Genet. Res., Camb. (1981), **37**, *pp.* 17–31
With 2 text-figures
Printed in Great Britain

The population genetics of the *H-2* polymorphism in European and North African populations of the house mouse (*Mus musculus* L.)

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(*Received* 22 *April* 1980, *and in revised form* 13 *June* 1980)

SUMMARY

Two hundred and two house mice (*Mus musculus* L.) from 29 populations in Europe and North Africa were typed for 16 H-2K and 17 H-2D antigens, each antigen defining a different allele. Among the 13 best characterized populations, 1 to 4 common and 3 to 20 rare antigens were observed. However, an average of 37% of the H-2K and 39% of the H-2D antigens remain to be identified. Ninety-four percent of the 50 mice tested were heterozygous for H-2K antigens and 89% for H-2D antigens. In 4 of the 8 populations tested, the most common H-2K and H-2D antigens occurred in the same individual more often than if randomly associated. Associations between common H-2K and H-2D antigens and excess heterozygosities may be the consequence of the small size and instability of populations composed primarily of related individuals. Estimates of the genetic distances between populations revealed that Danish, Egyptian, and several of the Orkney Island populations were related. These were the only populations in which metacentric chromosomes were not found. In contrast, populations which were antigenically different were also karyotypically different, regardless of taxonomic status of allozymic similarity.

1. INTRODUCTION

The H-2 complex, the major histocompatibility complex (MHC) of the house mouse, is a cluster of loci occupying a segment of chromosome 17 about 0·4 cm in length (for reviews, see Klein, 1975, 1979). Based on their phenotypic effects, the loci can be grouped into three classes: class I, composed of loci *K* and *D* coding for molecules with broad tissue distribution and molecular weight of some 44000 daltons; class II, exemplified by loci *A* and *E* and coding for molecules predominately expressed on B lymphocytes and macrophages and each consisting of two polypeptides, α (35000 daltons) and β (28000 daltons); and class III, consisting of at least one locus, *S*, coding for complement component C4. The order of these loci in the *H-2* complex is: *K*, *A*, *E*, *S*, and *D*. The products of these loci can be studied with appropriate antisera as serologically detectable antigens.

0016-6723/81/2828-8360 $01.00

The function of the class I and class II loci is thought to be the regulation of the immune response.

One of the most remarkable features of the *K*, *D*, and *A* loci is their extreme polymorphism. Preliminary estimates suggest that there are about 100 to 200 alleles at each of the class I loci, and some 50 alleles at the *A* locus; at the *E* locus, there may be only about 10 alleles (Duncan, Wakeland & Klein, 1979*a*, Klein *et al.* 1980). Furthermore, in excess of 95% of wild mice are heterozygous for *K*, *D*, and *A* alleles (Duncan, Wakeland & Klein, 1979*b*). These conclusions were based on H-2 antigenic frequencies determined for two populations of wild mice in North America. The data obtained from these samplings were, however, insufficient to draw any conclusions about the distribution of H-2 antigens in different geographical areas. To obtain such information on the H-2 antigenic polymorphism, we have recently initiated a systematic study of wild mice in Europe and North Africa. The serological typing results from the first phase of these studies will be described elsewhere (Götze *et al.* 1980); the genetic implications of these data are discussed in this communication. In particular, we analysed the patterns of antigenic variation within and between populations.

2. MATERIALS AND METHODS

(i) *Mice*

Wild house mice, *Mus musculus* L., were trapped in the localities indicated in Fig. 1. Mice at the different localities were caught by the following persons: Castletown, Caithness, Scotland (*CTC*), and the Orkney Islands (*ORK*) of Wyre, North Ronaldsay, Egilsay, Papa, and Eday, by Professors R. J. Berry and M. Newton, Department of Genetics and Biometry, University College London, London, England; Bjertrup (*BJE*), Hjortshøj (*HJO*), Hov (*HOV*), and Klank (*KLA*) near Aarhus, Denmark, by Professors J. P. Hjorth and J. T. Nielsen, Institute of Ecology and Genetics, University of Aarhus, Denmark; Santa Caterina (*SCA*), Zanica (*ZCA*), Ovada (*OVD*), and Citta Ducale (*CDL*) in Northern Italy by Drs F. Bonhomme and J. Britton-Davidian, Laboratoire d'Evolution des Vertébrés, Paléontologie et Génétique, Faculté des Sciences, Montpellier, France; La Roca (*LRA*), Moya (*MOY*), and La Alumina (*LAA*) near Barcelona, Spain, and Binisalem (*BNS*), Mallorca Island, Spain by Dr J. Vives, Hospital Clinico y Provincial, Barcelona, Spain; Ryazan and Astrachan (*MSW*), U.S.S.R., by Dr I. Egorov, Duke University Medical Center, Durham, North Carolina; Schwilău (*SWU*) near Lübeck, FRG, by Professors A. Gropp and H. Winking, Institut für Pathologie, Medizinische Hochschule Lübeck, Lübeck, FRG; Nahya (*CRO*) and Abu Rawash (*GZA*), Imbaba, Giza Governate, and El Faiyum Governate (*ELF*), Egypt, by Dr Harry Hoogstraal, U.S. Naval Medical Research Unit No. 3, Cairo, Egypt; Wendelsheim (*BNK*) and Reusten (*RST*) near Rottenburg am Neckar, Ammerhof (*AMM*) and Gniebel (*GLR*) near Tübingen, and Mochenwangen (*BNT* and *MCW*) near Ravensburg, F.R.G., by J.H.N. More specific information about the localities where mice were caught can be obtained from the authors.

