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Genic differentiation and origin of Robertsonian populations of the house mouse (*Mus musculus domesticus* Rutty)

JANICE BRITTON-DAVIDIAN[1], JOSEPH H. NADEAU[2], HENRI CROSET[1] AND LOUIS THALER[1]

[1] *Institut des Sciences de l'Evolution, CNRS UA 327, USTL, Place Eugene Bataillon, 34060 Montpellier Cedex*
[2] *The Jackson Laboratory, Bar Harbor, Maine 04609, USA*

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Summary

This paper examines the relation between chromosomal and nuclear-gene divergence in 28 wild populations of the house mouse semi-species, *Mus musculus domesticus*, in Western Europe and North Africa. Besides describing the karyotypes of 15 of these populations and comparing them to those of 13 populations for which such information was already known, it reports the results of an electrophoretic survey of proteins encoded by 34 nuclear loci in all 28 populations. Karyotypic variation in this taxon involves only centric (or Robertsonian) fusions which often differ in arm combination and number between chromosomal races. The electrophoretic analysis showed that the amount of genic variation within Robertsonian (Rb) populations was similar to that for all-acrocentric populations, i.e. bearing the standard karyotype. Moreover, divergence between the two types of populations was extremely low. These results imply that centric fusions in mice have not modified either the level or the nature of genic variability. The genetic similarity between Rb and all-acrocentric populations is not attributed to the persistence of gene flow, since multiple fusions cause marked reproductive isolation. Rather, we attribute this extreme similarity to the very recent origin of chromosomal races in Europe. Furthermore, genic diversity measures suggest that geographically separated Rb populations have *in situ* and independent origins. Thus, Rb translocations are probably not unique events, but originated repeatedly. Two models are presented to explain how the rapid fixation of a series of chromosomal rearrangements can occur in a population without lowering variability in the nuclear genes. The first model assumes that chromosomal mutation rates are between 10^{-3} and 10^{-4} and that populations underwent a series of transient bottlenecks in which the effective population size did not fall below 35. In the second model, genic variability is restored following severe bottlenecks, through gene flow and recombination.

1. Introduction

Karyotypic differentiation among closely related species is believed to be an important mechanism of speciation by reducing gene flow between karyotypically divergent populations (White, 1968). Reduction in gene flow is achieved through the selective disadvantage of heterokaryotypes brought about by abnormal meiotic processes. In the semi-species *Mus musculus domesticus*, chromosomal variability occurs only through centric fusion of acrocentric chromosomes (termed Robertsonian translocation) which results in a reduction of the diploid complement number (2n = 22 at the minimum).

Reprint requests to: Dr J. Britton-Davidian, Laboratoire de Génétique, Institut des Sciences de l'Evolution, USTL, Place E. Bataillon, 34060 Montpellier Cedex.

Robertsonian (Rb) populations of mice are distributed within geographically separated systems surrounded by populations of mice with 2n = 40 chromosomes (see Fig. 2). A system can be defined as an array of parapatric karyotypic races sharing a certain number of fusions (Capanna, 1982). However, each race is distinguished either by the arm combination or the number of translocations it possesses. The contact between Rb and all-acrocentric populations occurs as narrow hybrid zones occupied by chromosomally polymorphic populations (Spirito *et al.* 1980; Gropp *et al.* 1982; Capanna *et al.* 1985*a*).

The concept of chromosomal speciation has generated considerable theoretical debate over the years and led to a variety of different models (see review by Sites & Moritz, 1987). Two of these may account for the processes of chromosomal differentiation in mice.

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In the cumulative fusion model (Capanna *et al.* 1977; White, 1978), reproductive isolation results from the cumulative effects of several rearrangements, each of which is slightly underdominant, owing to the formation of trivalents at meiosis in heterozygotes (Fig. 1). The karyological analysis of Rb populations in Southern Germany (Adolph & Klein, 1983) supports the view that fusions are acquired successively by the same population. Within this region, which is occupied by a 2n = 38 Rb population homozygous for the fusion of chromosomes 4 and 12 termed the Rb(4·12) fusion, there are two other Rb populations with 2n = 36, each homozygous for a different additional translocation, Rb(5·15) and Rb(13·14) respectively. The second model, speciation through monobrachial homology (Baker & Bickham, 1986), involves the independent accumulation in two populations of Rb translocations with only one chromosome arm in common. [For example, Rb(6·7) and Rb(1·6) are monobrachially homologous fusions present respectively in Binasco and Bergamo, see Table 1.] This results in high levels of hybrid sterility owing to the formation of chains of chromosomes at meiosis (Fig. 1).

If the processes of chromosomal diversification can theoretically be easily understood, the amount of reproductive isolation they generate is not, due to the following paradox: to be efficient in reducing gene flow, a chromosomal rearrangement must confer a high selective disadvantage to the heterokaryotype, which in turn will substantially reduce its probability of becoming fixed within a deme. Attempts to measure the effects of the chromosomal barrier on gene flow have been conducted by means of biochemical surveys on a variety of mammalian groups. Frykman *et al.* (1983), summarizing electrophoretic studies of karyotypically variable mammals found that normally only small genetic differences exist between closely related parapatric karyotypic races. Such results, however, do not allow one to distinguish between two alternative hypotheses regarding the efficiency of the chromosomal barrier in reducing genetic exchange: (i) the absence of genetic differentiation could result from current gene exchanges in which case chromosomal rearrangements cannot be considered as barriers to gene flow; (ii) gene flow may be in fact reduced but the time frame since the onset of karyotypic differentiation has been too short to allow divergence in allele frequencies.

Rb populations of house mice appear well suited to test the effect of chromosomal rearrangements on gene flow and to investigate the origin of the phenomenon for several reasons: within the same genetic background (i.e. *M. m. domesticus*), populations have emerged showing different levels of chromosomal differentiation due to the fixation of a varying number of chromosomal rearrangements; the latter are always and only centric fusions which, if they do not change the alignment of genes on the translocated chromosomes, do modify their segregation pattern; these populations occur in widely separated geographic regions; the arrival of the house mouse in Europe can be dated; and, finally, data on hybrid sterility of heterozygotes for centric fusions are available.

Previous genetical studies of Rb populations of mice have shown the level of divergence to be slight (Britton-Davidian *et al.* 1980; Nash *et al.* 1983; Capanna *et al.* 1985*b*; Said *et al.* 1986). However, these estimates were limited to populations belonging to three geographically separate Rb systems. The data presented in this paper allow comparisons between genic and chromosomal differentiation both between adjacent Rb populations as well as between the latter and neighbouring all-acrocentric populations. Four types of questions will be addressed:

(i) Has the presence of centric fusions altered the variability of the loci they bring together?

(ii) Are levels of genic differentiation in accord with predicted degrees of hybrid sterility?

(iii) Do geographically isolated systems of Rb populations have independent origins?

(iv) What population structures necessary for fixation of rearrangements are compatible with the gene variability data?

2. Material and Methods

(i) *Mice*

Twenty eight populations of wild house mice were live-trapped in the localities indicated in Fig. 2.

