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Genetic variation of wild mouse populations in southern Germany

I. Cytogenetic study

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SUMMARY

Some 400 wild mice (*Mus domesticus*) from southern Germany (the triangle formed by the cities Tübingen, Heidenheim and Friedrichshafen) were karyotyped and, in 243 of them, the chromosome compositions were determined by banding techniques. Virtually all mice tested carried at least one pair of metacentric chromosomes; some mice had up to ten metacentric chromosomes. Based on their chromosome composition, five mouse populations could be distinguished. Population I was characterized by the diploid chromosome number of $2n = 38$ and the presence of two copies of metacentric chromosome *Rb(4.12)1Tu*. This translocation was also found in virtually all mice captured in southern Germany, almost always in a homozygous state. Mice of other populations had extra metacentric chromosomes *Rb(5.15)15Tu* (population II), *Rb(13.14)17Tu* (population III), *Rb(5.14)18Tu* (population IV) and *Rb(11.13)6Tu* (population V). In addition, rare variants (1 or 2 mice) were found in the different populations, which were heterozygous for additional metacentric chromosomes. Population V was quite heterogeneous in that it contained up to five metacentric chromosomes in addition to those mentioned. The number and the composition of these metacentric chromosomes varied from place to place. With the exception of population I, the individual populations occupied geographically distinct areas. Representatives of population I were found concentrated in one area, but, in addition, some were scattered over the entire studied region.

1. INTRODUCTION

Until 1969, geneticists and cytogeneticists believed that the karyotype of virtually all house mice (*Mus musculus* or *Mus domesticus*) consisted of 40 acrocentric chromosomes (Cox, 1926). Although animals with fewer than 40 chromosomes had now and then been found among laboratory mice (Evans, Lyon & Daglish, 1964; Leonard & DeKnudt, 1967; White & Tijo, 1967; Baranov & Dyban, 1971), they were regarded as rare exceptions. Even after Gropp, Tettenborn & Lehman (1969) discovered the first example of Robertsonian chromosomal

variation among wild mice in the Valle di Poschiavo in the Alps (the mice had 14 metacentric chromosomes with each metacentric derived by Robertsonian fusion from two acrocentrics), there was no reason to believe that the variation was more than a local curiosity. Later, however, Robertsonian variation of mouse chromosomes was found not only in other valleys of the Alps (Gropp *et al.* 1972) but also in other parts of the world: in various regions of Italy (Capanna *et al.* 1976; Capanna, Civitelli & Cristaldi, 1977), Sicily (Lehman & Radbruch, 1977), the Eolian Islands and Dalmatia (Gropp & Winking, 1981), Greece (Winking, Gropp & Bulfield, 1981), southern Germany, Spain, Scotland (Adolph & Klein, 1981), India (Chakrabarti & Chakrabarti, 1977) and an island of the Antarctic region (Robinson, 1978). These findings suggested that the Robertsonian variation among wild mice represented a widespread chromosomal polymorphism similar to that found in other rodents, for example, gerbils (Wahrman & Gourevitz, 1973), *Acomys* (Wahrman & Gotein, 1972) and mice of the *Leggada* group (Jotterand, 1972).

Studies carried out in the house mouse have been of two kinds. In the first kind, small samples of mice from different parts of the world were analysed without an attempt at systematic local coverage. In the second, large numbers of mice from a single region were obtained, but most of these were analysed by standard karyotyping while chromosome banding analysis was limited to only a few mice. The information obtained from both kinds of study is limited.

Little work has also been done in terms of combining chromosomal with genetic analysis. Britton-Davidion and his colleagues have studied enzyme variation in some of the populations with Robertsonian chromosomes and failed to find significant genetic distances between these populations and populations containing only acrocentric chromosomes (Britton-Davidion *et al.* 1980). However, these findings do not mean that differences do not exist; to find them, it may be necessary to use more variable markers than those used thus far.

In this and the accompanying communication, we have attempted to avoid some of the limitations of the previous studies. We have concentrated on one large geographical area (that of southern Germany), sampled this area systematically, and identified the Robertsonian translocations present in a large number of mice. In the accompanying communication (Figueroa *et al.* 1983) we studied the same mice for their variation at one of the most polymorphic gene complexes known – the *H-2* loci. The combination of the two approaches provides new information about the early differentiation of populations displaying Robertsonian variation.

2. MATERIALS AND METHODS

Mice. Wild mice were trapped using wooden or metal traps; the former were variants of the Longworth trap; the latter were of the Sherman type. Traps were usually set up in the evening and checked the next morning; a trapping period normally lasted 2 days. Captured mice were brought into our wild mouse colony, bathed in a solution of Neguvon® (Bayer) once a week for 3 weeks to rid them of external parasites and maintained in plastic cages supplied with nesting material

(mainly tissue paper). Their diet, the normal dry food used for laboratory mice (Alma), was supplemented with carrots and sunflower seeds. The room housing the mice was on a fixed 12-hour light–dark cycle.

