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A comparison of genetic variability at *X*-linked and autosomal loci in kangaroos, man and *Drosophila*

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SUMMARY

This paper tests the hypothesis that haplodiploidy or *X* linkage leads to less genetic variability. Although haplodiploid organisms exhibit a low level of genetic variability the wide variation existing between different diploid organisms implies that factors other than the genetical system could also be responsible. In order to test the hypothesis critically it is necessary to compare the level of genetic variability between *X*-linked and autosomal genes within a closely related group of organisms. For kangaroos, the ascertainment bias for *X*-linked loci has been removed by assuming the correctness of Ohno's law of conservation of the mammalian *X*, i.e. that genes found to be *X*-linked in man can be assumed to be *X*-linked in kangaroos. For Man and *Drosophila*, it has been assumed that the percentage of the karyotype which is *X* chromosome can be used as the expectation for the percentage of *X*-linked polymorphisms. No difference between the two classes of loci is evident in kangaroos and man for percentage polymorphism. The data however have confidence limits which would allow autosomal loci to have three times greater percentage polymorphism. In *Drosophila* the published data of Prakash show that autosomal loci are polymorphic about twice as frequently as are their *X*-linked counterparts. Thus there *may* be a modest reduction in percentage polymorphism as a result of *X*-linkage (i.e. haplodiploidy). No reduction in the number of alleles per locus or average heterozygosity at those loci which are polymorphic is evident in kangaroos, man, or *Drosophila*. More data on more *X*-linked enzymes are necessary to establish firmly that there is a real reduction in percentage polymorphism and to estimate its extent. The kangaroo data are incompatible with the hypothesis that a large fraction of the variability is maintained by simple overdominance since overdominance is very unlikely in the quasi-haploid genetical system which results from the paternal *X* inactivation mode of dosage compensation used by kangaroos. This is the first report on level of enzymic variability in marsupials. 17% of autosomal loci and 18% of *X*-linked loci are polymorphic, average heterozygosity is 4% for

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autosomal and 4% for X-linked loci and number of alleles per locus is 1·25 for autosomal and 1·21 for X-linked loci. These figures are somewhat lower than for eutherian mammals.

1. INTRODUCTION

It is sometimes said that haplodiploid organisms and X-linked loci should have less genetic variation than diploid organisms and autosomal loci (White, 1973; Crozier, 1977). Essentially there are three grounds for this assertion. In a haplodiploid system directional selection against a recessive is assumed to proceed more quickly because the hemizygote exposes alleles to selection more frequently. A formula which verifies this argument for simplified assumptions has been given by Morton (1971). A haplodiploid system has a smaller effective population size, provided there is not a large excess of the diploid or homogametic sex (Crozier, 1976). Under simple deterministic models, the conditions for balanced polymorphism at a haplodiploid locus are more restrictive (Bennett, 1958; Haldane & Jayakar, 1962; Cavalli-Sforza & Bodmer, 1971; Hartl, 1971).

The low level of allozymic variability which has been found in a number of haplodiploid insects such as the Hymenoptera suggests that this expectation is realized in nature (Metcalf, Marlin & Whitt, 1975; Pamilo, Varvio-Aho & Pekkarinen, 1978). However, there are such wide differences between different groups of organisms in the extent of allozymic variability (Lewontin, 1974) that it is also possible that the Hymenoptera have an intrinsically low level of variation for reasons other than their haplodiploid genetical system. In order to test more critically the hypothesis that haplodiploidy leads to less genetic variability, it is necessary to compare the level of variation for autosomal and X-linked loci within the one species or group of species. This is not straight-forward because the detection of X-linked genes by their peculiar mode of transmission biases any sample of X-linked loci towards those which exhibit more variability.

The purpose of this paper is to outline ways in which this bias can be circumvented, and then to analyse our own data and published data to test the hypothesis. Our own data are from kangaroos. Kangaroos are particularly appropriate for testing the hypothesis because unlike eutherian mammals their mode of dosage compensation is paternal X-inactivation (reviewed in Cooper *et al.* 1977). Only the mother's X is expressed in most tissues and so both sexes have haploid expression for their sex linked loci. The allele which is not expressed in the mother may nonetheless be transmitted to her progeny in the expected frequency of one half and there be expressed (Johnston & Sharman, 1975). Accordingly a deleterious or advantageous gene with recessive effect is exposed to selection more often than in either the usual X-linked or haplodiploid system, and much more often than in an autosomal system. It is very unlikely that a balanced polymorphism will exist at an X-linked locus with complete paternal X inactivation (Cooper, 1976). If directional or balancing selection are important, the difference between autosomal and sex linked loci in kangaroos should be considerable.

