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Sex reversal in a wild population of *Talpa occidentalis* (Insectivora, mammalia)

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Summary

Two sex-reversed males and eight intersexes have been found in a natural population of the mole species *Talpa occidentalis*. All individuals of karyotype 34,*XY* were normal males, while the 34,*XX* karyotype was found in normal females, intersexes and sex-reversed males. Small testes were present in *XX* males, and ovotestes in intersexes. Intersexes showed male antigen levels higher than for females and lower than for males (including *XX* males), as judged by cytotoxicity tests. The *X* chromosome of sex-reversed males and intersexes and the Y chromosome of males appeared morphologically normal.

1. Introduction

It has been generally considered that the *Y* chromosome is necessary for the development of the male phenotype in mammals. However, a number of cases have been described in which the female karyotype has been found in phenotypical males (see review by Lyon, Cattanach & Charlton, 1981).

Cattanach, Pollard & Hawkes (1971) describe a similar case in the mouse where the factor causing the so-called sex-reversal (*Sxr*) appears to show autosomal dominant inheritance. This case has been deeply studied in subsequent years (Cattanach & Bigger, 1976; Bennett *et al.* 1977; Winsor, Ferguson-Smith & Shire, 1978; Tres, 1978; Chandley & Fletcher, 1980; Evans, Burtenshaw & Brown, 1980; Lyon *et al.* 1981 and Singh & Jones, 1982).

Finally, Evans, Burtenshaw & Cattanach (1982) demonstrated that in 'sex-reversed' *XX* male mice, the sex-reversing factor (*Sxr*) is carried on a dark-staining body located at the distal end of the paternal *X* chromosome, and that this body can be transferred by regular crossing-over from a chromatid of the *Y* to a chromatid of the *X* in paternal meiosis. Cattanach *et al.* (1982) and Maclaren & Monk (1982) suggest that the *Sxr* factor is subject to the *X*-chromosome-inactivation process; they have shown that, when the *Sxr-bearing X* chromosome is rendered inactive in most, if not all, somatic cells (by the (T16H)*X*-autosome translocation), these individuals can develop as normal but sterile males, normal fertile females or intersexes.

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To our knowledge, the 'sex-reversal' condition has not previously been reported in a wild mammal population. This paper describes the case of a natural population of the mole *Talpa occidentalis* that in many aspects is similar to the situation in the mouse.

2. Materials and methods

A total of 54 moles (35 males, two of which were sex-reversed $2n = 34$, *XX* males, 11 females and 8 intersexes) were trapped live in a restricted area (less than 200 × 500 m) in the Vega de Granada, Spain.

(i) *Chromosome preparations*

Bone marrow preparations were made by the method of Lee & Elder (1980) with minor modifications: the animals were subcutaneously injected (1 ml per 100 g body weight) with a suspension of active yeast at 40 °C; after 14–18 h they were intraperitoneally injected (0·5 ml per 100 g body weight) with 0·02% colchicine (Sigma); after 45 min the moles were sacrificed by an overdose of ether and the bone marrow treated with 0·56% KCl at 37 °C for 15 min. The material was fixed in modified Carnoy's fixative (methanol-acetic acid. 3:1) for 40 min at 4 °C, with three subsequent changes of fixative. The preparations were made on cold wet slides from a height of 50–80 cm.

(ii) *Staining*

Slides were stained with 10% Giemsa (Merck) for 5 min. For G-banding, we followed the method of

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Burgos, Jiménez & Díaz de la Guardia, (1986); the slides were immersed in trypsin solution (0·025% in Ca^{2+}- and Mg^{2+}- free PBS solution) at 4 °C and then in 2 × SSC at 65 °C for 1–1·5 min, after which they were stained for 5 min in 10% Giemsa in phosphate buffer at pH = 6·8.

(iii) *Meiotic preparations*

For each male, the two testes were weighed together before processing. Meiotic preparations were made from one testis following the method of Ford & Evans (1969), except that 0·56% KCl was used for the hypotonic treatment.

(iv) *Histological preparations*

The other testis was fixed in Bouin's fluid, and standard histological sections of 5 μm thickness were made. Staining was performed with haematoxylin and eosin. The average diameter of the seminiferous tubules was based on 10 tubules scored with a micrometer eyepiece.

