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LINKAGE OF THE LOCI FOR GLUCOSE-6-PHOSPHATE DEHYDROGENASE AND FOR INOSINIC ACID PYROPHOSPHORYLASE TO THE X CHROMOSOME OF THE FIELD-VOLE *MICROTUS AGRESTIS*

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SUMMARY

It has been proposed that there are strong selective pressures which have acted during the evolution of mammals to conserve the linkage of genes on the X chromosome. If so, loci that are known to be X-linked in one mammalian species should be X-linked in others. The loci for glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49) and for inosinic acid pyrophosphorylase (E.C. 2.4.2.8) are known to be X-linked in a variety of mammals. The linkage of these loci to the X chromosome of the field-vole, *Microtus agrestis*, is indicated by the pattern of segregation of these loci in hybrid cells derived by fusion of mouse cells with vole lymphocytes.

INTRODUCTION

It has been proposed that there are strong selective pressures which have acted during the evolution of mammals to conserve the linkage of genes on the X chromosome (Ohno, Beak & Becak, 1964; Ohno, 1973). These genes need have no direct connexion with sex determination. Diploid cells from a normal male contain one X chromosome whereas those from a female contain two; somatic cells of the two sexes, whilst possessing two copies of each autosome, differ in their dose of X chromosomes. A mechanism has evolved which compensates for this difference in dose of X chromosomes in the two sexes by inactivating one of the two X chromosomes in cells of the adult female so that genes on only one X chromosome are expressed. Diploid cells from adult males and females therefore have the same number of active X chromosomes (for a discussion see Cook, 1973, 1974). Both copies of each autosome in a cell are believed to be active and the function of cells containing only one copy of a single autosome is thought to be so impaired that they usually die (for a discussion see Ohno, 1971). Therefore, X chromosomal genes differ from autosomal genes both in their regulation and in that they function normally when present in one effective dose. For these reasons it has been argued that selection during evolution has tended to conserve X chromosomal linkages but not autosomal linkages (Ohno *et al.* 1964; Ohno, 1973). If so, loci that are X-linked in one mammalian species should be X-linked in others. The locus for glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate:NADP oxidoreductase, E.C. 1.1.1.49) is linked to the X chromosome in the horse and

donkey (Trujillo, Walden, O'Neil & Anstall, 1965; Mathai, Ohno & Beutler, 1966), the mouse (Epstein, 1969), the hare (Ohno, Poole & Gustavsson, 1965), the kangaroo (Richardson, Czuppon & Sharman, 1971) and in man (Childs, Zinkham, Browne, Kimbro & Torbet, 1958) as is the locus for inosinic acid pyrophosphorylase (IMP: pyrophosphate phosphoribosyltransferase; hypoxanthine-guanine phosphoribosyltransferase; E.C. 2.4.2.8) in the horse (see Ohno, 1973), the mouse (Epstein, 1972) and man (Nyhan, 1968). These two loci are also probably linked to the X chromosome in the hamster (Westerveld, Visser, Freeke & Bootsma, 1972). Here evidence is presented for the X-linkage of the loci for glucose-6-phosphate dehydrogenase and for inosinic acid pyrophosphorylase in the field-vole, *Microtus agrestis*.

The linkage analysis involved the use of mouse-vole hybrid cells and depends upon the random loss of vole chromosomes from such hybrids. The methods used are similar to those used with human–mouse hybrids to establish the linkage relationships of human chromosomes (Nabholz, Miggiano & Bodmer, 1969; Miller, Cook, Khan, Shin & Siniscalco, 1971). Hybrids were made by using inactivated Sendai virus to fuse mouse cells deficient in inosinic acid pyrophosphorylase with vole lymphocytes that had been induced to multiply. The resulting heterokaryons were cultivated in a medium containing hypoxanthine, aminopterin and thymidine (HAT). In this medium, cells lacking inosinic acid pyrophosphorylase die (Szybalska & Szybalski, 1962) and as vole lymphocytes are inviable under these conditions only hybrid cells containing inosinic acid pyrophosphorylase grow. Metaphase spreads made from cells surviving in HAT medium contained mouse chromosomes and the large and easily distinguishable X chromosome of the vole; they contained few other vole chromosomes. Segregants which had lost inosinic acid pyrophosphorylase were obtained by growth in thioguanine (TG). In the presence of this drug only those cells lacking inosinic acid pyrophosphorylase grow (Szybalska & Szybalski, 1962). Linkage of vole inosinic acid pyrophosphorylase and glucose-6-phosphate dehydrogenase to the vole X chromosome was inferred from their co-segregation under these conditions.

MATERIALS AND METHODS

Media

All media (Flow Laboratories, Irvine, Scotland) were supplemented with 10% foetal calf serum and antibiotics (neomycin 30 μg/ml; kanamycin 30 μg/ml; streptomycin 200 μg/ml; mycostatin 40 units/ml). Media were generally changed once a week. Lymphocytes were grown in RPMI 1640 medium. Minimal essential medium (MEM; Eagle, Oyama, Levy, Horton & Fleischman, 1956) was the basis of the other media used. HAT and TG media were made by supplementing MEM with hypoxanthine (10^{-4} M), aminopterin (4×10^{-7} M), thymidine ($1{\cdot}6 \times 10^{-5}$ M), sodium pyruvate (10^{-3} M) and glycine (3×10^{-6} M) or thioguanine (1 μg/ml) respectively (Szybalska & Szybalski, 1962). A9, L, E2 and MAXY cells were grown in MEM. The name of a hybrid sub-line is followed by the letters TG if that sub-line was grown in TG medium; otherwise all hybrids were grown in HAT medium.

Cells

The mouse cells used were L cells (Earle, 1943) and a subclone, A9cl2a, of the A9 line. The A9 cells, which were initially obtained from Dr J. W. Littlefield, lack inosinic acid pyrophosphorylase (Littlefield, 1964). They were obtained from L cells by continued subcultivation

in the presence of 8-azaguanine; cells that have lost inosinic acid pyrophosphorylase cannot incorporate azaguanine or 6-thioguanine into nucleic acid and are thus resistant to the lethal effects of the antimetabolites.