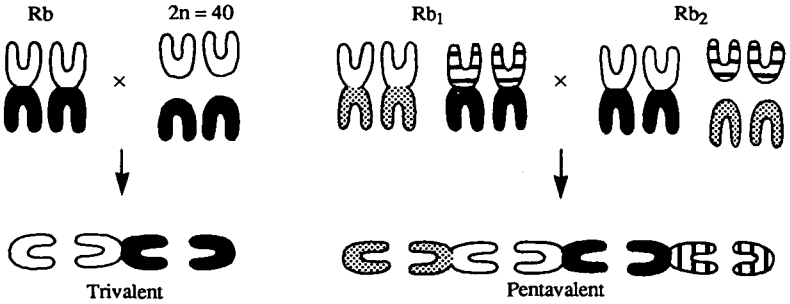

Fig. 1. Schematic representation of (*a*) trivalent and (*b*) pentavalent chain formation during meiosis of Rb hybrids (see text for explanation).

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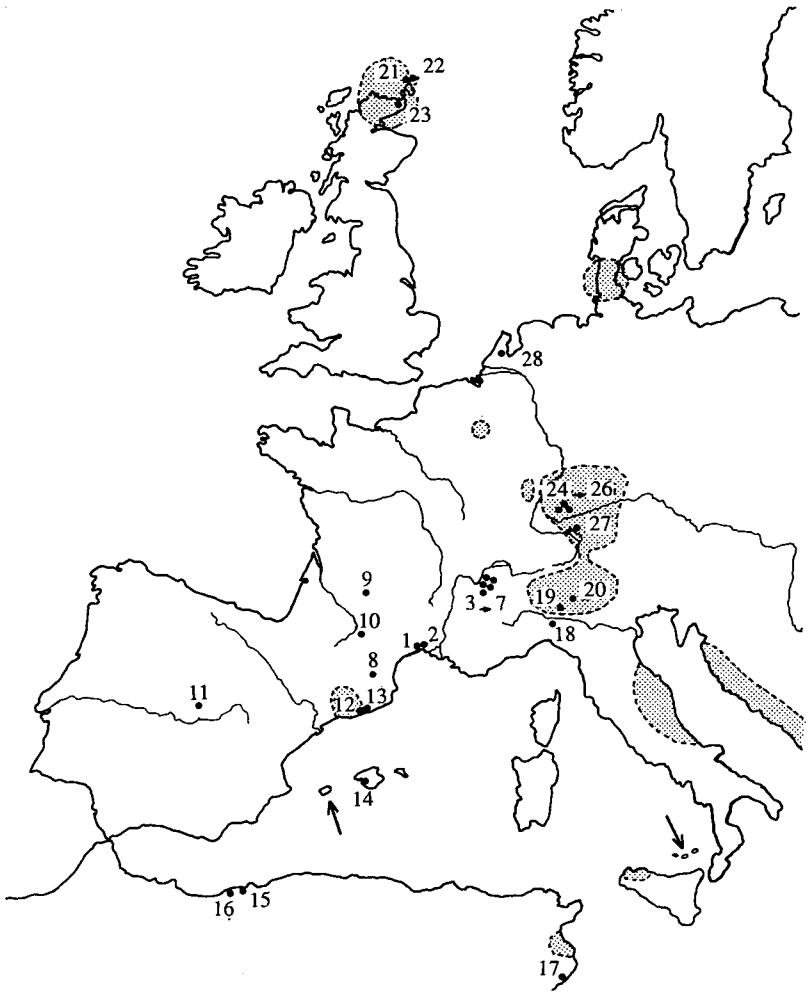

Fig. 2. Localities sampled. Arrows and shaded zones indicate areas inhabited by Rb populations. France: 1. ENSA, 2. Pages, 3. Haute Savoie, 4. Bourget, 5. Les Deux Guiers, 6. Le Marais, 7. Iseron, 8. Pratx, 9. Dordogne, 10. Toulouse. Spain: 11. Colmenar, 12. SP-1, 13. SP-2, 14. SP-3. Algeria: 15. Oran, 16. Bouzadjar. Tunisia: 17. Sfax. Italy: 18. Ovada, 19. Binasco, 20. Bergamo. Great Britain: 21. ORK-2, 22. ORK-1, 23. SCO. West Germany: 24. TUB-1, 25. TUB-2, 26. TUB-3, 27. RAV. Netherlands: 28. AMS.

Populations designated by upper cases correspond to the following localities: ORK-1: pooled samples from Ely, North Ronaldsay and Papa, ORK-2: Eday; SCO: Scotland (Great Britain); AMS: Amsterdam (The Netherlands); SP-1, SP-2: Barcelona, SP-3: Mallorca (Spain); TUB-1, TUB-2, TUB-3: Tübingen region, RAV: Ravensburg region (Federal Republic of Germany).

For additional information concerning localities and method of pooling populations, see Nadeau *et al.* 1981 (SP-1 = RPT, AVY and VDX; SP-2 = LRA and MOY; SP-3 = BNS and SJI; SCO = CTC), Adolph and Klein, 1983 (TUB-1 = BNK; TUB-2 = WRM, GLR, HLZ, HGL, BES and HRN; TUB-3 = BHL, KBG, SCH and HSN; RAV = BNF, BNT, WRS and MWN) and the authors.

(ii) *Karyological analysis*

The mice from Great Britain, West Germany, the Netherlands and three of the Spanish localities (SP-1, SP-2, SP-3) were analysed by S. Adolph at the Max Planck Institüt für Biologie in Tübingen, F.R.G. (Adolph & Klein, 1981). The arm composition of the metacentrics present in the Italian populations of mice was determined by Gropp *et al.* (1982). The remaining mice were karyotyped by J. B.-D. in Montpellier, France, from bone marrow samples following a slight modification of the 'air drying' technique after yeast stimulation (Lee and Elder, 1980). G-banding was performed following the method of Seabright (1971) and chromosome identification was determined according to Nesbitt and Francke (1973).

(iii) *Electrophoretic methods*

Tissue preparations, buffer systems, starch gels and stains were similar to those described in Selander *et al.* (1971) and Harris & Hopkinson (1976) [see Pasteur *et al.* 1987 for the complete list of techniques]. A total of 34 loci were scored; lactate dehydrogenase (*Ldh*-1 and *Ldh*-2), isocitrate dehydrogenase (*Idh*-1 and

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Idh-2), NAD-dependent malate dehydrogenase (*Mor*-1 and *Mor*-2), NADP-dependent malate dehydrogenase (*Mod*-1 and *Mod*-2), hemoglobin beta chain (*Hbb*), albumin (*Alb*-1), alcohol dehydrogenase (*Adh*-1), alpha-glycerophosphate dehydrogenase (*Gdc*-1), amino-aspartate transaminase (*Got*-1 and *Got*-2), esterase (*Es*-1, *Es*-2, *Es*-3, *Es*-5, *Es*-10 and *Es*-15), pyruvate kinase (*Pk*-1 and *Pk*-3), phosphoglucomutase (*Pgm*-1 and *Pgm*-2), 6-phosphogluconate dehydrogenase (*Pgd*), transferrin (*Trf*), superoxide dismutase (*Sod*-1), glucose phosphate isomerase (*Gpi*-1), carbonic anhydrase (*Car*-2), mannose phosphate isomerase (*Mpi*-1), glyoxalase (*Glo*-1), amylase (*Amy*-1), nucleoside phosphorylase (*Np*-1) and the regulator gene of *Ldh*-2 in red cells (*Ldr*-1). Symbols for loci follow those provided by Mouse NewsLetter (1985). Alleles were designated as mobilities relative to those present in the laboratory strain C57 BL/6J which were arbitrarily set as 100, except for *Hbb* and *Ldr*-1 for which the conventional alphabetic allelic designation was used.

