Esterase alleles of inbred mouse strains maintained in The Netherlands

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Summary

Fifty-seven mouse strains were examined for genetic variation at 21 esterase loci. Three new alleles were found: *Es-6^d* in strain A/WySna, *Es-11^e* in FTC/CpbU and *Es-18^e* in two WLL/BrA sublines. At most loci there was a single allele found in over 80 % of strains, with one or two rare alleles. However, the *Es-1, 3, 10, 13, 25* and *27* loci were much more polymorphic. Although several loci were linked on chomosomes 3, 8 and 9, linkage disequilibrium was only found between *Es-5* and *Es-11* (chromosome 8) and *Es-26* and *Es-27* (chromosome 3). There was also significant disequilibrium between *Es-1* and *3*, *Es-1* and *10*, and *Es-3* and *10*, which are on different chromosomes, suggesting that the 57 strains are not a random sample of inbred mouse strains. Fifty-four strains were closely related, with the *Es-7^b*, −*17^a*, −*18^a*, −*23^c* set of alleles, which are typical of *Mus musculus domesticus*. The three exceptional strains were MOL3 (*Mus musculus molossinus*), WLL/BrA (English–Norwegian origin) and TA2 (Chinese origin). There were 10 groups of strains which were identical at all loci. Sublines of the same strain were usually identical. Sometimes more distantly related strains, such as CBA/Bi, C3H/He, SM and DBA/Li, were identical, and in a few cases strains with no known common ancestry such as C58 and MAS were identical. Attempts to discriminate between a subset of 22 American and 15 European strains were unsuccessful, suggesting that the European strains add only in a quantitative manner to the gene pool of 'laboratory mice', whereas wild-derived strains such as MOL3 are genetically quite distinct from other laboratory mice.

1. Introduction

Allozyme proteins provide an important basis for identification and characterization of inbred mouse (Taylor, 1972) and rat (Festing & Bender, 1984) strains, especially when the origin of the strains is obscure. A knowlege of the genotype of each strain at allozyme loci is of practical importance both in genetic quality control and in choosing strains for research.

Several new inbred strains of independent origin were created in the Netherlands between 1940 and 1960 by Muhlbock in Amsterdam and Haagedoorn in Utrecht. Later, other strains of both American and non-American origin were introduced into the colonies of the Netherlands Cancer Institute. Although the

origin and genetic similarities of many American strains have been traced in detail (Potter & Klein, 1979; Staats, 1979; Roderick, Staats & Womack, 1981), fewer studies have been published for European strains, though Groen (1977) and Hilkens *et al.* (1981) have published information on some of them. Nor have these strains been compared with those of American origin.

The purpose of this study was to report on the esterase genotype at 21 loci of a large sample of inbred mouse strains of diverse origin, and to use the data to explore the genetic relationships between the strains. In particular, it was of interest to see whether the marked clustering of inbred strains of rats due to linkage disequilibrium at six esterase loci observed by Festing & Bender (1984) would be repeated among a large sample of inbred mouse strains where there are

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Table 1. *Origin of mouse strains used*

Strain	Previous name	From	In	Country where inbred
A/BrAf (old)	—	Bonser	1948	USA[a]
A/WySnA	—	Snell	1969	USA[a]
A2G/GA	—	Glaxo Labs	1957	USA/UK
ACR/A	—	Loustalot	1953	Europe?
AKR/FuRdA	—	Rudali	1958	USA[a]
BALB/cByA	—	Bailey	1972	USA[a]
BALB/cCdA	—	Claude	1976	USA
BALB/cCrglA	—	Nandi	1972	USA
BALB/cHeA	—	Heston	1964	USA
BFM2/A	—	Bonhomme	1984	France
CE/JA	—	Jackson Labs	1976	USA[a]
C57BL/LiA	—	Little	1931	USA[a]
C57BL/LiAU	—	REPGO-TNO	1953	USA
BIMA/A	—	Muhlbock	1959	(USA)
BIR/A	—	Muhlbock	1958	(USA)
C57BL/6ByA	—	Bailey	1972	USA
C57BL/10ScSnA	—	Snell	1968	USA
C58/JA	—	Jackson Labs	1976	USA[a]
CBA/BrA	—	Bonser	1948	USA[a]
C3H/HeAf(old)	—	Heston	1951	USA[a]
C3H/HeDiSnA	—	Snell	1969	USA[a]
C3H/BiU	—	Chester Beatty Inst.	1955	USA[a]
DBA/LiA	—	Little	1931	USA[a]
DD/HeAF	—	Heston	1970	Japan
DBA/LiAf(new)	—	Nikkels	1979	USA
FVB/NA	—	Rowe	1978	USA?
FTC/CpbU	CPB-FT/U	CPB-TNO	1950	NL[b]
GRS/A	—	Muhlbock	1959	NL[b]
GRS-Mtv-2⁻/A	—	Van Nie	1977	NL
HC/CpbU	CPB-H/U	CPB-TNO	1950	NL[b]
KC/CpbU	CPB-K/U	CPB-TNO	1950	NL[b]
LIS/A	—	Lieberherr	1955	NL[b]
LTS/A	—	Loustalot	1954	NL[b]
MAS/A	—	Maier	1955	NL[b]
MOC/CpbU	CPB-Mo/U	Inst. Trop. Hyg.	1957	NL[b]
MOL3/JA	—	Roderick	1977	(USA)
NC/CpbU	CPB-N/U	CPB-TNO	1950	NL[b]
NFS/NA	—	Rowe	1978	USA[a]
020/A	—	Korteweg	1931	NL[b]
OIR/A	—	Muhlbock	1959	NL[b]
PC/CpbU	CPB-P/U	CPB-TNO	1950	NL[b]
QC/CpbU	CPB-Q/U	Hagedoorn/Hirschfeld	1937	NL[b]
RC/CpbU	CPB-R/U	Hagedoorn	1949	NL[b]
RIII/SeAf(old)	—	Severi	1965	France
SC/CpbU	CPB-S/U	Hagedoorn	1949	NL[b]
SJL/JA	—	Jackson Labs	1977	USA
SL/NiA	—	Nishizuka	1977	Japan
SM/JA	—	Jackson Labs	1981	USA[a]
STS/A	—	Muhlbock	1955	NL[b]
TA1/A	—	Li Yi	1982	China
TA2/A	—	Li Yi	1982	China
TSI/A	—	Muhlbock	1958	NL[b]
VC/CpbU	CPB-V/U	Hagedoorn (?)	1949	NL[b]
WLL/BrA	—	Bonser	1948	UK[b]
WLL/BrAf(old)	—	Mulhbock	1952	UK
129/MA	—	Boyse	1975	USA[a]
129/SvSlA	—	Czarnomska	1978	USA[a]