(ii) *Typing reagents*

The production and specificity of antisera used as typing reagents are described in detail elsewhere (Duncan *et al.* 1979*a*, Götze *et al.* 1980). We presently have typing reagents recognizing approximately 60% of the naturally occurring antigens (Klein *et al.* 1980, Götze *et al.* 1980). The remaining antigens remain undefined (i.e. they do not react with any of the H-2K or H-2D antisera used) and hence appear as 'blanks' in our assay system.

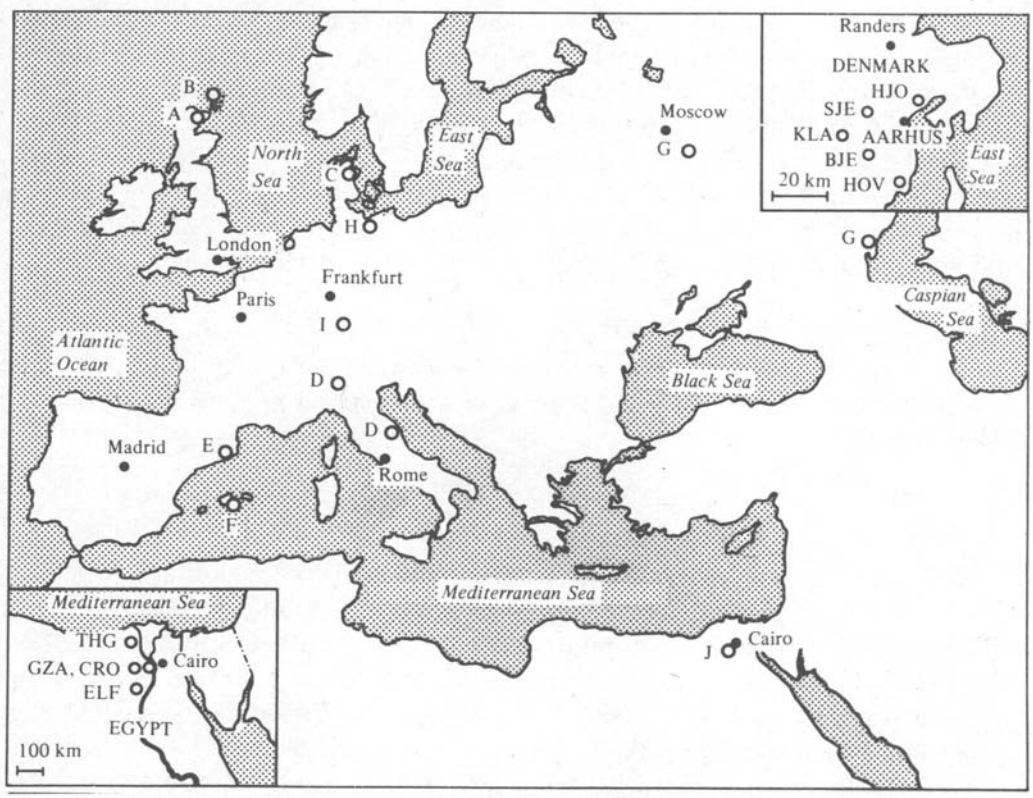

Fig. 1. Map of the locations in Europe, Russia, and North Africa from which mice were obtained. The general areas are the following: A, Castletown, Scotland; B, Orkney Islands; C, Aarhus, Denmark; D, northern Italy; E, Barcelona, Spain; F, Mallorca Island; G, Ryazan and Astrachan, U.S.S.R.; H, Lübeck, FRG; I, South Germany; J, Cairo, Egypt.

(iii) *Antigen typing*

To identify antigens encoded by the *H-2K* or *H-2D* loci, the two-stage complement-dependent microcytotoxicity test on Terasaki plates was used as previously described (Zaleska-Rutczynska & Klein, 1977; Götze *et al.* 1980).

(iv) *Heterozygosity testing*

Although H-2 antigens are dominant, 'blanks' are recessive and their presence can only be detected when an animal is homozygous for 'blank' alleles or among progeny of crosses between wild mice and inbred mice of known genotype. Since 'blanks' are common in wild mice (Duncan *et al.* 1979*a*, Götze *et al.* 1980), heterozygosity cannot be determined by direct typing of wild mice. For this reason, we studied heterozygosity by typing progeny of crosses between wild males and inbred females of known *H-2* genotype. Progeny were tested for segregation of H-2 antigens and the allozymes, complement component C3 (*C3*) and kidney catalase (*Ce-2*). The allozymic polymorphisms, which are encoded by loci linked to the *H-2* complex (da Silva *et al.* 1979; Hoffman & Grieshaber, 1976), were used to confirm the sampling of paternal chromosomes. Wild males suspected of having *t* alleles (as determined by mating the wild male to a *T*-bearing tester stock) were not included in the analysis because *t* alleles usually distort segregation of chromosome 17 to an extent that, in a small sample of progeny, only one of the two paternal chromosomes is found (Bennett, 1975; Klein & Hammerberg, 1977).

