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Genetic variation of wild mouse populations in southern Germany

II. Serological study

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SUMMARY

A total of 207 wild mice trapped at different localities in southern Germany were tested for the presence of antigenic determinants controlled by class I genes (*K* and *D*) of the *H-2* complex. The test was based on the complement-dependent killing of lymphocytes in the microcytotoxicity assay. Both private (allele-specific) and public (shared) determinants were tested using polyclonal and monoclonal antibodies. The results of the H-2 typing were in agreement with karyological typing which divided the sampled mice into 5 populations. Each population was characterized by a certain antigenic profile (occurrence of individual determinants at certain frequencies); the profiles of the individual populations were sufficiently unique to differentiate these populations but at the same time sufficiently similar to indicate common origin of the populations. The karyological typing of the same mice reveals that all 5 populations share 1 pair of metacentric chromosomes, *Rb(4.12)1Tu*, but that, in addition, each population has at least one metacentric chromosome differentiating it from other populations. We interpret these findings as evidence that all wild mice in southern Germany stem from a common stock in which the *Rb(4.12)1Tu* translocation became fixed and which subsequently differentiated into the individual populations. This differentiation is accompanied by the fixation of new Robertsonian translocations (different ones in different populations) and the acquisition of characteristic H-2 antigenic profiles.

1. INTRODUCTION

The cell-membrane molecules controlled by the *H-2* complex of the house mouse function as antigens when injected into *H-2* disparate individuals and trigger the production of specific antibodies (reviewed by Klein, 1981). Using such antibodies,

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one can distinguish serologically molecules controlled by different *H-2* alleles. Serological analysis of wild mice has revealed the *H-2* genes to be extremely polymorphic (reviewed by Klein & Figueroa, 1981). A given mouse population usually carries some 20–30 alleles at the *H-2K* locus and another 20–30 alleles at the *H-2D* locus. Different populations carry different alleles or the same alleles as other populations but in different frequencies. This geographical differentiation of mouse populations in terms of the *H-2* polymorphism makes this polymorphism uniquely suitable for the study of the relationships and origin of the different populations.

In this communication, we have used the *H-2* polymorphism to answer questions about the origin of Robertsonian chromosomal variation among wild mice. We have reasoned that if the various populations carrying metacentric chromosomes were related in origin, they should be more homogeneous in terms of their *H-2* polymorphism than are unrelated populations carrying only acrocentric chromosomes. We have determined, therefore, the *H-2K* and *H-2D* polymorphism of mice displaying Robertsonian chromosomal variation and compared it to the *H-2* polymorphism of populations lacking such variation.

2. MATERIALS AND METHODS

Mice. Wild mice were trapped at localities described in the accompanying paper (Adolph & Klein, 1983). They were maintained in the laboratory for several months during which time they were karyotyped and *H-2* typed. All inbred mice were produced in our animal colony.

Polyclonal H-2 antibodies. Alloantisera were produced by immunization in the strain combinations listed in Table 1. Cell suspensions, obtained from spleen, lymph nodes, and thymus of one donor were injected intraperitoneally into approximately 20 recipients. After five weekly injections, immunizations were alternated with bleedings, always in weekly intervals, the sera from individual bleedings were stored at −70 °C, tested at the completion of the immunization, and positive sera were pooled. Each antiserum was tested against a panel of cells carrying most of the known *H-2* alleles and its pattern of reactivity was determined. The specificity of each antiserum was restricted by appropriate absorptions: each antiserum was incubated twice with equal volumes of packed spleen and lymph node cells, each time for 30 min. The absorbed antiserum was then again tested against the panel of cells and its reactivity pattern determined. The specificity indicated in Table 1 is that obtained after absorption. The antisera were selected to detect private antigens characterizing the known alleles at the two class I *H-2* loci, *K* and *D*.

Monoclonal antibodies. A list of monoclonal antibodies used in this study appears in Table 2. Some of the antibodies detected private H-2 antigens, whereas others detected determinants shared by several allomorphs (products of allelic *H-2* genes). Most of the antibodies were obtained from ascites fluid produced by the inoculation of hybrid cell lines into mice; others were obtained from supernatants of lines grown in culture.

