ESTERASE GENETICS IN *MUS MUSCULUS*: EXPRESSION, LINKAGE, AND POLYMORPHISM OF LOCUS *Es-2*

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AMONG the vertebrates, the genetic control of esterases is best known in the mouse (*Mus musculus*). Four loci have been described which have been designated *Es-1* (POPP 1962; RUDDLE and RODERICK 1968); *Es-2* (PETRAS 1963; POPP 1967) *Es-3* (RUDDLE and RODERICK 1965); and *Es-5* (PETRAS and BIDDLE 1967). The locus reported as *Es-4* has been shown identical to *Es-1* (RUDDLE and RODERICK 1968). *Es-1* and *Es-5* have high activities in serum, whereas *Es-3* occurs with high activity in kidney tissue (RUDDLE and HARRINGTON 1967). *Es-2* has higher activity in the kidney than the serum. *Es-3* is further distinguished by its sensitivity to eserine sulfate (RUDDLE 1966). Variant phenotypes determined by alleles segregating at these loci have been detected by various electrophoretic techniques. Two bands migrating in the serum albumin region are determined by *Es-1*: *Es-1a* (fast) and *Es-1b* (slow). *Es-2*, which migrates in the prealbumin region, expresses two phenotypes: the presence (*Es-2b*) or absence (*Es-2a*) of esterase activity. *Es-3* expresses three phenotypes in the post albumin region: *Es-3a* (intermediate), *Es-3b* (fast), and *Es-3c* (slow). *Es-5* determines the presence (*Es-5b*) or absence (*Es-5a*) of activity in the post serum albumin region. In each case the alleles controlling the expression of these phenotypes act codominately. In heterozygotes, no evidence has been reported for the occurrence of heteropolymeric enzymes. It may be important that *Es-1*, *Es-2* and *Es-5* all occur on linkage group XVIII within approximately 10 recombination units of each other (POPP 1967; PETRAS and BIDDLE 1967).

In this paper, we present evidence for a new allele at locus *Es-2*, estimate the recombination frequency between *Es-2* and *Es-1*, report linkage studies on a number of biochemical markers and linkage group XVIII *Es-2* esterases, and present data on the distribution of *Es-2* variants in feral and inbred mice.

MATERIALS AND METHODS

The enzymes from plasma and kidney homogenates were prepared from animals 6 to 10 weeks old. Plasma was obtained from the retro-orbital sinus with heparin treated pipettes. Erythrocytes were removed by centrifugation. Enzyme extracts from kidneys were obtained as previously described (RUDDLE and RODERICK 1966; RUDDLE, SHOWS and RODERICK 1968) by homogenizing in a volume of distilled water twice that of the kidney. The homogenates were stored at -90°C for no more than two weeks before analysis. Before electrophoresis the kidney homogenates were centrifuged at $27,000 \times g$ for 1 hr at 4°C .

Electrophoresis was performed in a Tris-EDTA-borate buffer system (BOYER, FAINER and

FIGURE 1.—Zymogram of kidney esterases with α -naphthyl acetate as substrate. Variation in the mid-gel region is produced by allelic variants at the *Es-3* locus.

NAUGHTON 1963). The stock buffer at pH 8.6 was composed of Tris (0.9M), tetrasodium salt of EDTA (0.2M), and boric acid (0.5M). The stock buffer was diluted 1/20 for the gel buffer, 1/5 for the cathode bridge buffer, and 1/7 for the anode bridge buffer. Electrophoresis was carried out at 3°C for 18 hrs at 10 v/cm across the ge .

The esterases were stained using α naphthyl acetate and α naphthyl butyrate substrates by methods reported previously (RUDDLE and RODERICK 1965, 1966).

The RF/Al strain was kindly supplied by Dr. R. C. ALLEN, Oak Ridge National Laboratory. Wild populations of mice from North Carolina, Vermont, and Alberta were generously supplied by Drs. P. D. WEIGL, R. K. CHIPMAN, and P. K. ANDERSON, respectively. Inbred lines originating from wild mice of Peru, Skokholm Island, Israel, and San Francisco were given by Dr. MARGARET WALLACE (1968).

Immunoglobulin (*Ig-1*) analysis was performed by Mrs. LENORE HERZENBERG, Department of Genetics, Stanford University. Tufted (*tf*) linkage crosses were performed by Dr. DOROTHEA BENNETT, Department of Anatomy, Cornell University Medical School.

