Inheritance and Expression of Two Peptidases in the Wallaroo, *Macropus robustus* (Gould)

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Abstract

The formal genetics of two peptidase loci in *M. robustus* are presented. Both loci are autosomal but do not appear to be located on the same chromosome pair. Alleles at both loci are codominantly expressed in neonates, pouch young and adults. The application of autosomal loci segregating for three or more alleles in studies of embryonic gene expression is discussed.

*Additional keywords*: kangaroo, marsupial, autosomal inheritance.

Introduction

The mode of dosage compensation for sex-linked genes in female kangaroos differs radically from that of eutherian mammals. In the Eutheria compensation in somatic tissues is achieved by inactivation of one or other of the X-chromosomes in a pattern which is random from cell to cell (reviewed by Lyon 1972) while in oocytes (meiotic germ cells) both X-chromosomes are active (Epstein 1972; Gartler et al. 1972). In kangaroos the X-chromosome of paternal origin is preferentially inactivated in most somatic tissues (Johnston and Sharman 1975; Cooper et al. 1977) and this inactivation appears to extend to the germ-line cells. In the ovaries (containing 70% primary oocytes) of pouch young *Macropus robustus* heterozygous at the sex-linked glucose-6-phosphate dehydrogenase (*G6PD*) locus only the allele of maternal origin was expressed (Johnston et al. 1976). Extension of these studies to examine gene expression in more mature oocytes, fertilized eggs and early embryos requires detection of non-dose compensated, autosomally inherited enzyme variants to serve as comparisons with the sex-linked markers. We have therefore screened our pedigreed colony of *M. robustus* for 13 enzyme-coding loci, of which 11 proved to be monomorphic (Murray 1979). We present here the formal genetics of alleles at two peptidase loci and show that they are expressed codominantly in neonates, pouch young and adults. The value of autosomal loci segregating for three or more alleles in studies of embryonic gene expression is discussed.

Materials and Methods

The species *Macropus robustus* is currently acknowledged to contain four subspecific forms (Richardson and Sharman 1976), of which we have studied two. The two subspecies, *M. r. erubescens* and *M. r. robustus*, interbreed freely in captivity, producing viable offspring. Female hybrids may be successfully backcrossed to males of either subspecies and, in this paper, we show that hybrid males are fertile.
Electrophoresis for peptidases A and D was carried out on lysates of fresh or ethylene glycol-preserved (Vandeberg and Johnston 1977) erythrocytes, kidney homogenates or tail-tip biopsies from small pouch young. The extraction medium was 2% (v/v) 2-mercaptoethanol in distilled water (1 part packed erythrocytes or tissue : 2 parts extraction medium). The enzyme phenotype obtained from any individual was independent of the tissue used. Both peptidases were separated on Cellogel sheets (Chemetron, Milan) for 90 min with a potential gradient of 10–12 V cm⁻¹. The continuous separation and electrode buffer for peptidase A (substrate valine–leucine) was 0·1 m lithium borate–0·0024 m EDTA, pH 9·0, and for peptidase D (substrate phenylalanine–proline) 0·1 m Tris-citrate, pH 8·5. The peptidases were stained by incubating the Cellogel strips face-down in a mixture containing substrate and o-dianisidine (final concentrations 2 mg ml⁻¹), amino acid oxidase and peroxidase (final concentrations 0·3 mg ml⁻¹) in 0·1 m Tris-HCl, pH 7·4, containing 0·3 mm magnesium chloride.

Results

The peptidase-D locus is segregating for two alleles in the M. robustus colony. One allele codes for a faster migrating (F) variant and one for a slower (S) variant of the enzyme. The two alleles are codominantly expressed in heterozygotes. Progeny from parents of known genotype fall into classes and ratios concordant with autosomal Mendelian inheritance of the locus (Table 1). Both alleles are found in pure-bred euros and pure-bred wallaroos.

Table 1. Family data for the peptidase-D polymorphism in the Macquarie University M. robustus colony

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<thead>
<tr>
<th>Mating</th>
<th>No. of offspring</th>
<th>Mating</th>
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<td>SF × FF</td>
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The peptidase-A locus segregates for four alleles designated 1–4 in order of descending anodal migration. The enzyme exists as a dimer since a single heteropolymer band is evident in heterozygotes. Seven of the observed phenotypes are illustrated in Fig. 1, together with the assigned allelic constitution of each individual. As the founding population size of the colony was small and yet alleles 1, 2 and 3 are found in pure-bred euros and alleles 2, 3 and 4 in pure-bred wallaroos, it seems reasonable to suppose that all four alleles may be found in natural populations of both subspecies.

Extensive pedigree data involving 80 progeny from matings of known peptidase-A genotype were collected (Table 2) and the pedigree of 33 offspring from one male euro (e29) was constructed (Fig. 2). In all cases the phenotypic classes of progeny correspond to those expected on the basis of Mendelian autosomal inheritance of codominant alleles. The ages of individuals studied for both peptidases A and D ranged from 9 days post partum to adults. In heterozygotes of both sexes and all ages the gene products of both alleles stained on the gel with equal intensity, indicating that there is no age or sex influence on the expression of alleles (cf. sex-linked G6PD in this species).