Table 1. *H-2 alloantisera detecting private determinants used for the typing of wild mice*

Code designation	Recipient	Donor	*H-2*-haplotype combination; (recipient) donor	Absorbed by cells of *H-2* haplotype	Antigenic determinant detected
K304	(A.BY × B10.AKM)F_1	B10.A	(*b/m*)*a*	—	4
T117PI	(A.TL × DBA/2)F_1	A.TFR1	(*t1/d*)an1	*k*	9
T26	[A.CA × B10.A(2R)]F_1	B10.WB	(*f/h2*)*j*	*d, p, q, w13, w15*	15
K13PPI	(B10.A × A.SW)F_1	B10.P	(*a/s*)*p*	*d, f, j, q, r, w1, w13, w14*	16
K26	(C57BL/10 × A)F_1	B10.AQR	(*b/a*)*y1*	*r*	17
K-118	(C57BL/10 × C3H)F_1	B10.RIII(71NS)	(*b/k*)*r*	*s*	18
T30PI	(A.AL × DBA/2)F_1	A.TL	(*a1/d*)*t1*	*v, w26*	19
T21	(A × B10)F_1	B10.NZW	(*a/b*)*z*	*w13, w14, w24*	20
K356	(B10.A × C3H)F_1	B10.SM	(*a/k*)*r*	*b, w13*	21
T76	(DBA/2 × A.TL)F_1	A.AL	(*d/t1*)*a1*	*p, q, r, u, w1, w3, w23*	23
T37	(DBA/2 × B10.A)F_1	B10.M(11R)	(*d/a*)*ap1*	*p, w13*	26
K355	(B10.A × LP.RIII)F_1	B10.AKM	(*a/r*)*m*	*d, j, w1*	30
K548PIII	(A × B10.A)F_1	B10.D2	(*a*)*d*	*b, w15*	31
T28PI	(DBA/2 × B10.A)F_1	B10.OH	(*d/a*)*o2*	*w13*	32
K333PI	(B10.D2 × A)F_1	B10.A(5R)	(*d/a*)*i5*	*f, j, p, q, r, u, v, w7*	33
T29	(A × B10.CAS2)F_1	B10.SAA48	(*a/w17*)*w3*	*d, w18, w24, w27*	103
K561	(B10 × A)F_1	T/t^{12}	(*b/a*)*w28*	*f, s, w22, w29*	106
K560	(B10 × A)F_1	T/t^{w2}	(*b/a*)*w29*	*d, j, v, w13*	107
K559	(B10 × A)F_1	T/t^{w1}	(*b/a*)*w30*	*f, v, q, w27*	108
K541	(A × B10.D2)F_1	B10.CAS2	(*a/d*)*w17*	*w3*	111
T159	(B10.A × B10.PL)F_1	B10.NZW	(*a/u*)*z*	—	114
T48	(A × B10.CHR51)F_1	B10.STC90	(*a/w18*)*w15*	*u, w7, w16*	115
T167	(B10.A × B10.SAA48)F_1	B10.BUA16	(*a/w3*)*w22*	*k, w7, w24*	116
T152PI	(A × B10.BUA16)F_1	B10.BUA1	(*a/w22*)*w16*	*f, q, w27*	130
T147	(B10.M × B10.P)F_1	B10.CAA2	(*f/p*)*w11*	*d, w15*	131
T166	(B10.A × B10.BR)F_1	B10.WR7	(*a/r*)*wr7*	*s*	132
T131	(B10.G × A.TL)F_1	B10.CHA2	(*q/t1*)*w26*	*w8*	137
T149	(A × B10)F_1	B10.T7WF	(*a/b*)*w6*	*d, q, r, s, u*	143
T14	(A × B10)F_1	B10.CAS1	(*a/b*)*w23*	*f, r, s, u*	144
T169	(BALB/c × B10.P)F_1	B10.CHR51	(*d/p*)*w18*	*w14, w26*	147

Serological typing. To detect individual H-2 determinants in wild mice, the lymph-node lymphocytes of these mice were incubated with polyclonal or monoclonal H-2 antibodies in the presence of complement and the percentage of killed cells was estimated. The test was carried out in Terasaki plates (C. A. Greiner & Söhne, Nürtingen, F.R.G.) with a mixture of normal guinea-pig and normal rabbit serum as the source of complement. Dead and live cells were distinguished using an inverted phase-contrast microscope. The details of this two-stage microcytotoxicity test were previously described (Klein, Hauptfeld & Hauptfeld, 1975).