(v) *Sperm count*

To estimate the fertility of each male, sperm counts were made in epididymes as follows: both epididymes were carefully cut off and placed in Petri dish containing exactly 20 ml of phosphate buffer; they were then minced with scissors and gently squashed in a manual conical homogenizer; after this, the spermatozoa were suspended in the solution. Total number was estimated with a haemocytometer. In some cases (for epididymes of individuals with small testes) only 4 ml of phosphate buffer was used to suspend the spermatozoa.

(vi) *Serological assays*

For each animal both kidneys were used for cytotoxicity assays to estimate the relative levels of male specific antigens. The kidneys were homogenized with 0·9% NaCl and centrifuged at 3000 rev/min for 10 min. The supernatant was discarded and the sediment resuspended by strong agitation. The resulting thick suspension was distributed in several aliquots of 50 μl in Eppendorf tubes and immediately frozen at −20 °C until use. To obtain the active antiserum and to carry out the cytotoxicity tests we followed the method of Goldberg *et al.* (1971).

(vii) *Estimation of relative ages*

For each animal, an index which is in direct ratio to the age was obtained on the basis of dental wear. The skulls were stuffed and both m^1 were removed and measured according to Fig. 1 under a binocular microscope. The age index (A.I.) was obtained by the arithmetic mean of the results obtained by applying the formula A.I. = A/(B+C) to each m^1. This index is in direct proportion to dental wear and consistently, with the age of the animal.

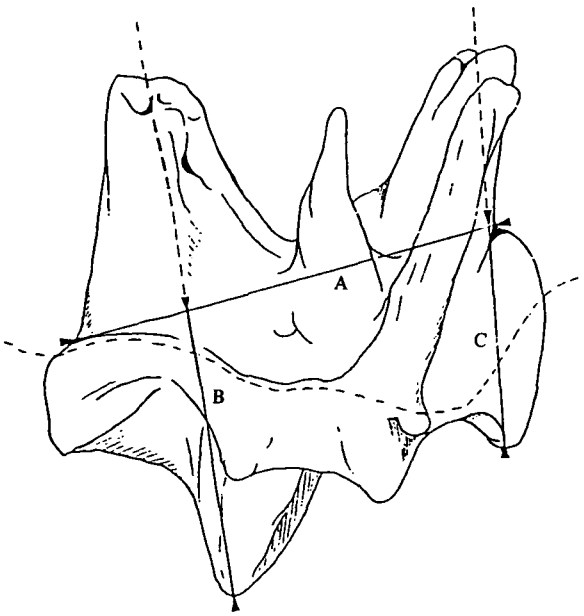

Fig. 1. Upper molar m^1 from *Talpa occidentalis* indicating parameters A, B and C used for estimation of relative ages.

3. Results

(i) *Anatomical and histological studies of the reproductive system*

In external appearance and size of accessory reproductive structures, all the *XY* males showed a normal male phenotype, but a marked variation was found in testicular weight (20–939 mg) and diameter of seminiferous tubules (56–160 μm). The estimated number of spermatazoa in epididymes varied from 19×10^6 to 43×10^6 in individuals showing an intermediate–high testicular weight (350–900 mg), and from 0 to $0{\cdot}2 \times 10^6$ in those of a low testicular weight (20–180 mg). These variations are probably due to low age in some cases and to seasonal fluctuations in others (Jiménez *et al.* in preparation). Similar variation has been observed in other species of insectivores such as *Sorex araneus* (see Saure *et al.* 1972).

Testes from both immature and adult *XY* males (Fig. 2*a* and 2*b*) appeared histologically normal, with more than one layer of germinative cells and a more or less evident lumen in the seminiferous tubules.

The two sex-reversed *XX* males showed an external phenotype similar to that of a normal male, but their testes were minute (25 and 40 mg), and similar in size to those of normal immature males. Both sex-reversed individuals showed a total absence of meiotic stages and spermatazoa.