(v) *Allozyme electrophoresis*

Starch and high voltage agarose electrophoresis of kidney catalase (*Ce-2*) and complement component C3 (*C3*) were used as described elsewhere (Nadeau & Klein 1980).

3. RESULTS

(i) *Antigenic frequencies within local populations*

We typed a total of 202 mice from 29 populations in Europe and North Africa (Tables 1 and 2). The individual mice were typed for 16 different H-2K and 17 different H-2D antigens, each antigen presumably identifying a different allele. Each antigen was present in some populations and absent in others. Six to 60% of the K antigens (mean 37%) and 0 to 61 % of the D antigens (mean 39%) remained unidentified ('blanks'). We arbitrarily divided the antigens into common (antigens occurring with frequencies both in excess of 10% and twice that of the next most abundant antigen) and rare antigens (all antigens which were not common). The 13 populations listed in Table 1 contained 1 to 4 common (mean 2·4) and 3 to 20 rare antigens (mean 10·3). Common antigens accounted for 23 to 89% of the detectable antigenic variation in each population.

(ii) *Population density*

In the house mouse, polymorphisms are often affected by both population structure and population stability (Lewontin & Dunn, 1960; Anderson, 1970; Selander, 1970), and a measure of these effects is population density (Selander 1970). We used trapping success (the number of mice caught per 100 traps set) as a measure of population density. Trapping success and population density should be related if trapping effort is sufficiently intensive. Our trapping effort

Table 1. *Frequencies of H-2K and H-2D antigens within populations for which 8 or more mice have been typed**

(Frequencies were estimated by $n_i/2N$ where n_i is the number of positive reactions for antigen i, N the sample size, and $2N$ the number of possible positive reactions in N mice. Bl = blank.)

	Population (N)												
	Scotland		Denmark			Germany		Italy		Spain	Egypt		
Antigen	CTC	ORK†	BJE	HJO	HOV	BNK	MCW	SCA	ZCA	LRA	CRO	ELF	GZA
N	(13)	(14)	(18)	(11)	(30)	(10)	(10)	(11)	(12)	(8)	(15)	(13)	(15)
K.15	0	0	0	0	0·02	0	0	0	0	0·06	0·20	0	0
K.16	0	0·04	0	0	0	0·35	0	0	0·13	0·06	0·03	0·08	0·07
K.17	0	0	0·06	0	0	0	0	0	0	0·06	0	0	0
K.19	0	0	0·08	0·23	0·05	0·05	0	0	0	0	0·10	0	0
K.20	0	0	0	0	0	0	0		0·04	0	0	0	0
K.21	0	0	0	0	0·03	0	0·20	0	0·08	0·13	0·10	0·03	0
K.23	0	0	0·03	0	0	0	0	0·05	0·38	0·13	0·03	0·08	0·03
K·26	0	0	0	0	0·05	0	0	0	0	0·13	0·03	0	0·13
K.31	0·15	0·29	0·22	0	0	0	0·05	0	0·04	0·06	0·03	0·15	0·20
K.33	0	0	0	0·09	0·03	0	0·10	0·18	0	0·06	0·03	0·15	0·03
K.103	0	0·07	0·06	0·05	0·03	0	0	0·09	0	0	0	0	0
K.108	0·08	0·07	0	0	0	0	0	0	0	0	0·07	0·03	0·10
K.109	0·39	0·07	0·17	0·14	0·17	0·10	0	0·05	0·04	0·19	0·23	0·19	0·13
K.113	0	0	0·03	0·05	0·03	0	0	0·05	0	0·06	0·03	0	0
K.115	0	0	0	0	0	0	0·05	0	0	0	0	0	0
K.116	0	0	0	0	0	0	0	0	0·04	0	0·03	0·03	0·03
Bl	0·38	0·46	0·35	0·44	0·59	0·50	0·60	0·58	0·25	0·06	0·09	0·26	0·21
D.2	0	0·04	0·08	0	0	0	0·05	0·05	0	0	0	0	0
D.4	0	0·07	0·25	0·09	0·05	0	0·10	0·05	0	0·31	0·10	0·08	0·10
D.9	0	0	0·03	0	0	0·10	0	0	0	0	0	0	0
D.30	0	0·07	0	0	0	0	0	0·05	0·13	0·06	0·03	0	0·03
D.32	0	0	0	0	0	0	0·05	0·05	0	0·19	0·07	0·08	0·10
D.106	0	0	0	0·09	0·05	0	0	0	0	0	0·03	0	0·03
D.107	0·04	0·07	0·25	0·14	0·25	0·10	0	0	0	0·25	0·27	0·12	0·10
D.110	0	0	0	0·05	0	0	0	0	0·04	0	0	0	0·03
D.111	0	0	0·03	0·09	0	0·05	0	0	0	0	0·10	0·12	0·13
D.112	0	0	0	0	0·02	0	0	0	0	0	0	0	0
D.114	0·27	0	0	0	0	0	0	0·14	0·23	0·19	0	0·03	0
D·117	0	0·11	0	0	0	0	0·05	0·05	0·	0	0·10	0·23	0·13
D.118	0·08	0·07	0·06	0·05	0·12	0·40	0	0·09	0	0	0·07	0	0·03
D.122	0	0·04	0	0	0	0	0·05	0	0	0	0·07	0·08	0·03
D.123	0	0	0	0	0	0	0·05	0	0	0	0·07	0	0
D.124	0	0	0	0	0	0	0·05	0	0	0	0·07	0	0
Bl	0·61	0·53	0·30	0·19	0·51	0·35	0·60	0·52	0·60	0	0·02	0·26	0·29