2. Results

(i) *Chromosomal variability*

Nine of the 28 populations studied showed chromosomal variability consisting in a reduction of the diploid number due to centric fusions. The number of Rb translocations present varied from one to nine pairs (2n = 38 to 2n = 22). No deviation from the standard 40 acrocentric karyotype was detected in the samples from France, Northern Africa, Spain (Colmenar, SP-2 and SP-3), Italy (Ovada) and Amsterdam (AMS). The arm composition of the translocations present in the Rb populations is given in Table 1. The Italian localities of Bergamo and Binasco were ascribable to the Cremona and Milano II groups, respectively, as determined by Gropp *et al.* (1982) in the Lombardy region.

Results show that the cytogenetically variant populations belong to four different Rb systems, each characterized by its own combination of translocations and in which certain populations are homokaryotypic (Bergamo, Binasco, ORK-2, TUB-1, TUB-2, TUB-3), while the remainder are polymorphic. The latter fall into different groups: the SP-1 sample pertains to a transition zone between Rb and all-acrocentric mice, the RAV sample is most likely the product of hybridizations between populations differing in the arm composition of the translocations (Adolph & Klein, 1983), whereas the SCO sample from Scotland belongs to a very complex Rb system in which 15 arm combinations are observed, most of which are present in heterozygous form (see Brooker, 1982).

(ii) *Genic variability*

Of the 34 nuclear loci studied electrophoretically, nine (*Adh*-1, *Gdc*-1, *Got*-1, *Idh*-2, *Mor*-1, *Mor*-2, *Pgd*, *Pk*-1 and *Sod*-1) showed no variation. The electrophoretic analysis confirmed that all populations studied regardless of karyotype belong to the Western European semi-species of house mice, *M. m. domesticus* (biochemical group Mus 1, Bonhomme *et al.* 1978).

From the allelic frequencies (Table 2), levels of genic variability (mean heterozygosity, percentage of polymorphic loci and mean number of alleles) were computed for each population (Table 3). Results show that all-acrocentric and Rb populations yield a comparable range of values (*t*-test, n.s. for P, H and A). The mean values of genic heterozygosity ($\bar{H} = 0{\cdot}09$) recorded in this study are similar to those reported elsewhere for this semi-species ($\bar{H} = 0{\cdot}09$ for continental populations, Berry & Peters, 1981; $\bar{H} = 0{\cdot}09$, Sage, 1981).

If overall levels of variability are similar in both karyomorphs, how do variability estimates compare at each locus? When the data are plotted (all-acrocentric versus Rb, see Fig. 3), results show that

Table 1. *Arm composition of metacentrics and chromosome number (2n) of the Rb populations sampled. The range of diploid numbers found in the chromosomally polymorphic populations are indicated*

Rb system	Locality	2n	Arm composition
Barcelona	12. SP-1	38–31	4·14, 5·15, 9·11, 12·13, 6·10*
Rhaeto-Lombardy	19. Binasco	24	16·17, 11·13, 10·12, 9·14, 5·15, 3·4, 2·8, 6·7†
	20. Bergamo	22	16·17, 11·13, 10·12, 9·14, 5·15, 3·4, 2·8, 7·18, 1·6†
N.E. Britain	21. ORK-2	34	4·10, 9·12, 3·14*
	22. SCO	36–34	4·10, 9·12, 6·13*
S.W. Germany	24. TUB-1	38	4·12‡
	25. TUB-2	38	4·12‡
	26. TUB-3	38	4·12‡
	27. RAV	37–35	4·12, 3·6, 11·13, 2·5, 8·17, 10·14‡

* Adolph and Klein, 1981
†. Gropp *et al.* 1982
‡ Adolph and Klein, 1983

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Table 2. *Allele frequencies at the 25 polymorphic loci. Locality numbers are identified in Fig. 1 and Table 3. The chromosomal localization (Chrom.) of the genes is indicated*

		Localities									
Locus		1	2	3	4	5	6	7	8	9	10
ALB-1	100	1·00	1·00	0·89	1·00	1·00	1·00	1·00	1·00	1·00	1·00
	98	—	—	0·11	—	—	—	—	—	—	—
AMY-1	100	1·00	1·00	0·94	1·00	1·00	1·00	1·00	1·00	0·19	0·85
	80	—	—	0·06	—	—	—	—	—	0·81	0·15
CAR-2	100	1·00	1·00	1·00	1·00	1·00	0·75	0·94	0·97	1·00	1·00
	120	—	—	—	—	—	—	—	0·03	—	—
	80	—	—	—	—	—	0·25	0·06	—	—	—
ES-1	94	1·00	1·00	1·00	1·00	1·00	1·00	0·94	1·00	1·00	1·00
	100	—	—	—	—	—	—	0·06	—	—	—
ES-2	100	1·00	1·00	1·00	0·79	0·47	0·29	0·67	1·00	1·00	1·00
	99·0	—	—	—	—	—	—	—	—	—	—
	98·5	—	—	—	—	—	—	—	—	—	—
	0	—	—	—	0·21	0·53	0·71	0·33	—	—	—
ES-3	102	1·00	0·67	0·89	0·96	1·00	1·00	1·00	1·00	0·93	1·00
	100	—	—	—	—	—	—	—	—	0·07	—
	80	—	0·33	—	0·04	—	—	—	—	—	—
	110	—	—	0·11	—	—	—	—	—	—	—
	180	—	—	—	—	—	—	—	—	—	—
ES-5	100	0·18	0·29	—	0·24	0·25	0·18	0·26	0·46	1·00	1·00
	0	0·82	0·71	1·00	0·76	0·75	0·82	0·74	0·54	—	—
ES-10	100	1·00	1·00	1·00	0·94	1·00	1·00	1·00	1·00	1·00	0·80
	60	—	—	—	0·06	—	—	—	—	—	0·20
ES-15	100	1·00	0·67	0·88	0·85	0·92	1·00	1·00	0·84	0·27	0·45
	90	—	0·33	0·12	0·15	0·08	—	—	0·16	0·73	0·55
GLO-1	100	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	0·78	1·00
	40	—	—	—	—	—	—	—	—	0·22	—
	45	—	—	—	—	—	—	—	—	—	—
	120	—	—	—	—	—	—	—	—	—	—
GOT-2	100	0·43	0·25	—	0·75	0·18	—	0·50	0·40	0·04	0·05
	50	0·57	0·75	1·00	0·25	0·82	1·00	0·50	0·60	0·96	0·95
	30	—	—	—	—	—	—	—	—	—	—
	N	7	6	9	24	14	6	9	20	24	10