The published data are for Man and *Drosophila*. Prakash (1973) has previously

discussed the question of the level of *X*-linked versus autosomal variation for part of these data and here we extend his analysis to include his more recent data.

2. MATERIALS AND METHODS

Animals. These were either from the captive colonies kept at Macquarie University and CSIRO Wildlife Canberra, or were from animals shot in the wild. Most of the data were obtained during the course of a search for sex linked enzymes in kangaroos (see Cooper *et al.* 1977 for a review).

Electrophoretic methods. These are described in Cooper, James & Woolley (1979), Meera Khan (1971) and Shaw & Prasad (1970).

Selection of an unbiased sample of X-linked loci

(*a*) *Kangaroos*

We have attempted to overcome the selection bias by using the conservative nature of the mammalian *X*. Ohno (1967) first proposed that genes found to be *X*-linked in one mammal would also be *X*-linked in others. A considerable body of evidence supports this proposal for marsupials and eutherians (Ohno, 1969; Lyon, 1974; Cooper *et al.* 1977). We have chosen four enzymes known to be *X*-linked in man, namely glucose-6-phosphate dehydrogenase (G6PD) (Beutler & Yoshida, 1973), phosphoglycerate kinase (PGK) (Chen *et al.* 1971), α-galactosidase (Kint, 1970), and ornithine transcarbamylase (OTC) (Campbell *et al.* 1971; Scott *et al.* 1972), and examined them for electrophoretically detectable polymorphism in kangaroos, in which no previous information on their inheritance was available. We have found that G6PD (Johnston & Sharman, 1975), PGK (Cooper *et al.* 1977) and α-galactosidase (Cooper *et al.* unpublished data) exhibit electrophorectic variation in kangaroos which shows their loci to be *X*-linked. This strongly supports Ohno's hypothesis. The discovery that the four loci are each *X*-linked in man was made from pedigrees in which absence or gross deficiency of the enzyme led to a highly deleterious condition. With the exception of G6PD the variant deleterious genes are very rare. Extensive electrophoretic studies of G6PD (Beutler & Yoshida, 1973) and PGK (Omoto & Blake, 1972) revealed that man is largely monomorphic for these two enzymes; variation at polymorphic frequency for both is confined to a few ethnic groups. It seems unlikely that normal variation at polymorphic frequencies in kangaroos and deleterious variation in man would be correlated.

Finally, most loci seem to exhibit rare variation, at least in electrophoretic mobility (Harris, 1970). Hence, provided Ohno's law of conservation of the *X* is valid, a sample of *X*-linked enzymes in Man ascertained through rare deleterious variation should constitute an unbiased sample of *X*-linked enzyme loci in kangaroos.

(*b*) *Man and Drosophila*

Prakash (1973) assumed that enzyme loci are distributed at random through the genome. Accordingly since 38 % of the euchromatin of the female *D. robusta* is in

the X (Carson, 1955), 38% of all detectable polymorphisms should be on the X if there is no difference between the two classes of loci in their incidence of polymorphism. We make the same assumption for Man and *Drosophila*. For Man 5% of the haploid karyotype is X (Ohno, 1967). For *D. persimilis* and *D. pseudoobscura* we have calculated from Figs. 1–6 of Tan (1935) that 38–40% of the salivary gland chromosome is X material.

3. RESULTS

(*a*) *Kangaroos*

The data are summarized in Table 1. Polymorphism is defined here as a frequency of greater than 1% for the least frequent alleles. 16·7% of autosomal loci exhibit polymorphism as opposed to 18·2% for X-linked loci (i.e. A/S = 0·92); and the figure for mean average heterozygosity of individuals is also very similar, 4·0% for autosomal loci and 4·2% for X-linked loci (i.e. AHA/AHS = 0·95). These values are somewhat lower than the 7·9% found for rodent species and are indeed lower than those found for most other diploid organisms (Lewontin, 1974). The 95% confidence limits for A/S and AHA/AHS, given at the foot of Table 1, make it unlikely that X-linked and autosomal loci differ in their percentage of polymorphic loci by more than a factor of two and in their mean average heterozygosity by more than a factor of three. These confidence limits were derived on the assumption that $\log_e$A/S and $\log_e$AHA/AHS are both normally distributed (see Table 1 for the definitions of A, S, AHA, and AHS). We are grateful to Professor Alan Robertson for testing this assumption by direct simulation of the distribution for percentage polymorphism. He found the confidence limits of the ratio of percentage polymorphic autosomal loci to percentage X-linked polymorphic loci (i.e. A/S) to be 0·45–3·3 as compared to 0·39–2·14 (Table 1) by our formula. Thus the assumption of normality is not entirely correct, but even so autosomal loci are unlikely to exceed X-linked loci by more than a factor of three. The mean number of alleles at X-linked loci was 1·26 and 1·21 at autosomal loci.