Field-voles (*Microtus agrestis*) were kindly supplied by Dr Clark from his colony in the Department of Agriculture, University of Oxford. Lymphocytes were obtained from the cervical lymph nodes of 3-month-old voles. A lymph node was teased open in RPMI 1640 medium to release about 5×10^6 lymphocytes. The lymphocytes were 'transformed' by growing the contents of the cervical lymph nodes of one animal in 5 ml of RPMI 1640 to which 0·1 ml of phytohaemagglutinin M (Difco Laboratories, Detroit, Michigan) had been added. By the third day, the phytohaemagglutinin had induced a rapid proliferation of lymphocytes. MAXY and E2 cells are lines of vole cells that have been grown continuously *in vitro* for over a year. Seven foetuses were removed separately from a pregnant vole that had mated 14 days previously. Each foetus, with the exception of the head, was minced up and disaggregated by treatment for 0·5 h at 37 °C in trypsin-EDTA solution (0·125% trypsin; 1 mM EDTA). A study of the metaphase chromosomes of two cultures (E2 and E3) a week later showed that they were derived from female and male embryos respectively. When E3 had been passaged 14 times a rapidly growing fibroblastic colony was isolated (E3a) and cloned again. This subclone is known as MAXY.

Autoradiography

Inosinic acid pyrophosphorylase activity can be conveniently detected in autoradiographs prepared from cells incubated in the presence of [^{3}H]hypoxanthine (Harris & Cook, 1969). Cells growing in HAT or TG were plated on to coverslips and grown for 2 days in HAT or MEM respectively. Coverslips, with cells attached, were then washed in phosphate-buffered saline (Dulbecco & Vogt, 1954) at 37 °C and incubated for 4 h in MEM at 37 °C in the presence of 2 μCi/ml of [^{3}H]hypoxanthine (generally labelled; 1 Ci/mmol; 7·8 mCi/mg; The Radiochemical Centre, Amersham, Bucks). Autoradiographs were then prepared and exposed for 2–4 days and the number of grains over nuclei counted. Under these conditions the counts for A9 cells (lacking inosinic acid pyrophosphorylase) and for L cells (possessing this enzyme) were less than 10 grains and greater than 100 grains respectively. Hybrids were scored as negative (lacking the enzyme) and positive (possessing the enzyme) by these criteria.

Enzyme studies

Inosinic acid pyrophosphorylase was assayed in cell homogenates by the method of Harris & Cook (1969). 1·4 mM thymidine triphosphate was added to the incubation mixture to suppress the effects of any nucleotidase activity (Fujimoto & Seegmiller, 1970). The species of origin of the inosinic acid pyrophosphorylase was determined by the electrophoretic technique of Cook (1970) using the 0·02 M phosphate buffer (pH 7·0) and conditions of electrophoresis as described by Shin, Khan & Cook (1971). Extracts of broken cells were subjected to low speed centrifugation (about 250 **g** for 5 min) to remove cell debris but not to ultracentrifugation and dialysis. Electrophoretic mobilities were unchanged by the omission of these procedures. Under these conditions of electrophoresis, the inosinic acid pyrophosphorylase activities of the mouse (L cells) and the vole (E2) may be resolved (Fig. 1, p. 98). Mouse (A9 cell) and vole (MAXY) glucose-6-phosphate dehydrogenase activities were resolved electrophoretically by the technique of Ratazzi, Bernini, Fiorelli & Mannucci (1967) (Fig. 2).

Preparation of chromosomes

Spreads of metaphase chromosomes were prepared by standard techniques and the centromeric regions stained to produce centromeric bands (c-bands) by the method of Dev, Miller, Allderdice & Miller (1972), except that slides were treated with the formamide solution for 30 min at 65 °C, then washed thoroughly with cold tap water and stained in 10% Giemsa in phosphate buffer (pH 6·8) for 2 h, and finally washed in de-ionized water. Metaphase chromosomes were also stained by the technique of Cooper & Hsu (1972). This differs from the method of Dev *et al.* (1972) essentially in that slides are treated for 18 h at 65 °C in 6 × sodium chloride,

sodium citrate solution rather than for 30 min. This causes the heterochromatic portion of the vole X and Y chromosomes to become deeply staining and brings out bands on the euchromatic arms of the chromosomes and at the centromeres.

RESULTS

Production of hybrids and derivatives

Vole cells were obtained from two males (27A and 27D) and one female (27b). Three days after the initiation of the culture of transformed lymphocytes, between 5×10^6 and 10^7 lymphocytes were fused with an equal number of A9 cells by the technique of Harris & Watkins (1965). Each fusion mixture was then plated in MEM into 5 bottles (numbered 1–5). Two days later the MEM was replaced with HAT

