Aust. J. Biol. Sci., 1977, **30**, 421–30

Studies on Metatherian Sex Chromosomes V.* Activity of the Paternally Derived Allele at the X-linked *Pgk-A* Locus in Some Tissues of the Kangaroos *Macropus giganteus* and *M. parryi*

J. L. VandeBerg,[A] *D. W. Cooper*[B] *and G. B. Sharman*

School of Biological Sciences, Macquarie University, North Ryde, N.S.W. 2113.
[A] Present address: Laboratory of Genetics, University of Wisconsin, Madison, Wisconsin, 53706, U.S.A.
[B] Person to whom reprint requests should be directed.

Abstract

In pretty-face wallabies (*M. parryi*) all adult males express either the N or the VP allozyme of the X-linked enzyme phosphoglycerate kinase, PGK-*A*, in all their tissues examined. Some females do likewise, but others express both. In about half of these latter females N is much stronger than VP and in the other half VP predominates. The more strongly expressed form is presumed to be of maternal origin although no pedigree data are available. This hypothesis is supported by data from a related species, the eastern grey kangaroo (*M. giganteus*). The tissues of four females of this species which were known to be heterozygous for the N and VE allozymes have been typed for their PGK-*A* phenotypes. Some tissues express only the maternally derived allozyme, i.e. they manifest complete inactivation of the paternally derived allele at this locus. The paternally contributed allozyme was detectable in other tissues, but it was usually much less pronounced than the maternal one. In no instance was the paternal allozyme more pronounced than the maternal one. Heterozygotes produced from reciprocal crosses were accordingly phenotypically different.

Introduction

It is now well established that sex chromosome dosage compensation in erythrocytes and lymphocytes of several kangaroo species is accomplished by inactivation of the paternal X chromosome (reviewed in Sharman 1973; Cooper *et al.* 1975*a*, 1975*b*). We have briefly reported a study of dosage compensation in other tissues of the pretty-face wallaby (*Macropus parryi*), in which two allozymes of the X-linked enzyme phosphoglycerate kinase, PGK-*A*, occur in nearly equal frequencies (VandeBerg *et al.* 1973). All males and some females expressed either the N or VP allozyme in erythrocytes, and the same allozyme in all tissues. The remaining females, however, expressed one allozyme in erythrocytes and in some tissues, but expressed both allozymes in other tissues. In all tissues in which both allozymes were present, the allozyme expressed in erythrocytes was much more active than the alternative one. We proposed that this latter class of females comprised heterozygotes in which the paternally derived allele was completely inactive in some tissues, and active to a lesser extent than the maternally derived allele in others. This hypothesis could not be tested directly, however, because the animals were shot in the wild and had unknown parentage. Here we give a full presentation of the data for the PGK-*A* phenotypes of heterozygous female *M. parryi* adults. We also describe the PGK-*A* phenotypes of tissues from four eastern grey kangaroos (*M. giganteus*) which were bred in our captive colony and were known to be heterozygous for the N and VE

* Part IV, *Aust. J. Biol. Sci.*, 1977, **30**, 115–25.

forms of PGK-*A*. The results support the hypothesis that the paternally derived *Pgk-A* allele is completely inactive in some tissues and partially active in other tissues.

Materials and Methods

Terminology

The gene symbols used are *Pgk-A*^{N}, *Pgk-A*^{VE}, and *Pgk-A*^{VP} which determine the N, VE, and VP allozymes respectively. Because of the parental source effect, the allele written first in the heterozygote is always the maternally derived one.

Animals

The *M. parryi* animals were shot under licence in a restricted area of New South Wales at Coombadjha (29°21′S., 152°31′42″E.), where they were in considerable numbers and considered to be an agricultural pest. Tissues were taken from each animal and frozen in dry ice within 1–3 h after death. Male N and VP animals served as controls for *M. parryi*. Data on reproduction in these specimens have been given by Maynes (1973).

Three of the *M. giganteus* females used in this study, SG52, SG60, and SG63, were from N♀ × VE♂ matings; and one, SG9, was from the reciprocal cross. At the age at which the tissues were studied all four *M. giganteus* animals expressed in their erythrocytes the same PGK-*A* type as their mother, and presumably carried the contrasting *Pgk-A* allele on their paternally derived X chromosome. When they were killed, SG60 was an adult, SG9 and SG52 were juveniles which had vacated the pouch, and SG63 was a very small pouch young. A preliminary report on SG63 has appeared previously (Cooper *et al.* 1971).