Table 2. *H-2 monoclonal antibodies detecting private or public determinants*

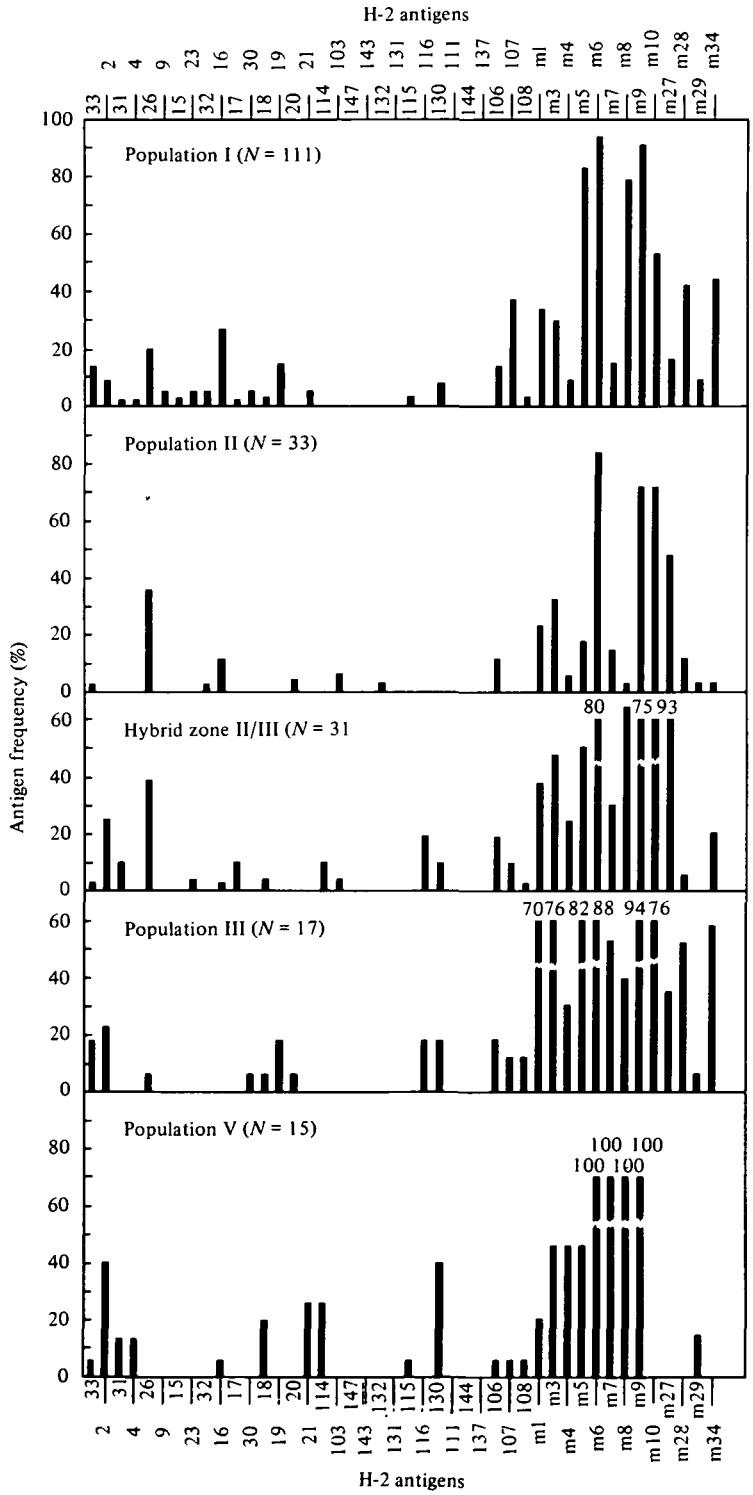
Antibody against determinant	Hybridoma	Immunization	Reference
H-2.m1	H116-22R7	BALB/c anti-CBA	a
H-2.m2	B22-249.R1	BALB/k anti-C57BL/6	a
H-2.m3	H100-5R/28	BALB/c anti-CBA	a
H-2.m4	H100-27.R55	BALB/c anti-CBA	a
H-2.m5	H100-30.R23	BALB/c anti-CBA	a
H-2.m6	H141-11	BALB/c anti-C57BL/6	a
H-2.m7	H141-29	BALB/c anti-C57BL/6	a
H-2.m8	H141-51	BALB/c anti-C57BL/6	a
H-2.m9	H142-23	BALB/c anti-CBA	a
H-2.m10	H142-45	BALB/c anti-CBA	a
H-2.m17	F5.21.37	(A.By × B10.A)F_1 anti-B10.Q	b
H-2.m18	T3.6	C3H/HeJ anti-RIII/2J	b
H-2.m21	T2.100.1	A.SW anti-B10.WOA105	b
H-2.m24	T1.48P	CBA anti-B10.A	b
H-2.m27	T1.80c1	CBA anti-B10.A	b
H-2.m28	T1.31c	CBA anti-B10.A	b
H-2.m29	T1.4c	CBA anti-B10.A	b
H-2.m34	T2.35	A.SW anti-B10.WOA105	b
H-2.m35	T93.104.9	(B10.SAA48 × A) anti-B10.CAS2	b

