variation. A more detailed examination of such a complex polymorphism will be made in a separate paper. The variations in heterochromatin found in this species can be classified into three types: (a) variation for the size of paracentromeric C-bands in the L_1 and L_2 chromosomes, (b) amplification of C-bands in the L_2 associated with a NOR, M_7 and S_8 chromosomes, and (c) additional C-bands, including small C-bands located interstitially on the X, M_4 and M_5 chromosomes, and larger C-bands on the M_4 , M_5 and M_6 chromosomes.

DISCUSSION

The amplification hypothesis could explain the origin of the majority of supernumerary heterochromatic chromosome segments analysed

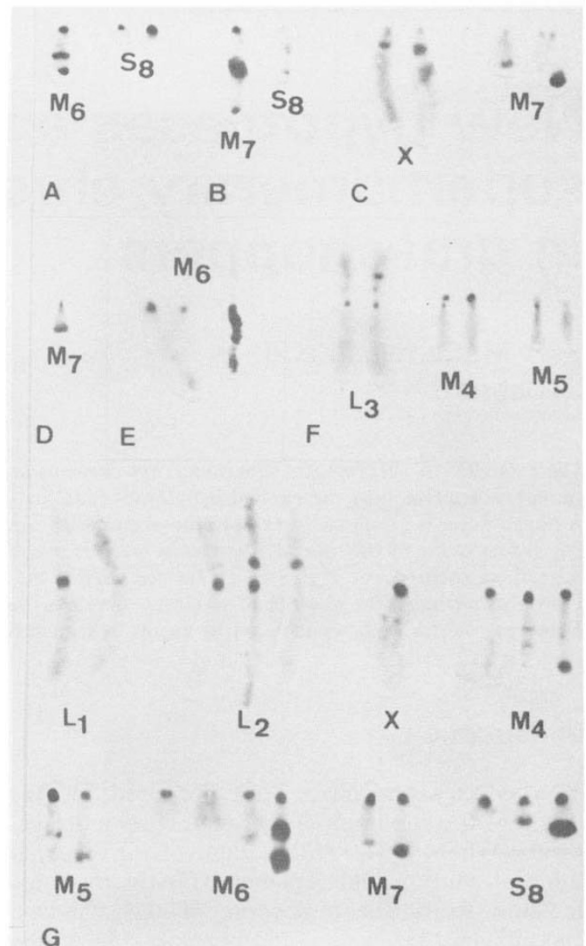


Figure 1 Supernumerary segments and C-band variations in gomphocerine grasshoppers. (A) *Euchorthippus pulvinatus*: unequal M_6 bivalent and heteromorphic S_8 pair. (B) *Stenobothrus festivus*: unequal M_7 and S_8 bivalents. (C) *Omocestus raymondi*: heteromorphic X chromosomes from a female embryo, M_7 chromosome with a small distal extra segment and M_7 bivalent with an unsegmented member and the other carrying a large extra segment. (D) *Chorthippus apicalis*: M_7 chromosome with a distal extra segment. (E) *Chorthippus binotatus*: euchromatic extra segment distally located on C-banded (left) and silver stained (right) heteromorphic M_6 bivalents. (F) *Chorthippus vagans*: C-band variations in L_3 , M_4 and M_5 chromosomes. Note the presence in the L_3 on the right of an extra C-band beyond the interstitial C-band on the short arm, and the absence of paracentromeric C-band in M_4 and M_5 homologues on the right. (G) *Chorthippus brunneus*: supernumerary segments and C-band variations in chromosomes from neuroblast embryo mitoses. L_1 : variation in size of paracentromeric C-band. L_2 : variation for the size of interstitial C-band on short arm and for presence or absence of paracentromeric C-band. X: interstitial extra C-band. M_4 : interstitial extra C-bands and subdistal extra segment. M_5 : interstitial and distal extra C-bands. M_6 : from left to right, basic chromosome, one lacking paracentromeric C-band, one carrying a grey extra segment and one possessing two darkly C-banded extra segments. M_7 : variation for the size of the distal C-band. S_8 : basic (left) and segmented chromosomes.

in this report, but it is especially consistent with the amplification of pre-existing C-bands observed in the S_8 of *E.pulvinatus*, the X of *O.raymondi* and the L_2 , M_7 and S_8 of *Ch.brunneus*. When an heterochromatic supernumerary segment is located in a chromosome region which is euchromatic in its partner chromosome, the amplification hypothesis needs to be coupled with the heterochromatinisation of the duplicated regions (John, 1973). However, two other types of chromatin variation found in our investigation seem to have arisen by other mechanisms, namely the disappearance of paracentromeric C-bands and the existence of euchromatic extra segments. On the one hand, the paracentromeric C-bands are absent from some chromosomes of *Ch.vagans* and *Ch.brunneus*. Because the presence of paracentromeric C-bands is the norm in gomphocerine chromosomes (Cabrero and Camacho, 1986a) and given that there is no change in morphology or size of these chromosomes (as would be expected in the case of a deletion), we propose that these paracentromeric C-bands have been lost by means of a process of transformation of heterochromatin into euchromatin, similarly to that reported for the paracentromeric C-bands in the neo-Y chromosome of the grasshopper *Stenacatantops angustifrons* (King and John, 1980). On the other hand, the existence of euchromatic extra segments in the M_6 chromosomes of *Chorthippus binotatus* (this paper) and *Omocestus bolivari* (Camacho *et al.*, 1984) contradicts all the existing hypotheses for the origin of extra segments. Although both extra segments are slightly different from one another in meiotic behaviour (that in *Ch.binotatus* associates distally with the unsegmented homologue, and that in *O.bolivari* does not show such association) both are similar in being euchromatic in nature. So, if they were composed of repeated chromosome material (such as would be expected from an origin by duplication or translocation), they must have been heterochromatinised like other chromosome repetitions (such as polysomic chromosomes) are in both species (Viseras and Camacho, 1984; Cabrero, 1985). The fact that the euchromatic extra segments in both species occupy a similar position (distal) in the same chromosome (the megameric M_6) seems to indicate that they could actually be a relic of an euchromatic region which formed part of the ancestral M_6 chromosome of the gomphocerine grasshoppers, a region which presumably has been lost in species other than *O.bolivari* and *Ch.binotatus*.

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