Control *M. giganteus* animals were also obtained from the captive colony at Macquarie University or shot in the wild. Tissues from VE and N males were used as hemizygous controls. No captive females known to be homozygous for *Pgk-A*^{N} were available for controls, but N females shot in the wild were used under the assumption that the majority would be homozygous because of the low frequency of the *Pgk-A*^{VE} allele.

Electrophoresis

Tissue samples were homogenized in a motor-driven glass homogenizer with a Teflon pestle using between one and seven parts by volume of ice-cold 0·01 M tris–citrate buffer, pH 7·3, to one part by weight of finely minced tissue. The variation in ratio of buffer to tissue was required to offset activity differences between tissues and between different developmental stages. Tissues such as liver, kidney, brain, heart and other red muscle have very similar PGK activity levels, and skeletal muscle has approximately twice as much PGK activity in juvenile and adult animals as do the other tissues mentioned. This was apparent from the staining intensity after electrophoresis, and it has also been shown by spectrophotometric assay of tissues from eutherian mammals (Scrutton and Utter 1968; Dalrymple *et al.* 1974). Accordingly, skeletal muscle was usually homogenized in seven parts buffer, and the other tissues mentioned in three parts buffer, giving 1/8 and 1/4 homogenates respectively. Smooth muscle was found to be particularly low in PGK activity, although this effect may have been caused partly or completely by the difficulty in achieving satisfactory homogenization of smooth muscle. It was therefore homogenized in one or two volumes of buffer per weight of tissue. The homogenates were centrifuged in a bench centrifuge at 4°C for 30 min to remove suspended particulate matter. The supernatant fractions were subjected to electrophoresis by the technique of Cooper *et al.* (1971). Intact erythrocytes were applied to the gel by the technique of VandeBerg and Johnston (1977).

Results

In Table 1 the data on the expression of N and VP in heterozygous *M. parryi* females are given in detail. Heart, skeletal muscle, diaphragm, tongue and one smooth muscle, the bladder, generally express both alleles in an asymmetric manner. As noted elsewhere (VandeBerg *et al.* 1973) we assume that the more strongly expressed form is controlled by the maternally derived allele, but direct evidence on

Table 1. PGK-*A* phenotypes in the tissues of 12 heterozygous female *M. parryi*

The allele or allozyme written first is presumed to be maternally derived. *Pgk-A*^{N}/*Pgk-A*^{VP}, *N* presumably from mother; *Pgk-A*^{VP}/*Pgk-A*^{N}, *VP* presumably from mother; N, strong expression of N; VP, strong expression of VP; N/(VP), strong N and weak VP; VP/(N), strong VP and weak N; (N), weak N; (VP), weak VP. All *Pgk-A*^{N}/*Pgk-A*^{VP} heterozygotes had N erythrocytes and all *Pgk-A*^{VP}/*Pgk-A*^{N} heterozygotes had VP erythrocytes

Tissue	Presumed *Pgk-A*^{N}/*Pgk-A*^{VP} heterozygotes						Presumed *Pgk-A*^{VP}/*Pgk-A*^{N} heterozygotes					
Animal No.:	5[A]	19	32	34	42	57[A]	7[A]	16[A]	23[A]	27[A]	30[A]	40
(a) Tissues which express both alleles												
Right auricle	(N)				N/(VP)	(N)		VP/(N)		VP/(N)		
Left auricle	N/(VP)				N/(VP)	(N)		VP/(N)				
Right ventricle, left ventricle, abdominal muscle[B]	N/(VP)	N/(VP)	N/(VP)	N/(VP)	N/(VP)	N/(VP)	VP/(N)	VP/(N)	VP/(N)	VP/(N)	VP/(N)	VP/(N)
Diaphragm	N					N	VP/(N)	VP/(N)	VP/(N)	VP/(N)	VP/(N)	
Tongue			N	N	N	N/(VP)				VP/(N)	VP/(N)	VP/(N)
Bladder	N	N	N	N	N	N	(VP)	VP/(N)	VP/(N)	VP/(N)	VP/(N)	VP
(b) Tissues which express only one allele												
Right kidney	N	N	N	N		N	VP	(VP)	VP	(VP)	VP	VP
Left kidney	N	N				N	VP	(VP)	VP	(VP)	VP	VP
Liver	N	N	N	N	N	N	VP	VP	VP	VP	VP	VP
Spleen	N					(N)	VP	VP				VP
Gut	N					(N)	VP	(VP)	(VP)	(VP)	(VP)	VP
Right and left ovary, right and left uterus[C]	(N)					N	(VP)	(VP)	(VP)	(VP)	(VP)	(VP)
Right lateral vagina	(N)										VP	VP
Median vagina	N	(N)				N	(VP)	(VP)	(VP)	VP	(VP)	(VP)
Left lateral vagina			N	(N)	(N)	N					VP	(VP)