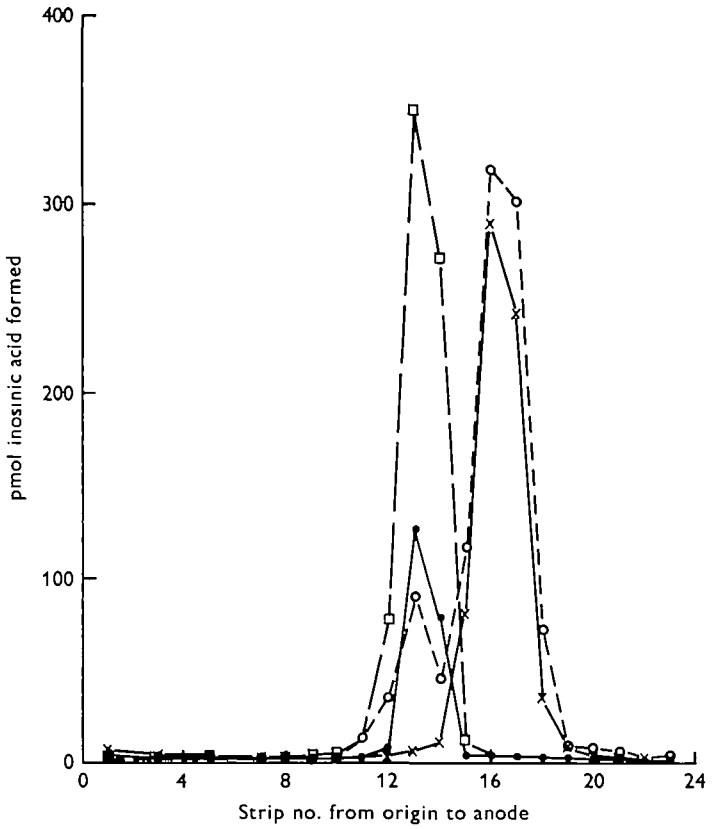

Fig. 1. Electrophoretic mobilities of inosinic acid pyrophosphorylase activities in mouse, vole and hybrid cell extracts. ×, L-cell extract; ●, vole, E2 extract; □, 27b3f extract; ○, mixture of extracts from L cells and vole E2 cells.

medium. Most of the cells died, but two to three weeks after fusion between 10 and 20 colonies per bottle became visible to the naked eye. No colonies arose in bottles containing fusion mixtures from which one or other of the parental cells had been omitted. Between 3 and 4 weeks after fusion, colonies were isolated from the bottles

and grown up separately in HAT medium. Clonal lines are designated by a lower case letter which follows the bottle number. Two cultures, 27A3 and 27A4, were not cloned. Thioguanine-resistant derivatives were obtained by plating into a bottle containing TG medium about 10^6 cells that had hitherto been growing in HAT medium. Most of the cells died, but after 2 weeks between 10 and several hundred colonies became visible. All the contents of the bottle were passaged as an uncloned culture.

Table 1. *Enzyme activities in hybrid clones*

	Hybrids grown in HAT			Hybrids grown in TG		
	Inosinic acid pyrophosphorylase			Inosinic acid pyrophosphorylase		
Hybrid	Autoradiography	Electrophoresis	Glucose-6-phosphate dehydrogenase	Autoradiography	Direct assay	Glucose-6-phosphate dehydrogenase
27A 2a	ND	Vole	Vole, mouse, H	—	—	Mouse
27A 3	ND	Vole	Vole, mouse, H	—	ND	Mouse
27A 4	+	Vole	Vole, mouse, H	ND	ND	ND
27D 2b	ND	Vole	Vole, mouse, H	—	ND	Mouse
27D 2c	ND	Vole	Vole, mouse, H	—	—	Mouse
27D 2d	ND	Vole	Vole, mouse, H	—	ND	Mouse
27D 2d*	ND	Vole	Vole, mouse, H	ND	—	Mouse
27b 3d	ND	Vole	Vole, mouse, H	—	ND	ND
27b 3e	+	Vole	Vole, mouse, H	—	ND	Mouse
27b 3f	ND	Vole	Vole, mouse, H	—	ND	ND
27b 3g	ND	Vole	Vole, mouse, H	—	ND	Mouse
27b 3h	ND	Vole	Vole, mouse, H	—	ND	Mouse
27b 4a	ND	Vole	Vole, mouse, H	—	ND	Mouse
27b 4c	ND	Vole	Vole, mouse, H	—	ND	Mouse
27b 4d	+	Vole	Vole, mouse, H	—	ND	Mouse
27b 4h	ND	Vole	Vole, mouse, H	—	ND	Mouse

ND, not done; H, heteropolymeric or hybrid enzyme present; +, inosinic acid pyrophosphorylase detectable; —, inosinic acid pyrophosphorylase absent.

Enzyme studies

Whereas A9 cells lacked inosinic acid pyrophosphorylase and died in HAT, all populations of cells arising from fusions between A9 and the vole lymphocytes and able to grow in HAT possessed inosinic acid pyrophosphorylase activity; this had the electrophoretic mobility characteristic of the vole fibroblast and not of the mouse (Fig. 1 and Table 1). These populations also contained glucose-6-phosphate dehydrogenase activities that possessed the electrophoretic mobilities characteristic of vole and mouse enzymes; in addition they contained a third activity with a mobility between that of the vole and the mouse (Fig. 2). A third or heteropolymeric and hybrid enzyme activity is only thought to arise when the sub-units of glucose-6-phosphate dehydrogenase from different species are mixed and assembled within a cell to give active polymeric enzyme (Harris, 1970). These findings indicate that single cells growing in HAT were hybrid in that they possessed both vole and mouse glucose-6-phosphate

dehydrogenase as well as vole inosinic acid pyrophosphorylase. In contrast, when derivatives of these hybrids able to grow in TG were examined, all possessed no inosinic acid pyrophosphorylase activity detectable by either of the 2 assay procedures, nor did they contain vole or heteropolymeric glucose-6-phosphate dehydrogenase activity (Table 1). This loss of inosinic acid pyrophosphorylase was irreversible in that 2×10^6 cells of 27A2a TG died when plated in HAT.

Chromosome studies

The chromosomes of the mouse A9 cell (Schwartz, Cook & Harris, 1971; Allderdice *et al.* 1973) and of the vole (Cooper & Hsu, 1972) have been extensively studied. Assignment of the species of origin of a particular chromosome in a hybrid was based on its morphology and whether or not it possessed a deeply staining centromeric band (c-band) (Dev *et al.* 1972). The small dot chromosomes and all the bi-armed chromosomes of the A9 are distinguishable on morphological grounds from vole chromosomes (Figs. 3, 4). Similarly, the huge vole X and Y chromosomes are readily recognizable (Fig. 4). Forty-six of the 48 vole autosomes are single-armed and many are indistinguishable on morphological grounds from those of the A9. However, all but two or three of the single-armed A9 chromosomes possess deeply staining c-bands (Fig. 3), whereas vole chromosomes have none (Fig. 4). As a result, all but two or three chromosomes in a mixture can be readily assigned to their species of origin even though the A9 is aneuploid.

