NOTES AND COMMENTS

A TANDEM FUSION ORIGIN OF A NEO XY SEX DETERMINING MECHANISM IN THE LONG-HORNED *CALLICRANIA SEOANEI* (BOL.)

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1. INTRODUCTION

ONLY seven species of Tettigonioida have been recorded with a neo XX - XY system derived from an XX - XO mechanism, including that of *Pycnogaster cucullata* (unpublished data).

All previous cases described have arisen through the usual process of centric fusion between an acrocentric X and an autosome. The present paper deals with the structure of a neo XY sex pair originating by tandem fusion in the species *Callicrania seoanei*. In this, unlike centric fusion systems, the sex bivalent divides equationally at first anaphase. An undescribed species (P45b) of Australian morabine (White *et al.*, 1967), is the only other species of orthopteroid insect where the neo XY system seems to stem from a tandem fusion.

Tandem fusion has also occurred in multiple sex chromosome systems of *Warramaba virgo* (White *et al.*, 1973). Post-reductional XY systems have also been cited in the mice of the genus *Apodemus* with the exception of *A. geisha* which invariably shows pre-reduction (see White, 1973, pp. 594). Similar mechanisms though perhaps not due to recent tandem fusion probably exist in the centipedes *Thereuonema hilgendorfi* and *Thereuopoda clunifera* (Ogawa, 1950, 1952; in White, 1973, pp. 605).

The acrocentric neo X in the Acridoidea *Dichroplus silveiraguidoi* could be derived by an inversion from a metacentric neo X , or possibly the original mutation was a tandem fusion of an autosome with the distal end of the X (see Hewitt, 1979).

2. MATERIALS AND METHODS

The Tettigonioid *Callicrania seoanei* (Bol.) is endemic to the Iberian peninsula where it is found on shrubs from northern Portugal and Spain to the Pyrenees (Gangwere and Morales Agacino, 1970, pp. 47).

All 20 individuals collected over the past two years from several populations in León (Spain): from Truchas, Nogarejas and Tera, had a neo XY sex chromosome mechanism. Two individuals collected from a population of Pajares (Santander) had an XO mechanism.

A 0.05 per cent solution of colchicine was injected into the abdomen of females eight hours before the gastric caeca were removed. Both pretreated gastric caeca and untreated testis material were submitted to

hypotonic treatment in 0.5 per cent potassium chloride for 15 min, and fixed in acetic-alcohol (3:1) for 2 hours. Conventional acetic orcein squash preparations were used as well as C-banding and silver-staining techniques. For C-banding we have employed the technique described by Sumner (1972) with slight modifications, including trypsin and 2XSSC. All these techniques produce positive results in species of related genera *Pycnogaster*, *Baetica* and *Steropleurus* (unpublished data). For NOR detections a silver impregnation technique was used (Bloom and Goodpasture, 1976; Goodpasture and Bloom, 1975).

3. RESULTS

(i) The chromosome complement

In these populations we have found two main karyotypes of *Callicrania seoanei* (fig. 1). A basic disomic karyotype consists of 22 chromosomes and XX (female) XO (male) sex chromosomes (fig. 1a). There is one large metacentric pair (L_1), five pairs of medium autosomes (M_2 - M_6) with a

FIG. 1.—The basic karyotypes of males and females of *C. seoanei*. a. An XO male karyotype. b. A neo XY male karyotype with two extra chromosomes (tetrasomy). c. Karyotype of neo XX female showing 2 to 4 extra chromosomes (Polysomy).

Plate I

FIG. 2.—a. Prophase nucleus obtained by C-banding technique. Note X_L segment and other positive heteropycnotic regions belonging to megameric and extra chromosomes (arrows). b. Idem. obtained by conventional acetic orcein stain. All heteropycnotic regions are localised inside nuclear envelope. c. Diplotene cell with an extra-chromosome (arrow). Note interstitial chiasma on neo XY sex pair. d. Metaphase I with the neo XY sex bivalent showing interstitial chiasma. e. Non-reductional segregation of the neo XY at anaphase I. f. Exceptional reductional segregation of the neo XY at anaphase I. g. Metaphase II cell with a neo XY chromosome (univalent). h. Equational segregation of the neo XY sex chromosome at anaphase II.

Plate II

FIG. 3.—Sex bivalent at diplotene (a–c) and prometaphase (d and e). Silver staining (f–l).
a and d. Interstitial chiasma.
b. Subterminal chiasma.
c and e. Terminal chiasma. In this case note that there is heteropycnotic material around the secondary constriction (arrow).
f. Silver stained prophase nucleus. Arrows denote the silver positive region. X_L shows intermediate stain.
g. Zygotene: nucleolus is indicated (arrow).
h. Negative heteropycnosis of the neo X (X_L) at metaphase I.
i. Post-meiotic reactivation of the NOR activity in spermatids nuclei.
j and k. Silver stained at diplotene. Dark region (nucleolus and NOR regions) is located on M_3 bivalent.
l. NOR activity during mitotic prophase.

terminal centromere (with the exception of the M_3), and five pairs of short chromosomes (S_7 - S_{11}). Secondary constrictions are observable on M_3 and M_4 bivalents. The X is a telocentric univalent and a megameric S-pair shows its heterochromatic content in early prophase nuclei as well as in C-banded preparations (see fig. 2a and b.).

The second basic karyotype differs from the former by the absence of the M_2 pair and includes a neo XX (female) XY (male) sex chromosome pair. In the neo XY populations seven males and one female were apparently polysomic in both the germ and the somatic line for the megameric short bivalent (Fig. 1b and c).