[A] Known to have been heterozygous because they gave rise to a pouch young with erythrocyte PGK-*A* type unlike their own. The remainder were presumed to be heterozygotes because of their tissue patterns.

[B] Right ventricle of 5 was N.

[C] Left uterus in 7 and left ovary of 23 not examined. Both uteri of 27 were VP.

this point is lacking for this species. The failure to find VP in the tongue, bladder, and diaphragm of the *Pgk-A*^{N}/*Pgk-A*^{VP} heterozygotes is probably a reflection of the technical difficulty of discerning a weak VP band behind a strong N band; the reverse pattern, strong VP behind N, is more easily typed.

All tissues from the *M. giganteus* control animals expressed only a single PGK-A allozyme—that expressed in their erythrocytes. Some tissues from each of the four known heterozygotes were phenotypically identical to the control tissues; but other tissues expressed, in addition to the maternal PGK-*A* type, another usually fainter band equal in mobility to the paternal type. A gel showing the phenotypes of some of the tissues of SG60 is shown in Fig. 1. Activity of the paternally derived allele is

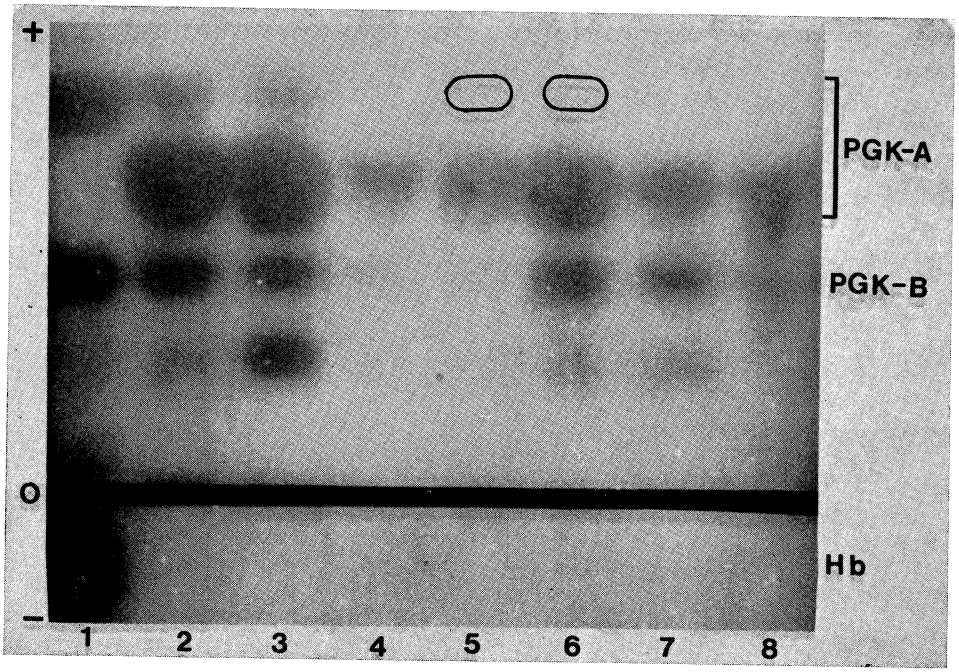

Fig. 1. Gel showing the PGK-*A* phenotypes of some tissues of SG60, a female *M. giganteus* from a N♀ × VE♂ mating. Explanations of the phenotype symbols are given in the subheading of Table 2. The values in parentheses indicate the ratio of tissue to buffer (w/v) used in homogenization. *1*, Erythrocytes applied as packed cells—VE standard; *2*, left auricle (1 : 3)—N/(VE); *3*, left retina (1 : 1)—N/(VE); *4*, left cornea (1 : 1)—N; *5*, ocular fluid from left eye (undiluted)—N/(VE)—the VE is very faint but clearly visible on original gel; *6*, left kidney (1 : 3)—N/(VE)—VE faint but clearly visible on original gel; *7*, right ear (1 : 1)—N; *8*, left ear (1 : 1)—N. The slowly migrating unlabelled band particularly evident in channel 3 is probably a sub-band of PGK-*B*. Faint VE bands are outlined in channels 5 and 6. Hb, haemoglobin.

