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Studies on Metatherian Sex Chromosomes. XII.* Sex-linked Inheritance and Probable Paternal *X*-inactivation of α-Galactosidase A in Australian Marsupials

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Abstract

An investigation of genetic variation in the electrophoretic mobility of the enzyme α-galactosidase A (EC 3.2.1.22) has been carried out for 33 species of Australian metatherian (marsupial) mammals. The results are compatible with the enzyme being sex-linked in macropodids (kangaroos and wallabies) and probably in dasyurids (marsupial 'mice', etc.), as it is in eutherian (placental) mammals. The results also suggest that the mode of dosage compensation for this locus is the same as for other sex-linked loci in kangaroos, i.e. paternal X inactivation, rather than the random X inactivation system of eutherian mammals. The bearing of the enzyme mobility data on phylogenetic relationships among macropodid species is discussed.

Introduction

The enzyme α-galactosidase A (α-D-galactosidase; α-D-galactoside galactohydrolase; EC 3.2.1.22; AGA-A) is sex-linked in man (Kint 1970; Grzeschik *et al.* 1972), mouse (Kozak *et al.* 1975; Lusis and West 1976), cattle (Heuertz and Hors-Cayla 1978) and rabbits (Echard *et al.* 1981) but seemingly not in horses and donkeys (Beutler and Kuhl 1972). With the exception of the two equid species, these results conform with Ohno's thesis that the mammalian X chromosome is an evolutionarily 'frozen' linkage group (Ohno 1967). Cloning of human cultured cells indicated that the locus is subject to dosage compensation by random X-inactivation (Romeo and Migeon 1970), although a family with non-random inactivation has been reported (Ropers *et al.* 1977). Since all of the species so far investigated are eutherians (placental mammals), it is worth while examining the genetic control of AGA-A in the nearest relatives of eutherians, namely the metatherians (marsupials). Metatherians resemble eutherians in carrying the genes for the enzymes glucose-6-phosphate dehydrogenase, phosphoglycerate kinase, and hypoxanthine phosphoribosyltransferase on their X chromosome (Johnston and Sharman 1975; VandeBerg *et al.* 1977; Graves *et al.* 1979; Donald and Hope 1981). However, they differ in two respects: (1) they have paternal X-inactivation as their mode of dosage compensation; and (2) they have some tissues which show incomplete dosage compensation (reviewed in Cooper *et al.* 1977*b*). Here we report data concerning these two questions for the *Aga-A* locus in marsupials.

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Materials and Methods

Animals

The origin of all macropodids, possum, and bandicoot species are given in Table 1. The origins of the species hybrids are shown in Table 2. With one exception, the origin and numbers of all dasyurid specimens are given in Cooper and Woolley (1983). These latter authors have given new generic names to some species previously assigned to *Antechinus*. These are *Pseudantechinus* (≡ *Antechinus*) *macdonnellensis*, *Dasykaluta* (≡ *Antechinus*) *rosamondae*, *Parantechinus* (≡ *Antechinus*) *apicalis*, and *Parantechinus* (≡*Antechinus*) *bilarni*. For ease of reference by the reader, the older names are used here. The *Dasyurus viverrinus* sample was obtained from Dr J. Nelson, Department of Zoology, Monash University, in 1971.