(ii) *The neo XY sex bivalent*

The structure and meiotic behaviour of this heteromorphic sex bivalent is shown in fig. 2 and 3. This system is presumably derived from a tandem fusion in a progenitor XO - XX system as in the karyotype of the Pajares population. This tandem fusion results from a distal break in a telocentric M-autosome and a proximal one in the X -chromosome. This gives a telocentric neo X , larger than the primitive X , combining the progenitor X (X_L limb) with one of a pair of autosomes (X_R segment) the partner of which becomes neo Y . The progenitor autosome was probably the M_2 in the XO state, but the X_R segment is shorter than its free partner neo Y presumably as a consequence of tandem fusion.

Neo X and neo Y retain an euchromatic appearance during meiotic prophase as well as their primitive homology. A single chiasma is formed (never 0 or 2) but its position is somewhat variable: it may be either interstitial or close to the centromere, but never in a distal position.

The X_L is heteropycnotic at first meiotic prophase and a secondary constriction is observable just between X_L and X_R (fig. 3). An individual (CsT δ 9) was found where the chiasma is only located proximally. In this case positive heteropycnotic material is around the secondary constriction, existing in a short heteropycnotic zone from the secondary constriction away to the X_R limb (fig. 3b and c).

The formation of one chiasma at meiotic prophase makes each chromatid, of the neo Y segregate with a chromatid of X_L ; thus the neo XY bivalent always undergoes an equational segregation (fig. 2e, g and h). Only two anaphase plates (in the Cs δ 9 individual) showed reductional segregation (fig. 2f), probably because of a failure in the chiasma formation in two meiotic cells.

(iii) *C-banding and silver staining*

Only the megameric (S-chromosome) and the extra (polysomic) chromosomes show small faint blocks of C-heterochromatin (fig. 2a). However, NOR-Ag staining revealed a substantial positive region at M_3 chromosome coinciding with the secondary constriction of this chromosome. The silver positive region is present in both homologues but shows a differential pattern of activity in each one; there may even be complete absence in one homologue (fig. 3j, k and l).

The activity of the NOR decreases during meiotic prophase and is not observable later than metaphase I (see fig. 3f, g and j). Postmeiotic reactivation is observed in spermatid nuclei (fig. 3i). A similar pattern of NOR activity was found in both *XO* and *XY* states. The X_L region of the neo *X* chromosome displayed negative heteropycnosis at metaphase I when stained with this Ag-technique (fig. 3h).

4. DISCUSSION

(i) *The neo XY structure*

Progressive heterochromatinization of the neo *Y* could be a characteristic of neo *XY* systems (Saez, 1963; White, 1973). This, in addition to several other mechanisms such as inversions, may cause loss of homology between the X_R (autosomal) region of the neo *X* and neo *Y* chromosome (Hewitt, 1979). In the case of the neo *XY* system in the undescribed species of morabine grasshopper (P45b) which arose by tandem fusion, the entire length of the neo *X* shows negative heteropycnosis. This heterochromatinization could provide a means of preventing recombination in its vicinity and would be particularly useful to maintain effectively isolated heterologous sex region.

Conventional stain and C-banding techniques demonstrate only small amounts of heterochromatin in the chromosome complement of *C. seoanei* (fig. 2a, b and c). Only the X_L region of the neo *X*, megameric pair and extra chromosomes are positively heteropycnotic during meiotic prophase. These results indicate that no heterochromatinization of the X_R occurs in spite of the absence of any centromere between X_R and X_L regions. This situation could be explained if this system had a recent origin as is deduced from its polytypic state.

In most of the neo *XY* systems, pairing segments are restricted to small distal segments of the *Y* and X_R , but in our case a single chiasma is found whose position varies from proximal to interstitial (fig. 2c and d, fig. 3). This chiasma can not be formed at the distal half position due in part to the loss of a distal fragment of the X_R limb when the tandem fusion occurred, and/or as a consequence of the fusion itself. Another consequence of this is the decrease in the number of chiasmata present in the neo *XY* bivalent of *Callicrania* in relation with those of M_2 at *XO* state.

The case of P45b (White *et al.*, 1967, pp. 275) is dissimilar, in that the only chiasma is either very close to the centromere or close to the distal end of the *Y*.

We have found an individual (CsT♂9) where the neo *XY* chiasma is only located proximally. In some way a heterochromatinization event has produced a short heteropycnotic segment in the distal region of X_R that is not formed in the partner neo *Y* (fig. 3b and c). A case is known in *Paratylotropidia beutenmulleri* (White, 1953; in White, 1973, pp. 610) where some heterochromatic blocks have developed in the X_R limb that are not homologous to those in the *Y*. In any case there is no evidence to suggest that the constriction has moved its position in this individual of *Callicrania*. The size relation between X_R and neo *Y* is not apparently altered.

(ii) *Pattern of NOR activity*

During the various stages of meiosis the active NORs of both *XO* and *XY* states had identical silver staining. At present all the evidence suggests that the method of staining of organizer regions (NORs) with silver nitrate shows essentially the regions of active ribosomal RNA synthesis (Miller *et al.*, 1976; Howell, 1977; Hausmann *et al.*, 1978; Hofgärtner *et al.*, 1979 etc.). This means that the pattern of RNA synthesis activity or the amounts of rDNA present at the NORs (Goodpasture and Bloom, 1975; Czaker, 1978) are not altered by the neo *XY* state.

We conclude that modifications between *XO* and *XY* states only affect the location and number of chiasmata in the chromosomes involved in the fusion. The absence of heterochromatinization in the *XY* sex bivalent and its polytypic states suggest a recent origin for the neo *XX-XY* system of *C. seoanei*.

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