PYCNOGASTER CUCULLATA (CHARP.): A POLYTYPIC SPECIES OF TETTIGONIOIDEA WITH *XO* AND NEO *XY* SEX DETERMINATION

JOSÉ FERNANDEZ-PIQUERAS, ANTONIO RODRIGUEZ CAMPOS, CARLOS SENTIS CASTAÑO and FRANCISCO WANDOSELL JURADO

Departamento de Genética, Facultad de Ciencias, Universidad Autónoma de Madrid, Mód. C-XV, Cantoblanco, Madrid-34, Spain

1. INTRODUCTION

THE superfamily Tettigonioidea (long-horned grasshoppers) comprises more than 3500 species of which about 100 have been studied cytologically. Most of these show an *XO–XX* sex chromosome mechanism and only six species are known to have a derived neo *XY–XX* system (Hewitt, 1979).

The Tettigoniid *Pycnogaster cucullata* (Charp.) is endemic to the Iberian Peninsula where it is found on clumps of Ilex. We have found populations of this species to be polytypic with respect of its sex chromosome system and this paper compares the structure of the *XO* and neo *XY* forms.

2. MATERIAL AND METHODS

We have studied seven populations of this species (fig. 1). The Truchas (Leon, Spain) population (49 males and one female) inhabits a marginal

FIG. 1.—Populations studied.

zone within the biogeographic region defined for the species and the individuals here differ morphologically from those collected in the central area of the species distribution (Morales Agacino, personal communication).

The individuals collected from the Morcuera population are similar to the Truchas, but this population is outside the range of this species (Gangwere and Morales Agacino, 1970).

Five populations have been collected in the Sierra de Gredos region, designated PyJP (nine males), PyP (ten males), PyA (five males), PyJA (five males) and PyJH (seven males).

A 0·05 per cent solution of colchicine was injected into the abdomen of males eight hours before the gastric caeca were removed. Both pretreated gastric caeca and untreated testis material of males were fixed for 2 h in alcohol–acetic (3 : 1) and then stored in 70 per cent alcohol. Conventional acetic orcein squash preparations were used together with C-banding preparations (Sumner, 1972).

3. RESULTS

(i) *The chromosome complement*

We have found two principal cytological races of *P. cucullata*. The basic karyotype of the Gredos populations (PyJP, PyJH, PyJA, PyA: $2n$-male- $= 28 + XO$ and PyP: $2n$-male- $= 28 + XO \pm 1B$) consists of 29 chromosomes (XO) in the male and 30 in the female (XX) (fig. 2a). Two types of M_2 chromosomes (M_2 and M'_2) have been seen with respectively one or two blocks of procentric C-heterochromatin (fig. 3b). A secondary constriction is present on this chromosome (fig. 2a and 3a and b).

The second basic karyotype of the Truchas (T) and Morcuera (M) populations ($2n$-male- $= 26 + XY \pm 1B$) differs from the former by the absence of the M_2 pair and includes a neo XX (female) neo XY (male) sex chromosome pair (fig. 2b). Two males from the T population also carried a small B-chromosome. The neo X chromosome is the largest in the complement and carries two secondary constrictions: one pericentromeric and the other in the distal section of its long arm (fig. 3c).

Although *P. cucullata* is characterized by a low heterochromatin content, there are three substantial blocks in the neo X chromosome (fig. 3d). One of them, the largest, is proximal to the centromere and on the short arm. The neo Y is slightly smaller than the short arm of neo X and slightly larger than the M_3 autosome. The centromeric and telomeric regions of the neo Y also exhibit minor C-bands (fig. 3d). A comparison of the two chromosome races thus suggests that the neo XY system has been derived by an M_2–X centric fusion in a progenitor XO–XX system similar to that in the Gredos populations. The precise size of the progenitor neo Y is open to question since the short (X_R) arm of the neo X is longer than its free partner Y.