Karyotyping. Wild mice and their hybrids with laboratory mice were karyotyped using cells from whole blood. Phytohaemagglutinin-(PHA)-stimulated peripheral lymphocytes were cultured for 48 h, according to a modified method of Triman, Davisson & Roderick (1975). The modifications consisted of bleeding the mice from the retro-orbital plexus and applying the colcemid block for only 2 h (final concentration of 80 ng/ml of the culture solution) and the hypotonic solution for only 15 min. Lymphocytes of some wild mice could never be stimulated with PHA, and these mice were then tested by the bone-marrow technique: 1 h after the I.P. injection of the 0·5 % colchicin solution, the bone-marrow cells were incubated for 25 min in warm 0·5 % KCl solution and fixed by applying several changes of the methanol–acetic acid solution. In both the whole-blood and the bone-marrow technique, the fixed material was dropped onto cold, wet slides from about 50 cm, and air-dried. After 2 days in the case of blood-culture preparations, and 1 week in the case of the bone marrow slides, G-banding was done using the technique of Seabright (1971). To verify some of the results obtained using these methods, somatic mitoses of wild-laboratory mouse hybrids or the meiotic chromosomes of the original wild or wild–wild hybrid males from different populations were analysed (Evans, Brackon & Ford, 1964).

3. RESULTS

The region. The area covered by this study is a triangle with apexes occupied by the cities of Tübingen, Heidenheim and Friedrichshafen (Bodensee) (Fig. 1); its size is approximately 500 km^2. However, we did not trap mice with the same intensity over the whole triangle. The most densely covered region was a 30 km wide band from the Neckar valley near Tübingen, across the Schwäbische Alb (Swabian Jura), to Ulm at the Danube river. Two smaller, densely covered trapping regions were the eastern and southern corners of the triangle (near the cities of Heidenheim and Ravensburg). The main geographical features of the region are the plateau of the Schwäbische Alb and its foothills descending to the Neckar valley. The Schwäbische Alb is a limestone terrace, rising steeply on the northwest side (a difference of about 400 m between the foot and the top of the terrace) and flattening out slowly towards the Danube river (a decrease in height of about 200 m per 45 km distance). The terrain is covered with deciduous forests along the rockfalls on the northwest side, and agricultural fields, small forested patches, and relics of the original dry meadows with juniper stands on the plateau. The region around Heidenheim borders in the north with a wooded area; that around Friedrichshafen is hilly, with large, rich meadows. All mice were trapped at agricultural sites inside farm buidings. We analysed mice from 28 trapping sites in the foothills of the Alb and the Neckar valley, from 30 trapping sites in the Alb mountain range and from 13 trapping sites south of the Danube. The distances

between trapping sites varied, ranging from neighbouring farm houses to distances of some 30 km. The trapping season lasted from May to October.

The sample. Altogether we caught approximately 700 wild mice of the species *Mus domesticus*; of these, we managed to analyse cytogenetically 402 mice – 243 mice by the banding technique and the rest only for chromosome number. The number of mice captured at individual trapping sites (farms) varied from 1 to 21.

The karyotype. With few exceptions (11 of 402 mice), all karyotyped mice had 38 or fewer chromosomes in their somatic cells, and carried at least one pair of metacentric chromosomes (199 mice had a diploid chromosome number of 38 and

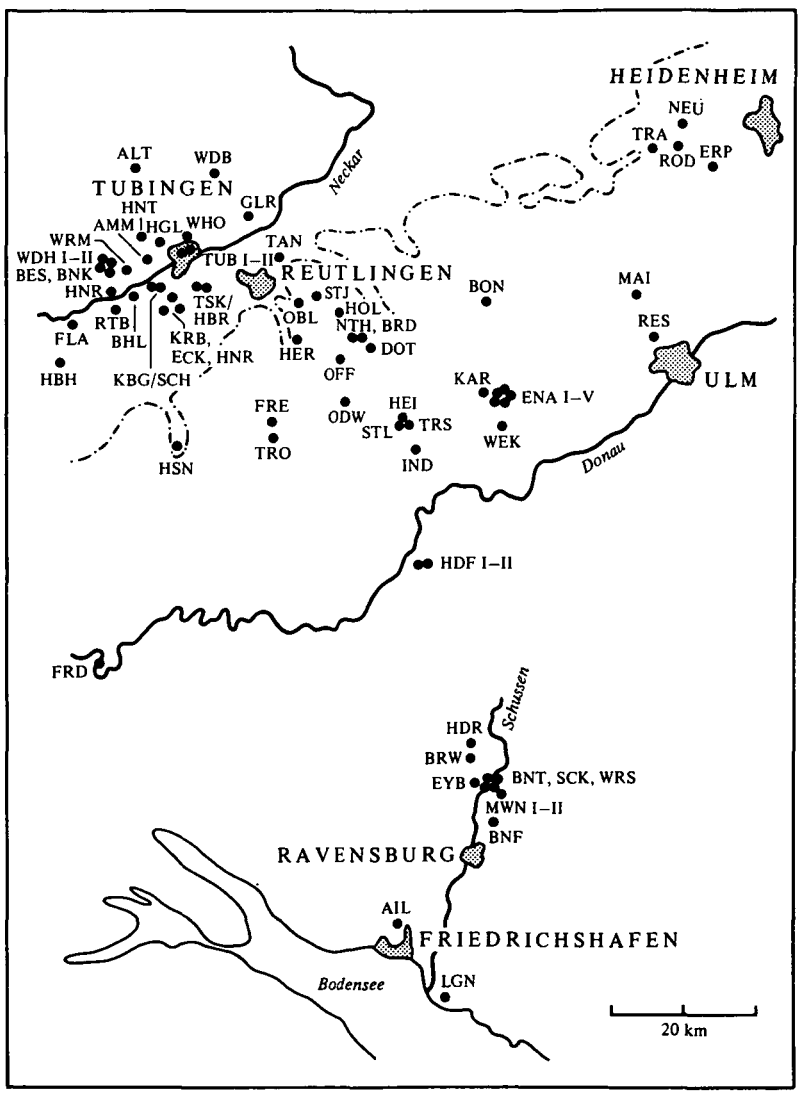

Fig. 1. Distribution of trapping sites in the region covered by this study. Abbreviations are explained in Tables 2 through 7.