a. Lemke, Hämmerling & Hämmerling (1979)
b. Figueroa & Klein (1981).

3. RESULTS

In the accompanying paper (Adolph & Klein, 1983) we have divided the wild mice in southern Germany into five populations, which we have designated populations I to V (see fig. 1 in Adolph & Klein, 1983). Each population occupies a defined geographical region and is characterized by the presence, in virtually all mice, of a particular metacentric chromosome often in a homozygous condition. (In this communication we describe the H-2 antigenic profiles of 4 of these

Fig. 1. Frequencies of class I H-2 antigens in the different populations of wild mice from southern Germany. *N* indicates the number of mice tested. Frequencies were calculated by dividing the number of mice positive for a given antigen by the total number of mice tested.



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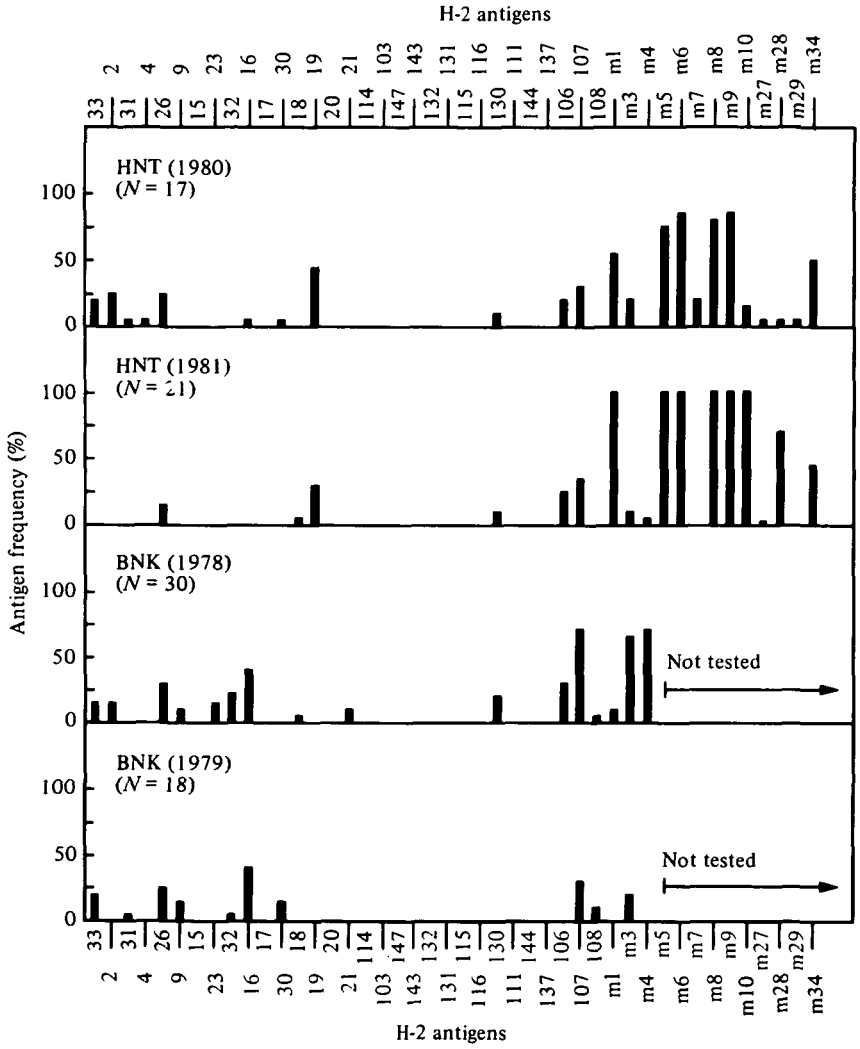