evident in auricle, retina, ocular fluid, and kidney but not in cornea or ears. Fig. 2 shows the phenotypes of some of the tissues of SG9. Activity of the paternally derived allele was apparent in leg muscle, ventricle, lens, tongue, and auricle but not in liver, bladder, lung, or ovary. A comparison between the phenotypes of three tissues of SG9 and a male (SG2) hemizygous for VE is shown in Fig. 3. The phenotypes of the erythrocytes of the two animals are shown to be VE. Lens and ventricle of SG2

were also VE, but the same two tissues of SG9 were VE/(N).* The gel in Fig. 4 compares the phenotypes of four tissues of SG9 and SG60, the heterozygous progeny of reciprocal crosses. Each of the four tissues of SG9 expressed the exact reciprocal phenotype of the corresponding tissue of SG60. Erythrocytes and liver expressed VE for SG9, and N for SG60; ventricle and leg muscle expressed VE/(N) for SG9, and N/(VE) for SG60. Although tissues expressing approximately an equal ratio of the activities of maternally derived : paternally derived alleles were chosen for the gels pictured, the ratio did vary from tissue to tissue of a particular animal, and was not consistent for every tissue among the four animals.

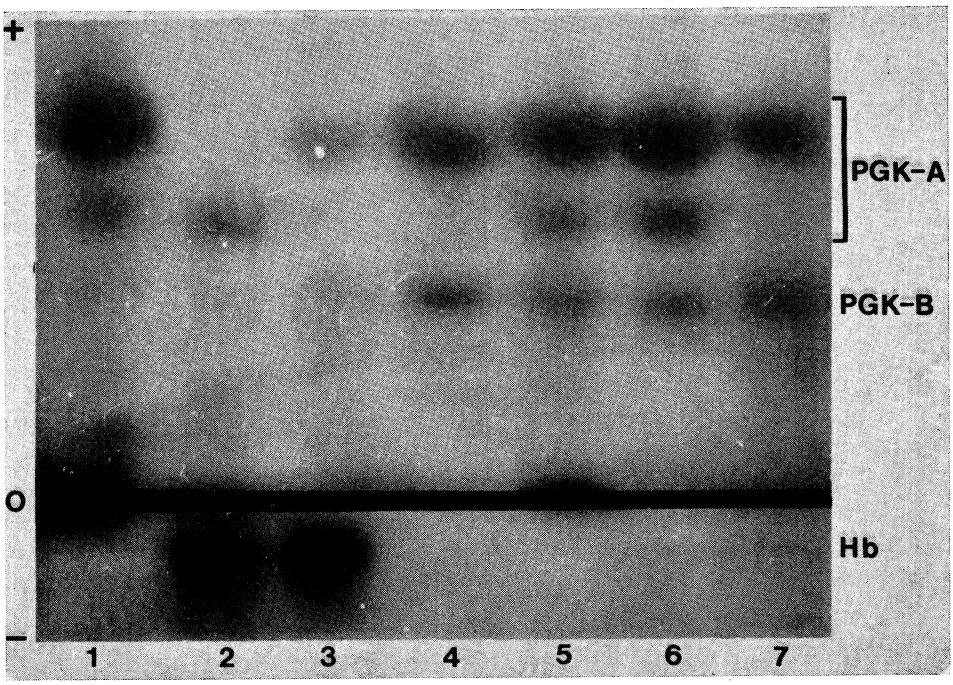

Fig. 2. Gels showing the PGK-*A* phenotypes of some tissues of SG9, a female *M. giganteus* from a VE♀×N♂ mating. All channels contain 1 : 3 (w/v) homogenates or lysates, except channel 6 which is a 1 : 4 (w/v) homogenate. *1*, Left leg muscle—VE/(N); *2*, erythrocyte lysate—N standard; *3*, erythrocyte lysate (SG9)—VE; *4*, liver—VE; *5*, right ventricle—VE/(N); *6*, right eye lens—VE/(N); *7*, bladder—VE. See subheading of Table 2 for phenotype symbols. Hb, haemoglobin.