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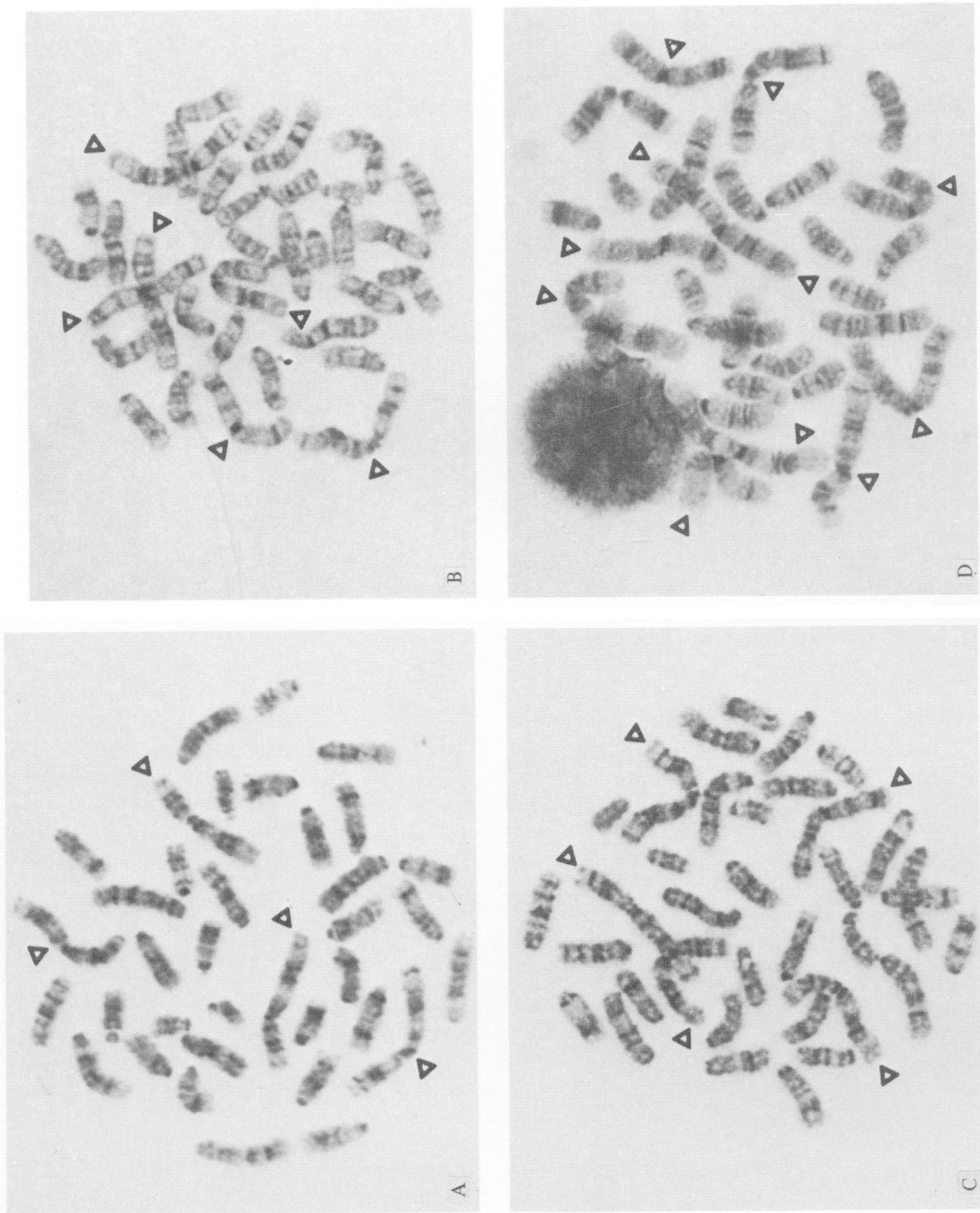

PLATE 1

Examples of metaphase plates with G-banded chromosomes. A, mouse HBR938 from population II. B, mouse OFF1127 from population II/III. C, mouse OFF1152 from population II/III. D, mouse AIL944 from population V.

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192 mice carried more than one pair of metacentric chromosomes, with the lowest $2n$ number equaling 30. Examples of the karyotypes found are shown in Plate 1). The metacentric chromosome found in almost all mice (240 of the 243 mice analysed by G-banding) was derived by the centromeric fusion (Robertsonian translocation) of the acrocentric chromosomes 4 and 12. We have designated this