(ii) *Chiasma frequency*

Mean chiasma frequencies at diplotene of the different populations are given in table 1. Significant differences exist between the XO and the neo
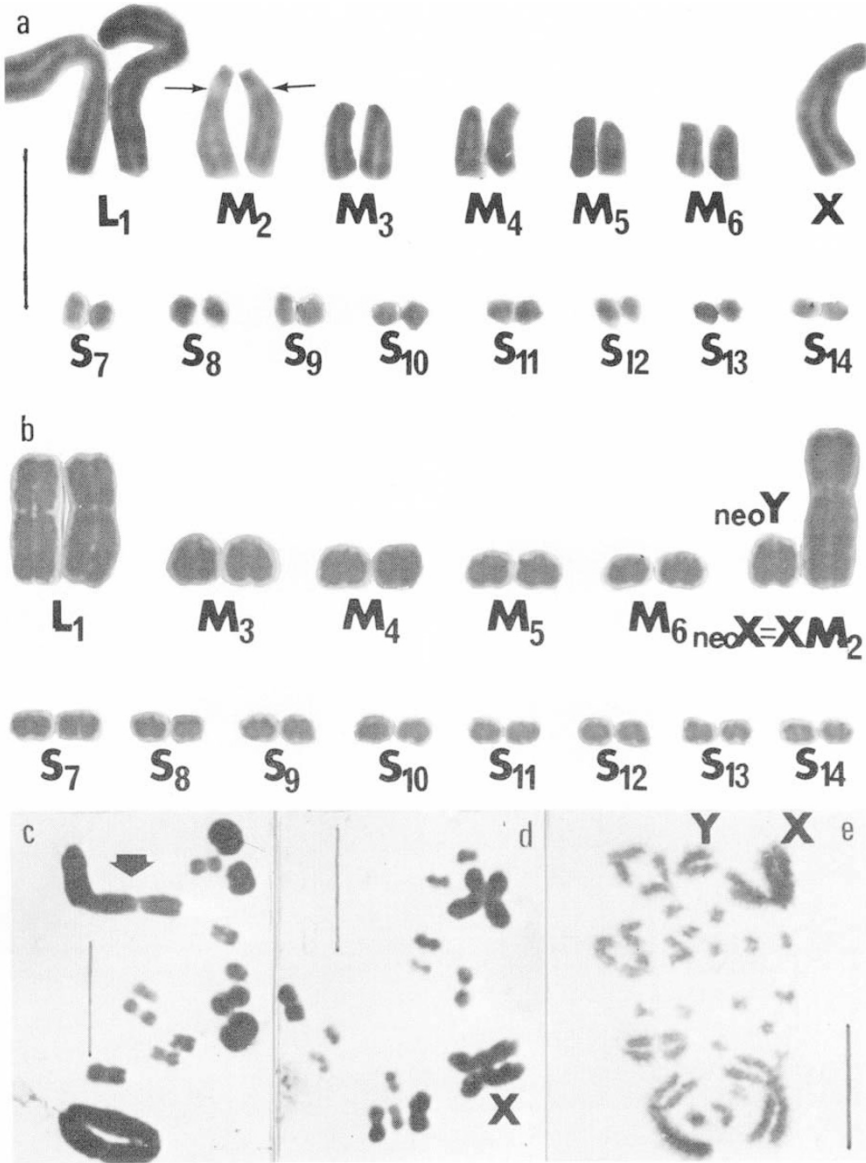
Plate I

FIG. 2.—a. The basic karyotype of *Pycnogaster cucullata* in the Gredos populations. Note the secondary constriction in the M_2 pair (arrow). b. Idem in the neo XY populations. Note the unequal size of the X_R limb and the neo Y chromosome. c. Metaphase I with the neo XY sex bivalent showing a single terminal association (arrow). d. Metaphase II with a neo X chromosome (arrow). e. Reductional segregation of the neo XY sex bivalent at anaphase I. Bars in all figures represent 10 μm.
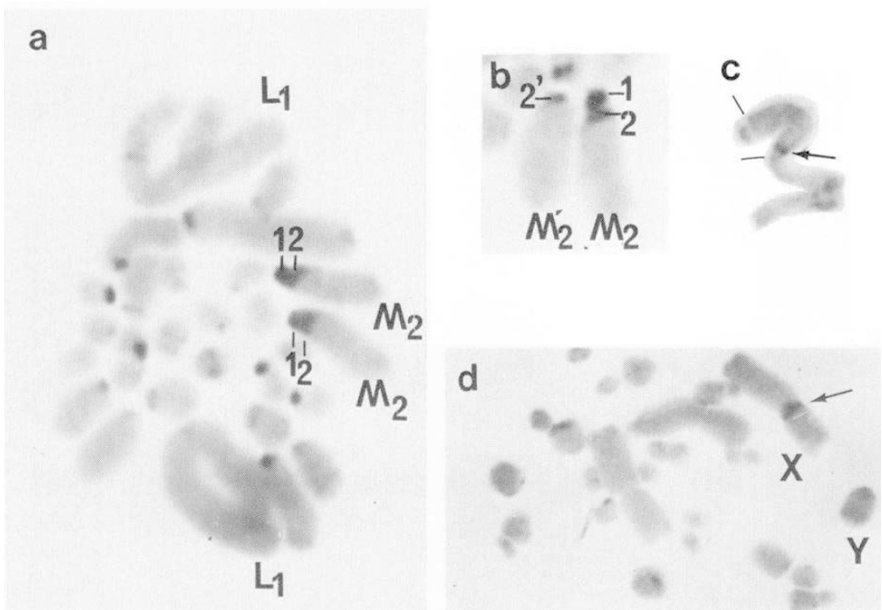
Plate II