The data for *M. giganteus* are summarized in Table 2. All tissues from SG60 and SG9 expressed predominantly the maternally derived *Pgk-A* allele. In three instances, however, the younger animals expressed both alleles equally; these were leg muscle and uteri from SG52, and uteri and ovaries combined for SG63. It was necessary to homogenize the uteri and ovaries of SG63 together because they weighed only 7·4 mg *in toto*. Consequently, the relative proportion of PGK-*A* activity contributed by these two organ types is unknown.

One unusual finding should also be noted. SG9 expressed both VE and N in its erythrocytes at 9 months of age, with the maternally contributed VE being the

* Explanations of phenotype symbols are given in the subheading of Table 2.

stronger band. This suggests that some heterozygotes may manifest both alleles in their blood during pouch life.

Discussion

The data from the four *M. giganteus* heterozygotes support the hypothesis that where expression of both alleles occurs the more weakly expressed one is from the father. The data from the *M. parryi* heterozygotes are consistent with this hypothesis, in that the numbers of animals with the N/(VP) and VP/(N) phenotypes are approximately equal (VandeBerg *et al.* 1973). While further data are desirable, the discussion below will assume the validity of this hypothesis for both species.

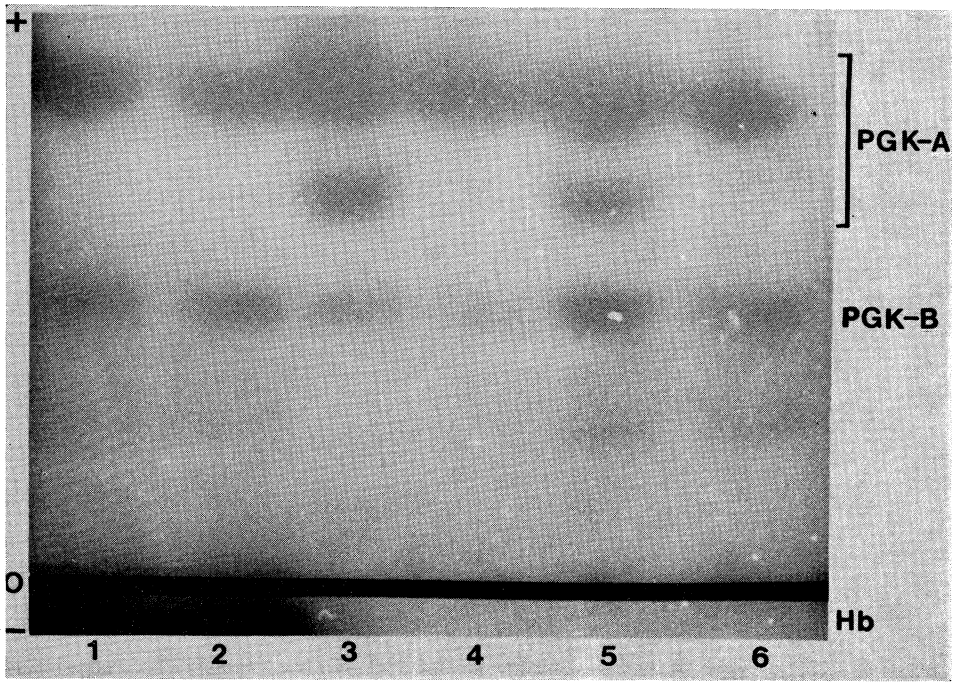

Fig. 3. Gel showing a comparison of the PGK-*A* phenotypes of three tissues of SG9 and a hemizygous VE male. Erythrocytes were applied as packed cells. The other tissues were homogenized in three parts buffer (w/v) except ventricle of SG2, which was homogenized in two parts buffer to compensate for activity loss during 30 months of frozen storage. The qualitative phenotypes of the tissues of SG9 have not changed during 1 year of frozen storage. *1*, SG9 erythrocytes—VE; *2*, SG2 erythrocytes—VE; *3*, SG9 right eye lens—VE/(N); *4*, SG2 lens—VE; *5*, SG9 right ventricle—VE/(N); *6*, SG2 ventricle—VE. See subheading of Table 2 for phenotype symbols. Hb, haemoglobin.