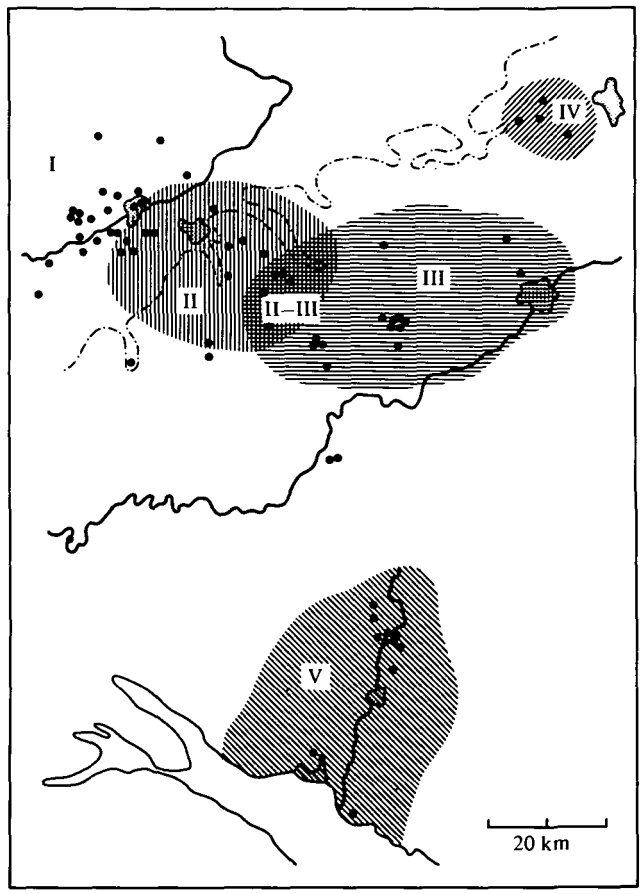

Fig. 2. Areas occupied by the five tested populations.

translocation *Rb(4 . 12)1Tu* (Adolph & Klein, 1981), where 'Tu' stands for Tübingen. Most of the mice were homozygous for this translocation (228 out of 243). Sixteen additional translocations were found in the studied region: *Rb(2 . 5)2Tu*, *Rb(3 . 6)3Tu*, *Rb(8 . 17)4Tu*, *Rb(10 . 14)5Tu*, *Rb(11 . 13)6Tu*, *Rb (1 . 5)19Tu*, *Rb(5 . 15)16Tu*, *Rb(13 . 14)17Tu*, *Rb(5 . 14)18Tu*, *Rb(3 . 8)20Tu*, *Rb(7 . 18)22Tu*, *Rb(11 . 16)26Tu*, *Rb(6 . 10)21Tu*, *Rb(8 . 10)23Tu*, *Rb(10 . 14)25Tu* and *Rb(9 . 14)24Tu* (some of these have been described previously; see Adolph & Klein, 1981). Based on their karyotypes, we divided the mice in the studied area into five populations, which, for simplicity, we shall refer to as population I through V (Fig. 2, Table 1).

Population I. The sample representing this population consisted of 144 mice from 25 trapping sites; of these, 48 mice were analysed by G-banding (Tables 1 and 2). The diploid chromosome number of these mice was 38 and the mice were homozygous for the metacentric chromosome *Rb(4 . 12)1Tu*, which was also present in all other populations. Population I occupied a continuous stretch of land along the Neckar valley west of Tübingen. In addition, mice belonging to this karyotype were found scattered throughout the entire region. For reasons explained in the *Discussion*, we consider population I to be the oldest metacentric chromosome-carrying mouse population of southern Germany, and view populations II through V as differentiating from it.

Table 1. *Frequencies* (%) *of the Robertsonian translocations in the different populations*

	Populations											
	I		II		II/III		III		VI		V	
Rob. translocation	Hom	Het	Hom	Het	Hom	Het	Hom	Het	Hom	Het	Hom	Het
Rb(4 . 12)1Tu	98	—	69	20	100	—	100	—	100	—	100	—
Rb(5 . 15)16Tu	—	—	51	23	73	16	—	—	—	—	—	—
Rb(13 . 14)17Tu	—	—	2	3	27	33	50	33	—	—	—	—
Rb(5 . 14)18Tu	—	—	—	—	—	—	—	—	17	33	—	—
Rb(11 . 13)6Tu	—	—	—	—	—	—	—	—	—	—	42	24
Rb(1 . 5)19Tu	—	2	—	—	—	—	—	—	—	—	—	—
Rb(3 . 8)20Tu	—	—	—	—	—	—	—	—	—	—	—	—
Rb(10 . 14)25;5Tu	—	—	—	—	—	2	—	—	—	—	—	17
Rb(7 . 18)22Tu	—	—	—	—	—	8	—	—	—	—	—	—
Rb(8 . 10)23Tu	—	—	—	—	—	2	—	2	—	—	—	—
Rb(11 . 16)26Tu	—	—	—	—	—	—	—	2	—	—	—	—
Rb(6 . 10)21Tu	—	—	—	—	—	—	—	—	—	6	—	—
Rb(3 . 6)3Tu	—	—	—	—	—	—	—	—	—	—	31	24
Rb(2 . 6)2Tu	—	—	—	—	—	—	—	—	—	—	14	10
Rb(8 . 17)4Tu	—	—	—	—	—	—	—	—	—	—	7	14
Rb(9 . 14)24Tu	—	—	—	—	—	—	—	—	—	—	4	4
Number of mice tested by G-banding	48		61		45		42		18		29	

Hom, homozygous; Het, heterozygous.