		Localities									
Locus		11	12	13	14	15	16	17	18	19	20
ALB-1	100	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00
	98	—	—	—	—	—	—	—	—	—	—
AMY-1	100	—	0·80	0·73	0·87	0·63	0·79	—	1·00	0·62	0·65
	80	1·00	0·20	0·27	0·13	0·37	0·21	1·00	—	0·38	0·35
CAR-2	100	1·00	0·89	0·90	0·80	1·00	1·00	0·95	1·00	0·98	1·00
	120	—	0·11	0·10	0·20	—	—	0·05	—	0·02	—
	80	—	—	—	—	—	—	—	—	—	—
ES-1	94	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00
	100	—	—	—	—	—	—	—	—	—	—
ES-2	100	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00
	99·0	—	—	—	—	—	—	—	—	—	—
	98·5	—	—	—	—	—	—	—	—	—	—
	0	—	—	—	—	—	—	—	—	—	—
ES-3	102	1·00	0·95	0·92	1·00	0·75	—	0·95	0·87	1·00	1·00
	100	—	—	—	—	—	—	—	—	—	—
	80	—	0·05	0·08	—	—	—	0·05	0·13	—	—
	110	—	—	—	—	0·25	—	—	—	—	—
	180	—	—	—	—	—	1·00	—	—	—	—
ES-5	100	—	0·18	1·00	0·36	0·21	0·24	—	0·32	0·47	0·68
	0	1·00	0·82	—	0·64	0·79	0·76	1·00	0·68	0·53	0·32
ES-10	100	1·00	1·00	1·00	0·75	1·00	1·00	0·15	0·96	0·96	0·78
	60	—	—	—	0·25	—	—	0·85	0·04	0·04	0·22
ES-15	100	0·79	0·50	0·50	0·50	0·19	0·29	0·35	0·86	0·70	0·87
	90	0·21	0·50	0·50	0·50	0·81	0·71	0·65	0·14	0·30	0·13

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Table 2 (*cont.*)

Locus		Localities 11	12	13	14	15	16	17	18	19	20
GLO-1	100	1·00	1·00	0·95	1·00	1·00	1·00	1·00	1·00	1·00	1·00
	40	—	—	—	—	—	—	—	—	—	—
	45	—	—	—	—	—	—	—	—	—	—
	120	—	—	0·05	—	—	—	—	—	—	—
GOT-2	100	—	0·22	0·33	0·33	0·44	1·00	0·95	0·10	0·16	—
	50	1·00	0·78	0·56	0·67	0·56	—	0·05	0·90	0·84	1·00
	30	—	—	0·11	—	—	—	—	—	—	—
	N	12	10	10	13	8	12	11	16	27	30

Locus		Localities 21	22	23	24	25	26	27	28	Chrom.
ALB-1	100	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	5
	98	—	—	—	—	—	—	—	—	
AMY-1	100	—	0·12	—	0·77	0·46	0·56	1·00	0·06	3
	80	1·00	0·88	1·00	0·23	0·54	0·44	—	0·94	
CAR-2	100	1·00	0·73	0·78	0·90	0·72	0·75	0·91	0·75	3
	120	—	0·27	0·22	0·10	0·28	0·25	0·09	0·25	
	80	—	—	—	—	—	—	—	—	
ES-1	94	1·00	1·00	1·00	0·99	0·81	1·00	1·00	1·00	8
	100	—	—	—	0·01	0·19	—	—	—	
ES-2	100	1·00	0·75	1·00	0·03	0·41	—	0·04	1·00	8
	99·0	—	—	—	0·37	0·45	1·00	0·82	—	
	98·5	—	—	—	0·60	0·14	—	0·14	—	
	0	—	0·25	—	—	—	—	—	—	
ES-3	102	1·00	1·00	0·96	0·99	0·82	0·56	0·86	0·50	11
	100	—	—	—	—	—	—	—	—	
	80	—	—	0·04	0·01	0·18	0·44	0·14	0·50	
	110	—	—	—	—	—	—	—	—	
	180	—	—	—	—	—	—	—	—	
ES-5	100	1·00	0·61	0·78	0·79	0·32	—	0·04	0·65	8
	0	—	0·39	0·22	0·21	0·68	1·00	0·96	0·35	
ES-10	100	1·00	1·00	1·00	0·98	0·62	1·00	1·00	1·00	14
	60	—	—	—	0·02	0·38	—	—	—	
ES-15	100	0·90	0·78	0·37	1·00	1·00	1·00	1·00	0·81	—
	90	0·10	0·22	0·63	—	—	—	—	0·19	
GLO-1	100	1·00	1·00	1·00	0·99	1·00	1·00	1·00	1·00	17
	40	—	—	—	—	—	—	—	—	
	45	—	—	—	0·01	—	—	—	—	
	120	—	—	—	—	—	—	—	—	
GOT-2	100	—	0·03	0·08	0·92	0·79	0·89	0·77	1·00	8
	50	1·00	0·97	0·92	0·08	0·21	0·11	0·23	—	
	30	—	—	—	—	—	—	—	—	
	N	6	18	27	43	21	13	13	10	

Locus		Localities 1	2	3	4	5	6	7	8	9	10
GPI-1	80	0·64	1·00	1·00	1·00	1·00	1·00	1·00	0·97	1·00	0·90
	100	0·36	—	—	—	—	—	—	0·03	—	—
	60	—	—	—	—	—	—	—	—	—	0·10
HBB	S	1·00	0·92	1·00	0·65	0·75	0·67	0·94	0·47	1·00	0·83
	D	—	0·08	—	0·35	0·25	0·33	0·06	0·53	—	0·17
IDH-1	100	—	0·58	0·67	0·23	—	—	0·19	0·47	0·79	0·65
	125	1·00	0·42	0·33	0·77	1·00	1·00	0·81	0·53	0·21	0·35
LDH-1	100	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00
	110	—	—	—	—	—	—	—	—	—	—
LDH-2	100	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00
	110	—	—	—	—	—	—	—	—	—	—
LDR-1	A	—	0·41	—	—	0·38	—	—	0·33	0·53	0·77
	B	1·00	0·59	1·00	1·00	0·62	1·00	1·00	0·67	0·47	0·23

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Table 2 (*cont.*)

Locus		Localities 1	2	3	4	5	6	7	8	9	10
MOD-1	140	—	—	—	—	—	—	—	—	—	—
	120	0·57	0·75	1·00	0·81	0·89	1·00	0·94	0·77	0·85	0·95
	100	0·43	0·25	—	0·19	0·11	—	0·06	0·23	0·15	0·05
MOD-2	110	0·29	—	—	0·47	0·71	0·83	0·44	0·10	—	—
	100	0·71	1·00	1·00	0·53	0·29	0·17	0·56	0·90	1·00	1·00
MPI-1	100	1·00	1·00	0·67	1·00	1·00	1·00	1·00	1·00	1·00	1·00
	120	—	—	0·33	—	—	—	—	—	—	—
	80	—	—	—	—	—	—	—	—	—	—
	60	—	—	—	—	—	—	—	—	—	—
NP-1	100	1·00	1·00	0·22	0·58	0·54	0·67	0·94	1·00	1·00	1·00
	90	—	—	0·78	0·42	0·46	0·33	0·06	—	—	—
PGM-1	100	1·00	1·00	1·00	0·98	0·93	0·92	1·00	0·97	1·00	1·00
	120	—	—	—	0·02	0·07	0·08	—	0·03	—	—
	80	—	—	—	—	—	—	—	—	—	—
PGM-2	100	1·00	1·00	1·00	1·00	0·32	0·25	1·00	1·00	1·00	1·00
	80	—	—	—	—	0·68	0·75	—	—	—	—
PK-3	100	1·00	0·75	1·00	1·00	1·00	1·00	1·00	0·89	1·00	0·85
	120	—	0·25	—	—	—	—	—	0·11	—	0·15
TRF	100	1·00	1·00	1·00	0·87	1·00	1·00	1·00	1·00	1·00	1·00
	110	—	—	—	—	—	—	—	—	—	—
	90	—	—	—	0·13	—	—	—	—	—	—
	N	7	6	9	24	14	6	9	20	24	10