Histological studies revealed a notable difference in structure of the testes of the sex-reversed and the normal males (Fig. 2*c*). The seminiferous tubules of the sex-reversed males were smaller in diameter (average

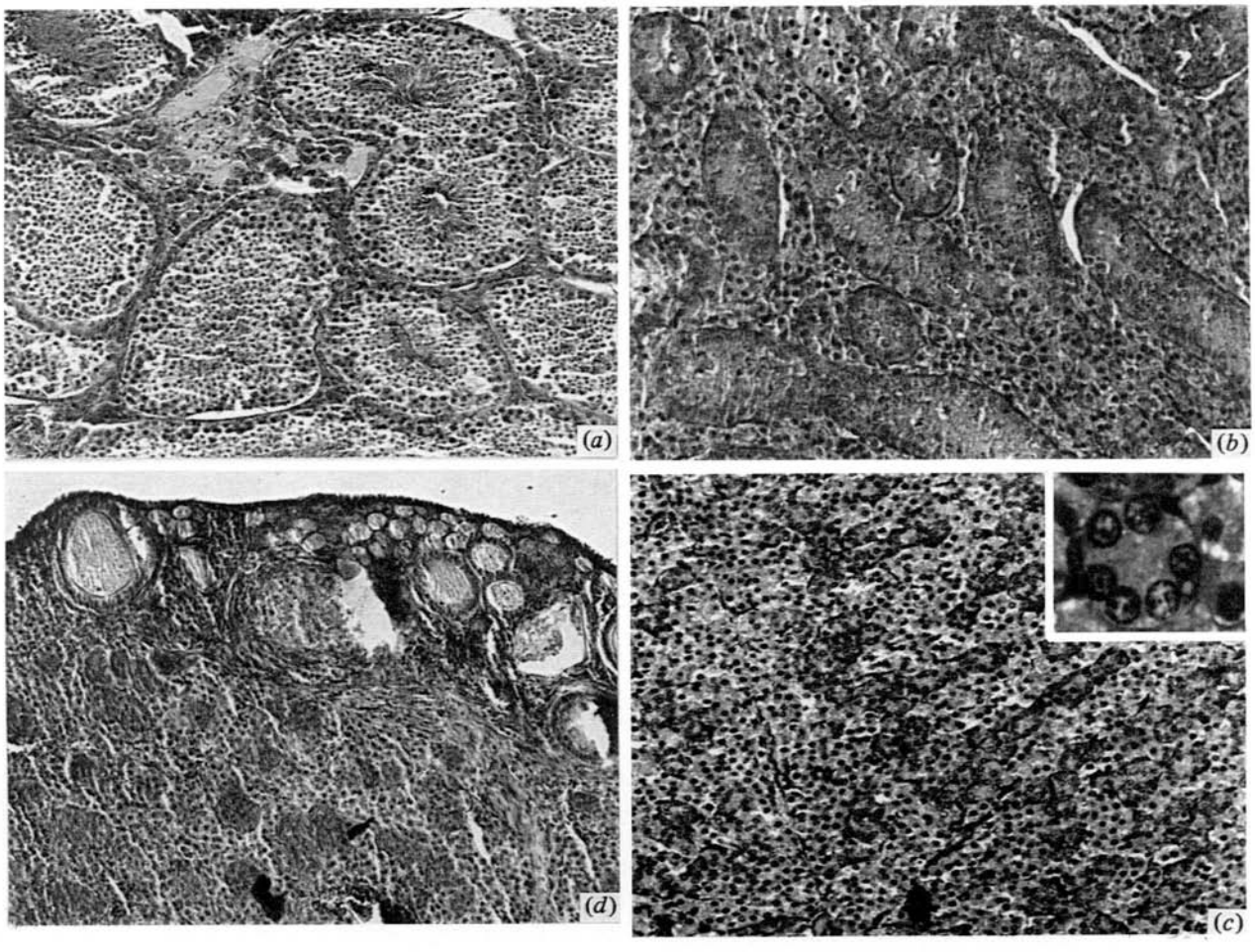

Fig. 2. Gonadal histological sections from several individuals. (*a*) Well-developed testis from a normal adult *XY* male. (*b*) Testis from a young XY male; this immature gonad shows higher numbers of interstitial Leydig cells and a lower diameter of seminiferous tubules devoid of spermatazoa (*c*) Testis from a 'sex-reversed' XX male showing clear dysgenic characteristics; here, most of the testis volume consists of interstitial tissue including some few isolated 'solid sexual cords' whose structure is shown at greater magnification. (*d*) Ovotestis from an intersex *XX* individual; evident ovarian follicles are visible in the periphery of the organ, which is filled with testicular tissue very similar to that described for the *XX* male.