Among the 402 mice from 71 trapping sites, we found only 15 mice from 4 trapping sites in which the *Rb(4 . 12)1Tu* was either in a heterozygous condition (12 mice) or was missing altogether (3 mice). These four exceptional trapping sites were clustered in the vicinity of the city of Tübingen. Two sites contributed only 1 mouse each; of the other two sites, one contributed 13 mice in one trapping season (8 homozygotes, 4 heterozygotes, and one unidentified); and the other 3 and 9 mice in two trapping seasons, 1 year apart [2 homozygotes, 7 heterozygotes, and 2 without the *Rb(4 . 12)1Tu* translocation]. In addition to *Rb(4 . 12)1Tu*, we also found, at three of the four sites, 17 mice carrying the *Rb(5 . 15)16Tu* translocation

(4 homozygotes and 13 heterozygotes), indicating that the sites are part of both population I and II (see below). The closeness of these sites to a city in which several research institutions with mouse colonies are located, combined with the fact that one site was near the city's garbage dump, suggests that the exceptional mice might have been descendents of a cross with laboratory mice. Alternatively,

TABLE 2. *Mice examined for Robertsonian translocations: Population I*

Locality	Trapping site*	Trapping year	No. mice	2*n*	No. G-banded samples	Zygosity status of translocations†	
						Rb(4.12)1Tu	*Rb(1.5)19Tu*
Waldenbuch	WDB	80	1	38	—	—	—
Altdorf	ALT	80	2	38	2	—	—
Gniebel	GLR	78	3	38	1	2	—
Hagelloch	HGL	79	2	38	1	2	—
Hohenentringen	HNT	80/81	38/26	38	13/6	2	—
Tübingen	WHO	80	2	38	1	2	—
	TUBI	80	1	38	—	—	—
Ammerhof	AMM	78	1	40	1	—	—
Wendelsheim	BES	78	2	38	—	—	—
	BNK	78	8	38	4	2	—
	WDH I	80	5	38	2	2	—
	WDH II	81	1	38	—	—	—
Wurmlingen	WRM	78	1	38	—	—	—
Rottenburg	HRN	78	4	38	—	—	—
	RTB	80	20	38	8	2	—
	RTB	80	1	37	1	2	1
Bühl	BHL	79	4	38	1	2	—
Kilchberg	KBG/SCH	78	1/1	38	—	—	—
Schwalldorf	FLA	81	6	38	2	2	—
Hart bei Haigerloch	HBH	80	3	38	2	2	—
Hausen	HSN	78	4	38	—	—	—
Fridingen a.D.	FRD	78	1	38	—	—	—
Heudorg (Riedl)	HDF I–II	78	1/1	38	—	—	—
Trochtelfingen	TRO	80	4	38	2	2	—
Total			144		48		

* Initials represent abbreviations of the farm's designation.
† 2, homozygote, 1, heterozygote for a given translocation.

the mice might have derived from wild immigrants or remnants of an all-acrocentric population.

We also found one rare variant in population I – 1 mouse heterozygous for the translocation *Rb (1.5)19Tu*. This translocation was found in a sample of 21 mice; all others in the sample had the typical *Rb(4.12)1Tu* karyotype.

Population II. The metacentric chromosome characterizing this population is *Rb*

(*5 . 15*)*16Tu* (Tables 1 and 3). It occurs either in a homozygous or heterozygous state, with most of the homozygotes found in the core of the population and the heterozygotes in its periphery. Among the 61 mice from 12 trapping sites analysed by G-banding, 11 were heterozygous for *Rb*(*5 . 15*)*16Tu*, 9 carried only the *Rb* (*4 . 12*)*1Tu* translocation, and the rest were *Rb*(*5 . 15*)*16Tu* homozygotes. All mice belonging to population II were *Rb*(*4 . 12*)*1Tu* homozygotes except for mice from the three trapping sites near Tübingen (see above). The centre of the population lies in the area of the Schwäbische Alb referred to as the Reutlinger Alb. How far north the population extends could not be determined; in the west and south it meets population I, in the east it meets population III in the middle of the Alb. In the eastern part, a third translocation, *Rb*(*13 . 14*)*17Tu*, which we consider characteristic of population III, emerges. From this part, we obtained 45 mice captured at five trapping sites (Tables 1 and 4). All mice had the *Rb*(*4 . 12*)*1Tu* translocation. Twenty-three mice from three of the five sites were homozygous for the *Rb*(*5 . 15*)*16Tu* translocation but 16 of them carried, in addition, the *Rb* (*13 . 14*)*17Tu* translocation in either homozygous or heterozygous condition. In the remaining 22 mice from the other two sites, the *Rb*(*5 . 15*)*16Tu* translocation segregated. These findings lead us to believe that populations II and III form a hybrid zone where they come together.

Rare variants also appear in this population: *Rb*(*8 . 10*)*23Tu* at two sites with one mouse each (see below), *Rb*(*3 . 8*)*20Tu* and *Rb*(*10 . 14*)*25Tu* at one site (3 mice) and *Rb*(*7 . 18*)*22Tu* in four mice from a third farm. All these variants occur in the hybrid zone.

Population III. As previously mentioned, this population is characterized by the occurrence of *Rb*(*13 . 14*)*17Tu*, in addition to *Rb*(*4 . 12*)*1Tu*. Whereas *Rb*(*4 . 12*)*1Tu* is always homozygous, *Rb*(*13 . 14*)*17Tu* heterozygotes and homozygotes are equally distributed over the region (Tables 1 and 5). The area covered by this population extends from the hybrid zone with population II down to the Danube in the southeast. The exact borders remain unidentified, but they probably lie roughly in the region where populations IV and V begin. For technical reasons, we were unable to search for hybrid zones between these populations. If they do exist, it is likely that they are of different character from that of the hybrid zone between populations II and III: since translocations *Rb*(*5 . 15*)*16Tu*, *Rb*(*13 . 14*)*17Tu* and *Rb*(*5 . 14*)*18Tu* would meet in these zones, hybrids derived from representatives of the two populations would have a chain of three paired metacentric chromosomes in their meiotic metaphases. A similar situation would occur in the putative hybrid zone between populations III and V. Animals in this zone would carry a chain of three paired metacentric chromosomes *Rb*(*11 . 13*)*6Tu* – *Rb*(*13 . 14*)*17Tu* – *Rb*(*10 . 14*)*5Tu*.