Locus		Localities 11	12	13	14	15	16	17	18	19	20
GPI-1	80	1·00	0·95	1·00	1·00	0·94	1·00	1·00	0·93	1·00	1·00
	100	—	0·05	—	—	0·06	—	—	0·07	—	—
	60	—	—	—	—	—	—	—	—	—	—
HBB	S	0·83	0·94	1·00	1·00	0·93	0·42	0·50	0·93	0·67	0·88
	D	0·17	0·06	—	—	0·07	0·58	0·50	0·07	0·33	0·12
IDH-1	100	1·00	0·40	0·68	0·64	0·31	0·25	—	0·18	0·25	0·32
	125	—	0·60	0·32	0·36	0·69	0·75	1·00	0·82	0·75	0·68
LDH-1	100	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00
	110	—	—	—	—	—	—	—	—	—	—
LDH-2	100	1·00	1·00	1·00	1·00	1·00	1·00	0·95	1·00	1·00	1·00
	110	—	—	—	—	—	—	0·05	—	—	—
LDR-1	A	1·00	0·33	—	0·58	—	—	0·32	0·38	—	0·20
	B	—	0·67	1·00	0·42	1·00	1·00	0·68	0·62	1·00	0·80
MOD-1	140	—	—	—	—	—	—	—	—	—	0·05
	120	0·79	0·75	0·63	0·72	0·33	0·25	1·00	0·53	0·56	0·92
	100	0·21	0·25	0·37	0·28	0·67	0·75	—	0·47	0·44	0·03
MOD-2	110	0·83	0·06	0·27	1·00	0·80	0·71	0·25	0·04	0·09	—
	100	0·17	0·94	0·73	—	0·20	0·29	0·75	0·96	0·91	1·00
MPI-1	100	1·00	1·00	1·00	1·00	1·00	1·00	0·90	1·00	0·88	1·00
	120	—	—	—	—	—	—	—	—	—	—
	80	—	—	—	—	—	—	0·10	—	—	—
	60	—	—	—	—	—	—	—	—	0·12	—
NP-1	100	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00
	90	—	—	—	—	—	—	—	—	—	—
PGM-1	100	1·00	0·89	0·95	0·70	1·00	0·73	1·00	1·00	0·84	0·97
	120	—	—	—	—	—	—	—	—	—	—
	80	—	0·11	0·05	0·30	—	0·27	—	—	0·16	0·03
PGM-2	100	1·00	0·81	0·96	1·00	0·94	1·00	1·00	1·00	1·00	1·00
	80	—	0·19	0·04	—	0·06	—	—	—	—	—
PK-3	100	1·00	1·00	0·80	1·00	0·81	0·88	1·00	1·00	0·73	1·00
	120	—	—	0·20	—	0·19	0·12	—	—	0·27	—
TRF	100	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	0·98	0·96
	110	—	—	—	—	—	—	—	—	0·02	0·04
	90	—	—	—	—	—	—	—	—	—	—
	N	12	10	10	13	8	12	11	16	27	30

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Table 2 (*cont.*)

Locus		Localities 21	22	23	24	25	26	27	28	Chrom.
GPI-1	80	1·00	0·96	1·00	—	0·32	0·28	0·75	0·94	7
	100	—	0·04	—	1·00	0·68	0·72	0·25	0·06	
	60	—	—	—	—	—	—	—	—	
HBB	S	1·00	1·00	0·82	0·90	0·94	1·00	1·00	1·00	7
	D	—	—	0·18	0·10	0·06	—	—	—	
IDH-1	100	—	—	0·76	0·58	0·93	0·50	0·58	0·83	1
	125	1·00	1·00	0·24	0·42	0·07	0·50	0·42	0·17	
LDH-1	100	1·00	1·00	1·00	0·98	1·00	1·00	1·00	1·00	7
	110	—	—	—	0·02	—	—	—	—	
LDH-2	100	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	6
	110	—	—	—	—	—	—	—	—	
LDR-1	A	1·00	0·76	0·58	—	—	—	—	—	6
	B	—	0·24	0·42	1·00	1·00	1·00	1·00	1·00	
MOD-1	140	—	—	—	0·01	0·28	0·25	—	—	9
	120	—	0·47	0·88	0·97	0·58	0·75	1·00	0·94	
	100	1·00	0·53	0·12	0·02	0·14	—	—	0·06	
MOD-2	110	0·38	—	—	0·96	0·43	0·50	0·67	0·29	7
	100	0·62	1·00	1·00	0·04	0·57	0·50	0·33	0·71	
MPI-1	100	1·00	0·97	1·00	1·00	1·00	0·94	1·00	1·00	9
	120	—	0·03	—	—	—	0·06	—	—	
	80	—	—	—	—	—	—	—	—	
	60	—	—	—	—	—	—	—	—	
NP-1	100	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	14
	90	—	—	—	—	—	—	—	—	
PGM-1	100	1·00	0·90	1·00	1·00	1·00	1·00	0·95	1·00	5
	120	—	—	—	—	—	—	—	—	
	80	—	0·10	—	—	—	—	0·05	—	
PGM-2	100	1·00	1·00	1·00	0·98	1·00	1·00	1·00	1·00	4
	80	—	—	—	0·02	—	—	—	—	
PK-3	100	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	9
	120	—	—	—	—	—	—	—	—	
TRF	100	1·00	1·00	1·00	0·97	0·96	1·00	1·00	1·00	9
	110	—	—	—	0·03	0·04	—	—	—	
	90	—	—	—	—	—	—	—	—	
	N	6	18	27	43	21	13	13	10	

the mean values of expected heterozygosity at each of the polymorphic loci follow roughly the same pattern of variability in Rb and all-acrocentric mice. Furthermore, at most of these loci, the same set of alleles is segregating in both karyotypic groups; the only exception is the *Es*-2 locus at which two new variants were found in the German Rb populations (TUB-1, TUB-2, TUB-3 and RAV) which are not known to exist elsewhere in this semi-species (Britton-Davidian, 1985). Unique rare variants have been scored at eight loci but they occur in all-acrocentric as well as in Rb populations: *Alb*-1^{98}, *Es*-3^{180}, *Glo*-1^{120}, *Glo*-1^{45}, *Glo*-1^{40}, *Got*-2^{30}, *Ldh*-1^{110}, *Ldh*-2^{110}, *Mpi*-1^{80}, *Mpi*-1^{60} and *Trf*^{90}. Although the mean number of mice per locality is higher in the Rb sample ($\bar{n} = 21{\cdot}1$) than the all-acrocentric one ($\bar{n} = 12{\cdot}6$), the distribution of the number of alleles per sample size is similar in both groups (see insert in Fig. 3). The *Trf* locus will be further discussed below for, although it is very rarely polymorphic in the genus *Mus*, the same rare allele (110) was recorded in three Rb systems (W. Germany, Italy and additional unpublished results from other Rb populations of the Barcelona region).