[a] 'American' strains used in discriminant function analysis.
[b] 'European' strains used as in (a) above.

also some close linkages among esterase loci. Several esterase loci are quite variable, providing alleles which can easily be recognized using gel electrophoresis or isoelectric focusing. While some loci are informative on subspecies divergence (Es-14, -17 and -18) others can be used to trace intra-specific variation within *Mus musculus domesticus*.

2. Materials and Methods

(i) *Mice*

The origin of all the inbred strains involved in this study is given in Table 1. A detailed history is only given for strains which have not been described in detail elsewhere (e.g. Festing, 1979; Hilgers *et al.* 1985; Hilkens *et al.* 1981; Staats, 1985; Van der Valk, 1981; Groen, 1977). The nomenclature of the Utrecht strains has been changed and this is indicated in the table.

Brief description of strains in Table 1 which are not described elsewhere:

BFM2/A F8. Origin: from Bonhomme to Amsterdam, 1984. This strain belongs to the 'brevirostris' subgroup of *Mus musculus domesticus*.

BIMA/A G10F58. Origin: Muhlbock, 1959. A partly congenic strain of C57BL/LiA following an outcross to C3H/HeA with cross-intercross matings with selection for large mammary glands to G10 then further selection with b × s mating (to F11).

BIR/A G12F61. Origin: Muhlbock, 1958. A partly congenic strain of C57BL/LiA following an outcross to DBA/LiA with cross–intercross matings with selection for resistance to the growth of a C57BL/LiA mammary tumour cell line transplanted *in vivo*.

FVB/NA F25. Albino: c. Origin: Rowe (NIH) to Amsterdam, 1978.

GRS-MTV-2⁻/A. G18F30. Origin: Van Nie, 1977. Partly congenic with GRS/A. Lacks the *Mtv-2* gene on chromosome 18, obtained by cross–intercross matings and selection for MTV antigen-negative milk following an outcross to C57BL/LiA.

KC/CpbU F?+82. Beige: *a, B, C, d, P*. Origin:

Table 2. *Summary of esterase markers tested, tissues, separation methods, and substrates applied*

Esterase symbol[a]	Tissue	Separation[b]	Substrate[b]	Reference
Es-1	Testis, plasma	PAGE	NASD-O-2	Popp & Popp (1962)
Es-2	Kidney	PAGE	αN-O-2; NASD-O-2	Petras (1963)
Es-3	Kidney, liver	PAGE	NASD-O-2	Ruddle & Roderick (1965)
Es-5	Plasma	PAGE	αN-O-2	Petras & Biddle (1967)
Es-6	Heart, testis	PAGE	αN-O-2	Petras & Sinclair (1969), Nash & Von Deimling (1982)
Es-7	Lung, tongue	PAGE, IEF	5BrI-O-2; αN-O-4	Chapman (1973) Lipps *et al.* (1979)
Es-9	Kidney, liver	PAGE	αN-O-2	Schollen *et al.* (1975)
Es-10	Erythrocytes	IEF	U-O-2	Peters & Nash (1976), Womack & Sharp (1976)
Es-11	Liver	PAGE	NASD-O-2	Peters & Nash (1977)
Es-13	Kidney	IEF	αN-O-2	Womack *et al.* (1978)
Es-14	Erythrocytes	IEF	αN-O-4 αN-O-ala	Britton-Davidian & Bonhomme (1979), Von Deimling & De Looze (1983)
Es-16	Kidney	IEF	5BrI-O-2	Von Deimling *et al.* (1981)
Es-17	Testis	IEF	NASD-O-2	Otto & Von Deimling (1983)
Es-18	Kidney	IEF	αN-O-2	Von Deimling (1981)
Es-22	Liver	PAGE	αN-O-2	Eisenhardt & Von Deimling (1982)
Es-23	Kidney	PAGE, IEF	5BrI-O-2	Von Deimling (1984)
Es-24[c]	Lung	PAGE	αN-O-2	Von Deimling (1982)
Es-25	Kidney	PAGE	5BrI-O-2	Von Deimling (1983)
Es-26	Liver	IEF	5BrI-O-2	Von Deimling *et al.* (1984)
Es-27	Plasma	PAGE	αN-O-2	Bonhomme and Selander (1978)
Esr	Plasma	PAGE	αN-O-2	Von Deimling *et al.* (1982)

[a] Gene symbols are used except for ES-24, for which no separate gene locus has been described.
[b] Abbreviations: IEF, isoelectric focusing; PAGE, polyacrylamide gel electrophoresis; NASD-O-2, naphthol AS-D acetate; αN-O-2, αN-O-4, α-naphthyl acetate, butyrate; 5BrI-O-2, 5-bromoindoxyl acetate; αN-O-ala, *N*-acetylalanine-α-naphthyl ester; U-O-2, 4-methylumbelliferyl acetate.
[c] ES-24 describes a variation in a carboxylesterase isozyme occurring in lung (Von Deimling, 1982) and in liver (Berning *et al.* 1985). The genetics of ES-24 are not clear but it was suggested that it is a hybrid aggregate of products of different but very closely linked loci within cluster 1 of the esterase gene region of chromosome 8, which apparently segregated like the product of a single gene (Von Deimling, unpublished). Hence, ES-24 has been defined only as phenotype. Nevertheless, ES-24 is a useful marker for cluster 1 loci.