Table 1. Origin of animals used in the study

Species	Origin	No.	Reference
Macropus eugenii	Kangaroo Island, S.A.	20	
Macropus parma	Northern New South Wales	5	Maynes (1977)
Macropus giganteus	Dunedoo, N.S.W., or bred in captivity at Macquarie University	50	
Macropus rufogriseus	Dunedoo, N.S.W.	10	
Macropus parryi	Northern New South Wales	43	VandeBerg *et al.* 1973, 1977
Macropus robustus	Captive colony at Macquarie University, based on animals from New South Wales, South Australia, and Western Australia	30	
Macropus agilis	Captive at Macquarie University	1	
Wallabia bicolor	Captive colony at Macquarie University	4	
Macropus rufus (≡ *Megaleia rufa*)	Captive colony at Macquarie University	7	
Thylogale thetis	Moonpar State Forest and Myall River State Forest, New South Wales	28	
Thylogale billardierii	Captive colony at Macquarie University based on Flinders Island, Bass Strait, animals	8	
Trichosurus vulpecula	Sydney metropolitan area	17	
Trichosurus vulpecula arnhemensis	Jabiru, N.T.	4	Kerle (unpublished data)
Trichosurus caninus	Cloud's Creek, N.S.W.	40	VandeBerg *et al.* (1979)
Pseudocheirus peregrinus	Sydney metropolitan area	2	
Perameles nasuta	Sydney metropolitan area	20	Close (1975)
Isoodon macrourus	Townsville and Mona Vale, Qld; Thursday Island, N.T.; Sydney metropolitan area	44	
Isoodon obesulus	Tasmania	1	
Potorous tridactylus	Captive colony at Macquarie University, based on Tasmanian and mainland animals	5	

Sample Preparation

Kidney samples were homogenized in 3 parts gel buffer by volume to 1 part tissue by weight and spun at 3000 rpm in the cold room for 10 min. *Fibroblast cultures* were prepared as in Cooper *et al.* 1977*a*. At least 10^6 cells are necessary to do an AGA-A typing. *Electrophoresis* was performed on Cellogel (Chemetron, Milan) as in Grzeschik *et al.* (1972) except that the buffer was 0·01 M phosphate, pH 7·0. *Identification of macropodid species hybrids*: metaphase spreads of chromosomes from leucocyte or fibroblast cultures were made to verify the identity of hybrid animals.

The locus symbol used is *Aga-A*.

Results

Fig. 1 (*left*) illustrates four of the mobilities found in macropodid species. Each pattern consists of one major band with a varying number (one to four) of faster minor bands. Fig. 1 (*right*) shows the polymorphisms seen in *Antechinus rosamondae*, a dasyurid species.

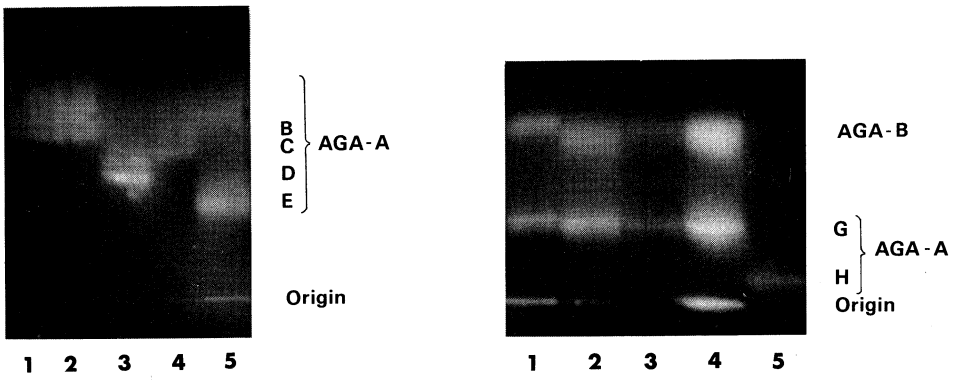

Fig. 1. *Left*, Four of the AGA-A mobilities found in macropodid species of marsupials. Channels 1 and 2 have B, 3 has D, 4 has C and 5 has E. *Right*, AGA-A polymorphism in *Antechinus rosamondae*. Channels 1–4 have mobility G and channel 5 has H. The fast migrating band labelled AGA-B is presumably α-galactosidase B, now thought to be α-*N*-acetylgalactosaminidase (see text). Migration is towards the top in both parts of the figure.