* Also typed were 15 mice from 10 other populations (see Table 2) and 2 mice suspected of having t-alleles: 2 from MSW (K.33, K.109, D.107), 2 from MOY (K.108, D.117), 2 from BNK (K.Bl, D.107 and K.26, D.107), and 1 from CDL (K.16, D.114).

† ORK represents the combined sample of mice from the islands of Wyre (3), North Ronaldsay (2), Egilsay (2), Papa (3) and Eday (4).

Table 2. *Heterozygosity of H-2K and H-2D antigens in wild mice*

Locality	Mouse no.	H-2 antigens (K, K/D, D)	Segregating H-2 antigens (K-D)*	No. of offspring tested
BJE	166	nt.†	19, 109–4	3
			(103)‡-bl	1
	169	31, bl/	31–107	5
		bl, 107	bl–bl	5
	170	nt.	31–bl	2
			(109)–2	3
	173	nt.	31–4	2
			bl–107	2
	175	19, 31/	31–4	4
		4, 106	19–106	2
	184	nt.	(16)–122	4
			116–9	3
HJO	123	nt.	(109)–(107)	6
			21–114	1
	126	nt.	109–bl	2
			bl–bl	2
	128	nt.	109–4	1
			bl–107	3
HOV	131	nt.	19–107	4
			106–118	2
	139	109, bl	109–bl	11
		bl, bl		
	144	nt.	21–112	1
			113–107	3
	145	nt.	21–107	1
			(33)–107	1
	148	nt.	109–107	4
			19–107	2
	149	109, bl/	bl–107	3
		bl, 107	109–bl	3
	154	nt.	23–4	3
			103–bl	1
	157	nt.	(113)–bl	2
			109–107	4
	158	nt.	109–107	3
			103–118	2
	159	nt.	109–107	3
			bl–bl	2
	162	103, bl/	103–118	3
		118, 107	bl–107	3
KLA	186	nt.	23–4	3
			103–bl	1
AMM	14	nt.	16–4	2
			109–bl	1
RST	38	nt.	16–118	1
			15–bl	2
BNK	26	16, 109/	16–118	6
		118, bl	109–bl	5
	259	nt.	21–107	1
			16–118	1
	266	26, bl/	26–107	7
		bl, 107	bl–107	3
	284	nt.	33–107	3
			113–107	5
	275	nt.	16–114	4
			26–107	3

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Table 2. (*cont.*)

Locality	Mouse no.	H-2 antigens (K, K/D, D)	Segregating H-2 antigens (K-D)*	No. of offspring tested
BNT	40	nt.	bl–bl	2
			109–bl	3
	69	nt.	bl–m2	5
			109–bl	2
GLR	74	33, 21/	33–(4)	1
		4, (32)	21–(32)	2
SWU	373	nt.	21–122	8
ZCA	212	nt.	21–122	3
			16, 103–bl	3
	215	m12, bl/	m12–114	1
		114, bl	bl–bl	6
	219	nt.	(109)–114	2
			bl–bl	2
	220	nt.	(109)–114	2
			16–bl	1
SCA	197	nt.	33–4, m2	2
			bl–m2	2
	210	nt.	113–bl	3
			33–122	3
OVD	228	nt.	103–bl	3
			103–4	1
	237	nt.	(109)–m2	3
			117–bl	1
CDL	239	nt.	109–bl	2
			bl–114	1
	240	nt.	16–4	5
			23, 26, 109–bl	1
	241	nt.	16–4	2
			109–bl	1
	242	nt.	109–bl	3
			bl–114	1
MSW	310	17, bl/	17–117	3
		106, 117	bl–106	3
	313	nt.	31–117	8
			(21)–(107)	6
LAA	319	16, 31/	16–4§	4
		4, 107	19–117	1
BNS	564	nt.	26, 31, 108–bl	4
			bl–bl	3
CRO	554	nt.	116–9	3
			109–107	2
	429	nt.	109, 23–bl	3
			113–107	2

* Sampling of paternal chromosomes was confirmed by segregation of the *H–2* linked *C3* and *Ce–2* polymorphisms whenever the male parent was heterozygous for these allozymic polymorphisms. Male SWU373 must have been an *H–2K*, *H–2D* homozygote because allozyme typing of his progeny showed segregation among progeny typed for the *H–2* polymorphism of both the *C3* and *Ce–2* loci.

† nt. = Animal died before testing.

