NOTES AND COMMENTS

POLYSOMY AND SUPERNUMERARY ISOCHROMOSOMES IN THE GRASSHOPPER *OMOCESTUS BOLIVARI* (CHOPARD)

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1. INTRODUCTION

POLYSOMY is not a frequent phenomenon in grasshoppers, particularly at the level reached by the M_4 -tetrasomy in *Chorthippus parallelus* (Hewitt, 1963; Southern, 1967; Hewitt and John, 1968; 1970). The polysomy has been regarded as a possible source of formation of B-chromosomes, especially those of some Pyrgomorphid grasshoppers (Hewitt, 1979). Supernumerary chromosomes are frequently found in grasshopper species, and they have been detected in about 80 species of Acridoids (see Hewitt, 1979). B-chromosomes of grasshoppers have been divided into four categories in relation to their sizes and centromeric position, one of these being the supernumerary isochromosomes which are usually quite stable in cell divisions (Hewitt, 1979). The present report describes the polysomy and the iso-B-chromosomes which we have found in *Omocestus bolivari*, a gomphocerine grasshopper.

2. MATERIAL AND METHODS

Adult individuals of *Omocestus bolivari* have been caught at three localities of Sierra Nevada (Spain). The sample from one of the localities (Campos de Otero: CO) consisted of 6 males and 3 females; that from Charca (CH) only 400 m. away consisted of 35 males. The sample from the more distant locality (Alto del Chorrillo: ACH) consisted of two males and two females. Testes were fixed in 1:3 acetic alcohol and subsequently squashed in acetic orcein. Females were treated with colchicine for 8 hours before fixation of ovarioles and gastric caecae, from which squashes were made in acetic orcein.

3. RESULTS

The standard complement of *O. bolivari*, like all other species of *Omocestus* so far examined cytologically, consists of three pairs of metacentric and five pairs of acrocentric autosomes together with an $XO\delta:XX\phi$ sex determining mechanism, so that $2n\delta = 17$ and $2n\phi = 18$. The autosome pairs can be divided in three size groups: 3 long (L_1-L_3), 3 medium (M_4-M_6) and 2 short (S_7 and S_8). The X-chromosome is the fourth in order of decreasing size (fig. 1a). In the primary spermatocytes the basic complement showed 11 bivalents plus the X-univalent. The M_6 bivalent is the megameric element.

(i) *The polysomy*

In the CO population we have observed polysomy for the M_4 autosome in the male germ line. Out of the six males examined one was completely M_4 -tetrasomic in the testes. Two other males were mosaic M_4 -tetrasomics, so that the number of M_4 elements varied between follicles but not among the cells within the same follicle. The three remaining males were normal disomics for the M_4 pair. In both the spermatogonial mitoses and during prophase I of meiosis, the extra M_4 's show differential heteropycnosis in relation to the normal M_4 elements. Thus in pachytene and diplotene cells the two extra M_4 are always paired forming a positively heteropycnotic bivalent (fig. 1b and c). Quadrivalents are not found. In 42 (60 per cent) of the 70 metaphase I cells examined the two extra M_4 formed a bivalent with a more terminalized chiasma than the normal M_4 bivalent (fig. 1d). In 26 (37 per cent) metaphase I cells the two extra M_4 were not associated (fig. 1e), and in 2 cells (3 per cent) both elements were observed with a secondary association. In consequence, in anaphase I the two extra M_4 can be observed going either to the same pole (in the majority of the cells) or to different poles (see fig. 1f and g). No variation for the number of M_4 elements was found in the three females studied; however, one of these did carry a medium sized metacentric supernumerary chromosome (fig. 1a). This was an isochromosome similar to the one found in males from the two other localities.

(ii) *The supernumerary isochromosomes*

In the CH population, situated close to Campos de Otero, polysomy for the M_4 chromosome was not detected in any of the 35 males analyzed; two males were however carriers of the metacentric iso-B-chromosome. The metacentric iso-B was also found in one of the two males taken from the ACH locality. In this male the meiotic behaviour of the supernumerary was studied. The iso-B is a mitotically stable element and shows positive heteropycnosis during prophase I (fig. 2a-c). In the majority of the cells at zygotene the iso-B can be observed associated with the X-chromosome (fig. 2a). This association is also seen at diplotene in 80 per cent of cells (fig. 2b). At metaphase I, however, no association of the two elements was observed. At diplotene and metaphase I the two arms of the iso-B are frequently found paired, and ring B-univalents can be observed in the majority of cells (fig. 2b-d).