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Table 3. *Allele distribution of esterases*

(8¹, 8²: esterase cluster 1, cluster 2 on chromosome 8.)

Chromosome Strain	Es-1 8^1	Es-2 8^2	Es-3 11	Es-5 8^2	Es-6 8^1	Es-7 8^2	Es-9 8^1	Es-10 14	Es-11 8^2	Es-13 9	Es-14 9
A/BrAf (old)	b	b	c	b	a	b	a	a	a	a	r
A/WySnA	b	b	c	b	d	b	a	a	a	a	r
A2G/GA	b	b	c	b	a	b	a	a	a	a	r
ACR/A	b	b	b	b	a	b	a	a	a	a	r
AKR/FuRdA	b	b	c	b	a	b	a	b	a	a	r
BALB/cByA	b	b	a	b	a	b	a	a	a	a	r
BALB/cCdA	b	b	a	b	a	b	a	a	a	a	r
BALB/cCrglA	b	b	a	b	a	b	a	a	a	a	r
BALB/cHeA	b	b	a	b	a	b	a	a	a	a	r
BFM2/A	b	b	b	b	c	b	d	a	a	a	r
CE/JA	b	b	c	b	a	b	a	b	a	a	r
C57BL/LiA	a	b	a	b	a	b	a	a	a	a	r
C57BL/LiAU	a	b	a	b	a	b	a	a	a	a	r
BIMA/A	a	b	a	b	a	b	a	a	a	a	r
BIR/A	a	b	a	b	a	b	a	a	a	a	r
B6/ByA	a	b	a	b	a	b	a	a	a	a	r
B10/ScSnA	a	b	a	b	a	b	a	a	a	a	r
C58/JA	b	b	c	b	a	b	a	a	a	a	r
CBA/BrA	b	b	c	b	a	b	a	b	a	a	r
C3H/HeAf(old)	b	b	c	b	a	b	a	b	a	a	r
C3H/HeDiSnA	b	b	c	b	a	b	a	b	a	a	r
C3H/BiU	b	b	c	b	a	b	a	b	a	a	r
DBA/LiA	b	b	c	b	a	b	a	b	a	a	r
DBA/LiAf(new)	b	b	c	b	a	b	a	b	a	a	r
DD/HeAf	b	b	c	b	a	b	a	b	a	b	r
FVB/NA	b	b	c	b	a	b	a	a	a	b	r
FTC/CpbU	b	b	b	a	a	b	b	b	e	a	r
GRS/A	b	b	b	b	a	b	a	a	a	a	r
GRS-Mtv-2⁻/A	b	b	b		a	b	a	a	a	a	r
HC/CpbU	b	c	b	b	a	b	a	b	a	a	r
KC/CpbU	b	b	b	b	a	b	a	b	a	a	r
LIS/A	b	b	c	b	a	b	b	a	a	b	r
LTS/A	b	b	c	b	a	b	b	a	a	a	r
MAS/A	b	b	c	b	a	b	a	a	a	a	r
MOC/CpbU	b	b	c	b	a	b	a	b	a	a	r
MOL3/JA	f	d	b	a	e	c	c	b	c	a	r
NC/CpbU	b	b	b	b	a	b	a	b	a	a	r
NFS/NA	b	b	a	b	a	b	a	a	a	b	r
020/A	b	b	c	b	a	b	a	b	a	a	r
OIR/A	b	b	c		a	b	a		a	a	r
PC/CpbU	b	b	b	b	a	b	a	b	a	a	r
QC/CpbU	b	b	c	b	a	b	a	b	a	a	r
RC/CpbU	b	b	c	b	a	b	a	b	a	a	r
RIII/SeAf (old)	b	b	a	b	a	b	a		a	a	r
SC/CpbU	b	b	c	b	a	b	a	b	a	b	r
SJL/JA	b	b	c	b	a	b	a	b	a	b	r
SL/NiA	b	b	c	b	a	b	a	b	a	b	r
SM/JA	b	b	c	b	a	b	a	b	a	a	r
STS/A	b	b	c	b	a	b	b	a	a	b	r
TA1/A	a	b	a	a	a	b	a	b	b	a	r
TA2/A	b	b	c	b	a	b	a	a	a	a	r
TSI/A	b	b	c	b	a	b	a	b	a	a	r
VC/CpbU	b	b	c	a	a	b	a	b	b	a	r
WLL/BrA	b	b	c	b	a	b	a	a	a	a	r
WLL/BrAf (old)	b	b	c	b	a	b	a	a	a	a	r
129/MA	b	b	c	b	a	b	a	b	a	a	r
129/SVS1A	b	b	c	b	a	b	a	b	a	a	r

Table 3 (*cont*)