‡ (.) = Cross-reaction. An antigen in the wild mouse which was serologically similar to but not identical with the antigens in parentheses.

§ Progeny of mating between wild parents and whose mother was typed.

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in each building or field usually consisted of 30–50 traps set for two nights. In South Germany and Switzerland, 133 mice were caught in a total of 4636 traps; a success rate of 3 mice per 100 traps. (An extensive trapping effort of more than 800 trap nights in eastern Switzerland failed to produce mice.) In comparison to the trapping success in such diverse habitats as the Antarctic island of South Georgia and chicken farms in Texas which were 15·4 and 46·5 mice per 100 traps, respectively (Berry, Bonner & Peters, 1979; Selander, 1970), South German and Swiss populations of house mice are characterized by very low population densities. In addition, the success rate is probably an overestimate of average population density since traps were only set in buildings or fields owned by people who believed that mice were present.

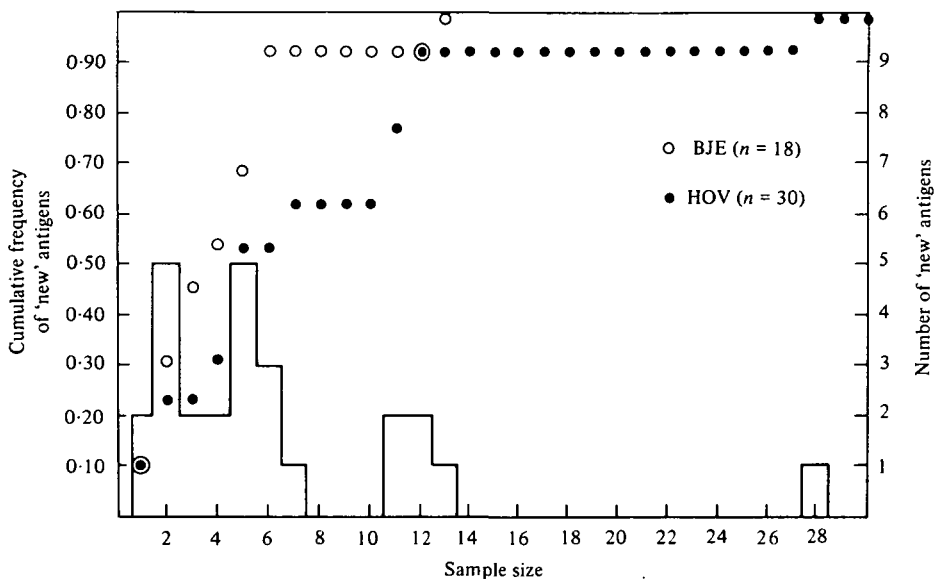

Fig. 2. The cumulative frequency of new antigens as a function of sample size at the BJE and HOV populations. Bars show the frequency of new antigens in each additional typed mouse for the combined samples of the BJE and HOV populations. Circles (open and closed) show the cummulative frequency of new antigens; new antigens being defined as those not found in mice previously tested from that population.

(iii) *Estimation of the sample size needed for characterization of local populations*

Our typing results enable us to estimate the number of mice needed to characterize a local population for the H-2 antigens present. The most direct way of obtaining such an estimate is to calculate the probability of finding new antigens as more and more mice are typed; new antigens being defined as those not found in previously tested mice from a given population. The analysis assumes that mice have been typed in a random sequence. This sequence can be obtained by ordering the typing results according to the number given each mouse when brought into the colony. Calculations carried out for the BJE (n = 18) and HOV

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(n = 30) populations indicated that 10–12 mice were sufficient to characterize about 90% of the antigens present in these local populations (Fig. 2). Similar results were obtained with samples of 10 or more mice from other populations. Ten to 12 mice are, on average, sufficient to estimate with reasonable accuracy both the number and frequency of alleles in local populations. Sampling error probably does not contribute significantly to variation of antigenic frequencies since the trapping effort, at least for the South German populations, was sufficiently intensive (see above) to ensure that most mice in each population were captured.

(iv) *Heterozygosity*

An important measure of polymorphism is the heterozygosity of the loci studied. Of the 60 males tested for heterozygosity, 7 were suspected of possessing a *t* allele, and 3 produced too few offspring for heterozygosity to be assessed with certainty. Of the 50 remaining mice, 94% were heterozygous for H-2K antigens and 89% were heterozygous for H-2D antigens (Table 2). Only one mouse, SWU 373, was homozygous for both H-2K and H-2D antigens. Within populations (Table 2), heterozygosity of H-2K or H-2D ranged from 60% (BNK) to 100%. In the largest sample from a single population (HOV, n = 11), only one *H-2K* and two *H-2D* homozygotes were observed. A comparison of the observed and expected heterozygosities indicated a nonsignificant excess of heterozygous (H-2K: observed = 0·94, expected = 0·84; H-2D: observed = 0·89, expected = 0·79). Expected heterozygosities were estimated by $1-\sum_i p_i^2$ where p_i is the average global frequency of allele i. Average global allelic frequencies are given by Götze *et al.* (1980). Expected heterozygosities were estimated in this manner because the exceptionally small size of many of the population samples (Table 2) precluded estimation of allelic frequencies within the local populations in which heterozygosity was tested.