All populations of cells growing in HAT medium and resulting from fusions between A9 and vole lymphocytes initially contained the marker chromosomes characteristic of A9 as well as the large vole X chromosome; this indicated that these cells were hybrid. Most hybrid clones contained few vole chromosomes even when examined between 47 and 49 days after fusion. The data presented in Table 2 on chromosome preparations made between 74 and 150 days after fusion indicate that these hybrids possessed most of the complement of bi-armed, dot and c-banded chromosomes of the A9 parent. Some colonies arising from fused cell populations growing in HAT initially contained cells with nuclei larger than either parental nucleus, but if such hybrids contained many vole chromosomes these were, in general, rapidly lost from the vole–mouse hybrids. In only one clone (27A2a) were about 15 vole chromosomes retained. Most of the metaphase spreads made from bulk populations of hybrids (27A3) and from 13 clones of hybrids contained the vole X chromosome characterized by its morphology and lack of c-band (Table 2, Fig. 6). This X chromosome was frequently to be found at the edge of the metaphase spread (cf. Comings, 1968). No vole Y chromosomes were detected in any hybrid spreads, and although a few spreads contained 2 vole X chromosomes, these were found as frequently in hybrids derived from male voles (series 27A and 27D) as from female voles (series 27b), and so probably arose by duplication.

Although most metaphase spreads in all hybrid populations contained the vole X chromosome it was not invariably present (Table 2). Furthermore, a percentage of spreads (between 0 and 50%) in 7 of the hybrid lines described in Table 2 contained a large metacentric chromosome that lacked a c-band, and this was initially thought

to arise from the vole X chromosome by deletion of a portion of the long arm (see later). The presence or absence of the vole X chromosome can be conveniently and rapidly scored at a low power of magnification in metaphase spreads or even in nuclei treated by the method of Cooper & Hsu (1972) (Figs. 5, 7). This treatment causes the

Table 2. *The chromosomal constitution of parental cells, hybrid cells and their derivatives*

		Mean numbers of chromosomes						
			Without c-bands			With c-bands		
Cell		Total	Large bi-armed	Single armed	Bi-armed	Single armed	Bi-armed	Dot
Vole lymphocytes								
Male		50	1	47	2	0	0	0
Female		50	2	46	2	0	0	0
A9cl2a		56·1 (0·5)	0	1·8 (0·2)	0·1 (0·04)	32·3 (0·5)	20·0 (0·3)	1·6 (0·1)
27A2a	HAT	97·9 (4·8)	0·7 (0·2)	17·6 (2·8)	0·2 (0·1)	51·8 (3·0)	25·3 (2·2)	2·2 (0·4)
	TG	50·6 (1·7)	0	2·1 (0·2)	0·2 (0·1)	27·9 (1·4)	19·4 (0·9)	1·0 (0·2)
27A3	HAT	76·6 (4·8)	0·9 (0·1)	4·7 (0·6)	0	47·9 (3·2)	22·0 (1·8)	1·2 (0·2)
	TG	73·2 (5·0)	0	8·6 (1·1)	0·5 (0·3)	42·0 (3·6)	21·0 (1·6)	1·2 (0·3)
27D2b	HAT	64·8 (4·6)	0·8 (0·1)	4·1 (0·5)	0·5 (0·2)	35·3 (3·3)	21·8 (1·3)	2·3 (0·3)
	TG	63·2 (2·8)	0	3·0 (0·3)	0·1 (0·1)	33·3 (2·3)	25·5 (1·1)	1·2 (0·3)
27D2c	HAT	68·4 (2·1)	0·9 (0·2)	4·1 (0·3)	0	40·2 (1·8)	21·5 (0·9)	1·6 (0·3)
	TG	46·7 (1·7)	0	2·4 (0·4)	0·1 (0·1)	26·7 (0·7)	15·8 (1·4)	1·6 (0·3)
27D2d	HAT	61·8 (1·5)	0·6 (0·2)	4·8 (0·4)	0·5 (0·2)	36·3 (1·0)	17·9 (1·0)	1·7 (0·2)
	TG	56·7 (1·4)	0	2·8 (0·4)	0	30·3 (1·2)	21·7 (0·7)	1·9 (0·3)
27D2d*	HAT	58·1 (1·3)	0·8† (0·1)	3·2 (0·4)	0	35·1 (1·5)	17·5 (1·1)	1·5 (0·2)
	TG	64·8 (1·5)	0	3·8 (0·4)	0·2 (0·2)	39·8 (0·9)	19·8 (0·9)	1·2 (0·2)
27b3d	HAT	51·1 (1·4)	0·9 (0·1)	3·0 (0·2)	0	26·8 (1·0)	18·6 (0·8)	1·8 (0·1)
27b3e	HAT	57·1 (3·8)	0·5 (0·2)	3·0 (0·5)	0·1 (0·1)	34·0 (2·9)	18·2 (1·0)	1·4 (0·3)
	TG	58·3 (0·9)	0	3·6 (0·4)	0·1 (0·1)	34·6 (0·8)	18·5 (0·5)	1·6 (0·2)
27b3f	HAT	54·1 (1·3)	1·0 (0·2)	3·6 (0·6)	0·3 (0·2)	29·5 (1·0)	18·3 (1·0)	1·3 (0·2)
27b3g	HAT	57·9 (0·8)	0·9 (0·1)	2·4 (0·2)	0·4 (0·2)	35·2 (1·2)	17·0 (0·8)	1·9 (0·2)
	TG	53·6 (1·9)	0	2·9 (0·4)	0	30·9 (1·3)	18·3 (0·8)	1·6 (0·2)
27b3h	HAT	57·0 (2·6)	0·8 (0·1)	2·3 (0·5)	0	31·6 (1·5)	20·3 (1·6)	2·0 (0·2)
	TG	54·8 (1·3)	0	3·1 (0·5)	0·2 (0·2)	30·7 (1·1)	19·1 (0·8)	1·7 (0·3)
27b4a	HAT	50·0 (1·7)	0·8 (0·1)	2·0 (0·5)	0·2 (0·2)	26·0 (2·2)	19·2 (1·7)	1·7 (0·3)
	TG	53·1 (1·1)	0	2·8 (0·3)	0·2 (0·1)	29·4 (0·8)	19·0 (0·8)	1·9 (0·4)
27b4c	HAT	57·3 (4·7)	1·0 (0·2)	3·1 (0·5)	0·7 (0·4)	34·8 (3·0)	16·0 (1·2)	1·6 (0·3)
	TG	57·1 (1·5)	0	3·0 (0·5)	0·2 (0·1)	32·0 (1·6)	19·7 (0·5)	1·3 (0·2)
27b4d	HAT	54·7 (1·1)	0·9 (0·1)	1·4 (0·4)	0·1 (0·1)	30·9 (0·7)	20·3 (0·7)	1·1 (0·2)
	TG	55·9 (0·9)	0	3·3 (0·3)	0·2 (0·1)	31·3 (1·0)	18·6 (0·3)	2·0 (0·2)
27b4h	HAT	54·7 (1·7)	0·8 (0·1)	2·0 (0·3)	0·3 (0·2)	32·3 (1·4)	17·4 (1·0)	1·8 (0·2)
	TG	49·3 (1·8)	0·1 (0·1)	1·0 (0·3)	0·3 (0·3)	20·6 (1·5)	25·9 (1·7)	1·1 (0·2)