Chromosome Strain	Es-16 3	Es-17 9	Es-18 19	Es-22 8¹	Es-23 8²	Es-24 8¹	Es-25 ?	Es-26 3	Es-27 3	Esr 6
A/BrAf(old)	a	a	a	d	c	A	a	b	r	a
A/WySnA	a	a	a	d	c	H	a	b	r	a
A2G/GA	a	a	a	d	c	A	a	a	r	a
ACR/a	a	a	a	d	c	A	a	c	s	a
AKR/FuRdA	a	a	a	d	c	F	a	c	s	a
BALB/cByA	a	a	a	d	c	A	a	a	r	a
BALB/cCdA	a	a	a	d	c	A	a	a	r	a
BALB/cCrglA	a	a	a	d	c	A	a	a	r	a
BALB/cHeA	a	a	a	d	c	A	a	a	r	a
BFM2/A	a	a	a	c	c	E	b	a	r	a
CE/JA	a	a	a	d	c	A		a	r	
C57BL/LiA	a	a	a	d	c	A	b	a	r	a
C57BL/LiAU	a	a	a	d	c	A	b	a	r	a
BIMA/A	a	a	a	d	c	A	b	a	r	a
BIR/A	a	a	a	d	c	A	b	a	r	a
B6/ByA	a	a	a	d	c	A	b	a	r	a
B10/ScSnA	a	a	a	d	c	A	b	a	r	a
C58/JA	a	a	a	d	c	A	b	a	r	a
CBA/BrA	a	a	a	d	c	A	b	a	r	a
C3H/HeAf (old)	a	a	a	d	c	A	b	a	r	a
C3H/HeDiSnA	a	a	a	d	c	A	b	a	r	a
C3H/BiU	a	a	a	d	c	A	b	a	r	a
DBA/LiA	a	a	a	d	c	A	b	a	r	a
DBA/LiAf (new)	a	a	a	d	c	A	b	a	r	a
DD/HeAf	a	a	a	d	c	A	b	b	r	o
FVB/NA	a	a	a	d	c	A	b	c	s	a
FTC/CpbU	a	a	a	h		A	a	a	r	
GRS/A	a	a	a	d	c	A	b	a	r	a
GRS-Mtv-2⁻/A	a	a	a	d	c	A	b			
HC/CpbU	a	a	a	d		A	a	a	r	a
KC/CpbU	a	a	a	d	c	A	b	a	s	a
LIS/A	a	a	a	h	c	A	b	a	r	a
LTS/A	a	a	a	h	c	A	b	a	r	a
MAS/A	a	a	a	d	c	A	b	a	r	a
MOC/CpbU	a	a	a	d	c	A	b	a	s	a
MOL3/JA	a	b	b	a	a	C	b	a	r	o
NC/CpbU	a	a	a	d	c	A	b	a	r	a
NFS/NA	a	a	a	d	c	F	b	a	r	a
020/A	a	a	a	d	c	A	b	c	s	a
OIR/A	a	a	a	d	c	A	b			
PC/CpbU	a	a	a	d	c	A	b	a	r	a
QC/CpbU	a	a	a	d	c	A	a	a	s	a
RC/CpbU	a	a	a	d	c	A	b	a	r	a
RIII/SeAf(old)	a	a	a	d	c	A	b	a	r	a
SC/CpbU	a	a	a	d	c	A	a	a	r	a
SJL/JA	a	a	a	d	c	F	b	c	s	a
SL/NiA	a	a	a	d	c	A	b	a	r	a
SM/JA	a	a	a	d	c	A	b	a	r	a
STS/A	a	a	a	h	c	A	b	a	r	a
TA1/A	a	a	a	d	c	A	b	b	r	
TA2/A	a	a	b	d	C	A	b	a	r	a
TSI/A	a	a	a	d	c	A	a	a	r	a
VC/CpbU	a	a	a	d		A	a	b	r	
WLL/BrA	a	a	c	d	c	A	b	a	r	a
WLL/BrAf(old)	a	a	c	d	c	A	b	a		a
129/MA	a	a	a	d	c	F	b	a	r	a
129/SvSlA	a	a	a	d	c	F	b	a		a

CPB-TNO in about 1950. To Vet. Fac. Utrecht in 1973. Small litter-size and excitable.

MOC/CpbU F?+61. Albino: *c*. Origin: Inst. Trop. Hygiene Amsterdam to CPB-TNO in about 1957. To Vet. Fac. Utrecht in 1973. Carries brachypodism gene.

MOL/3JA F?. Agouti. Origin: *Mus musculus molossinus* inbred by Roderick at the Jackson Laboratory. Genetically distinct from common inbred strains of laboratory mice.

NCU/CpbU F?+80. Pink-eyed-dilute: *a, b, C, D, p*. Origin: CPB-TNO in 1950 to Vet. Fac. Utrecht in 1973.

OIR/A G12F90. Origin: Muhlbock, 1959. A strain partly congenic with 020 developed by cross–intercross matings with selection for resistance to a transplanted mammary tumour of 020 following an outcross to DBA/LiA.

(ii) *Determination of esterase phenotypes*

References to the technical procedures for determination of the esterase phenotypes are summarized in Table 2. At least two animals of each strain were used. Two strains (DD/HeAf and MOL3/JA) which did not express *Es-5* were tested for *Esr* by breeding, using SK/Cam and ESR/Fre as test strains (Von Deimling, Otto & Reske-Kunz, 1982). Strain WLL/ BrA(old) was crossed with C57BL/10Sn and with PWK/Ph to clarify the nature of a new variant in the lysosomal esterases *Es-13/Es-18*. The nomenclature used by Hilkens *et al.* (1981) is generally followed. The allele designations of *Es-6* are those proposed by Nash & Von Deimling (1982). Gene symbols were used for designation of the esterases except for Es-24 (see footnote to Table 2), where no separate gene locus has been described.