(v) *Geographic distribution of H-2 haplotypes*

The geographic distribution of *H-2* haplotypes defined by progeny testing (Table 2) is given in Table 3. Many of these haplotypes involve combinations of common and rare antigens. Two of the most striking observations are that most of the haplotypes in each population are unique (unique haplotypes being defined as those in which the combination of K and D antigens occurs only once within or among populations) and that certain combinations of antigens were found in widely separated populations (e.g. K.16–D.4 in South Germany and Spain, K.21–D.122 in North Germany and Italy, and K.116–D.9 in Denmark and Egypt). The distribution of these haplotypes was studied by comparing the frequency with which unique haplotypes were found within and among populations. Fewer unique combinations are expected within local populations because of both the marked differences in antigenic frequencies between populations (Tables 1 and 5, and see below), and the ubiquity of a few antigens within each population (Table 1). In contrast to expectations, the frequency of unique haplo-

Table 3. *Geographic distribution of K-D antigenic combinations*

(The genetic composition of each combination was determined by progeny testing (Table 2). Also included are combinations from mice suspected of having a *t* allele.* Subscripts give the observed number of haplotypes if greater than 1. bl = blank and (.) = cross-reaction.)

	D antigen, Population																		
	Denmark				Germany						Italy				U.S.S.R.	Spain		Egypt	Frequency of unique
K antigen	BJE	HJO	HOV	KLA	AMM	RST	BNK	BNT	GLR	SWU	ZCA	SCA	OVD	CDL	MSW	LAA	BNS	CRO	haplotypes†
15	--	--	—	--	—	bl	—	—	—	—	—	—	—	—	—	--	—	—	--
16	--	--	—	--	4	118	118_2, 114	—	—	—	bl_2	—	—	114, 4_2	—	4	—	--	0·40
(16)	122	—	—	—	—	--	—	—	—	—	—	—	—	—	--	--	—	--	-
17	107	--	—	—	—	—	—	—	—	—	—	—	--	—	117	-	--	—	--
19	4, 106	--	107_2	—	—	—	—	—	—	—	—	—	—	—	—	117	—	—	0·80
21	—	114	107, 112	—	—	—	107	—	(32)	122_2	122	—	—	—	--	--	—	—	0·63
(21)	--	—	—	—	—	—	—	—	—	—	—	—	—	—	(107)	—	—	—	--
23	-	--	4	4	—	—	—	—	—	—	—	—	—	bl	—	--	—	bl	0·67
26	—	--	—	—	—	—	107_3	—	—	—	—	—	--	bl	--	--	bl	--	0·50
31	107, 4_2, bl	—	—	—	—	—	—	—	—	—	—	—	—	—	117	-	bl	—	0·80
33	-	—	—	--	—	—	107	—	(4)	--	—	4, m2, 122	—	—	107_2	--	—	--	0·71
(33)	—	--	107	—	—	—	—	--	—	—	—	—	—	—	--	—	—	—	--
103	--	--	118_2, bl	bl	—	--	—	--	—	—	bl	bl	4, bl	—	—	—	--	--	0·67
(103)	bl	—	--	—	—	—	—	—	—	—	--	--	—	—	—	—	—	--	--
106	--	—	118	—	—	—	—	--	—	—	—	—	—	—	—	—	--	—	--
108	--	--	—	—	—	—	—	—	—	—	--	—	—	—	—	117	bl	--	--
109	4	4, bl	107_4, bl_3	—	bl	—	bl	bl_2, 114	—	—	—	—	--	bl_4	107_2	--	—	bl, 107	0·36
(109)	2, bl	(107)	—	—	—	—	—	--	—	—	114_2	—	m2	—	—	—	——	—	0·83
113	-	—	107	—	—	—	107	--	—	—	—	bl	--	—	—	--	—	107	0·50
(113)	--	--	bl	—	—	—	—	—	—	—	—	—	—	—	—	—	—	-	-
116	9	—	--	—	—	—	--	—	—	—	—	—	—	—	--	—	--	9	--
117	—	—	—	—	—	—	—	—	—	—	—	—	bl	—	—	--	--	—	--
m12	--	—	—	—	—	—	—	--	—	—	114	—	—	—	—	--	—	--	—
bl	bl, 107	bl, 107	bl, 107_2	--	—	—	107_2	bl, m2	—	—	bl_2	m2	—	114_2	106	--	bl	--	0·42
Frequency of unique haplotypes†	0·93	1·00	0·70	--	—	—	0·67	1·00	—	--	0·86	1·00	1·00	0·75	0·75	-	1·00	1·00	

* The haplotypes in mice with t-alleles are given in the footnote to Table 1.

† Haplotypes in which one antigen was 'blank' were counted only once in each frequency estimate.

types within population (mean 0·89) was significantly higher than the frequency among populations (mean 0·63; $t = 4{\cdot}52$, $P < 0{\cdot}001$). This observation means that particular haplotypes were more likely to be found again in a population different from that in which the haplotype was originally observed.