M. giganteus heterozygotes resemble *M. parryi* heterozygotes in the pattern of expression or non-expression of the presumed paternally derived allele, but with some exceptions. For directly comparable tissues the uteri of SG9 and SG52 and the combined urogenital tract of SG63 had expression of both N and VE but none of the *M. parryi* showed any expression in their uteri or vaginae. SG60 was the only animal in either species to express both alleles in kidney. Conversely the $Pgk\text{-}A^{VP}/Pgk\text{-}A^{N}$

heterozygotes of *M. parryi* all showed expression of both in the bladder whilst none of the *M. giganteus* individuals did so. There is thus some indication that the pattern of expression and non-expression may be species specific. But since the samples from the two species were obtained under different conditions and were not run at the same time, we cannot rule out the possibility that these differences merely reflect variations in the sensitivity of detection of the more weakly staining allozyme.

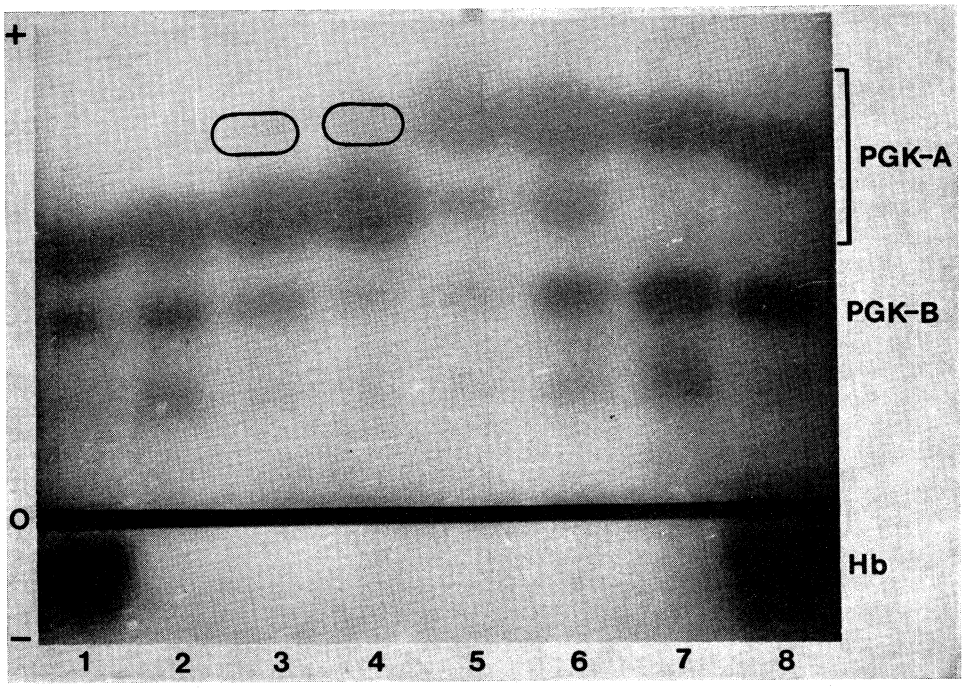

Fig. 4. Gel showing a comparison of the PGK-*A* phenotypes of four tissues of SG9 and SG60, the heterozygous progeny of reciprocal crosses. Erythrocytes were applied as packed cells. The values in parentheses indicate the ratio of tissue to buffer (w/v) used in homogenization. *1*, SG60 erythrocytes—N; *2*, SG60 liver (1 : 3)—N; *3*, SG60 right ventricle (1 : 3)—N/(VE); *4*, SG60 right leg muscle (1 : 7)—N/(VE); *5*, SG9 right arm muscle (1 : 7)—VE/(N); *6*, SG9 left ventricle (1 : 3)—VE/(N); *7*, SG9 liver (1 : 3)—VE; *8*, SG9 erythrocytes—VE. See subheading of Table 2 for phenotype symbols. Faint VE bands are outlined in channels 3 and 4. Hb, haemoglobin.

The tissues of the *M. giganteus* and *M. parryi* heterozygous females can be categorized into three groups as follows (see also Tables 1 and 2):

(1) tissues in which there is no detectable paternal X activity (observed at all ages);
(2) tissues which express predominantly the maternally derived allele, but to a lesser degree the paternally derived one (observed at all ages in *M. giganteus* but only in adult *M. parryi*; pouch young *parryi* had low PGK-*A* activity and so weak expression of the paternal allele may have gone undetected);
(3) tissues which express both alleles equally (observed only in the pouch young and the one juvenile of *M. giganteus*).