(iii) *Statistical methods*

Frequency distributions of alleles among the sample of 57 strains were prepared. As 10/19 polymorphic loci were on chromosome 8, 2/19 on chromosome 3 and 2/19 on chromosome 9, two-way tables were prepared to look for linkage disequilibrium. As the table cell numbers were unusually small, 2×2 tables were prepared, and Fisher's exact test was used to test any deviation from independence. The alleles were coded for numerical analysis. A matrix of similarities between every pair of strains was calculated by giving a score of 1 if a pair of strains were identical at a locus, and zero otherwise (Dunn & Everitt, 1982).

Two clustering techniques were used to study the relationships among the strains. Multidimensional scaling using Principal Coordinate analysis was used to produce a two-dimensional map showing the relationship between the strains with the minimum amount of distortion. Full details of the technique are given by Dunn & Everitt (1982), Blackith & Reyment

(1971) and Maxwell (1977). An identical method was used by Festing & Bender (1984) to analyse similar data on inbred strains of rats. The technique involves extraction of the latent roots and vectors of the similarity matrix in order to present the data with a reduced dimensionality. Hierarchical cluster analysis using single-linkage and average-linkage criteria was also used. The relative merits of these two methods were discussed by Dunn & Everitt (1982).

Discriminant function analysis based on six selected loci (*Es-3, -10, -13, -25, -26* and -27) was used to study two subsets of the 57 strains (Table 1). The aim was to predict group membership from a knowledge of the (coded) genotype at each of the six loci. A total of 15 strains were classified *a priori* as being of American, and 20 strains as being of European origin (Table 1). Where there were multiple samples of the same subline, only a single representative strain was chosen. The classification was somewhat arbitary in some cases. For example, strain SJL was inbred in the USA, but from 'Swiss' stock, so was excluded from both groups.

3. Results

The distribution and chromosome location of esterase alleles in the 57 strains included in this study are shown in Table 3. *Es-14* and *Es-16* were invariant in this sample of strains. Numerical analyses were therefore based on the remaining 19 loci.

(i) *New alleles*

Three new alleles were noted as follows: $Es\text{-}6^d$ (A/WySnA), $Es\text{-}11^e$ (FTC), and $Es\text{-}18^c$ *(WLL/BrAf)*.

(ii) *Frequency distribution of alleles*

The frequency distribution of the alleles at each of the 19 polymorphic loci is shown in Table 4. At most loci (e.g. *Es-2, 5, 6, 7, 9, 11, 17, 18, 22, 23* and *Esr*) there was a common allele observed in 86–98% of the strains, with the remaining strains having a rare allele (frequency being expressed relative to this sample of strains). Such loci are useful for identifying individual strains, but are of limited value in looking at broad relationships between groups of strains. However, there was a more equal distribution of alleles at the *Es-1, 3, 10, 13, 24, 25, 26* and *27* loci. Fifty-four strains showed a close genetic relationship, sharing the $Es\text{-}7^b$, $\text{-}17^a$, $\text{-}18^a$ and $\text{-}23^c$ alleles.

(iii) *Strains with identical alleles*

There were a total of 10 clusters of two or more strains which were identical at all 19 loci. These are shown in Table 5. Most of these were different sublines of the same strain, which are expected to be similar. However, there were some surprises. Strains MAS and C58 were identical although they have no known common ancestry. Similarly with CE and TSI. Strains

Table 4. *Distribution of alleles at 19 esterase loci among 57 mouse strains*

(Allele 1 is the most frequent, 2 the next most frequent, etc. among the sample of 57 strains.)

Es locus	Allele in order of frequency					Not done
	1	2	3	4	5	
1	49	7	1	—	—	—
2	55	1	1	—	—	—
3	34	13	10	—	—	—
5	51	4	—	—	—	2
6	54	1	1	1	—	—
7	56	1	—	—	—	—
9	51	4	1	1	—	—
10	28	27	—	—	—	—
11	53	2	1	1	—	—
13	49	8	—	—	—	—
17	56	1	—	—	—	—
18	53	2	2	—	—	—
22	51	4	1	1	—	—
23	53	1	—	—	—	3
24	49	5	1	1	1	—
25	41	15	—	—	—	1
26	45	5	5	—	—	—
27	45	8	—	—	—	4
Esr	49	2	—	—	—	6

CBA, C3H, SM and DBA are known to have some common ancestry, but it was still surprising that they were identical at all loci, and they have no known relationship with OIR or RC, which fell in the same cluster.

(iv) *Linkage disequilibrium*

Significant linkage disequilibrium between linked loci was found only between *Es-5* and *Es-11* on chromosome 8 ($P < 0.001$), and between *Es-26* and *Es-27* on chromosomes 3 ($P < 0.001$). In the former case there were 51 strains which were *Es-5^b*, *Es-11^a* and four strains (FTC, MOL3, TA1 and VC) which were *Es-5^a*, *Es-11$^{not\ a}$*, but there were no strains with the remaining two combinations of alleles.

With *Es-26* and *Es-27* there was a significant absence of any strains which were *Es-26^c*, *Es-27^r*, and more strains than expected which were *Es-26^r*, *Es-27^s*.

Interpretation of these results is complicated by the observation that there was also some significant disequilibrium between unlinked loci. Thus there was significant disequilibrium between *Es-1* and *Es-3* ($P < 0.01$), with a complete absence of strains which were *Es-1^a*, *Es-3^c* (4 strains expected), some mild disequilibrium ($P < 0.05$) between *Es-1* and *Es-10*, and more significant disequilibrium between *Es-3* and *Es-10* ($P < 0.001$) with only a single strain (TA1) having the *Es-3^a*, *Es-10^b* type whereas 6 strains would have been expected.

Interpretation of frequency data of this type depends on the assumption that the strains examined are a random sample of inbred mouse strains. Although this is true in the sense that the strains were not chosen for study on the basis of their esterase phenotypes, the inclusion of several sublines of C57BL, BALB/c, etc., may be biasing the frequency observations.