The rare variants of population III were *Rb*(*11 . 16*)*26Tu* found in one mouse only, and *Rb*(*10 . 8*)*23Tu*, which was also found in population II.

Population IV. The translocation characterizing this population is *Rb*(*5 . 14*)*18Tu* (Tables 1 and 6). Homozygotes and heterozygotes for this translocation occur in approximately equal numbers in this population. The population consisted of 24

TABLE 3. *Mice examined for Robertsonian translocations: Population II*

Locality	Trapping sites*	Trapping year	No. mice	2n	No. G-banded samples	Zygosity status of translocations†		
						Rb(4.12)1Tu	*Rb(5.15)16Tu*	*Rb(13.14)17Tu*
Tübingen	TUBII	80/81	1/1	38	1/1	2	—	—
			1/1	38	1/1	1	1	—
			1/6	39	1/4	1	—	—
			—	—	1	—	1	—
			1	40	1	—	—	—
Eckhof	ECK	80	1	38	1	2	—	—
			1	37	1	2	1	—
Kressbach	KRB	80	1	35	1	2	2	1
			3	36	3	2	2	—
			4	37	3	2	1	—
			3	38	2	1	1	—
			2	39	2	1	—	—
	HNF	80	1	36	1	2	2	—
Märingen	TSK	78	1	36	—	—	—	—
	HBR	80	1	36	1	2	2	—
Tannenhof	TAN	81	7	36	3	2	2	—
			2	37	1	2	1	—
St Johann	STJ	80	1	37	1	1	2	—
	OBL	80	1	34	1	2	2	2
			1	35	1	2	2	1
			15	36	14	2	2	—
Bleichstetten	HOL	81	3	36	1	2	2	—
Holzelfingen	HER	80	3	36	2	2	2	—
Trochtelfingen	FRE	81	2	36	2	2	2	—
			8	37	4	2	1	—
			9	38	5	2	—	—
Total			82		61			

* Initials represent abbreviations of the farm's designation.
† 2, homozygote; 1, heterozygote for a given translocation.

Table 4. *Mice examined for Robertsonian translocations: hybrid zone between populations II and III*

Trapping site*	Trapping year	No. mice	2n	No. G-banded samples	Zygosity status of translocations†						
					Rb(4.12)1Tu	*Rb(5.15)16Tu*	*Rb(13.14)17Tu*	*Rb(7.18)22Tu*	*Rb(10.14)25Tu*	*Rb(3.8)20Tu*	*Rb(8.10)23Tu*
					Dottingen						
DOT	81	1	33	1	2	2	2	1	—	—	—
		4	34	2	2	2	2	—	—	—	—
				2	2	2	1	1	—	—	—
		5	35	3	2	2	1	—	—	—	—
		6	36	2	2	2	—	—	—	—	—
				2	2	1	1	—	—	—	—
				1	2	1	—	1	—	—	—
		1	37	1	2	1	—	—	—	—	—
		1	38	1	2	—	—	—	—	—	—
					Offenhausen						
OFF	80	2	34	2	2	2	2	—	—	—	—
		1	35	1	2	2	—	—	1	—	—
	81	1	35	1	2	2	1	—	—	—	—
		2	35	2	2	2	—	—	—	1	—
		1	36	1	2	2	—	—	—	—	—
					Gächingen						
NTH	80	1	34	1	2	2	2	—	—	—	—
		1	35	1	2	2	1	—	—	—	—
BRD	80/81	7/2	34	4/2	2	2	2	—	—	—	—
		3/4	35	3/2	2	2	1	—	—	—	—
		2/3	36	1/2	2	2	—	—	—	—	—
					Ödenwaldstetten						
ODW	80	1	36	1	2	1	—	—	—	—	1
		6	37	2	2	1	—	—	—	—	—
				1	2	—	1	—	—	—	—
		4	38	3	2	—	—	—	—	—	—
Total		59		45							

* Initials represent abbreviations of the farm's designation.
† 2, homozygote; 1, heterozygote for a given translocation.

TABLE 5. *Mice examined for Robertsonian translocation: Population III*

Locality	Trapping site*	Trapping year	No. mice	2n	No. G-banded samples	Zygosity status of translocations†			
						Rb(4.12)1Tu	*Rb(13.14)17Tu*	*Rb(8.10)23Tu*	*Rb(11.16)26Tu*
Steighöfe	HeI	80	1	38	1	2	—	—	—
	STJ	80	1	37	1	2	1	—	—
	TRS	80	3	37	2	2	1	—	—
			1	36	1	2	2	—	—
Indelhausen	IND	80	2	36	1	2	2	—	—
					1	2	1	1	—
			2	37	2	2	1	—	—
			5	38	4	2	—	—	—
Ennahofen	ENA I–V	81	3	36	1	2	2	—	—
			5	37	3	2	1	—	—
Briel	WEK	81	1	36	1	2	2	—	—
			1	37	1	2	1	—	—
Karlshof	KAR	81	1	35	1	2	2	—	1
			4	36	4	2	2	—	—
			1	37	1	2	1	—	—
			1	38	1	2	—	—	—
Mähringen/Ulm	RES	81	4	36	4	2	2	—	—
			5	37	2	2	1	—	—
			1	38	1	2	—	—	—
Tomerdingen	MAI	81	1	37	1	2	1	—	—
Feldstetten	BON	81	11	36	8	2	2	—	—
Total			54		42				

* Initials represent abbreviations of the farm's designation.
† 2, homozygote, 1, heterozygote for a given translocation.

mice from four trapping sites. Twelve mice from two farms carried only translocation *Rb(4.12)1Tu*; mice from the other two sites carried *Rb(5.14)18Tu* and, as a rare variant, *Rb(6.10)21Tu*. This population occupies the southern border of the wooded area of the 'Ostalb', between the cites of Geislingen and Heidenheim.