Metaphase preparations were treated by the modified method of Dev *et al.* (1972) and chromosomes categorized by morphology and c-band. Bizarre chromosomes (e.g. ring and dicentric chromosomes) are included in the total. The standard error of the mean is given in brackets. 10 and 20 and 50 metaphase spreads were counted for each of the hybrids, male and female vole lymphocytes, and A9cl2a respectively. The variable numbers of polyploid metaphases in the different populations were not counted or included in the table.

†, the large bi-armed chromosomes of 27D2d* HAT were metacentric and were probably isochromosomes of the short arm of the vole X chromosome.

heterochromatic portion of the vole X chromosome – the long arm and a quarter of the short arm – to become densely staining, and leads, in addition, to the development of bands on the short arm (Figs. 5, 7). (This banding pattern of the short arm of the vole X chromosome differs from that published by Cooper & Hsu (1972), mainly in the absence of the telomeric band. This seems to be a characteristic of the Oxford colony of voles.) One hundred spreads from each of 3 hybrids (27A3, 27b4d and 27b3e) were initially examined in this way; 93, 89 and 73 spreads respectively from each hybrid contained the large heterochromatic vole X chromosome; in the other spreads no portion of the heterochromatic region of the X chromosome could be detected. Clearly the vole X chromosome is not stably integrated into the chromosomal complement of the hybrid cell.

When metaphases from 4 of the original clones were examined 1 month later by the method of Cooper & Hsu (1972) (these are designated by an asterisk), one clone (27b4d*) still contained the normal vole X chromosome, whereas the other three (27D2d*, 27D2b*, 27D3f*) contained a new chromosome whose banding pattern indicated that it was an iso-chromosome of the short arm of the vole X chromosome (Fig. 8). For example, of 50 spreads examined from 27D2d*, 40 contained the iso-chromosome and 10 lacked any heterochromatic material characteristic of the vole X chromosome. The large metacentric chromosomes seen in hybrid metaphases treated by the method of Dev *et al.* (1972), which were thought to arise from the vole X chromosome by deletion of a portion of the long arm, were probably these iso-chromosomes. They therefore arise at a high frequency, and hybrid cells carrying them are probably at a selective advantage as they rapidly replace in the population those that carry the normal vole X chromosome.

The short-arm of the vole X chromosome was present in all the hybrids grown in HAT, as were the vole enzymes glucose-6-phosphate dehydrogenase and inosinic acid pyrophosphorylase. Study of Table 2 indicates that few, if any, vole chromosomes lacking c-bands could be present in these hybrids, for the hybrid metaphases do not contain significantly more chromosomes without c-bands than do those of the A9 parent cells. The assignment of the locus for inosinic acid pyrophosphorylase activity to a particular chromosome can be strengthened if that chromosome is absent in derivatives of the hybrid that are able to grow in a medium that selects for the loss of that activity, i.e. in TG. A comparison of the chromosomal constitution of the hybrids grown in HAT with that of their derivatives grown in TG shows that it is only the vole X chromosome that is consistently absent in cells growing in TG (Table 2). In the case of clone 27D2d* it was the iso-chromosome of the short arm of the vole X chromosome that was absent in derivatives able to grow in TG. One hundred metaphase spreads made from each of five hybrid populations growing in TG (27A3 TG, 27b3h TG, 27b4c TG, 27D2b TG, 27b3g TG) were treated by the method of Cooper & Hsu (1972) and in none of them could any heterochromatic material from the vole X chromosome be detected.

CONCLUSIONS

When mouse A9 cells deficient in inosinic acid pyrophosphorylase are fused with vole lymphocytes, hybrid cells are obtained in HAT medium that contain an inosinic acid pyrophosphorylase activity possessing the electrophoretic mobility characteristic of the vole. They also contain glucose-6-phosphate dehydrogenase activities which are electrophoretically similar to both vole and mouse. Metaphase spreads of these hybrids contain the chromosome complement of the A9 cell, and few, if any, vole chromosomes are present, other than the large X chromosome. It can be inferred from these results that the loci for vole glucose-6-phosphate dehydrogenase and inosinic acid pyrophosphorylase are linked to the vole X chromosome. This conclusion is strengthened by the results of an analysis of hybrids grown in 6-thioguanine: these hybrids have lost the vole enzymes as well as the vole X chromosome. There were no exceptions to this co-segregation of the loci coding for the expression of the 2 vole enzymes and the vole X chromosome. The loci coding for these enzymes appear to be linked to the X chromosome in the vole as well as in the horse and donkey (Trujillo *et al.* 1965; Mathai *et al.* 1966), the mouse (Epstein, 1969, 1972), the hamster (Westerveld *et al.* 1972), the hare (Ohno *et al.* 1965), the kangaroo (Richardson *et al.* 1971) and man (Childs *et al.* 1958; Nyhan, 1968). The linkage of these genes on the X chromosome has thus been conserved during the evolution of mammals (Ohno *et al.* 1964; Ohno, 1973).