It appears then, from this genetical analysis that all-acrocentric and Rb house mouse populations share a comparable amount of genic variability and a similar allelic distribution at most of the 34 loci studied. Thus, the structural modification brought about by centric fusions has had no effect on the level or nature of genic variability.

(iii) *Genic differentiation and hybrid sterility*

The decrease in fertility of chromosomal heterozygotes can be estimated from the pairing configuration of chromosomes during meiosis. Three possibilities exist, trivalents, chains and rings, the first two of which will be discussed (Fig. 1).

Trivalents are formed in all heterozygotes between Rb populations and 40-chromosome populations and in some heterozygotes between different Rb populations (i.e. ORK-2 and SCO; TUB-1, -2, -3 and RAV, Table 1). The decrease in fertility associated with trivalents is 0–4% for one trivalent (Winking, 1986) and 51–77% when 7–9 fusions are involved (Winking & Gropp, 1976). Chains are present in

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Table 3. *Genic variability estimates for the 34 loci studied in the all-acrocentric and Rb populations sampled. P = % of polymorphic loci at the 0·95 level; H = expected heterozygosity computed according to Nei (1978); A = mean number of alleles per locus; N = number of mice (deviation from the Hardy–Weinberg distribution was tested, when applicable, at all the polymorphic loci using Chi-square; no significant departures were recorded)*

Locality	2n	*N*	*P*	$\bar{H}$	$\bar{A}$
France					
1. ENSA	40	7	15	0·07	1·15
2. Pages	40	6	26	0·11	1·26
3. Haute-Savoie	40	9	21	0·06	1·21
4. Le Bourget	40	24	32	0·12	1·32
5. Les Deux Guiers	40	14	32	0·12	1·32
6. Le Marais	40	6	23	0·09	1·23
7. Iseron	40	9	29	0·08	1·29
8. Pratx	40	20	26	0·11	1·26
9. Dordogne	40	24	21	0·07	1·21
10. Toulouse	40	10	29	0·08	1·29
Spain					
11. Colmenar	40	12	12	0·04	1·12
12. SP-1	38–31	10	41	0·12	1·41
13. SP-2	40	10	32	0·12	1·35
14. SP-3	40	13	29	0·13	1·29
Algeria					
15. Oran	40	8	35	0·12	1·35
16. Bouzadjar	40	12	26	0·10	1·26
Tunisia					
17. Sfax	40	11	29	0·08	1·29
Italy					
18. Ovada	40	16	26	0·08	1·26
19. Binasco	24	27	32	0·12	1·32
20. Bergamo	22	30	23	0·08	1·26
Great Britain					
21. ORK-2	34	6	6	0·02	1·06
22. ORK-1	40	18	23	0·09	1·23
23. SCO	36–34	27	23	0·08	1·23
West Germany					
24. TUB-1	38	43	20	0·08	1·23
25. TUB-2	38	21	38	0·16	1·44
26. TUB-3	38	13	26	0·11	1·26
27. RAV	37–35	13	23	0·07	1·26
Netherlands					
28. AMS	40	10	26	0·08	1·26
All-acrocentric N_{total}	239	Mean ± S.D.	25 ± 3	0·09 ± 0·01	1·26 ± 0·03
Robertsonian —	190	—	26 ± 8	0·09 ± 0·03	1·27 ± 0·08

heterozygotes between populations showing monobrachial homology of fusions. This occurs when the metacentrics in the two populations have only one chromosome arm in common (i.e. Binasco/Bergamo, Table 1). Impairment of fertility is very severe leading in some cases to full sterility in males and a minimum of 24–43% sterility in females (Gropp, 1974; Gropp & Kolbus, 1974). These data suggest that a substantial amount of genic isolation can be reached when either (i) up to 9 metacentrics are fixed (i.e. between Bergamo and Ovada which differ by 9 fusions and Binasco and Ovada which differ by 8, Table 1), or (ii) when chains are present in the heterozygotes (a heterozygote between Binasco and Bergamo has one pentavalent chain).

Divergence and differentiation estimates were computed from electrophoretic data by means of Nei's distance (Nei, 1978) and Wright's F_{ST} index (Wright, 1965) in the light of these estimates of hybrid sterility. Only neighbouring populations were compared to eliminate possible biases due to macrogeographic differentiation patterns. Results show that in all cases (Table 4), whether between all-acrocentric and Rb populations (Rb/40) or between the latter (Rb/Rb), genic differentiation is no larger than that between adjacent populations of all-acrocentric mice (Isere transect, localities 3–7). The observed levels of genic differentiation are then not related to the amount of genic isolation expected by the degree of hybrid sterility.

4. Discussion

(i) *Genic similarity*

Chromosomally differentiated house mice exhibit a considerable amount of genic similarity. Persistence

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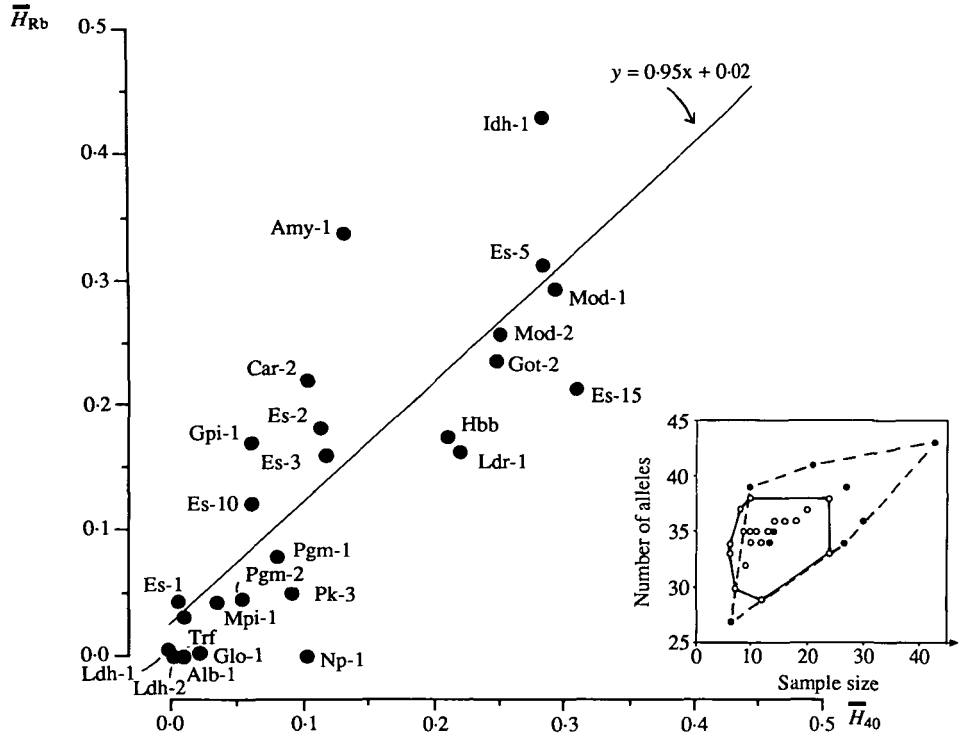

Fig. 3. Correlation of heterozygosity values for polymorphic loci between all-acrocentric ($\bar{H}_{40}$) and Rb populations ($\bar{H}_{Rb}$). Coefficient of correlation, $r = 0{\cdot}817$ ($r = 0$, $P < 0{\cdot}001$). The insert shows the distribution of the number of alleles per sample size for the 25 polymorphic loci. Black circles represent Rb populations and empty circles, the all-acrocentric ones. Polygones are drawn corresponding to the extreme distributions for both chromosomal groups.

