Heterochromatin variants in *Baetica ustulata* (Orthoptera: Tettigoniidae) analysed by C and G banding

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Extensive variation in heterochromatin exists in natural populations of *Baetica ustulata*, as deduced following the application of C and G banding techniques. These techniques enable us to describe nine C-heterochromatin variants for the M chromosomes (M_3 , M_4 and M_5) and to distinguish between the response of several classes of heterochromatin to G-banding. Different populations of this species are very different for the C-heterochromatin variants, which is due to their geographical isolation, the low mobility of these insects and the extensive intrapopulation variation which, as we propose, may be increased by meiotic recombination. Three classes of response to G banding show the different C-heterochromatic regions analysed. Firstly, a contrary response is shown by the nucleolar associated heterochromatin interstitially located in the long arm of L_2 chromosome so that it C-bands darkly but G-bands lightly. Secondly, a parallel response to both techniques is demonstrated by centromeric heterochromatin. Thirdly, the large heterochromatic blocks in the M chromosomes show dark C-banding but “grey” G-banding, being those in M_3 and M_4 surrounded by small dark G-bands.

INTRODUCTION

Intraspecific C-heterochromatin variation has been observed in many species of plants and animals. One of the possible significations of the polymorphisms for C-heterochromatin content rests on the influence that C-positive blocks have on both chiasma formation in the nearby regions to their location in the chromosome and mean cell chiasma frequency (Hewitt, 1979; John, 1983; Navas Castillo *et al.*, 1985). However, the characterisation of the different C-band variants is a necessary previous condition before generalising the results obtained from particular cases. In this sense, the heterochromatin of the standard complement seems to be heterogeneous (see Rocchi, 1982), and the supernumerary heterochromatin may show different responses to the same C-banding technique (Camacho *et al.*, 1984). In order to characterise the nature of heterochromatin variants it is advisable to use several different techniques which may discriminate between them. Although C-banding is easily obtained in orthopteran chromosomes, G-banding has only been well defined for the B chromosomes of *Chortoicetes terminifera* (Webb, 1976; Webb and Nehaus, 1979) and, with less definition, in standard chromosomes

of *Warramaba virgo* (Webb *et al.*, 1978) and *Melanoplus sanguinipes* (Zhan *et al.*, 1984).

Baetica ustulata is the only species of this monospecific genus, and is endemic in the Iberian Peninsula, living only in Sierra Nevada (Granada) at altitudes ranging from 2500 m to 3450 m (Pascual, 1978). This species has been studied cytologically by Fernández-Piqueras *et al.* (1984) in populations from the North side of Sierra Nevada, and they found an extensive polymorphism for C-heterochromatin. In this paper we analyse one population sample from the South side of Sierra Nevada using C and G banding techniques.

MATERIALS AND METHODS

Eleven males and seven females were caught in Alto del Chorrillo (2700 m altitude), which is situated at the South side of Sierra Nevada (Granada, Spain). Testes were fixed in ethanol-acetic acid (3:1). Females were injected with 0.05 per cent colchicine in insect saline for 6 hours prior to fixation of the ovarioles. C-banding was conducted as described by Camacho *et al.* (1984) and G-banding with trypsin followed by a brief treatment

with $2 \times \text{SSC}$ at 60°C . This G-banding technique was recently been put forward for mammal chromosomes by our colleagues M. Burgos, R. Jiménez and Dr R. Díaz de la Guardia (submitted for publication).

RESULTS

The chromosome complement of the ephippigerid *Baetica ustulata* consists of $2n = 24 + \text{XO}/\text{XX}$ chromosomes (fig. 1) and the 12 autosomal pairs may be divided into three size groups: Two long (L_1 and L_2), three medium ($M_3 - M_5$) and seven short pairs ($S_6 - S_{12}$). Both pairs of L autosomes are metacentric while the remainder, including the X, are telocentric. The size of the X chromosome is intermediate between that of the L and M autosomes. The C-banding pattern consists of small paracentromeric C-bands in all chromosomes, and there is an interstitial C-band in the long arm of L_2 chromosome which coincides with the only active NOR in this species (fig. 2). In addition there are prominent C-blocks located interstitially, subterminally and distally in the M chromosomes (fig. 1).

Fernández-Piqueras *et al.* (1984) have reported extensive variation for the C-blocks in the M chromosomes. In our population sample, collected from the South side of Sierra Nevada, there is also variation for the C-blocks in these chromosomes.

Some are similar and others are different from those observed by Fernández-Piqueras *et al.* (*loc cit.*) in North side populations. The C-heterochromatin variants for the M autosomes found in our population are shown in fig. 3. They were two for the M_3 , three for the M_4 and four for the M_5 . The most frequent type of M_3 (A-variant) carries two C-blocks, one subterminal and the other distal. This latter block is slightly larger than the subterminal one. The other M_3 variant (B) carries only the subterminal C-band.

The three types of M_4 chromosomes carry two C-blocks, one of which is subterminal the other distal. The A-variant possesses two C-blocks of similar size separated by an euchromatic short region, the B-variant is similar but with larger C-blocks, and the C-variant has a small subterminal C-block plus a larger distal one.

The M_5 is the most variable chromosome in our population caused by the presence of supernumerary chromosome segments. We found four different types: One lacking any distal C-heterochromatin (A), one carrying a small distal C-band (B), the third carrying a large distal C-block (C), and the fourth (D) carrying a distal C-block double the size of that found in C.

The G-banding technique gives a characteristic G-banding pattern in the L and M autosomes (fig. 4). The two L_1 chromosomes show a similar pattern of dark bands and light interbands which coincide

Figures 1 and 2 C-banded karyotype of a M_4-A/M_4-B heterozygous male (1), and silver stained pachytene cell showing the location (arrowhead) of the nucleolus organiser region (NOR) in *Baetica ustulata*. NU: nucleolus.