The vole X chromosome seems to be rather unstably integrated into the chromosome complement of the mouse A9 cell in these hybrids, for it is frequently lost. Furthermore, iso-chromosomes derived from the short arm of the vole X chromosome arise at a high frequency and hybrid cells carrying them must be at a selective advantage, for on culture they rapidly replace those that carry the normal vole X chromosome. The selective advantage may result from the duplication of active and necessary genes in the euchromatic portion of the short arm, and the loss of redundant and heterochromatic material on the long arm. One clone carrying the iso-chromosome contained vole inosinic acid pyrophosphorylase and glucose-6-phosphate dehydrogenase, thus suggesting that the loci for these activities are carried on the short arm of the vole X chromosome.

These hybrid cells contain, in addition to the chromosome complement of the mouse, part or all of the X chromosome material of the vole. As much of this extra vole material is heterochromatic, these hybrids should prove useful in cytological, physical and chemical studies on heterochromatin – and this is the reason why they were made.

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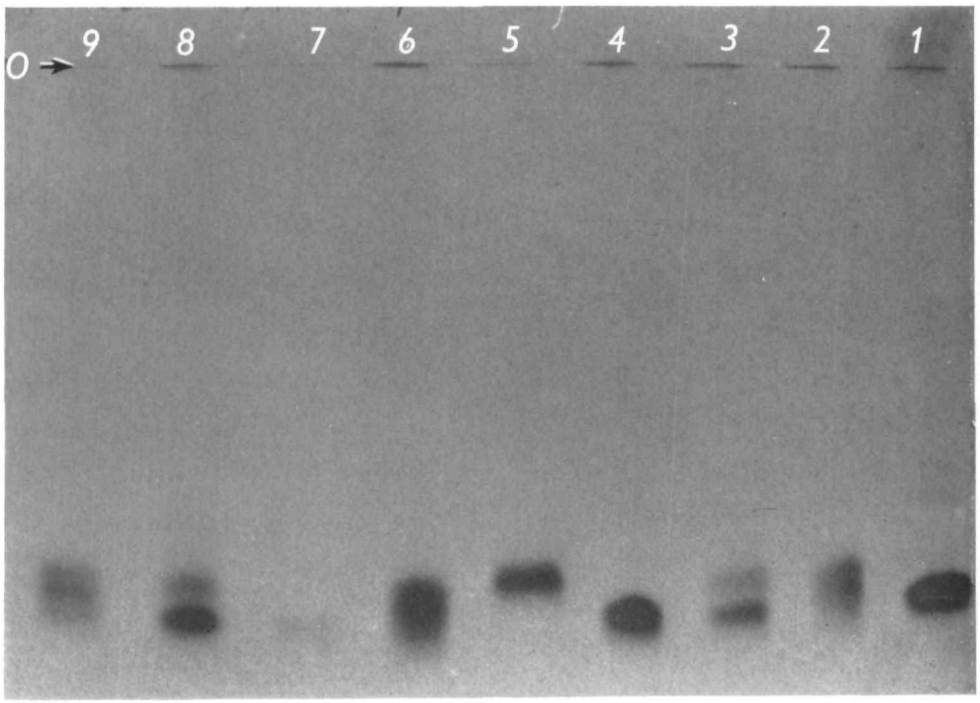
Fig. 2. Electrophoretic mobilities of glucose-6-phosphate dehydrogenase activities in mouse, vole and hybrid cells. Cell extracts were applied to 9 channels at the origin (*O*) on Cellogel, subjected to electrophoresis, stained for glucose-6-phosphate dehydrogenase activity and photographed. The glucose-6-phosphate activity produces a dark blue band. Channel *1*, A9cl2a; channel *2*, 27A2a HAT; channel *3*, mixture of A9cl2a and MAXY; channel *4*, 27b3h TG; channel *5*, MAXY; channel *6*, 27A2a HAT; channel *7*, 27b3f TG; channel *8*, a mixture of A9cl2a and MAXY; channel *9*, 27A2a HAT.

Fig. 3. Metaphase chromosomes of an A9 cell treated by a modification of the method of Dev *et al.* (1972). Nearly all the chromosomes have a c-band.