(*b*) *Man*

For Man an examination of published data also suggests that sex-linked polymorphisms may occur as frequently as autosomal ones. The limited data available are in agreement with the expectation of 5% X-linked polymorphisms (Table 2) ($\chi^2_1 = 0{\cdot}19$, $0{\cdot}6 < P < 0{\cdot}7$). McKusick's (1975) figures show that there are 93 X-linked loci out of 1124 human loci at which variation of any kind has been recognized. This is a percentage of 8·1 and is significantly higher than 5% ($\chi^2_1 = 23{\cdot}8$, $P < 0{\cdot}001$). As McKusick says this excess of X-linked loci probably reflects the greater likelihood of detecting an X-linked as opposed to an autosomal recessive.

(*c*) *Drosophila*

Prakash (1973) examined data for *D. robusta* and came to the conclusion that percentage polymorphism and average heterozygosity were less at X-linked loci than at autosomal loci. Further data for *D. persimilis* and *D. pseudoobscura* which

Table 1. *A comparison of the average heterozygosity of individuals and proportion of species polymorphic for autosomal and X-linked genes in ten species of kangaroos and wallabies**

(*a*) Autosomal loci	No. of species polymorphic in all populations examined	No. of species polymorphic in at least one population	No. of species monomorphic	Average† heterozygosity	Mean and range of no. of individuals examined
Albumin	2	1	5	(0·127)	76, 25–157
Haemoglobin	0	0	10	—	97, 26–203
Transferrin	3	0	6	(0·111)	167, 17–274
LDH *A*	0	0	7	—	66, 18–100
LDH *B*	0	0	7	—	66, 18–100
6PGD	1	2	7	(0·061)	75, 23–138
GPI	2	1	6	(0·059)	63, 15–172
PGM	1	0	8	(0·08)	63, 15–172
MDH *A*	0	0	9	—	36, 11–84
SOD	0	0	9	—	35, 20–58
PGK *B*	2	1	6	(0·079)	62, 12–127
Total autosomal polymorphisms	11	5	Total autosomal loci monomorphic 80	AHA = 0·040 S.E. = 0·0150	73
(*b*) X-linked loci					
α-galactosidase	2	0	6	0·072	32, 10–58
G6PD	0	1	9	(0·019)	97, 26–203
OTC	0	0	5	—	29, 12–36
PGK *A*	3	0	7	0·078	69, 13–140
Total sex-linked polymorphisms	5	1	Total sex-linked loci monomorphic 27	AHS = 0·042 S.E. = 0·019	57

A = the proportion of autosomal loci exhibiting polymorphism = 0·167, S.E. = 0·038
S = the proportion of sex-linked loci exhibiting polymorphism = 0·182, S.E. = 0·067.
AHA = autosomal average heterozygosity. AHS = sex-linked mean average heterozygosity.
The ratio A/S = 0·92 with 95% confidence limits of 0·39 and 2·14. These confidence limits are obtained using the relationship variance ($\log_e$(A/S)) = variance (A) ÷ A^2 + variance (S) ÷ S^2. From this the confidence limits of $\log_e$A/S are obtained and then A/S itself by taking antilogarithms. Similarly AHA/AHS = 0·95 with 95% confidence limits of 0·30 and 3·22. The validity of these confidence limits is dependent upon the assumed normality of the distribution of $\log_e$(A/S) and $\log_e$(AHA/AHS).

* The species of kangaroo and wallaby were (M = *Macropus*): *M. giganteus*, *M. fuliginosus*, *M. parryi*, *M. rufogriseus*, *M. robustus*, *M. rufus*, *M. parma*, *M. eugenii*, *Wallabia bicolor* and *Thylogale thetis*. Data on transferrin in *M. giganteus* and *M. fuliginosus* from Kirsch & Poole (1972) and in *M. robustus* and *M. rufus* from Richardson (1970). All the remainder unpublished data of the authors. Evidence for sex linkage of PGK summarized in Cooper *et al* (1977) and for G6PD in Johnston & Sharman (1975). *X*-linkage of α-galactosidase in kangaroos reported here for the first time. LDH *A* = lactate dehydrogenase A, LDH *B* = lactate dehydrogenase B, 6PGD = 6 phosphogluconate dehydrogenase, GP1 = phosphoglucoisomerase, PGM = phosphoglucomutase, MDH *A* = malate dehydrogenase A, SOD = superoxide dismutase. See text for remaining enzyme abbreviations.