FIG. 3.—a. The C-band pattern in a C-mitotic metaphase of the *XO* form. b. The two M_2 types found in the *XO* state. c. An early diplotene neo *XY* sex bivalent. Note the presence of a subterminal chiasma, a proximal C-positive block (arrow) and two secondary constrictions (bars). d. The C-band pattern of the neo *XY* state.

TABLE 1

Mean cell chiasma scores in the different populations of Pycnogaster cucullata. The neo XY chiasma is added to the M total

Populations	Chromosomal types	Individuals	Mean cell chiasma
PyT	XY	10	$16 \cdot 65 \pm 0 \cdot 3$
	XY + B	2	$18 \cdot 35 \pm 0 \cdot 07$
PyM	XY	9	$15 \cdot 52 \pm 0 \cdot 35$
PyP	XO	8	$18 \cdot 34 \pm 0 \cdot 68$
	XO + B	2	$19 \cdot 45 \pm 0 \cdot 77$
PyJP	XO	9	$18 \cdot 40 \pm 0 \cdot 67$
PyA	XO	5	$18 \cdot 42 \pm 0 \cdot 67$
PyJA	XO	6	$18 \cdot 56 \pm 0 \cdot 64$
PyJH	XO	7	$18 \cdot 41 \pm 0 \cdot 96$

XY forms ($P < 0 \cdot 005$). There is also a significant difference between karyotypes and a t-test shows that this is due to the B-chromosome individuals of the Truchas population ($t_{1,2} = 2 \cdot 61 < 0 \cdot 05$). A Snedecor's F distribution revealed that the B-chromosomes in *P. cucullata* significantly decrease the between cell variance ($P < 0 \cdot 01$).

4. DISCUSSION

In *P. cucullata* there is an obvious inequality in length between the neo Y and its homologous arm (X_R) in the neo X. This inequality is related to the presence of a proximal C-block and a secondary constriction in the X_R arm which is not represented in the neo Y (fig. 3c and d). Most of the recorded cases of neo XY systems postulate a neo Y that has been modified by deletion or sometimes by duplication and translocation following the centric fusion event. By contrast in two of the six neo XY mechanisms described in the superfamily Tettigonioidea the Y is considerably larger than the X_R arm (Hewitt, 1979). The present neo Y chromosome may have arisen from either of the two M_2 types. If the neo Y were to have originated from the smaller of these (M_2') (fig. 3b), which only posseses one C-band, this would involve loss of the secondary constriction together with part of the C-band so accounting for the smaller size of this band on the neo Y.

The chiasma difference between the XO and the XY populations of *P. cucullata* cannot simply be an effect of the fusion differences that distinguishes them, since this difference is due to the higher number of chiasmata present in both L and M bivalents of the Gredos populations. Nevertheless, the unfused progenitor of the neo Y/X_R (the M_2 bivalent) does make a contribution to this difference forming both ring and rod bivalents in all the Gredos populations, and these include obviously interstitial chiasmata. By contrast, in Truchas and Morcuera, the neo XY never forms a ring bivalent.

In *Podisma pedestris* the frequency of proximal chiasmata, in the neo XY forms, has been reduced probably as a direct consequence of the fusion itself (Hewitt and John, 1972). Thus in both Podisma and Pycnogaster there is a reduction in the chiasma frequency of the autosome involved in the fusion coupled with a repositioning of chiasmata to distal sites in the product of the fusion (fig. 3c).

Chiasma formation may or may not be affected by B-chromosomes (John, 1973; Schroeter and Hewitt, 1974). Both responses have been found in Pycnogaster. Here only the B-chromosomes of the Truchas population increased the mean chiasma frequency. The decreased between-cell variance for chiasma frequency suggests a stabilising role for the B-chromosomes in populations. The polytypic state of this species suggests that the neo XY system may be of recent origin.

According to Gangwere and Morales Agacino (1970) the central area of this species is Central Portugal. If this is so, it is possible that the two neo XY populations (Truchas and Morcuera) represent two different lines in the expansion of a more central original neo XY.

5. REFERENCES

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