Table 4. *Associations between the most frequent H-2K and H-2D antigens in each population. Sample sizes are given in Table 1*

Population	Antigens (K–D)	P	χ^2	D
CTC	109–117	NS	0·48	−0·11
BJE	31–4	<0·05	5·62*	+0·17
	31–107	NS	0·22	+0·06
HJO	19–107	NS	0·50	−0·13
HOV	109–107	NS	0·14	+0·07
BNK	16–118	<0·05	15·17*	+0·21
SCA	33–114	<0·05	4·58*	+0·17
ZCA	m12–114	<0·05	8·85*	+0·24
CRO	109–107	NS	0	−0·02

(vi) *Antigenic associations*

In wild mice whose progeny were not tested, we could not assign antigens to haplotypes but could only determine whether a given K antigen occurred in random combination with a given D antigen in individuals. We selected the most common H-2K and H-2D antigens and used Fisher's exact probability test to measure the degree of nonrandom association. Of the nine combinations tested in eight populations, four were non-randomly associated (Table 4). These associations could be of two types: coupling (++ and −−) or repulsion (+− and −+). The direction and magnitude of the associations are given by $D = f(++)f(--) - f(+-)f(-+)$ where f(..) denotes the frequency of a particular antigenic combination. Six of the nine pairs showed a positive association and all of the nonrandom associations were caused by the tendency for common antigens to occur in the same individual more often than if randomly associated.

(vii) *Genetic distance between populations*

The genetic distance of the *H-2K* and *H-2D* polymorphisms between populations was estimated by the method of Nei (1972) and the results are given in Table 5. The two major observations were: First, the genetic distances between neighbouring populations were similar. Genetic distances between the three Danish populations were small. This same pattern was also observed among the three Egyptian populations. The most significant exception was the distance between the BNK and MCW populations which are about 150 km apart but which did not share a single antigen. Second, the genetic distances between the Danish and Egyptian populations were similar to those observed between neighboring populations in either Denmark or Egypt.

Table 5. *Genetic distance between populations**

Population	ORK	BJE	HJO	HOV	BNK	MCW	SCA	ZCA	LRA	CRO	ELF	GZA
CTC	0·55	0·52	0·62	0·34	0·61	1·39	0·38	0·52	0·32	·053	0·72	0·65
ORK	—	0·15	0·57	0·44	0·57	0·47	0·51	0·80	0·43	0·36	0·18	0·15
BJE	—	—	0·21	0·18	0·63	0·57	0·53	0·96	0·19	0·22	0·33	0·20
HJO	—	—	—	0·12	0·51	0·77	0·47	1·43	0·37	0·21	0·52	0·41
HOV	—	—	—	—	0·43	0·79	0·47	1·30	0·21	0·13	0·65	0·34
BNK	—	—	—	—	—	∞	0·78	0·82	0·76	0·49	0·73	0·54
MCW	—	—	—	—	—	—	0·44	1·10	0·36	0·45	0·29	0·51
SCA	—	—	—	—	—	—	—	0·38	0·33	0·54	0·32	0·49
ZCA	—	—	—	—	—	—	—	—	0·36	0·97	0·67	0·73
LRA	—	—	—	—	—	—	—	—	—	0·17	0·34	0·22
CRO	—	—	—	—	—	—	—	—	—	—	0·36	0·20
ELF	—	—	—	—	—	—	—	—	—	—	—	0·11

* To account for blanks in the distance estimates, Nei's method was modified in the following manner:

$$I = j_{xy}/(j_x j_y) \text{ where } j_{xy} = (\Sigma x_i Y_i)/(1-(\mathrm{bl}_x + \mathrm{bl}_y)/2),\ j_x = (\Sigma x_i^2)/(1-\mathrm{bl}),$$

and $j_y = (\Sigma y_i^2)/(1-\mathrm{bl})$; and where x_i, y_i are the frequencies of allele i in populations x and y, bl_x, bl_y are the frequencies of blanks in populations x and y, and the genetic distance (D) is given by $D = -\log I$. Allelic frequency (i) in each population was estimated by $1-\sqrt{1-f}$ where f is the phenotypic frequency.

4. DISCUSSION

This study demonstrates that the antigenic composition of each population is unique, that the common antigens in each population occur in the same individuals more often than expected, that most mice are heterozygous for these antigens, that most haplotypes in each population are unique, and that the patterns of antigenic similarity between populations are complex.

The high frequencies of H-2K and H-2D heterozygotes (Table 2, and Duncan *et al.* 1979*b*), the highest heterozygosities yet reported for any locus in the house mouse, exceeded Hardy-Weinberg expectations by about 10%. This excess must, however, be considered in the context of the method by which observed and expected heterozygosities were estimated. Expected heterozygosities were estimated by $1-\sum_i p_i^2$, where p_i is the average global frequency of allele i among populations, while the observed heterozygosities were estimated from the pooled sample of mice listed in Table 2. Because gene and genotype frequencies differed between populations (Tables 1 and 5), a comparison of observed and expected heterozygosities should have revealed a deficiency of heterozygotes due to the Wahlund effect. The observed *H-2* heterozygosities were therefore opposite to the expected direction. This excess could be the result of either heterosis or of samples of small populations composed primarily of related individuals (Levene, 1949; Kirby, 1975). Selander (1970) observed that the heterozygosities of both the esterase-3 and haemoglobin β-chain encoding loci were negatively correlated with population density of house mouse populations in Texas. An excess of heterozygotes was observed in low density, unstable populations, while a deficiency was observed in high density, stable populations. Thus *H-2* heterozygosities may be

affected in the same manner as allozymic polymorphisms by population structure, density, and stability. These effects do not preclude the action of natural selection on the H-2 polymorphism since the effects could be enhanced or moderated by selection.