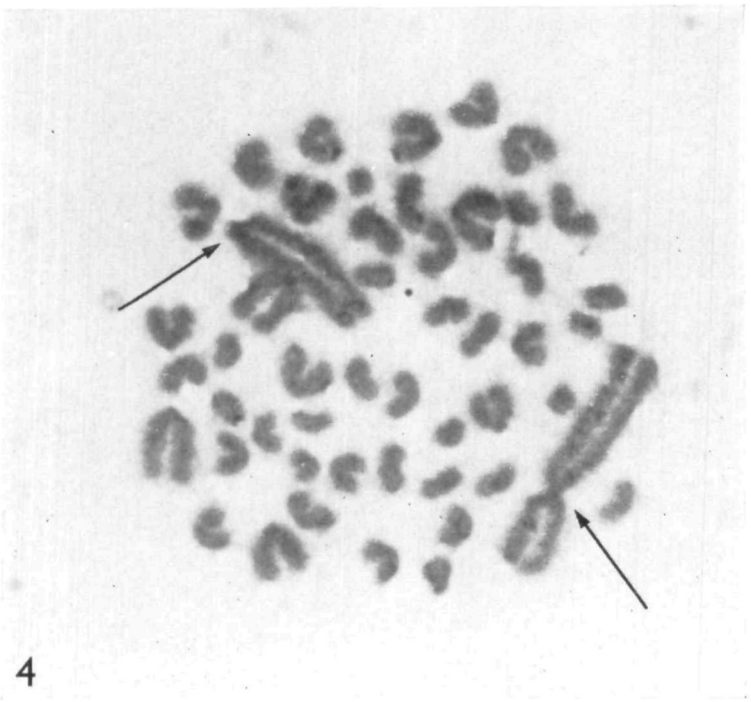
2



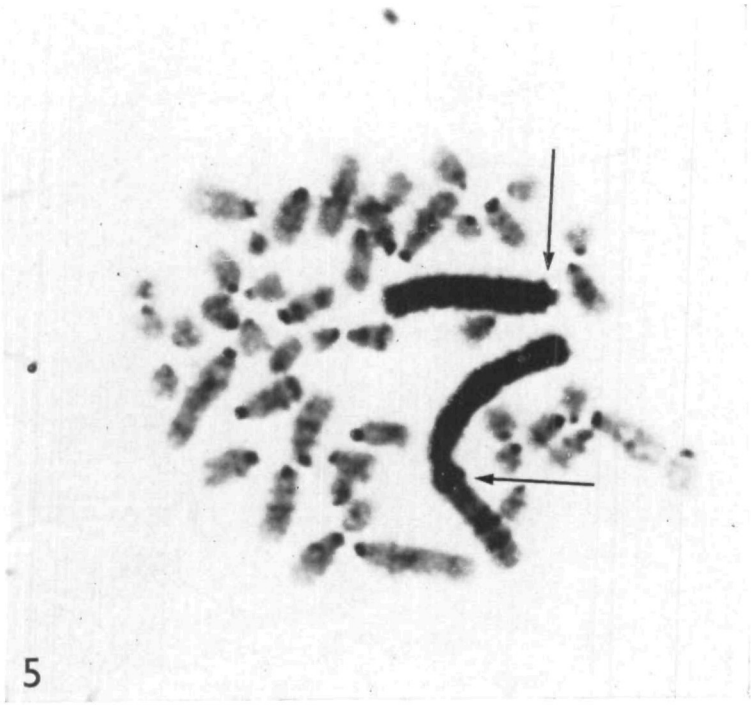
3

Fig. 4. Metaphase chromosomes of a vole lymphocyte, which had been induced to proliferate with phytohaemagglutinin, and treated by a modification of the method of Dev *et al.* (1972). None of the chromosomes possess c-bands. The 2 arrows point to the centromeres of the X (bi-armed) and Y (single-armed) chromosomes.

Fig. 5. Metaphase chromosomes of a MAXY cell treated by the method of Cooper & Hsu (1972). The heterochromatic regions of the X and Y chromosomes are densely stained and bands are visible on all the chromosomes. The MAXY cell line is aneuploid and possesses one metacentric marker chromosome. The 2 arrows point to the X (bi-armed) and Y (single-armed) chromosomes.