† The parenthesis means that significant gene frequency variation between populations existed in at least one species. For such species the heterozygosity for each population within the species was computed and mean value for the species obtained, weighting each population equally. For the *X*-linked loci it is not possible to detect heterozygotes phenotypically because of the paternal *X* inactivation system of kangaroos. For these loci expected values for the frequency of heterozygotes in females were calculated from the gene frequencies on the assumption of Hardy–Weinberg equilibrium.

could also be used to examine this question have now been published (Prakash, 1977*a*, *b*). The data on the numbers of polymorphisms, mean proportion of heterozygotes at loci which exhibit polymorphisms and mean number of alleles at such loci are shown in Tables 3, 4 and 5 respectively. On the assumption that loci are

Table 2. *Number of autosomal and X-linked polymorphisms in Caucasian populations for four classes of loci*

Class of locus (Reference	Autosomal polymorphisms	*X*-linked polymorphisms
Blood groups (Race & Sanger, 1975)	15	1(Xg)
Serum proteins (Cooper, 1978)	10	1(Xm)*
Erythrocyte enzymes (Harris & Hopkinson, 1972)	20	0
Other (McKusick, 1975)	5†	1(colour-blindness)
Total	50 (96·3 %)	3 (5·5 %)

* There is considerable doubt that this is in fact an *X*-linked polymorphism (see Cooper, 1978). If it is excluded the figure for percentage of *X*-linked polymorphisms becomes 4 %, which is also consistent with the expected 5 % ($\chi^2_1 = 0{\cdot}28$, $0{\cdot}5 < P < 0{\cdot}7$).

† PTC tasting, eye colour, excretion of beetroot pigment, excretion of asparagus odour in urine, hair colour.

distributed evenly over the genome, there is a clear deficiency of *X*-linked polymorphisms. If $a:1$ is the ratio of the probability that an *X*-linked locus will be polymorphic to the same probability of the same event for an autosomal locus, the value of a can be obtained from the relationships

$$\frac{a\,x\,\%\text{ genome } X \text{ linked}}{a\,x\,\%\text{ genome } X \text{ linked} + \%\text{ genome autosomal}} = \frac{\text{observed } X\text{-linked polymorphisms}}{\text{all observed polymorphisms}}$$

$$= 0{\cdot}23 \text{ (Table 3).}$$

Hence $a = 0{\cdot}49$, i.e. *X*-linked loci are polymorphic only about half as frequently as autosomal loci in *Drosophila* species.

There is no indication that *X*-linked polymorphic loci have less heterozygosity or fewer alleles than autosomal loci (Tables 4 and 5). It should be noted that these are not mean heterozygosities or average number of alleles over all *X*-linked or all autosomal loci. These values cannot be obtained, since we do not know if monomorphic loci are *X*-linked or autosomal. The mean heterozygosity at *X*-linked polymorphic loci in kangaroos is 0·23 and at autosomal polymorphic loci it is 0·24, values which are much the same as those in *Drosophila* (Table 4). The mean number of alleles at both *X*-linked and autosomal polymorphic loci in kangaroos is two, because with one exception (albumin in *M. rufogriseus*) all the kangaroo polymorphisms had two alleles.

Table 3. *Number of X-linked and autosomal polymorphisms in three species of* Drosophila. (Data of Prakash*)

Species	*X*-linked	Autosomal
pseudoobscura	7	19
persimilis	4	13
robusta	4	18
Totals	15 (23 %)	50 (77 %)

χ_1^2 (expected = 38 % for *X*-linked loci) = 6·14, 0·02 > *P* > 0·01.

The figure of 38 % for expected number of polymorphic *X*-linked loci is derived from the length of the salivary gland chromosomes for *Pseudoobscura* and *persimilis* given in Tan (1935) and from the euchromatic length of salivary gland chromosomes of *robusta* given by Carson, 1955 (cited by Prakash, 1973).

* Summarized from Prakash *et al.* (1969), Prakash (1973), Prakash (1977*a*, *b*).