Table 2. α-Galactosidase-A (AGA-A) types in kangaroo species hybrids

Parental species[A]	AGA types of parental species[B]	Ref. No. (origin of hybrid)	Sex of hybrid	Material of hybrid examined	AGA type of hybrid
M. robustus × *W. bicolor*[C]	E × C	OB1 (Macquarie)	Male	Fibroblasts	E
M. rufogriseus × *M. giganteus*[D]	C × D	RG1 (Macquarie)	Male	Fibroblasts, kidney	C
M. rufus × *M. fuliginosus*[E]	E × D	G405 (Div. Wildlife Res., Canberra)	Female	Fibroblasts	E
M. giganteus × *M. agilis*[F]	D × C	(Dr T. Kirkpatrick, Queensland Dept. of Primary Industry, Warwick, Qld)	Female	Fibroblasts	D
M. rufogriseus × *M. agilis*[G]	C × C	RA1 (Macquarie)	Female	Fibroblasts, kidney	C
M. robustus robustus × *M. rufus*[H]	E × E	OK1 (Macquarie)	Female	Fibroblasts	E

[A] Female parent written first: W = *Wallabia*; M = *Macropus*.
[B] Type of female parent written first. In no case was the actual parent examined.
[C] Chromosomes examined by D.W.C. (unpublished data).
[D] Referred to in Murray *et al.* (1979).
[E] Listed in Calaby and Poole (1971) as *Megaleia rufa* × *Macropus fuliginosus*. Chromosomes examined by D.W.C. (unpublished data).
[F] Not karyotyped.
[G] G6PD type described in Johnston *et al.* (1978).
[H] G6PD type described in Richardson *et al.* (1971), karyotype given in Donald and Cooper (1978).

Dasyurids and bandicoots typically showed two regions of activity. Both forms varied in mobility between species. Although the faster form is well defined in this figure, it was weak or smeared in many samples and no regular typing for it was possible; all data presented concern the more cathodal of the two isozymes.

Table 2 shows data on the inheritance of AGA-types in macropodid species hybrids. Where the two parental species differed the hybrid offspring, two males and two females, each have only the maternal allozyme. In particular no evidence of expression of the paternally derived allozyme could be seen in the fibroblast cultures of the two female hybrids of this kind. However, the activity in these cultures was rather weak. The data are consistent with the hypothesis of sex linkage with paternal X-inactivation for the inheritance of the enzyme.

Table 3. Family data for AGA-A allozymes in *A. rosamondae* and the Ningbing Antechinus

Species	Parental phenotypes		No. of litters	Numbers and phenotypes of offspring	
	Mother	Father		Females	Males
Antechinus rosamondae	G	G	3	9G	8G
	G	—	2	6G	6G
	H	—	1		1H
	—	—	1	1H	1G,3H
Ningbing Antechinus	—	—	3	4D	1D,2F

Particular note should be taken of the fact that reciprocal crosses, C × D and D × C, gave different results, which is incompatible with hypotheses involving autosomal dominance. Limited family data for inheritance of allozymic differences within two dasyurid species given in Table 3 are likewise consistent with the hypothesis of sex linkage with paternal X-inactivation, although they do not exclude hypotheses of dominant inheritance.

Table 4. Species with AGA-A polymorphisms

Species	Sex	Number of variants in each sex			Totals
Macropus parryi		B	C	D	
	♂		27	1	28
	♀	1	13	1	15
Thylogale thetis		D	E	F	
(*a*) Myall Creek	♂	3		2	5
State Forest	♀	6	2	2	10
(*b*) Moonpar State Forest	♂	4			4
and Macquarie University	♀	9			9
Antechinus rosamondae		G	H		
	♂	13	2		15
	♀	13	2		15
Ningbing Antechinus		D	F		
	♂	3	3		6
	♀	0	2		2
Antechinus macdonnellensis		E	G		
	♂		2		2
	♀	1	3		4

Data on the frequency of the allozymes in the five species within which polymorphisms were discovered are given in Table 4. The triple-banded dimeric pattern (i.e. the pattern expected of a heterozygote—see Rebourcet *et al.* 1975) was not found, and there was no

indication of any differences in frequency between the sexes. These features are also consistent with sex linkage and paternal X-inactivation.