Figure 3 C-heterochromatin variants for M_3 , M_4 and M_5 chromosomes.

in their position and sequence. The same is apparent in the L_2 chromosomes but, here, one of the light interbands in the long arm coincides in position with the dark C-band associated to the NOR (see also fig. 5). The C-heterochromatin blocks in the M chromosomes show differential response to G-banding, so that some C-regions G-band darkly and others only lightly (figs. 6–10). The M_3 shows in the region of the C-heterochromatic blocks, a G-banding pattern consisting of two unequal “grey” interbands surrounded by three small dark bands, one being proximal, one sited between the two interbands (which presumably coincides with the euchromatic region between both C-blocks) and one distally located (figs. 6 and 7). A similar G-banding pattern shows the terminal region of the M_4 possessing two C-heterochromatic blocks. However, the two “grey” interbands are only slightly unequal in size (figs. 4–9), and a short euchromatic region exists beyond the latter dark G-band (figs. 6–9). The extra segment in the M_5D chromosome shows the same greyish response to G-banding that the C-blocks in the M_3 and the M_4 , but in this case it is not surrounded by dark G-bands (figs. 4 and 6). However, where this extra heterochromatic seg-

ment is less condensed a series of small bands and interbands are apparent (see fig. 7).

In an individual carrying two different M_4 chromosome variants (A and C) we observed incurved pairing configuration in pachytene cells (fig. 10), which indicates that homologous pairing is specific for the dark G-bands, but inspecific for the light interbands.

Meiotic segregation of the distal C-block in M_5 heteromorphic bivalents is always equational due to the formation of one chiasma in the region between the C-blocks and the centromere (figs. 11 and 12). In the M_3 bivalents we have occasionally observed chiasma formation in the euchromatic region between the C-blocks (see fig. 6), which might be a source of new chromosome variants when they occur in heteromorphic M_3 (and perhaps M_4) bivalents.

DISCUSSION

The present report analyses a population sample from the South side of Sierra Nevada, while Fernández-Piqueras *et al.* (1984) studied individuals from the North side of Sierra Nevada. Both localities are sited very far and completely isolated one another, given the low mobility of these apterous insects. In the North side populations, Fernández-Piqueras *et al.* (1984) found one type of M_3 and four types of M_4 and M_5 chromosomes on the basis of C-banding pattern. In the South side population, we have found two types of M_3 , three types of M_4 and four types of M_5 chromosomes, some of which are similar but others are different to those in the North side populations: The two types of M_3 in the South side are different to the only type in the North side (compare our fig. 3 with fig. 1a,b in Fernández-Piqueras *et al. loc cit.*). The M_4 type A in South side population and that type A in North side populations seems to be the same but, however, the two other M_4 variants in the former are different to the three other ones in the latter. Two out of the four M_5 variants found in both populations are the same (A and B), but the other two are different. In summary, these populations show more differences than similarities for the C-heterochromatin variants in the M chromosomes, because out of a total of 14 variants, three were present in both North and South side populations and 11 are only in one of them. This chromosomal difference between populations may be expected given their geographic isolation, the restricted movement of these animals and the high intrapopulation

Figures 4–10 G-banding in spermatogonia (4 and 5) and primary spermatocytes (6–10) of *Baetis ustulata*. (4) Mitotic metaphase of a male heterozygous for the M₅-B and M₅-D chromosome variants. Note the similarity in G-banding of both members of each of L₁, L₂ and M₄ chromosome pairs. Arrowheads indicate the NOR in L₂. (5) Partial mitotic prometaphase showing the sequence of bands and interbands in the terminal half of the M₄, and the position of the NOR (arrowhead) in the L₂. (6–9) Partial diplotene (6) and pachytene cells (7–9) showing the G-banding response of the C-heterochromatic blocks in the M₃, M₄ and M₅ chromosomes. (10) Incurved pairing configuration in pachytene of a male heterozygous for the M₄-A and M₄-C chromosomes.

Figures 11 and 12 C-banded primary spermatocytes at metaphase I (11) and anaphase I (12) showing the C-blocks in M₃, M₄, M₅ chromosomes, and the interstitial C-band in the long arm of the L₂ chromosome corresponding to the NOR (arrowhead). Note in (12) the equational division of the M₅ heteromorphic bivalent.

variation. An explanation for the latter is that it might be increased by meiotic recombination. Although C-heterochromatin has been reported as impeding chiasma formation in adjacent chromosome segments (John, 1981; 1983; Camacho *et al.*, 1984; Navas-Castillo *et al.*, 1985), in *Baetica ustulata* the euchromatic regions pairs homologously with preference over heterochromatic ones (fig. 10) and a chiasma may occasionally form in the short euchromatic regions between two C-block (fig. 6). Whenever this occurs in heteromorphic bivalents, new intermediate C-heterochromatin variants would be expected to arise.

The comparison of C and G banding patterns demonstrates several interesting facts. Firstly, while the NOR located interstitially in the long arm of the L₂ chromosome C-bands darkly it only forms a light G-band. In contrast, the centromeric heterochromatin of all chromosomes stains heavily

with both C and G banding. This indicates substantial differences in the nature of centromeric and nucleolar associated heterochromatin. Secondly, as the G-banding technique reveals, the C-heterochromatic blocks in M₃ and M₄ chromosomes show a significant difference compared with the supernumerary segment of the M₅. While the former are surrounded by darkly G-banded chromomeres, the extra segment of M₅ does not. These chromomeres may act as stabilising structures and regulate the coiling properties of their heterochromatic regions lying nearby.

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