25 μm) and less complex than those of the immature or adult normal males (55 and 160 μm, respectively). In normal adults there were only small groups of Leydig cells. In immature animals these groups of interstitial cells were larger than in adults, but in the *XX* males Leydig cells were most numerous, making up most of the testis volume. This testis histology is unlike that of *SxrXX* male mice, which have fairly normal testis tubules, though devoid of spermatogenic cells, but it is very similar to that of *XXY* males of other insectivorous species such as *Sorex araneus* (Searle, 1984).

The intersexes showed a female external appearance and a vagina was visible. Internally, these individuals presented an uterus and ovotestes, containing both ovarian and testicular tissue (Fig. 2*d*). The ovarian follicles were located at the periphery, while most of the ovotestis volume was filled by testicular stroma very similar to that described for the sex-reversed males. Meiotic activity was absent.

(ii) *Cytogenetic studies*

(*a*) *Mitotic chromosomes.* Only tow karyotypic forms have been found in this population, corre-

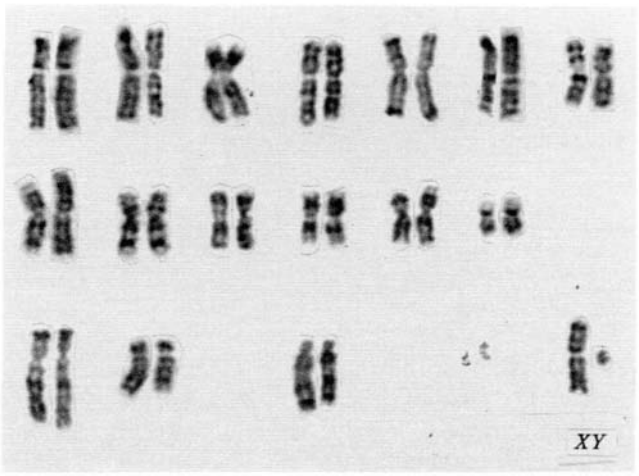

Fig. 3. Representative G-banded karyotype from an *XY* male.

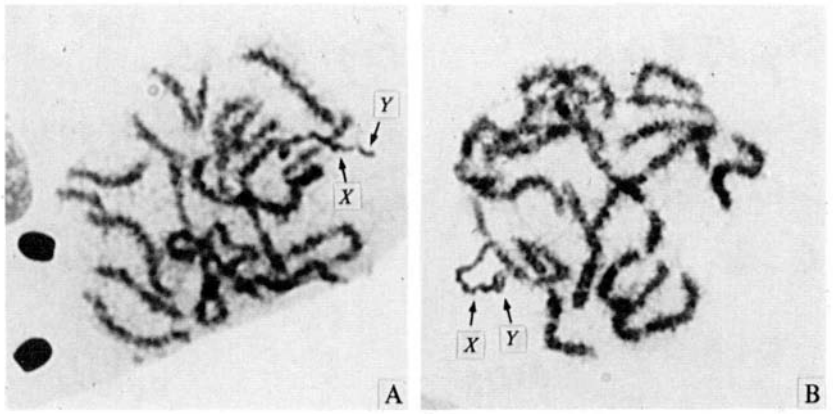

Fig. 4. Pachytene cells from a fertile *XY* male showing (*a*) terminal association between *X* and *Y* sex chromosomes, and (*b*) lateral association.

sponding to the male and female karyotypes described for this species (Jiménez *et al.* 1984*a*). All individuals showing a 34,*XY* male karyotype (Fig. 3) were invariably normal males. However, the 34,*XX* female karyotype was found in three kinds of individuals: normal fertile females, sex-reversed males and intersexes.

It is interesting to point out that while the karyotype ratio does not deviate significantly from the 1:1 expected ratio (33 *XY*:21 *XX*, $0{\cdot}1 < P < 0{\cdot}2$), the sex ratio is not in agreement with this proportion (35 males:11 females, $P < 0{\cdot}01$). There is a significant decrease in the number of females and a corresponding rise in numbers of *XX* males and *XX* intersexes.