Population V. This population is the most heterogeneous of the five populations tested, and is concentrated in the southern corner of the triangle studied. Most of the mice from this area were already described in a previous publication (Adolph & Klein, 1981) but, in addition, we analysed 26 mice from six new trapping sites. Altogether we obtained mice from 11 trapping sites, nine of them located in a region of about 30 km² north of Ravensburg; two sites were located near Lake Constance.

In addition to *Rb(4.12)1Tu*, which was always homozygous, the mice carried also the *Rb(11.13)6Tu* translocation, which was homozygous in 13 and heterozygous in seven of the 29 mice typed (Tables 1 and 7). The other translocations occurring in this region were: *Rb(3.6)3Tu*, *Rb(2.5)2Tu*, *Rb(10.14)5Tu*, *Rb(8.17)4Tu* and *Rb(9.14)24Tu*. *Rb(3.6)3Tu* was quite frequent; *Rb(2.5)2Tu* occurred sporadically throughout the area, as did *Rb(8.17)4Tu*. *Rb(10.14)5Tu* occurred only in a heterozygous state and only in the Mochenwangen population (near Ravensburg). Mice from the two trapping sites near Lake Constance were homozygous for *Rb(4.12)1Tu*, *Rb(2.5)2Tu*, *Rb(3.6)3Tu* and *Rb(11.13)6Tu;* the mice differed from one another in that mice from one site were homozygous for *Rb(8.17)4Tu* and mice from the other site were either homo- or heterozygous for *Rb(9.14)24Tu*. These were also the mice with the highest number of metacentric chromosomes (10, $2n = 30$) found in southern Germany.

Despite the heterogeneity found in this area (mice from one trapping site carried from three to seven metacentric chromosomes and some of the mice were heterozygous for up to four different metacentric chromosomes), there was a clear increase in the number of metacentric chromosomes from two in the most northern part of the population, to five pairs in the most southern part. Mice from different trapping sites always shared some metacentric chromosomes. The population may represent a hybrid zone between population I and another, as yet unidentified, population homozygous for all the metacentric chromosomes found in this region, a situation similar to that observed by Spirito and his co-workers (1980) in central Italy.

Stability of karyotypes. Our study has extended over 4 years, and at some trapping sites we were able to obtain samples in consecutive years. A comparison of the results obtained in different years (Tables 2 through 7) suggested that homogeneous populations remained stable (e.g. HNT) and that heterogeneous populations either retained the number and the composition of metacentric chromosomes involved (e.g. BRD) or acquired new metacentric chromosomes and lost some of the old ones (e.g. OFF, pop. V). This variation might, of course, be due to sampling error and to segregation of metacentric chromosomes in consecutive generations. Another indication of stability is the fact that mice from adjacent farm buildings (average distance 0·5–1 km) show the same chromosomal pattern.

TABLE 6. *Mice examined for Robertsonian translocations: Population IV*

Locality	Trapping* site*	Trapping year	No. mice	2*n*	No. G-banded samples	Zygosity status of translocations†		
						Rb(4.12)1Tu	*Rb(5.14)18Tu*	*Rb(6.10)21Tu*
Trasenberg	TRA	81	3	38	2	2	—	—
Neuseehalden	NEU	81	1	35	1	2	2	1
			1	36	1	2	2	—
			2	37	2	2	1	—
Dudelhof	ROD	81	1	36	1	2	2	—
			4	37	4	2	1	—
			3	38	2	2	—	—
Erpfenhausen	ERP	81	9	38	5	2	—	—
Total			24		18			

* Initials represent abbreviations of the farm's designation.
† 2, homozygote; 1, heterozygote for a given translocation.

Table 7. *Mice examined for Robertsonian translocations: Population V*

Trapping site*	Trapping year	No. mice	2n	G-banded samples	Zygosity status of translocations†						
					Rb(4.12)1Tu	*Rb(11.13)6Tu*	*Rb(3.6)3Tu*	*Rb(2.5)2Tu*	*Rb(8.17)4Tu*	*Rb(10.14)5Tu*	*Rb(9.14)24Tu*
					Halderhof						
HDR	80	10	38	6	2	—	—	—	—	—	—
		5	37	2	2	1	—	—	—	—	—
		1	36	1	2	2	—	—	—	—	—
		1	35	—	—	—	—	—	—	—	—
					Bärenweiler						
BRW	80	1	36	1	2	2	—	—	—	—	—
		2	38	2	2	—	—	—	—	—	—
					EYB						
EYB	80	1	33	1	2	2	1	—	1	1	—
					Mochenwangen						
BNT	78	2	35	1	2	2	1	—	—	—	—
				1	2	1	2	—	—	—	—
		1	34	1	2	2	—	2	—	—	—
MWN1	78	1	37	1	2	—	1	—	—	—	—
		1	35	1	2	1	1	1	—	—	—
	79	1	33	1	2	2	1	—	1	1	—
SCK	79	2	33	2	2	2	2	—	—	1	—
WRS	78	1	34	1	2	1	1	1	—	1	—
	80	2	34	1	2	2	2	—	—	—	—
					Sulpach						
MWN2	78	2	34	1	2	1	2	—	1	—	—
				1	2	1	1	1	1	—	—
					Baienfurt						
BNF	78	1	34	1	2	—	2	—	2	—	—
		1	35	—	—	—	—	—	—	—	—
					Langenargen						
LGN	80	1	30	1	2	2	2	2	2	—	—
					Ailingen						
AIL	80	1	30	1	2	2	2	2	—	—	2
		1	31	1	2	2	2	2	—	—	1
Total		39		29							

* Initials represent abbreviations of the farm's designation.
† 2, homozygote; 1, heterozygote for a given transplantation.