In order to study X-B segregation patterns at anaphase I we have scored 120 secondary spermatocytes (at prophase II and metaphase II); of these 26 were $n = 8$, 30 $n = 8 + X$, 31 $n = 8 + B$ and 33 $n = 8 + X + B$ (fig. 2e-g). These numbers are not significantly different from those expected assuming random segregation at anaphase I ($\chi^2 = 0.92$, $P = 0.8-0.9$). Finally, we have observed equational division of the iso-B in the first meiotic division in prophase II cells (fig. 2h).

4. DISCUSSION

(i) *Polysomy in the male germ line*

The M_4 polysomy observed in *Omocestus bolivari* closely resembles that found in *Chorthippus parallelus* (Hewitt and John, 1968). In both species

Plate I

FIG. 1.—Standard karyotype, iso-B-chromosomes, and M₄-tetrasomy in *O. boliviari*. (a) Karyotype of a female from CP population with one iso-B. (b)-(g) M₄-tetrasomy in the germ line of some males from CO population. (b) and (c), pachytene and diplotene cells showing precocious condensation of extra M₄ bivalents; (d) and (e), metaphase I cells showing the two extra M₄ forming one bivalent and two univalents, respectively; (f) and (g), anaphase I cells showing the extra M₄ elements going to opposite poles and to the same pole, respectively.

Plate II

FIG. 2.—Iso-B-chromosomes in *O. bolivari*. (a)-(d) Primary spermatocytes showing X-B association and ring B-univalent formation. (a), zygotene cells; (b) and (c), diplotene cells; (d), metaphase I cell. (e)-(g) X-B segregation at the first meiotic division, as it is inferred from the chromosomal constitution of the secondary spermatocytes. (e), prophase II cell with $n = 8 + X + B$; (f) and (g), metaphase II cells with $n = 8 + B$ and $n = 8 + X$, respectively. (h) Equational division of the iso-B at the first meiotic division, shown by the presence of a single B-chromatid in this prophase II cell.

the existence of mosaic individuals indicates that the polysomy probably originated as a result of mitotic instability of M_4 elements in the mitoses which gave rise to the different follicles in the testis. This must imply a differential survival of the different products of such instability since monosomic or nullisomic cells have not been found.

In *Chorthippus jucundus* we have found a M_4 -tetrasomic diplotene cell in a male which was disomic in all other cells (Camacho *et al.*, unpublished). This indicates that in this species also the M_4 has a tendency to mitotic instability.

(ii) *The iso-B-chromosomes*

The supernumerary metacentric chromosome found in *O. bolivari*, can be included in the category of the iso-B-chromosomes because of the meiotic pairing of its two arms and their similar length.

In *Myrmeleotettix maculatus* interstitial chiasmata in the ring B-univalents have been observed (John and Hewitt, 1965). In *O. bolivari* we have not observed this, but the persistence of the ring B-univalents at metaphase I indicates that presumably the association between the two arms is by homology. The formation of ring univalents seems to be a general characteristic of the supernumerary isochromosomes of grasshoppers, and they have been observed also in other species of grasshoppers, e.g., *Melanoplus femur-rubrum* (Stephens and Bregman, 1972) and *Phaulacridium marginale* (Westerman, 1975).

The regularity in the X-B segregation and the mitotic stability seem to characterize the iso-B's of grasshoppers. However, the only two cases where an accumulation mechanism caused by a preferential segregation in females has been demonstrated, are for iso-B's of two species of grasshoppers: *M. maculatus* (Hewitt, 1973) and *M. femur-rubrum* (Lucov and Nur, 1973).

At present we have no evidence that there exists any relation between the M_4 -polysomy and the origin of the iso-B's in *O. bolivari*.

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