(*b*) *Meiotic chromosomes.* Those individuals showing well-developed reproductive structures had a high rate of meiotic activity as observed in meiotic preparations. Abundant pachytene and diplotene figures and some metaphase I cells were present. In addition, large numbers of spermatids and spermatozoa were visible. As observed by Jiménez *et al.* (1984*b*), both *X* and *Y* sex chromosomes appear unfolded and positively heteropycnotic or isopycnotic in pachytene–diplotene cells. This allows us to determine the relative location of the *X* and *Y* chromosomes, and demonstrates frequent end-to-end contact (Fig. 4*a*) although in some cases this association seems to be lateral (Fig. 4*b*). The existence of a regular chiasma between sex chromosomes can therefore be assumed. In no case was a sex chromosome–autosome association observed.

(iii) *Male-antigen levels*

To determine the relative levels of male-specific antigens, also called SDM-antigens (serologically detectable male antigens), three cytotoxicity tests were performed.

The first test (Fig. 5*a*) shows that male-specific antigen levels were lower in the female than in the four tested males, including one sex-reversed 34,*XX* male.

Fig. 5*b* shows the results obtained in a second test where four intersexes were compared with a male and a female. For the intersexes, antigen levels were higher than in the female and lower than in the male.

Finally, Fig. 5*c* shows levels of male-specific antigen in four females, a male and the same two intersexes mentioned above. Levels of antigen were very variable in the females.

4. Discussion

With respect to the mole population studied here, five characteristics are worthy of mention. (1) All specimens were trapped in a natural population; (2) females, intersexes and males of identical sex chromosomal constitution were found; (3) intersexes showed different levels of male antigen and ovotestes of variable size and maleness; (4) all *XX* males and intersexes were sterile; (5) the frequency of affected individuals in this population was 0·178.

The existence of males, females and intersexes of similar sex-chromosome constitution as well as serological and histological results are similar to the case of *Sxr* mice (Cattanach *et al.* 1971, Bennett *et al.* 1977). In mice, the sex reversal is the result of a non-reciprocal cross-over in which male *Y*-DNA sequences (*Sxr*) are transferred by chiasma to the *X* chromosome. As was pointed out by Mclaren & Monk (1982), intersexes as well as sex-reversed males represent varying degrees of a common masculinizing event, so that in each individual the sex phenotype is related to the proportion of cells in which the X*Sxr* has been inactivated.

Such a mechanism for sex reversal could explain the findings in our Spanish moles, where in addition male antigen levels shown by intersexes and sex-reversed males are in agreement with this hypothesis (Figs. 5*a*, *b* and *c*). Furthermore, such a mechanism could explain the maintenance of the high frequency of affected individuals in a wild population as well as the deviation of the sex ratio with respect to the karyotype ratio.