Since the karyotype of M. robustus contains only seven pairs of autosomal chromosomes (Sharman 1961), there is a reasonable probability that any two loci studied
may be linked on the same chromosome. Possible linkage between the loci for peptidase A and D was tested by examining the 33 progeny of euro e29 who inherited the peptidase $A^1$ and peptidase $D^5$ alleles from his mother and $A^3$ and $D^F$ from his father. Progeny were included in this analysis only if the genotype of their dam allowed unambiguous determination of the allelic contribution made by e29. Of 17 suitable progeny nine inherited the parental combinations $A^1-D^5$ (2) and $A^3-D^F$ (7) while eight received recombinant gametes $A^1-D^F$ (6) and $A^3-D^5$ (2). The percentage recombination between the two loci was 47%, not significantly different from independent assortment of two loci on different homologous chromosome pairs. However, these data indicate an unusual phenomenon, namely the non-random transmission of peptidase-D alleles by the male euro e29 who passed the $F$ allele to 13 of the 17 progeny examined ($\chi^2 = 4.764$; $P < 0.05$ when tested against the null hypothesis of equal allelic transmission).

![Electrophoretic phenotypes and assigned allelic compositions of peptidase A in M. robustus.](image)

**Fig. 1.** Electrophoretic phenotypes and assigned allelic compositions of peptidase A in *M. robustus.* (a) Adult female e100; (b) adult male e29; (c) adult male w34; (d) adult male we51; (e) juvenile female we126; (f) 25-day-old male e71; (g) 9-day-old male ez/e//w127.

**Discussion**

The results presented here show that the mode of inheritance of loci coding for peptidases A and D in *M. robustus* conforms with the expected Mendelian predictions for two autosomally coded, codominantly expressed loci which are not linked. The expression of allelic activity does not appear to be either age-, sex-, or tissue-dependent.

The indication that there may be non-random transmission of peptidase-D alleles was unexpected. This distortion of segregation ratio cannot be attributed to an inbreeding effect as e29 is the offspring of two unrelated euros, nor to embryonic or neonatal mortality of progeny inheriting the peptidase-$D^5$ allele. The regular breeding pattern of *M. robustus* allows us to determine when confirmed matings fail to result in pouch young. There are insufficient failed matings by e29 to account for the discrepancy. We lack sufficient numbers of offspring from other individuals to determine if non-random transmission is a regular feature of the chromosome pair bearing the peptidase-D locus. However, asymmetry is also evident in the transmission of one particular sex chromosome in another line of *M. robustus* within the colony. An X-chromosome of euro morphology (Sharman 1971) but bearing the $G6PD-F$ allele characteristic of wallaroos (Richardson *et al.* 1971) has been transmitted to 12 of 15 offspring from females heterozygous for this chromosome and a normal euro $G6PD-S$-bearing X ($\chi^2 = 5.4$; $P < 0.025$).
Preliminary experiments have indicated that 10-day-old *M. robustus* blastocysts express only the *G6PD* allele of maternal origin (Robinson, Johnston, Murray and Briscoe, unpublished results). This may indicate that:

1. the blastocysts were male and possessed only the X-chromosome of maternal origin; or
2. the paternally derived X-chromosome was preferentially inactivated; or
3. the entire paternal haploid set was inactive in the blastocyst; or
4. the enzyme activity of the blastocyst resulted from long-lived messenger RNA of maternal origin.

The last possibility is particularly intriguing as kangaroo blastocysts may be retained in embryonic diapause for many months, presumably in a state of low metabolic activity. We intend to differentiate between alternatives (2)–(4) using the variation at the peptidase-A locus described here. Matings are being established of female euros

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| homzygous for *G6PD-S* and heterozygous for peptidase A 1/3 with male wallaroos hemizygous for *G6PD-F* and homozygous for peptidase A 2/2. For alternatives (2)–(4) above the *G6PD* and peptidase typings of the resulting blastocysts will be:

- (2) Peptidase A 1/2 or 2/3 *G6PD-S*;
- (3) Peptidase A 1 or 3 *G6PD-S*;
- (4) Peptidase A 1/3 *G6PD-S*.

Later matings utilizing females heterozygous for *G6PD* may indicate the time of reactivation of X-chromosomes which were inactive in the mother but which become active when transmitted via the egg cell.

The rationale of this experiment should be generally applicable to studies of gene expression in early embryos of organisms which possess autosomal loci segregating for three or more electrophoretically detectable alleles.

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**Fig. 2.** Pedigree illustrating the inheritance of peptidase A in *M. robustus*. □ Male. ○ Female. e, Euro; w, wallaroo; ew, F₁ hybrid with euro mother; ew/e, backcross hybrid with ew mother and e father (see Johnston and Sharman 1975 for details). S, G6PD slow; F, G6PD fast. Outlined box illustrates the first evidence that a F₁ hybrid male is fertile.
Acknowledgments

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