Fig. 2. Frequencies of class I H-2 antigens at two trapping sites in consecutive years. *N* indicates the number of mice tested. Frequencies were calculated by dividing the number of mice positive for a given antigen by the total number of mice tested.

populations; mice from population IV were not available in sufficient numbers for H-2 typing.) In addition, we also describe the antigenic profile of a population probably representing a hybrid zone between populations II and III (Figs 1 and 2).

Population I (111 mice typed). This population occupies the region around the city of Tübingen and is karyotypically characterized by homozygosity of the metacentric chromosome *Rb*(4 . *12*)*1Tu*. Antigenically, the population was characterized by the presence of private H-2 determinants H-2D . 2, 4, 9, 30, 32 and 130 and H-2K . 15, 16, 17, 18, 19, 21, 23, 26, 31, 33 and 115. Of these antigenic

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determinants H-2K . 16, 19, 26 and 33 were most frequent (with frequency of 10 % or more). Among the public determinants, most frequent were determinants H-2 . m5, m6, m8, m9 and m10, each occurring at a frequency above 50 %.

Population II (33 mice typed). This population occupies the outskirts of the Schwäbische Alb, the mountain range east of Tübingen. Mice composing this population carry the same pair of metacentric chromosomes as mice in population I, but in addition are also heterozygous or homozygous for another metacentric chromosome, *Rb(5 . 15)16Tu*. Serologically, the mice are characterized by the presence of private determinants H-2D . 32, H-2 K . 16, 20, 26, 33, 103 and 132. Of these, determinants H-2K . 16 and 26 occur at frequencies higher than 10 %. Of the public determinants present in this population, only determinants H-2 . m6, m9, m10 and m27 occur in more than 50 % of the mice.

Population III (17 mice typed). Eastwards population II passes imperceptibly into population III in which the mice carry, in addition to the *Rb(4 . 12)1Tu* pair, the metacentric chromosome *Rb(13 . 14)17Tu*, in either heterozygous or homozygous condition. The mice carry private determinants H-2D . 2, 30 and 130 and H-2K . 18, 19, 20, 26, 33 and 116, of which H-2D . 2 and 130 and H-2K, 19, 33 and 116 occur at frequencies above 10 %. Highly frequent in this population are also public determinants H-2 . m1, m3, m5, m6, m7, m9, m10, m28 and m34, all of which occur at frequencies above 50 %.

Population II/III (31 mice typed). This is the hybrid population in the region where populations II and III meet, Karyotypically, the population is characterized by the presence of *Rb(5 . 15)16Tu* (the representative chromosome of population II) and *Rb(13 . 14)17Tu* (the representative chromosome of population III). Serologically, the population is characterized by private determinants H-2D . 2, 114 and 130 and H-2K . 16, 17, 18, 23, 26, 31, 33, 103 and 116, and by high frequency of public determinants H-2 . m6, m8, m9, m10 and m27. Of the private determinants, most frequent are determinants H-2D . 2, 114 and 130 and H-2K . 17, 26, 31 and 116.

Population V (15 mice typed). This population, occupying the region around the city of Ravensburg near Lake Constance (Bodensee), is karyologically quite heterogeneous, containing several different metacentric chromosomes. However, the mice in this region are homozygous for *Rb(4 . 12)1Tu* and most also carry the chromosome *Rb(11 . 13)6Tu*. The serological typing reveals the presence of private determinants H-2D . 2, 4 and 114, and H-2K . 16, 18, 21, 31, 33, 115 and 130. Because of the small sample size, antigen frequencies are less informative here.

Temporal variation. To determine how the antigenic profiles of mice at a given locality change from year to year, we typed mice from two localities in two consecutive years. The antigen frequencies (Fig. 2) revealed remarkable stability: the profiles for the same locality in the two consecutive years are almost superimposable.