(v) *Similarity matrix*

An abbreviated similarity matrix in which the strains have been ordered to correspond with the single-linkage cluster analysis is given in Table 6. This clearly shows that most strains are quite similar, usually being identical at more than 80% of loci, but that there are a few strains which differ more markedly from the others, and a single strain MOL3 which is quite different from all other strains. The most dissimilar pair of strains were MOL3 and FVB, which differed at 18/19 of the polymorphic loci, being similar only at *Es-25*.

(vi) *Cluster analysis*

No distinct and clear-cut clusters of strains were identified either by single-linkage or by average-linkage cluster analysis. The single-linkage method tended to emphasize the similarities between most strains, and the great dissimilarity of MOL3, and gave a better-ordered similarity matrix than the average-linkage method. However, it produced some large primary clusters in which strains differed at one or no loci, whereas the average-linkage method produced

Table 5. *Strains which are identical at all esterase loci.*

Cluster	Strains
1	BALB/cBy, BALB/cCd, BALB/cCrgl, BALB/cHe
2	C57BL/LiA, C57BL/LiAU, BIMA, BIR, C57BL/6By, C57BL/10ScSn
3	GRS, GRS-Mtv-2⁻
4	NCU, PC
5	C58, MAS
6	WLL, WLL/Old
7	CE, TSI
8	CBA/Br, C3H/HeAf, C3H/HeDiSn, C3H/BiU, DBA/Li, DBA/LiAf (new), OIR, RC, SM
9	129/Ma, 129/SvSl
10	LIS, STS

Table 6 *Abbreviated similarity matrix.*

(A '9' indicates 90–100 % similarity between the two strains across all 19 loci.)

|	
A/Br	–
A2G	9–
TSI	89–
CE	899–
SM	8899–
RC	88999–
OIR	999999–
020	7788889–
MOC	78899999–
MAS	898999988–
DBA/LiAf	8899999899–
DBA/LiA	88999998999–
C3H/BI	889999989999–
C3H/HeDi	8899999899999–
C3H/HeA	88999998999999–
CBA/Br	889999989999999–
C58	8989999889999999–
129/MA	78899998889999998–
129/SvSl	788999989899999989–
WLL	8888889789888888988–
WLL/Old	88888898898888889889–
TA2	888888978988888898899–
SL	7889999888999999888888–
SC	88998887888888888887779–
QC	889988989888888888878788–
PC	7889999888999999888888888–
NC	78899998889999998888888889–
KC	778888989888888888878787899–
GRS	8888889789888888988888877998–
GRS-	887888988988888897788877779999–
BALB/cHe	8988888778888888887788878888788–
BALB/cCr	89888887788888888877888788887889–
BALB/cCd	898888877888888888778887888878899–
BALB/cBy	8988888778888888887788788888788999–
RIII	88899998899999998888888888998999999–
C57BL/10	78788887788888888778887778878888889–
C57BL/6By	787888877888888888778877788788888899–
BIR	7878888778888888877888777887888888999–
BIMA	78788887788888888778887778878888889999–
C57BL/Li	787888877888888887788877788788888899999–
C57BL/LiAU	7878888778888888877888777887888888999999–
ACR	88777788777777777677776787788988887777777–
FVB	777777988877777778777878777777877777777778–
DD	7778889777888888777777887777776666766666667–
SJL	66777788877777777886768777776766667666666787–
LTS	787888877888888887788877777787777787777776766–
STS	7777777678777777787777787677677777777777767769–
LIS	77777776787777777877778767767777777777777677699–
AKR/Fu	778877888777777778867677877767777776666668768655–
A/Wy	88777776677777777777776776667777777766666676666667–
NFS	777777767877777788877787677787888888888888677777766–
HC	7888887777888888777777788888878888877777777666666766–
VC	87887787777777777766667777766777776555555565755557757–
TA1	66677776667777776666566667766766667777777557555555658–
BFM2	66666665676666666766666655776776666766666666555766577744–
FTC	567666655566666655555556677666666666555555554547665447755–
MOL3	1122221122222222222212211332211111222222210212111111122223–
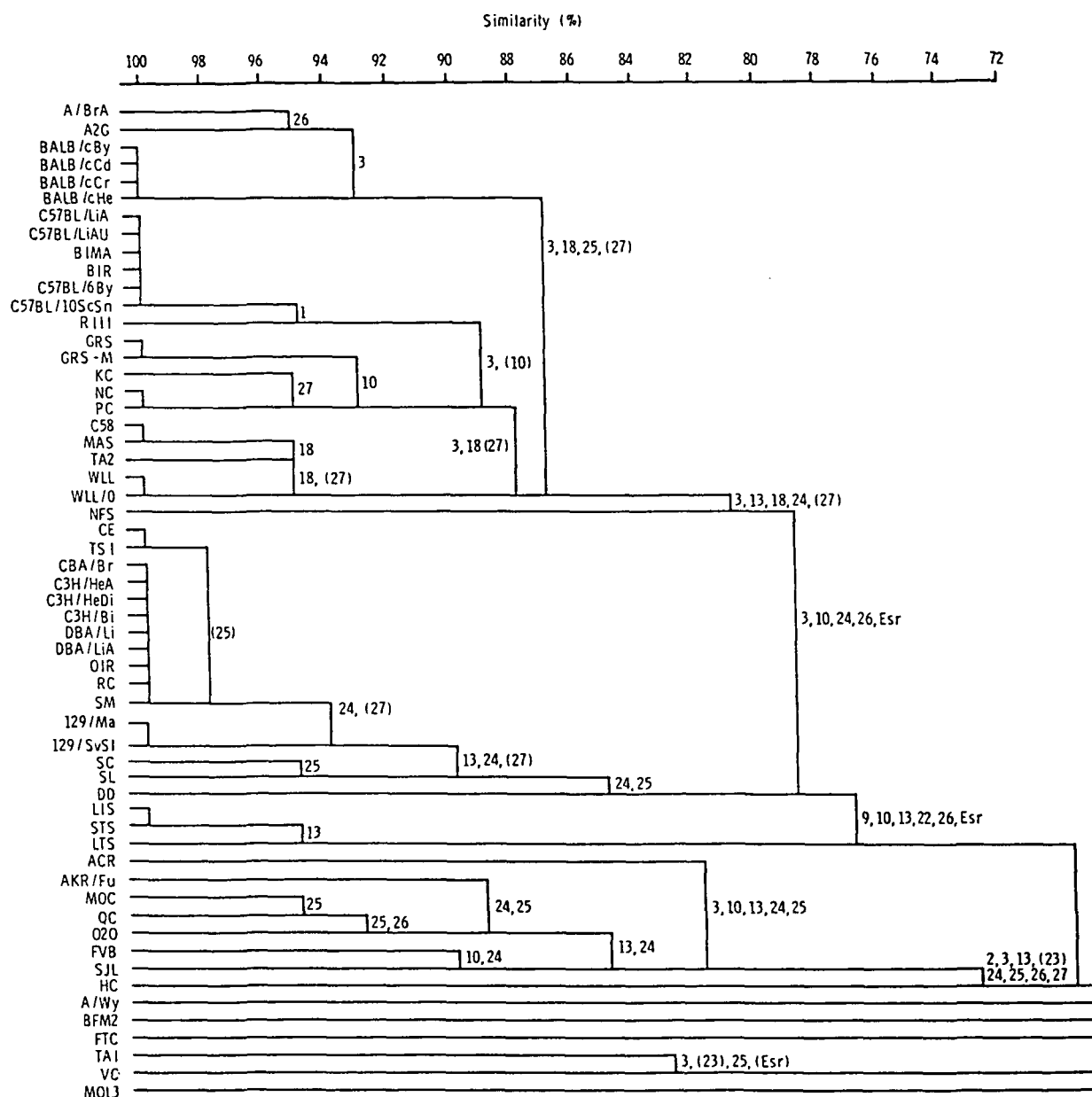
Fig. 1. Average linkage cluster analysis. The numbers show the loci at which the strains differ. For example, BALB/cCr and BALB/cHe do not differ, but both differ from A2G at the *Es-3* locus. Numbers in parentheses are loci at which one or more of the strains were not typed. Strains at the bottom of the chart (HC down to MOL3) were unclustered at the lowest levels shown in the chart.