The enzyme shows a fairly high degree of genetic variability. Nearly one-third (5/18) of the species for which at least five individuals were examined showed polymorphism. Eight different mobilities designated A (the fastest) to H (the slowest) were recognized. The mobilities of individual species are shown in Table 5. In general these allozymes were easily separable. However, in some runs two usually separable forms failed to separate. In particular macropodid B and C sometimes failed to separate as did D and E. The reason for this behaviour is unknown. Within each of the eight recognized categories some variation between samples from different species was noted in some runs. Since these differences were not easily reproducible, no attempt has been made to subdivide the eight mobilities further.

Electrophoresis and staining of homogenates of tissues other than cultured fibroblasts and kidney gave weak and smeared bands which were unsuitable for allozyme typing.

Table 5. Electrophoretic mobilities of AGA-A in some Australian marsupial species

Species given in brackets indicate that this species is polymorphic. Species followed by a question mark probably have this mobility, but direct comparison with the reference sample was not possible

Mobility	Species[A]
A	*P. tridactylus*
B	*M. eugenii*, *M. parma*, (*M. parryi*)
C	(*M. parryi*), *M. rufogriseus*, *M. agilis*, *W. bicolor*, *T. vulpecula*, *T. vulpecula arnhemensis*, *S. crassicaudata*, *A. stuartii*, *A. bellus*(?)
D	*M. giganteus*, *M. fuliginosus*, *P. peregrinus*, (Ningbing Antechinus), *T. stigmatica*, (*T. thetis*), *Petrogale* spp.[B], (*M. parrvi*)
E	*M. rufus*, *M. robustus*, [*A. macdonnellensis* (*tanami*)], (*T. thetis*), *T. billardierii*, *D. viverrinus*(?)
F	(Ningbing Antechinus), *A. bilarni*, *Dasyurus cristicauda*, *A. apicalis*, (*T. thetis*), *P. nasuta*(?)
G	(*A. rosamondae*), *A. macdonnellensis*, [*A. macdonnellensis* (*tanami*)], *I. obesulus*(?)
H	(*A. rosamondae*), *I. macrourus*(?)

[A] Generic names given in Table 1.

[B] One individual each of *Petrogale inornata* (16A chromosome race) and *P. godmani* were examined. All other rock wallaby species so far examined have the same mobility for this enzyme (D. A. Briscoe and G. B. Sharman, personal communication).

Discussion

The results obtained are consistent with the hypothesis of X-linkage and paternal X-inactivation for *Aga-A* in metatherian mammals. It thus becomes the fourth locus which is known to be X-linked in both metatherians and eutherians, providing further evidence in support of Ohno's (1967) postulate that the sex chromosomes in the two groups are at least partly homologous. This homology is probably not complete, since it has recently been found that the enzyme steroid sulfatase, which is X-linked in eutherians is probably autosomal in kangaroos and dasyurids (Cooper *et al.*, unpublished data).

The only direct evidence for paternal X-inactivation comes from the two female hybrids in which only the maternally derived allozyme was detectable. The single-band nature of the polymorphism in the five species where it was found and the lack of sex differences in frequency of the allozymes are both consistent with paternal X-inactivation, but they are also consistent with other hypotheses. The variation observed in the three dasyurid species (*A. rosamondae*, Ningbing Antechinus and *A. macdonnellensis*) is the first indication that this group of marsupials have paternal X-inactivation. Hitherto the data have all been from macropodids and phalangerids (VandeBerg *et al.* 1979 and previous references therein).

Although their fibroblast AGA-A activities were weak, the results for the two female hybrids also suggest that there might not be any activity for the paternally derived allele in fibroblasts. These results require confirmation with cultures with more activity, preferably for a polymorphic allozyme system within a species in order to rule out the possibility that the behaviour of the locus is different in species hybrids. If confirmed the failure of the paternally derived *Aga-A* allele to be expressed in cultured fibroblasts would contrast with the *Gpd* and *Pgk-A* loci (Johnston *et al.* 1978; Cooper *et al.* 1977*a*), both of which have expression of the paternally derived allele in such cells, although probably to differing degrees. Differential behaviour of the three loci would suggest that the marsupial X chromosome is not coordinately controlled, unlike the eutherian X. Elsewhere we have argued that instead it is subject to a piecemeal system of dosage compensation, as appears to operate in *Drosophila* (Cooper *et al.* 1977*b*; VandeBerg *et al.* 1983).