RESULTS

Phenotypic expression: The bands of Es-2 esterases prepared from plasma or kidney extracts migrate in the extreme anodal position. Three phenotypes exist: Es-2a which possesses no detectable esterase activity, Es-2b which migrates fastest, and Es-2c a new variant form described here for the first time which migrates more slowly than Es-2b, but faster than Es-1a (Figures 1 and 2). The activities of Es-2b and Es-2c are not detectably different.

Es-2 esterases derived from plasma and kidney extracts differ in their electrophoretic appearance (Figures 1 and 2). Note that in kidney extracts the expressions of each allele are composed of three sub-bands. For a given allele the single band of the serum has the same mobility as the middle sub-band of the kidney.

Genetic control of Es-2 esterases: Alleles *Es-2^a* and *Es-2^b* were first reported by PETRAS (1963). We made genetic crosses to determine whether the new phenotypic variant (*Es-2c*) was controlled by an allele of the *Es-2* locus (Table 1). Mating No. 2 should produce some *Es-2bc* animals unless the *Es-2b* and *Es-2c* phenotypes were produced by alleles or by extremely closely linked genes. In mating No. 1 the *Es-2c* phenotype was always accompanied by an *Es-2b* phenotype of half strength, a result which would be unlikely unless the two phenotypes were produced by alleles. In the progeny of Mating No. 3 phenotypes *Es-2c* and *Es-2b* were always half strength when they were together in the same animal (Figure 1). The segregation ratios of the three crosses confirm the allelic interpretation.

The three *Es-2* alleles are expressed codominantly. Animals of genotype *Es-2^a/Es-2^a* show no detectable Es-2 activity with the staining procedures we used. Animals of genotype *Es-2^a/Es-2^b* have $\frac{1}{2}$ the staining activity of *Es-2^b* homozygotes. The same contrast is apparent when the phenotype of *Es-2^a/Es-2^c* is compared with that of homozygous *Es-2^c*.

Linkage studies: The crosses in Table 1 also permitted the measurement of recombination frequency between *Es-1* and *Es-2*. In each of the matings the frequency is estimated by recombination in Parent 2 only. The matings are such that a direct estimate of recombination can be made from the numbers of offspring in the various phenotypic classes. In a total of 244 progeny there were 27 recombinants giving a recombination frequency of 11.1% which is in close agreement with POPP's value of 11.0% (POPP 1967). The expected recombinant types occurred with approximately equal frequency.

FIGURE 2.—Zymogram of serum esterases with α -naphthyl butyrate as substrate.

Linkage tests were also made with markers whose linkage associations are presently unknown: autosomal glucose-6-phosphate dehydrogenase (*Gpd-1*) (RUDDLE *et al.* 1968), isocitrate dehydrogenase (*Id-1*) (HENDERSON 1966), NADP dependent malate dehydrogenase (*Mdh-1*) (HENDERSON 1966; SHOWS and RUDDLE 1968), lactate dehydrogenase regulator (*Ldr-1*) (SHOWS and RUDDLE 1968), phosphoglucumutase (*Pgm-1*) (SHOWS, RUDDLE and RODERICK 1969),

TABLE 1
Genetic studies of *Es-1* and *Es-2*

Mating	Parent 1	Parent 2	Phenotypes of progeny										Totals	
			Es-1: Es-2:	Nonrecombinants					Recombinants					
				a/a b/b	a/b b/c	b/b a/c	a/b a/b	b/b a/a	a/a b/c	a/b b/b	b/b a/b	a/b a/c		b/b a/a
1.	$\frac{Es-1^a Es-2^b}{Es-1^a Es-2^b}$ (C57BL/6J)	$\frac{Es-1^a Es-2^b}{Es-1^b Es-2^c}$ (C57BL/6J $\times$ PL/J)F ₁	35	55	.	.	.	6	3	.	.	.	.	99
2.	$\frac{Es-1^b Es-2^a}{Es-1^b Es-2^a}$ (RF/Al) (Israel) (Skokholm)	$\frac{Es-1^a Es-2^b}{Es-1^b Es-2^c}$ (C57BL/6J $\times$ PL/J)F ₁	.	.	44	54	.	.	.	4	8	.	.	110
3.	$\frac{Es-1^b Es-2^a}{Es-1^b Es-2^c}$ (Offspring from Mating 2)	$\frac{Es-1^a Es-2^b}{Es-1^b Es-2^a}$	.	5	5	12	7	.	.	2	0	1	3	35
			$N_{nr} = 217$			$N_r = 27$			$N_t = 244$			Recombination frequency = 11.1%		