An analysis of antigenic associations demonstrated that common antigens usually occurred in the same individual in many but not all populations. These associations could be the result of linkage disequilibrium, nonrandom mating, heterosis, or sampling of families. Linkage disequilibrium probably did not contribute significantly to the associations because the finding of larger number of different haplotypes in each population suggests that common antigens did not usually occur on the same chromosome. In addition, if linkage disequilibrium was responsible for the associations, then the frequency of unique haplotypes should be lower in populations in which common antigens were strongly associated. This relation was not observed. Mate preferences in laboratory tests are partly controlled by loci linked to the *H-2* complex (Yamazaki *et al.* 1976, 1978), but whether wild mice behave similarly is unknown. Most probably, the observed associations result from breeding systems in which only a few individuals in each population produce most of the progeny and in which families remain as units for only a few generations. Population samples would then consist largely of related individuals. Such a breeding system would account for common antigens, excess heterozygosity, and variability of antigenic associations among populations. This system would also be consistent with low population density and would not preclude certain patterns of selection.

Polymorphisms such as those of the *H-2K* and *H-2D* loci can uniquely characterize individual populations and therefore provide very useful markers for studying the differentiation of populations. Based on morphological and allozymic characteristics, mice included in this study belong to at least two subspecies of *Mus. musculus* L. The Danish populations of mice (BJE, HJO, HOV, and KLA) belong to the subspecies *M.m. musculus* while all other mice probably belong to the subspecies *M.m. domesticus* (Schwarz & Schwarz, 1943; Ursin, 1952; Selander, Hunt & Yang, 1969; Hunt & Selander, 1973). If antigenic and phylogenetic similarities are correlated, as they are in man (Cavalli-Sforza, 1973; Piazza & Viganotti, 1973), then populations of *M.m. musculus* should be similar to each other and uniformly different from populations of *M.m. domesticus;* this is clearly not the case. Some of the shortest distances were between populations of different subspecies while some of the greatest distances were between populations of the same subspecies (Table 5).

Since the antigenic similarity and taxonomic relatedness of these populations are discordant, the relation between antigenic and karyotypic distance between populations was examined. These comparisons revealed a perfect correlation between antigenic and karyotypic constitution. While the standard karyotype of the house mouse has 40 acrocentric chromosomes, karyotypic analyses of mice from populations included in this study demonstrated that the only populations with this karyotype were the Danish, Egyptian, and most of the Orkney Island

populations. Each of the other populations had unique combinations of metacentric and acrocentric chromosomes (Gropp *et al.* 1972, Capanna *et al.* 1976; S. Adolph and J. Klein, in preparation). To determine whether this relation accounts for the pattern of variation, we performed a one way analysis of variance of the relation between karyotypic similarity and genetic distances. Distances were listed in one of three categories: (*a*) distances between populations with only acrocentric chromosomes, (*b*) distances between populations with both acrocentric and metacentric chromosomes, and (*c*) distances between populations with only acrocentric chromosomes and those with both acrocentric and metacentric chromosomes. The mean genetic distances between populations in each of these categories were: (*a*) $0{\cdot}29 \pm 0{\cdot}16$, (*b*) $0{\cdot}79 \pm 0{\cdot}41$, and (*c*) $0{\cdot}62 \pm 0{\cdot}24$. The relation between karyotypic and genetic similarity was highly significant ($F_{2,63} = 18{\cdot}67$, $P < 0{\cdot}001$). Thus populations of mice with only acrocentric chromosomes were antigenically similar while populations of mice which were karyotypically different were also antigenically different – *regardless of taxonomic status.* Paradoxically, *H-2* polymorphisms show a different degree of differentiation among chromosomal races than allozymic polymorphisms. Britton-Davidian *et al.* (1979) demonstrated that genetic distances based on the polymorphisms of 29 allozyme-encoding loci were independent of karyotypic differences among chromosomal races in Italian populations of house mice. The solution to this paradox can only be obtained by more extensive genetic and morphological studies of these chromosomal races and should reveal the modes by which these populations differentiate.

We are grateful to all the people who sent mice to us and for the excellent technical assistance of Ms E. Illgen, Ms D. Smith and Mr S. Forster. We also thank Margaret and Andreas Würstle for their help and hospitality in our fieldwork in Mochenwangen. H. Wolff kindly provided access to computer facilities. J.H.N. is a recipient of a Fellowship from the Max-Planck-Gesellschaft, Munich. E.K.W. is a recipient of the U.S. Public Health Service Research Fellowship 2F32 A105531-02. This study was supported in part by grants from NIH AI 14736 and the Volkswagen Foundation to J.K., and from the Deutsche Forschungsgemeinschaft, Forschergruppe Leukämie to D.G.

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