Table 4. *Genic differentiation according to the nature of the barrier to gene flow in Rb and all-acrocentric populations. Numbers in parenthesis refer to the localities used in the pairwise computations (see Table 3 for correspondence)*

Nature of barrier	2n	Localities	F_{ST}	D_{NEI}
Geographic				
Regional	40	France (1–10)	0·336	0·05
	40	Spain (11, 13, 14)	0·303	0·04
	40	Algeria (15, 16)	0·158	0·04
		Mean	0·266	0·04
Local	40	Isere (3–7)	0·244	0·03
	38	Tubingen (24–26)	0·206	0·05
		Mean	0·255	0·04
Chromosomal				
Trivalents	Rb/40	Bergamo/Ovada (18, 20)	0·073	0·01
	Rb/40	Binasco/Ovada (18, 19)	0·061	0·01
	Rb/40	SP-1/SP-2 (12, 13)	0·097	0·02
		Mean	0·077	0·01
	Rb/Rb	TUB-1/RAV (24, 27)	0·265	0·07
	Rb/Rb	TUB-2/RAV (25, 27)	0·119	0·04
	Rb/Rb	TUB-3/RAV (26, 27)	0·073	0·02
		Mean	0·152	0·04
Chains	Rb/Rb	Binasco/Bergamo (19, 20)	0·054	0·01

of gene flow cannot be ruled out and may likely be the factor responsible for the genic similarity of populations differing by a small number of fusions since hybrid sterility in these cases is slight. However, this no longer holds true for populations such as the three parapatric Italian populations, two of which (Bergamo and Binasco) are known to be nearly totally isolated through monobrachial homology and are also substantially isolated from the all-acrocentric mice (Ovada) by a high number of fusions, and yet yield the smallest levels of genic differentiation.

Neither do similar selective pressures appear to

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account strongly for the genetic similarity of these mice populations as suggested by the following arguments: (i) local populations of commensal mice show the same level of genic diversity as do distant ones (see diversity analysis below); (ii) mice from nearby commensal and feral localities exhibit only slight genic differentiation ($D = 0{\cdot}042 \pm 0{\cdot}027$, $n = 7$, Southern France, Britton-Davidian, 1985).

Time scale. The absence of genic differentiation may, however, be related to time since isolation. Genic differentiation in isolated populations is known to be time-related, the accumulation of genic differences being a function of the mutation rate and the fixation probability (Nei, 1971; Wilson *et al.* 1985). Fossil remains attributed to *Mus musculus* have been dated back to the Middle Pleistocene in Eastern Europe; however, they are no longer found in subsequent rodent rich deposits until the Upper Holocene at which time the presence of mice is once again recorded in Central Europe (Janossy, 1961). Thus the onset of the Rb process cannot be dated earlier than about 10000 years. In the case of the Italian populations, an even later date is advanced by Capanna (1982), who states that human settlements were established in the Alpine regions only 3500 years B.C. which would yield a rate of fixation of centric fusions roughly equivalent to 1 in every 600 years. The three Italian populations would then have reached a substantial degree of genic isolation only 1200 years ago when an 8th fusion was fixed in the putative ancestral Rb population (Rb(6·7) for Binasco and either Rb(7·18) or Rb(1·6) for Bergano, see Table 1). Such an event led to the isolation of the Bergamo and Binasco populations not only from the neighbouring all-acrocentric mice but also between them through monobrachial homology.

Our results suggest that, in those cases in which fixation of Rb fusions has led to a considerable level of post-mating isolation (50–100%), the genic similarity observed should be attributed to a very recent common ancestry rather than to the persistence of gene flow. The involvement of a small time scale in the Rb process was also suggested by Ferris *et al.* (1983), based on the low degree of mt DNA divergence between Rb and all-acrocentric mice. Furthermore, our data indicate that chromosomal divergence can proceed much faster than genic differentiation.

(ii) *Origin of Rb populations*

The Rb phenomenon in mice consists of an array of geographically separated systems composed of one to several parapatric Rb races which share one or more fusions. The phylogenetic relatedness of Rb populations within a polytypic system can be easily accounted for by the model of cumulative fusions within a single ancestral population as postulated by Capanna *et al.* (1977) and White (1978). Additional arguments for this view have been provided by serological data of H-2 polymorphisms (Figueroa *et al.* 1982) and chromosomal data (Corti *et al.* 1986).

However, the presence of the same Rb translocations in populations belonging to different geographic systems questions the relatedness of the different systems to one another. Two alternative views have been proposed based on the multiple or unique generation of Rb translocations.

(1) In the first view supported by the cladistic analysis of chromosomal phylogenies in mice (Larson *et al.* 1984; Corti *et al.* 1986), geographically separated Rb systems originated independently and *in situ*, suggesting that fusions may have multiple origins. In support of this view, several data are proposed based on the distribution of Rb translocations (see Tichy & Vucak, 1987 for an updated version of the chromosomal composition of the different Rb populations): (i) a total of 80 fusions have been reported in wild house mice, 45 of which are unique to distinct Rb systems. The remaining 35 fusions are common to 2, at the least, and 5, at the most, geographically separated systems; (ii) Rb translocations present in one or several systems also occur as rare fusions in other regions (i.e. Rb(10·11) in the Apennines and Denmark, V. Bolomier, unpublished observations), Rb(3·8) in Denmark, Lombardy, the Apennines and Great Britain; (iii) several geographically remote Rb systems share a large number of fusions (i.e. Scotland and the Apennines share four fusions), whereas nearby systems may not (the Rb populations in Thebes and Northwestern Peloponnesus); (iv) finally, one Rb population can share as many as six fusions with other populations dispersed throughout Europe and North Africa (i.e. the Northwestern Peloponnesus population with Lombardy, Southern Germany, the Apennines, Tunisia and the Lipari Islands). Additional indications that individual chromosomal rearrangements may not be unique events have received recent support in other rodent genera (Qumsiyeh *et al.* 1988; Rogers *et al.* 1984) in which the most accurate interpretation involves the convergent occurrence of fusions/fissions and pericentric inversions.