primary clusters that did not differ at any loci, and on which it was easier to show differences between clusters. Accordingly, the average-linkage clustering criterion was chosen to display the relationships among the strains (Fig. 1). At the highest level of similarity, there were 10 clusters of 2 or more strains, and 22 unclustered strains. At the 80 % similarity level there were five clusters involving 52 strains and five unclustered strains.

(vii) *Principal-coordinate analysis*

The first two latent roots of the similarity matrix produced by the principal coordinate analysis accounted for only 34 % of the total variation, suggesting

that it is difficult to give a true presentation of such complex data in a two-dimensional chart. However, a plot of these two principal coordinates is shown in Fig. 2, as the results are relatively easy to interpret.

Most strains were included in a single cigar-shaped cluster, with the length of the cigar (the first principal coordinate (PC)) representing variation at the polymorphic loci *Es-1, 2, 10* and *27,* and the dorso-ventral fatness (3rd PC) of the cigar represented by variation in *Es-25.* The thickness of the cigar (2nd PC) represents varation in the number in rare alleles at other esterase loci. Thus MOL3 and FTC both have an accumulation of rare or unique alleles.

Thus, the principal components analysis supports the cluster analysis in showing that there are no
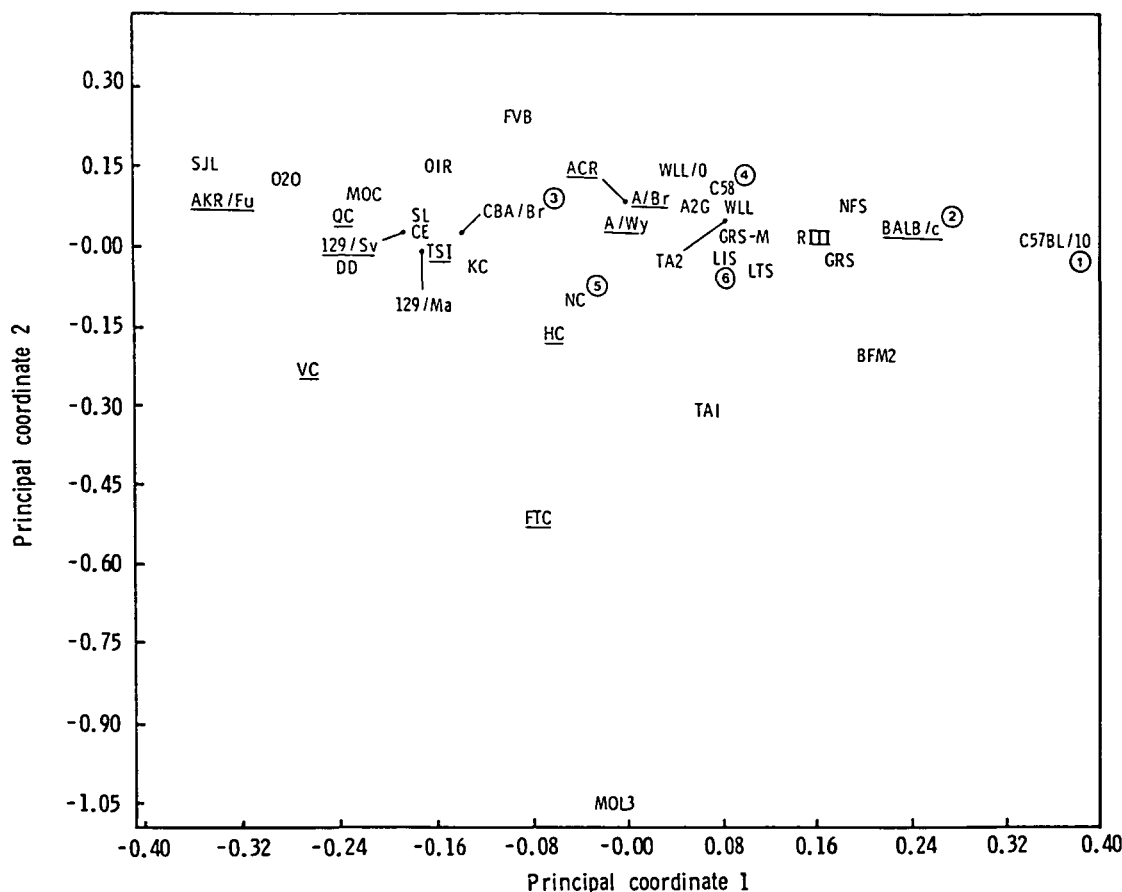
Fig. 2. Principal Coordinate analysis. Principal coordinate 1 represents variation at the *Es-1, -2, -10* and *-27* loci. A high positive value is associated with a high probability that a strain is *Es-1ᵃ, Es-3ᵃ, Es-10ᵃ* and *Es-27ʳ*, whereas a low negative value gives a high probability that the strain is *Es-1ᵇ, Es-3ᶜ, Es-10ᵇ* and *Es-27ˢ*. Strains which are underlined are *Es-25ᵃ*, and are separated by the 3rd principal coordinate (i.e. the plane of the paper). The second principal coordinate represents the probability of having rare alleles at the other loci, with a low negative value representing a high probability of many rare alleles (e.g. MOL3). (1) Includes C57BL/Li, C57BL/LiA, C57BL/6By, BIMA and BUR. (2) Includes all other BALB/c sublines. (3) Includes C3H/HeA, C3H/HeDi, C3H/Bi, DBA/Li, DBA/LiA. (4) Includes MAS. (5) Includes PC. (6) Includes STS.

distinct clusters of strains which can be distinguished on the basis of their esterase phenotypes. Most strains only differ from each other as a result of substitutions at one or more of the *Es-1, 3, 10, 25* and *27* loci. However, there are a few strains which differ from the others as a result of one or more rare alleles at other esterase loci, with MOL3, FTC, TA1, VC and BFM2 being particularly distinct.

(viii) *Comparison of American and European strains*

As the American strain C58 and the European strain MAS were identical at all loci, it is clear that no mathematical method will give clear discrimination between the two groups. In fact, there was only a single locus where differences in allele frequency between the two groups approached statistical significance. Among American strains, 0/15 had the *Es-3ᵇ* allele, whereas 6/20 European strains carried this allele ($P = 0.06$ using Fisher's exact test). Discriminant function analysis classified 24/35, or 69% of strains correctly. This is little better than the 50% which would be expected by chance. It is concluded