Although some variation was observed within and between species for the faster of the two zones of enzyme activity in dasyurids, no worth-while data could be collected. It is possible that this enzyme represents α galactosidase-B, which has recently been shown to be α-*N*-acetylgalactosaminidase (reviewed in Schram and Tager 1981).

The mobilities observed for macropodid species show similarities which are consonant with the generally accepted view of their relationships (Table 5). The two species of grey kangaroo share a mobility (D), which is also found in the *Thylogale* and *Petrogale* species. Since these species are very widely separated the D mobility might therefore be the original ancestral mobility in the macropodid group. The euros and wallaroos (*M. robustus*) and red kangaroo share a mobility (E); these species are regarded as forming a unified group within the macropodids (Kirsch 1977; G. B. Sharman, personal communication). Likewise the closely related *M. eugenii* and *M. parma* (see Maynes 1977) also have a shared mobility (B).

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References

Beutler, E., and Kuhl, W. (1972). Biochemical and electrophoretic studies of α-galactosidase in normal man, in patients with Fabry's disease, and in Equidae. *Am. J. Hum. Genet.* **24**, 237–49.

Calaby, J. H., and Poole, W. E. (1971). Keeping kangaroos in captivity. *Int. Zool. Yb.* **11**, 5–12.

Close, R. L. (1975). Sex chromosome loss and enzyme activity during bandicoot development. Ph.D. Thesis, Macquarie University, North Ryde, N.S.W.

Cooper, D. W., Edwards, C., James, E., Sharman, G. B., VandeBerg, J. L., and Graves, J. A. M. (1977*a*). Studies on metatherian sex chromosomes. VI. A third state of an X-linked gene: partial activity for the paternally derived *Pgk-A* allele in cultured fibroblasts of *Macropus giganteus* and *M. parryi*. *Aust. J. Biol. Sci.* **30**, 431–43.

Cooper, D. W., Johnston, P. G., Sharman, G. B., and VandeBerg, J. L. (1977*b*). The control of gene activity on eutherian and metatherian X chromosomes: a comparison. In 'Reproduction and Evolution'. Proc. 4th Symp. Comp. Biol. Reprod. (Eds J. H. Calaby and C. H. Tyndale-Biscoe.) pp. 81–7. (Australian Academy of Science: Canberra.)

Cooper, D. W., and Woolley, P. A. (1983). Confirmation of a new species of small dasyurid marsupial by electrophoretic analysis of enzymes and proteins. *Aust. J. Zool.* **34**, 743–51.

Donald, J. A., and Cooper, D. W. (1977). Studies on metatherian sex chromosomes. III. The use of tritiated uridine-induced chromosome aberrations to distinguish active and inactive X chromosomes. *Aust. J. Biol. Sci.* **30**, 103–14.

Donald, J. A., and Hope, R. M. (1981). Mapping a marsupial X-chromosome using kangaroo-mouse somatic cell hybrids. *Cytogenet. Cell Genet.* **29**, 127–37.

Echard, G., Gellin, J., Benne, F., and Gillois, M. (1981). The gene map of the rabbit (*Oryctolagus cuniculus L.*). Synteny between the rabbit gene loci coding for HPRT, PGK, G6PD and GLA: their localisation on the X chromosome. *Cytogenet. Cell. Genet.* **29**, 176–83.

Graves, J. A. M., Chew, G. K., Cooper, D. W., and Johnston, P. G. (1979). Marsupial–mouse cell hybrids containing fragments of the marsupial X-chromosome. *Somatic Cell Genet.* **5**, 481–9.

Grzeschik, K. H., *et al.* (1972). X-linkage of human α-galactosidase. *Nature New. Biol.* **240**, 48–50.

Heuertz, C., and Hors-Cayla, M. C. (1978). Carte génétique des bovins par la technique d'hybridation cellulaire. Localisation sur le chromosome X de la glucose-6-phosphate deshydrogenase, la phosphoglycerate kinase, l' α-galactosidase A et l' hypoxanthine guanine phosphoribosyl transferase. *Ann. Genet.* **21**, 197–202.