TABLE 8. *Frequencies of the dominant translocation and comparison to the Hardy–Weinberg distribution. The frequencies are calculated only for those trapping sites at which the respective translocations occurred*

Rob. translocation	Population	Number of		Frequencies	Number of mice found			Number of mice expected*		
		Mice	Trapping sites		Hom†	Het	—	Hom	Het	—
Rb(4.12)1Tu	I through V	243	54	0·97	228	12	3	228	13	3
Rb(5.15)16Tu	II and II/III	106	54	0·70	64	21	21	52‡	45	9
Rb(13.14)17Tu	II, II/III, III	106	20	0·41	31	20	51	18‡	51	37
Rb(11.13)6Tu	V	29	11	0·53	12	7	10	8‡	14	7

* According to Hardy–Weinberg distribution.

† Het, heterozygous; Hom, homozygous; —, without translocation.

‡ Significant deviation from Hardy–Weinberg distribution (χ^2-test, $P = 0{\cdot}01$).

4. DISCUSSION

Interpretation of chromosomal variation in mice of southern Germany. We believe that the key to understanding the chromosomal polymorphism in the house mice of this region is population I, characterized by the $2n$ number of 38 chromosomes and the presence of the *Rb(4.12)1Tu* translocation. This translocation apparently managed to spread through the whole of southern Germany and to become fixed. We base this conclusion on the following observations. First, with the exception of only a few mice (less than 7 % of our sample) that might have been descendants of migrants from other areas (see *Results*), all mice were homozygous for the *Rb(4.12)1Tu* translocation. Second, the original population seems to be preserved west of Tübingen, where no additional metacentric chromosomes could be found. The same fusion of chromosomes 4 and 12 was found by Grop and co-workers along the Rhine valley in Switzerland (Basel, Chur, Albula valley) and even on the other side of the main Alpine chain in northern Italy (Gropp *et al.* 1972). Third, all the populations containing additional metacentric chromosomes were surrounded by areas in which mice with the original karyotype could still be found together with mice carrying additional metacentric chromosomes. These areas may represent zones in which translocations characteristic of populations II through V diffuse into population I.

We suggest that populations II through V are in the process of differentiating from population I. This differentiation takes place by the acquisition of additional translocations, some of which are on the way to becoming fixed in the individual population.

Although we are unable to give fixed dates for the beginning of the differentiation of population I into populations II through V, we suggest that this differentiation is of rather recent origin. We base this conclusion on the observation that the populations carrying more than the one pair of metacentric chromosomes are still not fixed for the additional translocations. Although each of these new populations has acquired a characteristic new metacentric chromosome, up to 50 % of the mice are still heterozygous for this new translocation. However, there is a clear trend toward homozygosity suggesting that the populations evolve toward fixation of these translocations (Table 8).

Origin of the Robertsonian translocations. What do our observations tell us about the way Robertsonian translocations emerge in populations? Our data are compatible with the hypothesis that most of the metacentric chromosomes in the individual populations arose *de novo*, as a result of chromosomal mutations. If the additional metacentric chromosomes were brought into the populations by migrant mice, one would expect to see the common *Rb(4.12)1Tu* translocation more frequently in a heterozygous state than it actually occurs. However, the main indication for the *de novo* emergence of metacentric chromosomes is the presence of the rare variants in the populations. The metacentric chromosomes *Rb(7.18)22Tu*, *Rb(8.10)23Tu* and *Rb(11.16)26Tu* are not present in the surrounding populations, yet they occur in mice carrying translocations characteristic for

a given region. It is, therefore, inconceivable that these translocations had been introduced into southern Germany from some other geographical region. Some of the rare variants can be found outside Germany, together with translocations not found in our populations. For example, *Rb(1.5)19Tu* was found in northern Scotland (Brooker & Berry, 1981) and *Rb(6.10)21Tu* in Spain (Adolph & Klein, 1981). Nevertheless, it is extremely unlikely that these distant populations are the source of the rare variants found in southern Germany.

The situation might be different in population V. Of the six segregating translocations found in this population, five are also found in south-eastern Europe: *Rb(11.13)*, *Rb(3.6)* and *Rb(9.14)* in the Alpine region and *Rb(8.17)* and *Rb(10.14)* in mice from Zadar (Gropp & Winking, 1981) and Sicily (Lehman & Radbruch, 1977). It is, therefore, possible that a connection of population V to the Alpine system exists, and that, perhaps, population V is a hybrid zone between population I and a population belonging to the Alpine system. Gropp and his co-workers (1972) have suggested that conditions exist in populations with fixed metacentric chromosomes which may be conducive to the generation of new translocations. Such conditions may have existed in northern and central Italy and may have led to the accumulation of the maximal possible number of metacentric chromosomes in mice of this region. Similar conditions may also exist in southern Germany, where in population I, with one pair of metacentrics, we found one rare variant; in the region where populations II and III meet, we found four different variants; and in populations III, with two pairs of metacentric chromosomes, we found two rare variants.

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