Table 4. *Mean proportion of heterozygotes at X-linked and autosomal polymorphic loci in three species of* Drosophila. (Data of Prakash*)

Species	Polymorphic *X*-linked	Polymorphic autosomal
pseudoobscura	0·216 (0·106)	0·184 (0·033)
persimilis	0·350 (0·094)	0·220 (0·061)
robusta	0·157 (0·066)	0·211 (0·044)

Values in parenthesis are standard errors of means.

* Summarized from Prakash *et al.* (1969), Prakash (1973), Prakash (1977*a*, *b*).

Table 5. *Mean number of alleles at polymorphic X-linked and polymorphic autosomal loci in three species of* Drosophila. (Data of Prakash*)

Species	Polymorphic *X*-linked	Polymorphic Autosomal
pseudoobscura	3·85 (1·81)	3·10 (0·241)
persimilis	4·00 (1·08)	3·46 (0·333)
robusta	2·75 (0·478)	3·22 (0·263)

Values in parenthesis are standard errors of means.

* Summarized from Prakash *et al.* (1969), Prakash (1973), Prakash (1977*a*, *b*).

4. DISCUSSION

Before attempting to draw any conclusions, two reservations about the data must be spelled out. Firstly assumptions have had to be made to remove the bias due to ascertainment in recognizing *X*-linked loci. For kangaroos the main assumption was the validity of Ohno's law of the conservation of the mammalian *X*, and for Man and *Drosophila*, it was the randomness of distribution of enzyme loci

between X and autosomes. We have no independent check on the validity of either of the assumptions. Secondly the numbers of X-linked loci in all species are small, and there is a possibility that they may not be typical of X-linked loci as a whole. It is clear that glycolytic and Kreb's cycle enzymes, hydrolytic enzymes and larval proteins in *D. robusta* and other *Drosophila* species have different levels of polymorphism (Prakash, 1973). Unfortunately there are too few X-linked enzyme loci to make any comparison within these three subdivisions worthwhile.

If either of the main assumptions made here are valid, then the method(s) used to remove the ascertainment bias could be applied to other groups of organisms to obtain further tests of the hypothesis.

These reservations aside, the overall conclusion to be drawn from the data is that there may be a reduction in the proportion of X-linked loci which are polymorphic, although this has not been decisively demonstrated. For kangaroos and Man, the proportions are nearly equal, but the 95 % confidence limits (see footnote to Table 1) for the larger body of data from kangaroos are such that autosomal loci could have up to three times more polymorphisms per locus on average. The *Drosophila* data suggest that X-linked loci are polymorphic half as frequently as autosomal loci. However, the departure from the null hypothesis of equality is only just significant (Table 3).

For the loci which are polymorphic there is no indication that X-linked loci have a lower average heterozygosity or mean number of alleles. Prakash (1973) concluded that in *D. robusta* there was less heterozygosity at X-linked polymorphic loci, but this suggestion is not borne out by his additional data.

The levels of polymorphism in kangaroos at X-linked and autosomal loci are similar. These data are incompatible with the notion that a large fraction of polymorphisms is maintained by simple overdominance, since this is unlikely in an X-linked, paternal X inactivation system (Cooper, 1976). Paternal X inactivation results in a quasi haploid system. Our results agree with the observations of Milkman (1973), showing that polymorphism is comparatively frequent in haploid *Escherichia coli*.

It is possible that population size affects variability at X-linked and autosomal loci in kangaroos similarly. Newsome (1977) has shown that after sexual maturity there is an unequal sex ratio in the red kangaroo (*M. rufus*), one of the species studied in this investigation. It is unclear how much of the difference is due to human predation, i.e. to the larger male animals being selectively shot by hunters, and how much is due to other causes. The observed male:female ratio after sexual maturity was 0·34. Using the formula of Crozier (1977) this gives a ratio of effective population size at X-linked loci to effective population size at autosomal loci of 0·90. If an excess of females in the breeding population of kangaroos is the rule, differential effects of population size upon variability at the two classes of loci may not be great.

This study should be regarded as a first attempt to estimate the extent to which haplodiploidy results in a reduction in genetic variability, if it does at all. Further studies on a larger scale involving more X-linked loci are necessary to confirm

that there is such a reduction, and to estimate its magnitude. The extent of the contribution that haplodiploidy makes to the reduction in genetic variability in Hymenoptera and other haplodiploid organisms is still open to question. Given the wide variation in genetic variability between diploid organsims it is possible that other factors are more important.

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