Johnston, P. G., and Sharman, G. B. (1975). Studies on metatherian sex chromosomes. 1. Inheritance and inactivation of sex-linked allelic genes determining glucose-6-phosphate dehydrogenase variation in kangaroos. *Aust. J. Biol. Sci.* **28**, 567–74.

Johnston, P. G., Sharman, G. B., James, E. A., and Cooper, D. W. (1978). Studies on metatherian sex chromosomes. VII. Glucose-6-phosphate dehydrogenase expression in tissues and cultured fibroblasts of kangaroos. *Aust. J. Biol. Sci.* **31**, 415–24.

Kint, J. A. (1970). Fabry's disease: alpha-galactosidase deficiency. *Science (Wash., D.C.)* **167**, 1268–9.

Kirsch, J. A. W. (1977). The comparative serology of Marsupialia, and a classification of marsupials. *Aust. J. Zool. Suppl. Ser.* **52**, 1–52.

Kozak, Ch., Nicols, E., and Ruddle, F. H. (1975). Gene linkage analysis in the mouse by somatic cell hybridization: assignment of adenine phosphoribosyl transferase to chromosome 8 and α-galactosidase to the X chromosome. *Somatic Cell Genet.* **1**, 371–82.

Lusis, A. J., and West, J. D. (1976). X-linked inheritance of a structural gene for α-galactosidase in *Mus musculus*. *Biochem. Genet.* **14**, 849–55.

Maynes, G. M. (1977). Biology of the parma wallaby *Macropus parma* Waterhouse 1846. Ph.D. Thesis, Macquarie University, North Ryde, N.S.W.

Murray, J. D., McKay, G. M., and Sharman, G. B. (1979). Studies on metatherian sex chromosomes. IX. Sex chromosomes of the greater glider, *Marsupialia petauridae*. *Aust. J. Biol. Sci.* **32**, 375–86.

Ohno, S. (1967). 'Sex Chromosomes and Sex-Linked Genes' [Monographs on Endocrinology No. 1]. (Springer-Verlag: Berlin.)

Rebourcet, R., Weil, D., Van Cong, N., and Frezal, J. (1975). α-galactosidase: a dimeric enzyme dependent on a structural locus on the X-chromosome. *Cytogenet. Cell Genet.* **14**, 406–8.

Richardson, B. J., Czuppon, A. B., and Sharman, G. B. (1971). Inheritance of glucose-6-phosphate dehydrogenase variation in kangaroos. *Nature New Biol.* **230**, 154–5.

Romeo, G., and Migeon, B. R. (1970). Genetic inactivation of the α-galactosidase locus in carriers of Fabry's disease. *Science (Wash., D.C.)* **170**, 180–1.

Ropers, H. H., Wienker, T. F., Grimms, T., Shroetter, K., and Bender, K. (1977). Evidence for preferential X-chromosome inactivation in a family with Fabry disease. *Hum. Genet.* **36**, 289–97.

Schram, A. W., and Tager, J. M. (1981). The specificity of lysozomal hydrolases: human α-galactosidase isoenzymes. *Trends Biochem. Sci.* **6**, 328–30.

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 New Biol.* **243**, 47–8.

VandeBerg, J. L., Cooper, D. W., and Sharman, G. B. (1977). 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*. *Aust. J. Biol. Sci.* **30**, 421–30.

VandeBerg, J. L., Johnston, P. G., Cooper, D. W., and Robinson, E. S. (1983). X-chromosome inactivation and evolution in marsupials and other mammals. 37 pp. Proc. 4th Int. Isozyme Congr. Austin, Texas, June 1982. (In press.)

VandeBerg, J. L., Thiel, J. E., Hope, R. M., and Cooper, D. W. (1979). Expression of PGK-A in the Australian brush-tailed possum, *Trichosurus vulpecula* (Kerr) consistent with paternal X inactivation. *Biochem. Genet.* **17**, 325–32.

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