On the other hand, there are several points of

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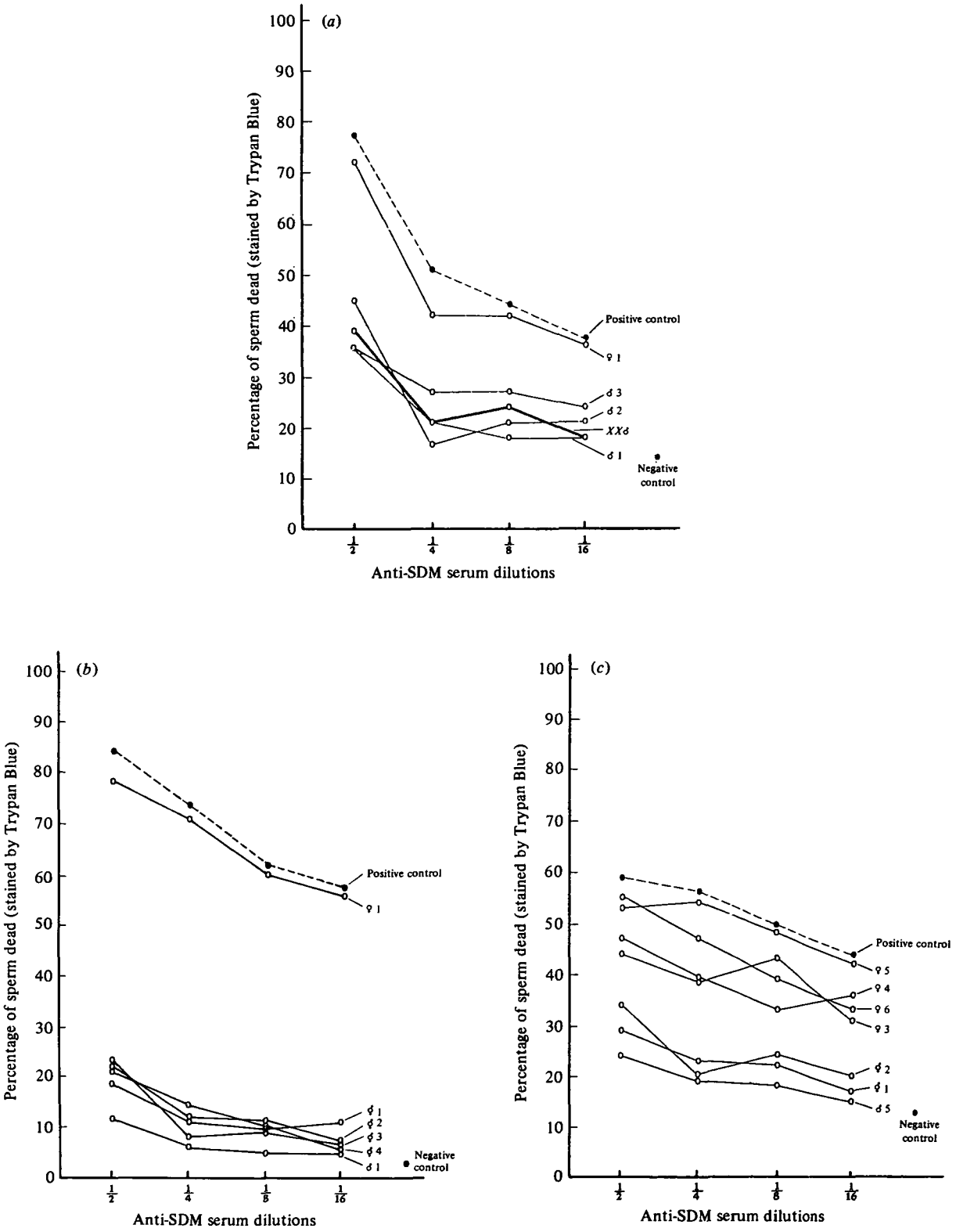

Fig. 5. Demonstration of male antigens on homogenized kidneys from several individuals analysed. (*a*) A female (♀1), three *XY* males (♂1, ♂2 and ♂3) and one *XX* male; all males, including the *XX* one, show similar antigen levels higher than those of the female. (*b*) Test comparing four intersexes (♀♂1, ♀♂2, ♀♂3 and ♀♂4) with a male (♂4) and a female (♀2); a noteworthy finding was that intersexes showed male antigen levels very similar to one another and close to those of the male but different from those of the female. (*c*) Test made with a male (♂5), two intersexes (♀♂1 and ♀♂2) and four females (♀3, ♀4, ♀5 and ♀6); note the significant dispersion shown by females. In all cases, the dotted line represents the positive control (unabsorbed antiserum, diluted from 1/2 to 1/16); the negative control in each test represents the percentage of sperm dead due to unspecific causes (suspension of spermatazoa mixed with complement only).

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disagreement between the two cases. Thus, while in mice the *Sxr* factor is cytologically visible, in moles no such body is detectable either on the *X* chromosomes of the *XX* males or on the *Y* of the *XY* males. In any case, as demonstrated by de la Chapelle *et al.* (1984) in *XX* men, this does not imply that interchange has not taken place. Furthermore, unlike the *Sxr* mice, where *XYSxr* male carriers are characterized by their lower testicular weight, in the mole such male carriers (if they exist) could not be identified on this basis because of the above mentioned seasonal fluctuations in testicular weight.

Another possibility is the translocation of *Y*-DNA sequences to autosomes. However, two reasons contradict this hypothesis: (1) the absence of sex chromosome–autosome associations as seen in meiotic preparations; and (2) the gradual expression of male characteristics is not compatible with such a mechanism if several different translocations are not produced.

Finally, the scarcity of genetic data about the mode of inheritance of this mutation makes any explanation of *XX* maleness based on gene mutation in the absence of *Y*-DNA sequences in the genome premature. However, a dominant condition for this mutation seems not to be compatible with the high frequency of affected individuals in the population, particularly in view of the fact that they are all sterile.

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