There are tendencies for particular tissue types to fall into one or another of the three categories. For example, striated and cardiac muscles tend to express the

Table 2. PGK-*A* phenotypes in tissues of female *M. giganteus* known to be heterozygous

The phenotypes were placed in five classes: (1) N, meaning only N; (2) N/(VE), meaning predominantly N with some VE; (3) N=VE, meaning equal quantities of N and VE; (4) VE/(N), meaning predominantly VE with some N; (5) VE, meaning only VE. Some of the tissues of SG52 expressed only faint PGK activity and the phenotypes are therefore indicated in parentheses. Some of the organs of SG63 were too small to be subdivided for homogenization and are therefore bracketed (i.e. they were homogenized together). Note that there were no instances in which the paternally derived allele was predominantly active, but approximately equal activity of both parental alleles was observed in three instances

Tissue type / Animal No.: / Age:	SG60 4·5 years	SG9 14·5 months	SG52 10–12 months	SG63 35 days
Erythrocyte phenotypes of parents (♀×♂):	N×VE	VE×N	N×VE	N×VE
Blood				
Erythrocytes	N	VE	N	N
Leucocytes		VE		
Lymph nodes		VE	N	
Lymph glands			N	
Striated muscle				
Legs	N/(VE)	VE/(N)	N=VE	N
Arms	N/(VE)	VE/(N)		
Abdominal wall	N/(VE)	VE/(N)		
Neck	N/(VE)	VE/(N)		
Tongue	N/(VE)	VE/(N)		
Diaphragm	N/(VE)	VE/(N)		N
Cardiac muscle				
Auricles	N/(VE)	VE/(N)	N	N/(VE)
Ventricles	N/(VE)	VE/(N)	N/(VE)	
Smooth muscle				
Oesophagus	N/(VE)	VE		
Stomach	N	VE		
Duodenum	N	VE		N
Small intestine	N	VE		N
Rectum	N/(VE)	VE		
Bladder	N	VE	N	N
Vaginae	N	VE		
Uteri	N	VE/(N)	N=VE	
Reproductive tissue				N=VE
Ovaries	N	VE	(N)	
Follicle cells	N			
Fallopian tubes			(N)	
Mammary tissue			(N)	
Internal organs				
Lungs	N	VE	(N)	N
Brain	N/(VE)	VE/(N)	N/(VE)	N
Spleen	N	VE	N	N
Kidneys	N/(VE)	VE	N	N
Liver	N	VE	N	N
Outer ears	N	VE		
Eyes				
Lenses	N/(VE)	VE/)N)		N
Retinas	N/(VE)	VE/(N)		
Corneas	N	VE		
Ocular fluid	N/(VE)	VE/(N)		

paternally derived allele, whereas smooth muscles do not. But exceptions do occur when the different tissues of a single tissue type are compared for a given animal, e.g. smooth muscle for SG60 and SG9. There is also some variation between animals, e.g. kidneys in SG60 express both allozymes but only one is expressed in the other animals. The possible biological significance of the relationship between tissue type and the extent of the expression of the paternally derived allele is obscure.

The question of whether the paternally derived allele is expressed in the germ line is of particular interest, because genetic data indicate that it can be transmitted to the next generation in the expected 50% frequency and there be expressed in an entirely normal manner (VandeBerg *et al.* 1977). SG63, the 35-day-old pouch young, manifested both alleles in its reproductive tissue. At this stage of development in a related species, the tammar wallaby (*M. eugenii*), oocytes comprise a considerable part of the total reproductive tissue (Alcorn 1975). It is therefore tempting to interpret this observation on SG63 to mean that both *Pgk-A* alleles are active in oocytes. This may be so, but the possibility remains that the paternal allozyme is really being contributed by the somatic tissue of the reproductive organs, and that the paternal allele is inactive in oocytes. Examination of oocytes uncontaminated by significant quantities of somatic cells is necessary to resolve this question.

It is obvious that the decision as to whether the paternally derived allele is to be partly active or inactive must for some tissues be made after the three primary germ layers of the embryo are formed. For example, skeletal muscle and blood are both mesodermal in origin but in adults at least skeletal muscle regularly expresses the paternally derived allele whilst blood does not. There is tissue-to-tissue and animal-to-animal variation for tissue of endodermal origin, such as liver and gut. Both tissues of ectodermal origin, brain and eye (with the exception of the cornea), expressed the paternally derived allele.