4. DISCUSSION

When one considers the serological data in their entirety, one comes to the conclusion that, on the one hand, the five tested populations (four 'pure' populations and one hybrid population) are similar to one another and, on the other hand, they differ from one another. The similarity of the populations is indicated by the appearance in the different populations of the same determinants and by the estimates of the genetic distance. As an example of the former, one can point out the fact that four of the five populations carry the determinant H-2K . 26 at relatively high frequency (the absence of H-2 . 26 in population V might be a sampling error). Similarly, the four populations share the determinant H-2K . 33, which is relatively frequent in some (I, III) and less frequent in other (II, II/III). Other determinants, at least partially shared by some populations, are H-2D . 2 and H-2K . 16, 18, 19, 115, 116 and 130.

Table 3. *Genetic distances among populations**

Populations	II	II/III	III	V	HNT 1980	HNT 1981	BNK 1978	BNK 1979
I	0·28	0·25	0·20	0·51	0·09	0·13	0·03	0·06
II	—	0·10	0·56	1·24	0·33	0·43	0·27	0·27
II/III	—	—	0·27	0·36	0·19	0·34	0·23	0·34
III	—	—	—	0·34	0·07	0·17	0·03	0·39
V	—	—	—	—	0·39	1·01	0·52	0·79
HNT 1980	—	—	—	—	—	0·08	0·18	0·24
HNT 1981	—	—	—	—	—	—	0·20	0·38
BNK 1978	—	—	—	—	—	—	—	0·09

* According to Nei's method modified by Nadeau *et al.* (1981).

The genetic distances of H-2K and H-2D polymorphisms among the German populations calculated from the serological data appear in Table 3. The mean value of $0{\cdot}41 \pm 0{\cdot}32$ ($0{\cdot}28 \pm 0{\cdot}15$ if population V is not considered) is indicative of their similarity. When distant populations of chromosomal races of *M. domesticus* are compared, the mean value is $0{\cdot}60 + 0{\cdot}36$ (Götze *et al.* 1980, Nadeau *et al.* 1981). Such comparisons show very clearly that the five populations in southern Germany are more related to one another than any one of them is to populations in other countries.

Yet, the similarities notwithstanding, the five populations are different from each other. This difference is apparent in the presence of different determimants in different populations, in the different frequencies of determinants shared by the populations and also from the estimation of genetic distance. For example, determinant H-2D . 9 was found only in population I, determinant H-2K . 103 only in populations II and II/III, determinant H-2 . 132 only in population II, and so on. An example of different frequency distributions is provided by the determinant H-2K . 33, which is relatively frequent in populations I and III and far less frequent

in populations II and II/III. In fact, it is possible that more thorough sampling would reveal more extensive sharing of determinants than we observed, and that most of the differences would become differences in frequencies. Such differences are strongly expressed in the profiles of the public H-2 determinants defined by the monoclonal antibodies. Although virtually all these determinants are present in all five populations, their frequency distributions are characteristic of individual populations (Fig. 1) and change relatively little from year to year. Population II/III, which karyotypically seems to represent a hybrid zone between populations II and III, appears to be also in terms of H-2 typing in between these two populations: it carries some determinants of population II (e.g. H-2K . 16 and 103) and others of population III (e.g. H-2D . 2 and H-2K . 18, 116 and 130). However, it also carries some determinants not found in either II or III (i.e. H-2K . 17 and 31 and H-2D . 114). The presence of the new determinants indicates either a sampling error (i.e. the determinants are present in II or III but have been missed because of insufficient sample size), the influence of an unidentified third population, or radiation of mice from II/III into II and III.

The estimates of genetic distances support these conclusions (Table 3). When mice from the same locality were tested in two consecutive years, D vaues of 0·08 for HNT and 0·09 for BNK were obtained, giving D estimates that are among the smallest reported. The D value obtained when HNT is compared with BNK is 0·25, which corresponds with the value for neighbouring populations (Nadeau *et al.* 1981). A very similar D value is obtained when population I is compared with populations II, II/III or III, population II with II/III and population II/III with population III. The D value of 0·56 when population II is compared with III puts the population II/III between the two populations – a finding that is in agreement with the karyotyping data.