that, although the frequency of some alleles may differ between stocks of different origin, there is no particular set of features by which stocks of European and American origin can be unequivocally distinguished.

3. Discussion

The main aim of these studies was to present data on the esterase alleles at 21 loci present in a large sample of inbred mouse strains of diverse origin, and to explore the genetic similarities among the strains based on these loci. Two of the loci were monomorphic in this sample of 57 strains, and several others were nearly so. Thus, no fewer than 54 strains were identical at loci *Es-7, -17, -18* and *-23*.

There was no evidence that the strains fell into distinct clusters, apart from some clusters of very closely related sublines and congenic strains, which were often identical at all loci. Nor could strains of American and European origin be unequivocally distinguished from one another, even though there appeared to be deficiency of the *Es-3ᵇ* allele in American strains. These findings are in marked

contrast with those of Festing and Bender (1984), who studied 46 inbred strains of rats, and found distinct clustering into two approximately equal groups due to linkage disequilibrium among esterase loci. In these studies little linkage disequilibrium was found among the common laboratory strains even though 10/19 of the polymorphic loci were on chromosome 8, and there were also pairs of linked loci on chromosomes 3 and 9. What little linkage disequilibrium was oberved tended to involve genetically highly distinct strains such as MOL3, FTC, VC and TA1.

These differences between rat and mouse strains may reflect the very different histories of domestication, or a real difference in functional activity of esterases in the two species. Mice have been domesticated for thousands of years (Festing & Lovell, 1981), and most strains of laboratory mice are believed to be descended from these pet and fancy mice. They certainly seem to be very different from most wild mice. Thus, it is possible that the common laboratory strains are descended from a relatively large homogenous gene pool which has had plenty of time to reach linkage equilibrium even for closely linked loci. In contrast, the rat has only been domesticated since the eighteenth century, following its migration to Europe from the Far East. According to Lindsey (1979), rat baiting was popular in France and England as early as about 1800, and rats were trapped for this sport. Records indicate that albinos were found and removed for show purposes and/or breeding, and their descendants then found their way into the laboratory. In these circumstances there may not have been time for any closely linked groups of genes to reach equilibrium in this species. Alternatively, it is possible that in rats (but not in mice) there is some mechanism which suppresses crossing-over or that certain combinations of esterase alleles are maintained by a selective advantage, so leading to the observed disequilibrium.

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