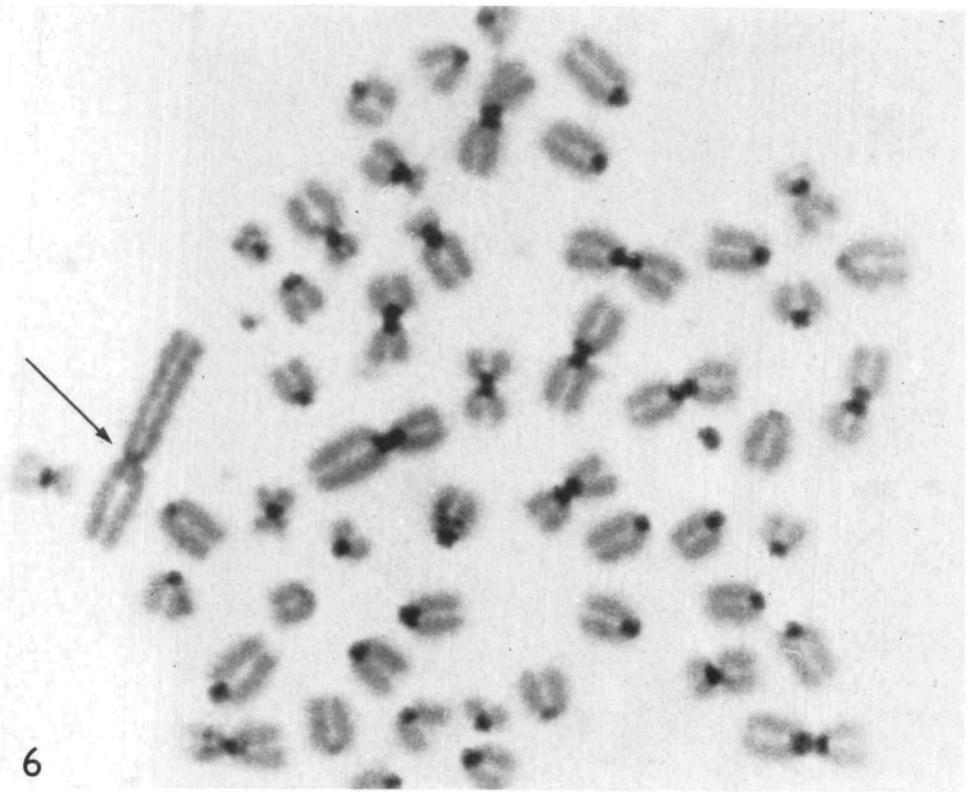
4



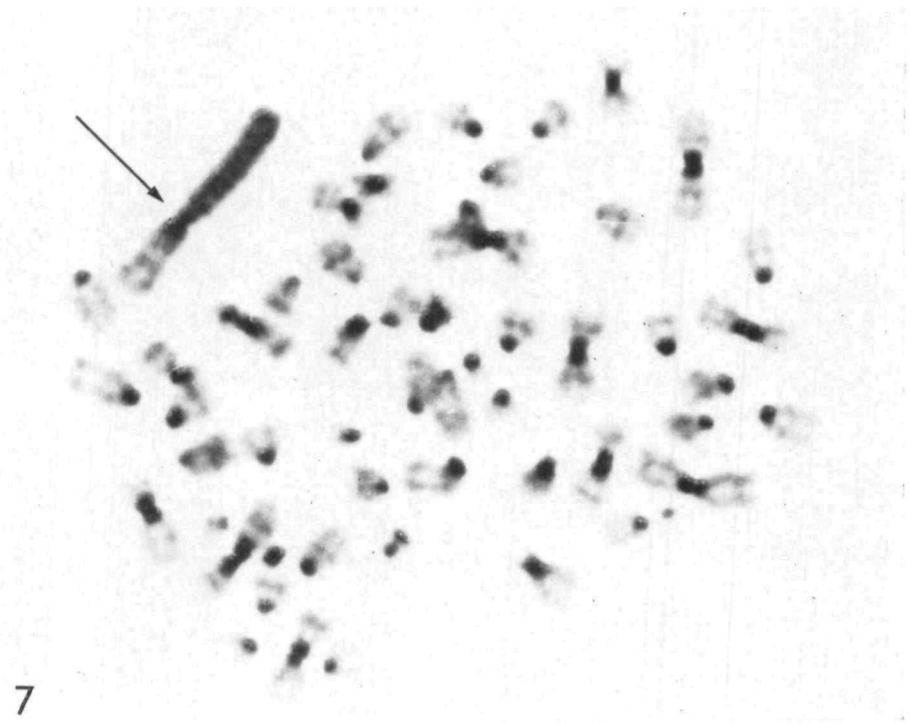
5

Fig. 6. Metaphase chromosomes of a cell from a hybrid clone (27b4d) treated by a modification of the method of Dev *et al.* (1972). The large vole X chromosome is arrowed; its long arm and one quarter of its short arm are slightly more densely staining than the remainder.

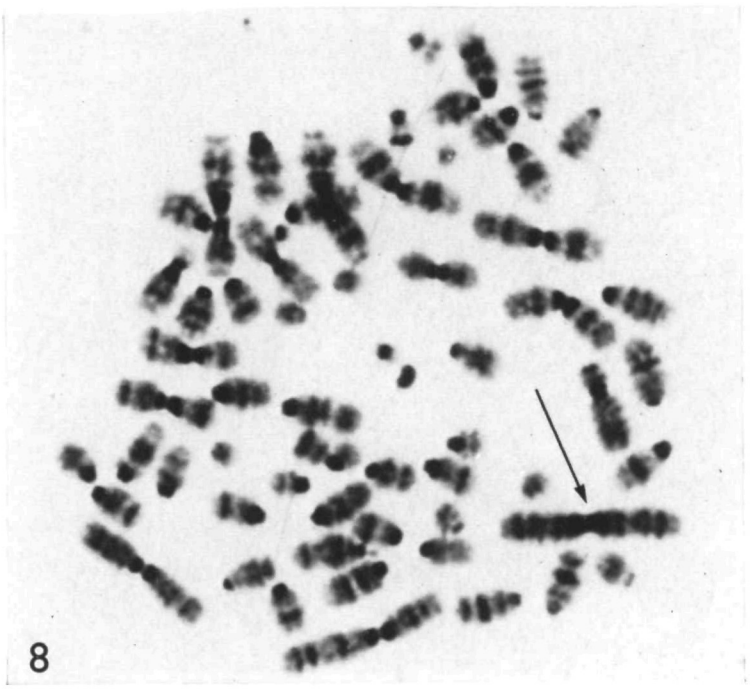
Fig. 7. Metaphase chromosomes of a cell from a hybrid clone (27b4d) treated by the method of Cooper & Hsu (1972). The heterochromatic vole X chromosome is clearly visible (arrowed).



6



7



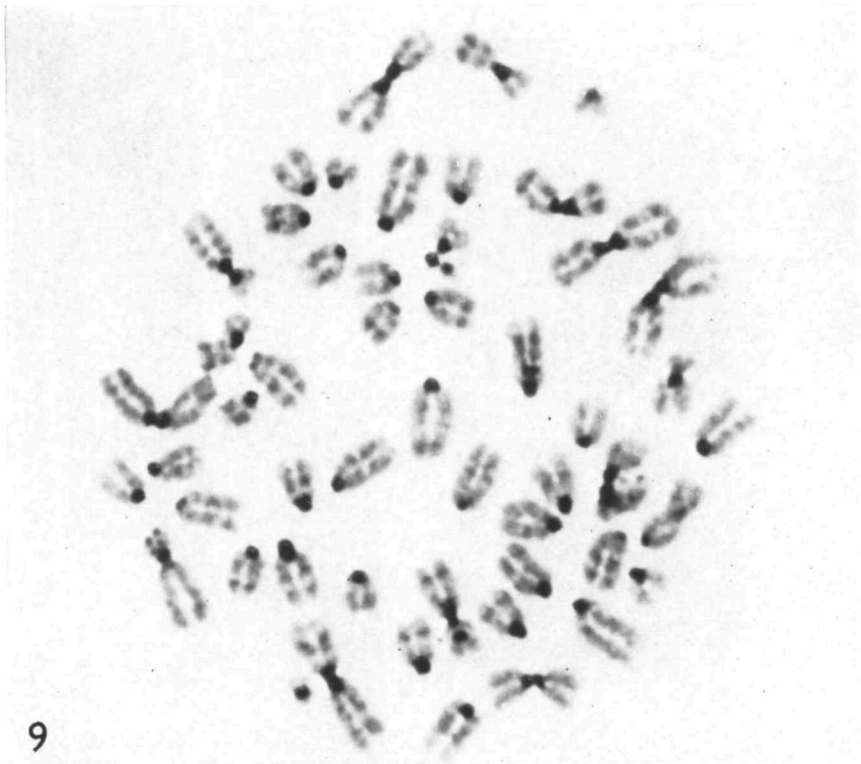


Fig. 8. Metaphase chromosomes of a cell from a hybrid clone (27D2 d*) treated by the method of Cooper & Hsu (1972). The iso-chromosome of the short arm of the X chromosome of the vole is arrowed.

Fig. 9. Metaphase chromosomes of a cell from a hybrid clone (27b3 g TG) treated by the method of Cooper & Hsu (1972). No chromosomes or heterochromatic material characteristic of the vole X chromosome can be detected.