In summary, the serological data suggest that all mice in southern Germany derive from the same stock which is now in the process of geographical differentiation. The sampled populations provide examples of this differentiation process. There are probably more differentiating populations in this region, which we have not detected because our sampling was not exhaustive. However, we feel that such an additional sampling would not change the picture obtained.

These conclusions are in full agreement with those which we reached in the accompanying paper (Adolph & Klein, 1983) on the basis of the chromosomal studies. In this paper, we propose that population I, characterized by the pair of metacentric chromosomes *Rb(4 . 12)1Tu*, is the population from which the other populations in this region have evolved by accumulation of additional Robertsonian translocations. The chromosomal studies show that all the mice in southern Germany share the *Rb(4 . 12)1Tu* translocation and that mice in different local populations have accrued one or more additional translocations. Similarly, the serological analysis reveals sharing among the different populations of a basic antigenic profile and acquisition by the individual populations of unique allomorphs. An important question is: what is the origin of the additional allomorphs? They can either be brought into the populations by immigrant mice, or they could

represent differentiation of the original population (i.e. this population contained a certain, relatively large number of *H-2* haplotypes, some of which were retained by all populations while others became restricted to the various local populations). We favour the latter possibility because we find it otherwise difficult to explain why virtually all mice in southern Germany are homozygous for the *Rb(4.12)1Tu* translocation; immigration of mice with different karyotypes would have left tracks, of which we find no sign (for discussion, see Adolph & Klein, 1983). It thus appears that some long time ago, the Rb(4.12)1Tu translocation arose in southern Germany by chromosomal mutation or immigration of mice and spread through the population by selection or drift. Subsequently, the German populations began to differentiate into a series of local, but not completely isolated populations. This differentiation process, which apparently continues to this day, leads both to the fixation of new Robertsonian translocations (different ones in different regions, each translocation probably arising de novo and in situ, so to speak) and to the differentiation of H-2 antigenic profiles.

The present study demonstrates the potential of the serological approach for population genetics. The *H-2* polymorphism provides a potent tool for the analysis of the origin and dynamics of natural mouse populations. When combined with other approaches, such as the karyotypic analysis in the accompanying paper, it can be used to answer questions about the evolution of mouse populations, questions that would be difficult to answer by most other approaches.

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5. REFERENCES

ADOLPH, S. & KLEIN, J. (1983). Genetic variation of wild mouse populations in Southern Germany. I. Cytogenetic study. *Genet. Res., Camb.* **41**, 117–134.

FIGUEROA, F., DAVIS, W. C. & KLEIN, J. (1981). Ten new monoclonal antibodies detecting antigenic determinants on class I H-2 molecules. *Immunogenetics* **14**, 177–180.

GÖTZE, D., NADEAU, J., WAKELAND, E. K., BERRY, R. J., BONHOMME, F., EGOROV, I. K., HJORTH, J. P., HOOGSTRAAL, H., VIVES, J., WINKING, H. & KLEIN, J. (1980). Histocompatibility-2 system in wild mice. X. Frequencies of H-2 and Ia antigens in wild mice from Europe and Africa. *Journal of Immunology* **124**, 2675–2681.

KLEIN, J. (1981). The histocompatibility-2 (H-2) complex. In *The Mouse in Biochemical Research*, vol. 1 (ed. H. L. Foster, J. D. Small and G. Fox) pp. 119–158. New York: Academic Press.

KLEIN, J. & FIGUEROA, F. (1981). Polymorphism of the mouse H-2 loci. *Immunological Reviews* **60**, 23–57.

KLEIN, J., HAUPTFELD, V. & HAUPTFELD, M. (1975). Evidence for a fifth (G) region in the H-2 complex of the mouse. *Immunogenetics* **2**, 141–150.

LEMKE, H., HÄMMERLING, G. J. & HÄMMERLING, U. (1979). Fine specificity analysis with monoclonal antibodies of antigens controlled by the major histocompatibility complex and by the Qa/TL region in mice. *Immunological Reviews* **47**, 175–206.

NADEAU, J. H., WAKELAND, E. K., GÖTZE, D. & KLEIN, J. (1981). The population genetics of the H-2 polymorphism in European and North African populations of the house mouse (*Mus musculus* L.). *Genetical Research* **37**, 17–32.