The recombination frequencies of the three mating types are as follows: Parent 1 $\delta \times$ Parent 2 ϕ gave 73 progeny with 7 recombinants for a recombination frequency of 9.6%. Parent 1 $\phi \times$ Parent 2 δ gave 26 progeny with 2 recombinants for 7.1%. The total recombination frequency for Mating 1 was 9.1%. Mating 2: Parent 2 from mating were females and the recombination frequency was 10.9%. Mating 3: Of 26 progeny from Parent 1 $\phi \times$ Parent 2 δ , 5 were recombinants for a frequency of 17.8%. From a mating of Parent 1 $\delta \times$ Parent 2 ϕ , 7 animals were obtained with one recombinant (14.3%). The total recombination frequency for Mating 3 was 17.1%. The total recombination frequency for the three matings was 11.1%.

glucose-phosphate isomerase (*Gpi-1*) (DeLORENZO and RUDDLE 1969), the immunoglobulin heavy chain (*Ig-1*) (HERZENBERG, WARNER and HERZENBERG 1965), and tufted (*tf*). No linkages were found between *Es-2* and any of these loci. Also no linkages were apparent between any pairs of these loci.

Distribution of phenotypes in feral and inbred mice: Wild populations of mice were examined from North Carolina (NC), Vermont (VT), and Alberta, Canada (AL). The NC sample contained 9 *Es-2b*, 9 *Es-2bc*, and 6 *Es-2c* animals. The VT sample of 35 animals were all of the *Es-2b* type. The AL sample contained 10 *Es-2b*, 12 *Es-2bc*, and 3 *Es-2c* animals. The polymorphic nature of this and other esterase loci is reported in detail elsewhere (RUDDLE *et al.* in preparation). Thirty inbred strains of mice were examined for their phenotypic expression. Only PL/J possessed the *Es-2c* esterase. *Es-2b* was found in the strains 129/J, A/HeJ, AKR/J, BALB/cJ, BUB/BnJ, C3HeB/FeJ, C57BL/6J, C57BL/KsJ, C57BL/6J-ob, C57BR/cdJ, C57L/J, C58/J, CBA/J, CBA/CaJ, CE/J, DBA/1J, DBA/2J, DE/J, DW/J, LG/J, LP/J, MA/J, NZB, P/J, RF/J, SEA/J, SJL/J, ST/6J, and SWR/J. Four highly inbred strains recently derived from the wild in Israel, San Fran-

cisco vicinity, Skokholm Island, and Peru described by WALLACE (1968) were also examined. Israel and Skokholm lines were *Es*-2a, whereas Peru and San Francisco were *Es*-2b. The *Es*-2a phenotype has also been reported in RFM/Un and RF/Al (POPP 1967).

DISCUSSION

POPP (1967) discovered the linkage association between *Es*-1 and *Es*-2 in linkage group XVIII. Our recombination frequency of 11.1% between these loci is in close agreement with his finding of 11.0%. PETRAS and BIDDLE (1967) found a slightly lower recombination frequency of 7.7%. They reported a linkage of similar magnitude between a new esterase locus (*Es*-5) and *Es*-1. POPP (1965) also discovered the tight linkage between *Es*-1 and oligosyndactyly (*Os*). Our recombination frequency of 0.6% for these loci (RUDDLE and RODERICK 1968) is in general agreement with his 2%. In further experiments, however, we have noted an even lower frequency; out of a total of 218 animals we have still detected only one recombinant for a frequency of .46%.

It is probably significant that *Es*-3 which is not linked to linkage group XVIII (and whose linkage has not yet been determined) specifies esterases which differ in several ways from those specified by *Es*-1, *Es*-2, and *Es*-5. *Es*-3 is inhibited by 10^{-3} M eserine sulfate (RUDDLE 1966) and is largely restricted to kidney tissue (RUDDLE and HARRINGTON 1967). The other esterase loci control esterases which are eserine resistant and are found in the serum. It is possible that the linkage of *Es*-1, *Es*-2 and *Es*-5 may reflect a physiological association of the loci. The linkage also suggests a common evolutionary origin.

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SUMMARY

We have reported a new allele *Es*-2^c which produces a band migrating more slowly than *Es*-2b but faster than *Es*-1a. The new allele *Es*-2^c is expressed co-dominantly with *Es*-2^b and shows half strength when heterozygous with the silent allele *Es*-2^a.—Our estimate of the frequency of recombination between *Es*-1 and *Es*-2 is 11.1% which is in close agreement with POPP's (1967) estimate of 11.0%. No linkage associations were found between *Es*-2 and *Gpd*-1, *Id*-1, *Mdh*-1, *Ldr*-1, *Pgm*-1, *Gpi*-1, *Ig*-1, and *tj*.—PL/J is the only inbred strain known to have the new allele, although it occurs in feral populations from North Carolina and Alberta.

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