It is not valid to deduce that all loci on the kangaroo X chromosome behave like the *Pgk-A* locus. The only other X-linked locus which has been extensively investigated in kangaroos is that for the enzyme glucose-6-phosphate dehydrogenase (G6PD, locus symbol *Gpd*) (Johnston and Sharman 1975). No expression of the paternally derived *Gpd* allele is detectable in any tissues of *M. robustus* females heterozygous for the F and S allozymes of this enzyme (Sharman and Johnston 1973). Whether this reflects a genuine difference between the two loci, or whether it reflects the greater difficulty of detecting slight expression of a *Gpd* allele is at present not entirely settled, although the former hypothesis is probably the correct one (Johnston *et al.*, unpublished data).

Our results show that female kangaroos could occasionally have a selective advantage for heterozygotes at their sex-linked loci, although they are less likely to do so than eutherian mammals. If such selective advantage is sufficiently common, it may be that the manifestation of the paternally derived allele is a response to the same kind of selection pressures which have brought about random X inactivation in eutherian females. An alternative possibility is that selection has favoured a dosage effect in females, irrespective of whether the alleles are the same or not.

Acknowledgments

This work was supported by grants from the Australian Research Grants Committee and Macquarie University to D.W.C. and G.B.S. and was presented in a Ph.D. thesis to Macquarie University by J.L.V.

References

Alcorn, G. T. (1975). Development of the ovary and urinogenital ducts in the tammar wallaby *Macropus eugenii* (Desmarest, 1817). Ph.D. Thesis, Macquarie University.

Cooper, D. W., Johnston, P. G., Murtagh, C. E., Sharman, G. B., VandeBerg, J. L., and Poole, W. E. (1975*a*). Sex-linked isozymes and sex chromosome evolution and inactivation in Australian marsupials and monotremes. In 'Isozymes'. Vol. III. 'Developmental Biology'. (Ed. C. L. Markert.) pp. 559–73. (Academic Press: New York.)

Cooper, D. W., Johnston, P. G., Murtagh, C. E., and VandeBerg, J. L. (1975*b*). Sex chromosome evolution and activity in mammals, particularly kangaroos. In 'The Eukaryote Chromosome'. (Eds W. J. Peacock and R. D. Brock). pp. 381–93. (Australian National University Press: Canberra.)

Cooper, D. W., VandeBerg, J. L., Sharman, G. B., and Poole, W. E. (1971). Phosphoglycerate kinase polymorphism in kangaroos provides further evidence for paternal X-inactivation. *Nature (London) New Biol.* **230**, 155–7.

Dalrymple, R. H., Cassens, R. G., and Kastenschmidt, L. L. (1974). Glycolytic enzyme activity in developing red and white muscle. *J. Cell Physiol.* **83**, 251–7.

Johnston, P. G., and Sharman, G. B. (1975). Studies on metatherian sex chromosomes. I. Inheritance and inactivation of sex linked allelic genes determining glucose-6-phosphate dehydrogenase variation in kangaroos. *Aust. J. Biol. Sci.* **28**, 567–74.

Maynes, G. M. (1973). Aspects of reproduction in the whiptail wallaby, *Macropus parryi*. *Aust. Zool.* **18**, 43–6.

Scrutton, M. C., and Utter, M. F. (1968). The regulation of glycolysis and glyconeogenesis in animal tissues. *Annu. Rev. Biochem.* **37**, 249–302.

Sharman, G. B. (1973). The chromosomes of non-eutherian mammals. In 'Cytotaxonomy and Vertebrate Evolution'. (Eds A. B. Chiarelli and E. Capanna.) (Academic Press: London.)

Sharman, G. B., and Johnston, P. G. (1973). X chomosome and X-linked gene inactivation in marsupials. *Genetics* **74**, s250.

VandeBerg, J. L., Cooper, D. W., and Sharman, G. B. (1973). Phosphoglycerate kinase *A* polymorphism in the wallaby, *Macropus parryi*: activity of both X chromosomes in muscle. *Nature (London) New Biol.* **243**, 47.

VandeBerg, J. L., Cooper, D. W., Sharman, G. B., and Poole, W. E. (1977). Studies on metatherian sex chomosomes. IV. X linkage of PGK-*A* with paternal X inactivation confirmed in erythrocytes of grey kangaroos by pedigree analysis. *Aust. J. Biol. Sci.* **30**, 115–25.

VandeBerg, J. L., and Johnston, P. G. (1977). A simple technique for long term storage of erythrocytes for electrophoresis. *Biochem. Genet.* **15**, 213–15.

Manuscript received 29 March 1977