(2) The alternative view is based on the assumption that fusions are unique events, the probability of the same fusion occurring twice by chance being very small (Sage, 1981). Based on the intense sea traffic that has promoted migration of mice, particularly along the Mediterranean coast, Tichy & Vucak (1987) argue that the Rb process originated in a limited number of geographic areas from which the translocations spread through chromosome flow to the regions in which they are now present. Once these fusions become established in a new geographic area, additional unique fusions accumulate and become characteristic of the geographic system. These hypotheses can be tested using the genetic information presented here. If all Rb systems have a common origin, they will appear genically more related to each other than to all-acrocentric populations. On the other hand, if

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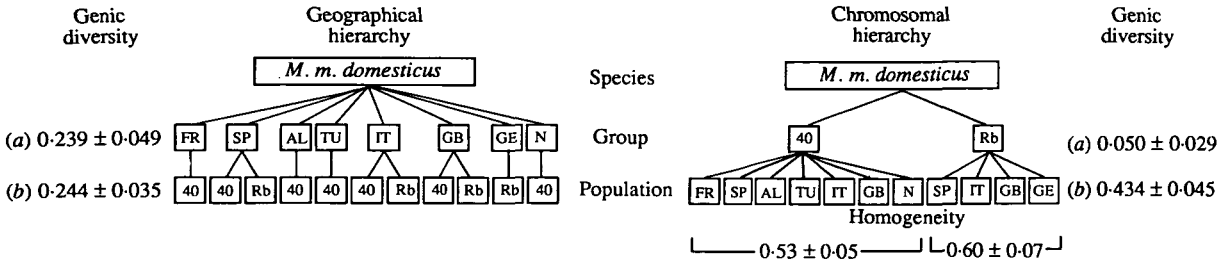

Fig. 4. Schematical representation of the hierarchical classification using a geographical or chromosomal criterion for grouping data (capital letters refer to countries of of origin of samples; 40 = all-acrocentric and Rb = Robertsonian populations). Two measures of diversity for 25 polymorphic loci are indicated relative to the total genic diversity in the taxon: (*a*) intergroup diversity and (*b*) interpopulation intragroup diversity (standard errors are indicated).

geographically isolated Rb systems developed independently, they will be more closely related to neighbouring all-acrocentric populations.

Diversity analysis. Genic diversity indexes (Lewontin, 1972) were computed and compared by assembling the data in two different arrays (see Fig. 4):

(1) geographical groups in which samples were pooled according to their geographic origin, regardless of their chromosome number;

(2) karyotypic groups formed by pooling all Rb populations in one group and all-acrocentric mice in the other regardless of their geographical origin.

For populations grouped by chromosomal affinities, the highest level of diversity is that of the within group value ($0{\cdot}434 \pm 0{\cdot}045$), while it is very low between the 40-chromosome and Rb groups ($0{\cdot}050 \pm 0{\cdot}029$). So, chromosome differentiation does not contribute to diversity in this semi-species. Because a bias in this computation could occur if one of the groups (Rb or all-acrocentric) was much more diverse than the other, the interpopulation diversity index relative to that of the group was calculated. Results show that both groups share on the average a similar level of homogeneity (all-acrocentric = $0{\cdot}53 \pm 0{\cdot}05$, Rb = $0{\cdot}60 \pm 0{\cdot}07$). On the other hand, in the geographical array, the within group value of diversity drops to $0{\cdot}244 \pm 0{\cdot}035$ which is similar to that between groups ($0{\cdot}239 \pm 0{\cdot}049$). That diversity between chromosomal races from the same region is smaller than that between regions is also in agreement with data for H-2 polymorphisms ($5{\cdot}76 \pm 0{\cdot}63$ and $9{\cdot}93 \pm 0{\cdot}93$ respectively; Nadeau *et al.* 1988). In other words (i) Rb systems do not share a common genic constitution differentiating them from all-acrocentric populations and (ii) Rb populations belonging to different Rb systems are more similar genically to neighbouring all-acrocentric populations than to each other. Such data argue in favour of the *in situ*, multiple origin of Rb populations.

Mutation rates. In regard to these results, a comment must be made on recent hypotheses concerning the origin of the Rb process. To account for the rapid rate of generation of Rb translocations as well as the distribution of the Rb populations and disparity in the number of fusions between populations, Winking (1986) following Sage (1981), Patton and Sherwood (1983) and Moriwaki *et al.* (1984) suggests that a mutator activity may be implicated in the genesis of the phenomenon. Considering that the process of Rb accumulation is complete in populations from Italy whereas it is in progress elsewhere, he further postulates that the mutagenic agent would have first been introduced into populations of Central and Northern Italy from which it spread to other regions of Europe through passive transport of mice by man. Such a hypothesis might be substantiated by our results at the *Trf* locus. The fact that the same rare variant (Trf^{110}) exists in three geographically separated Rb systems (at localities 19, 20, 24, 25 and unpublished results for an additional population from the Rb systems in Barcelona) might be considered as an indication of a common source of introduction of the mutagenic agent. However, similar electrophoretic properties of this protein variant do not necessarily equate with molecular identity and common ancestry, so further information is needed to confirm this observation.

Similarly, recent data from natural and experimental studies show that chromosomal mutation rates may be elevated in hybrid zones (Shaw *et al.* 1983). Such a mechanism may account for the five rare fusions occurring in hybrids between two differentiated Rb mice populations in Southern Germany (see Adolph and Klein, 1983). These observations encourage further investigations to ascertain the causes and rates of chromosomal mutations in nature.

An independent origin for the different Rb systems does not rule out the possibility that introgression of fusions may have occurred. However, the sharing of a common gene pool between Rb populations and the neighbouring all-acrocentric mice indicates that chromosomal flow would be very limited in extent and in any case cannot account for the numerous fusions common to different systems. This further suggests then that Rb translocations have multiple independent origins.

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(ii) Genic variability and population structure

The processes involved in the fixation of chromosomal rearrangements have been under considerable debate in recent years. In most models and simulations presented, the most favourable outcome even if selective forces are operating lies in population bottlenecks which cause sampling error to override negative heterosis due to abnormal meiotic effects (Lande, 1979; Futuyma & Mayer, 1980; Hedrick, 1981; Walsh, 1982). If fixation of chromosomal rearrangements requires strong genetic drift and/or inbreeding, one would expect a corresponding decrease in the genetic variability of such populations (Nei *et al.* 1975). The study of the Rb populations presented here has shown that chromosomal differentiation has not reduced the level of heterozygosity even though up to 9 founding events would be necessary to reach the chromosomal complement of some Rb populations.

Two hypotheses can be proposed to account for the absence of such a decrease in variability in the Rb house mouse populations.

(1) The size of the bottlenecks is large enough to prevent losses of genic variability. Fixation rates of chromosomal variants in bottlenecks of varying sizes was investigated by Chesser & Baker (1986). In simulations involving an initial founding population of 50 individuals, they show that no fixation of a chromosomal variant was scored in 1000 breeding periods even when no meiotic disadvantage was present. However, non-random processes such as meiotic drive, selective advantage of the homozygous mutant, or high mutation rates, which have been shown to increase the probability of fixation, may be occurring. These first two factors do not appear to apply to chromosomal differentiation in mice for the following reasons: meiotic drive which has been shown to occur for some Rb fusions, in all cases but one, favours the production of gametes without the translocated chromosome (Gropp & Winking, 1981); the exception is that of Rb(9·12) which arose spontaneously in a wild-derived laboratory strain of Peru-Coppock mice (Harris *et al.* 1986); additionally, the level of selective advantage needed by the new homokaryotype to achieve fixation is considered as probably unrealistically high by Hedrick (1981) and Walsh (1982). The third factor, high mutation rates, was investigated by comparing the observed rate of fixation of Rb translocations in mice with that computed according to Lande (1979) for varying mutation rates, selective disadvantage coefficients and bottleneck sizes (Fig. 5). Results show that, for the range of selection coefficients chosen ($0{\cdot}001 < s < 0{\cdot}05$), a mutation rate between 10^{-3} and 10^{-4} is sufficient to insure a fixation rate comparable to that observed, in large-sized bottlenecks (i.e. > 35 founding individuals which will produce almost no loss of variability in nuclear genes, see Sirkkomaa, 1983). So,

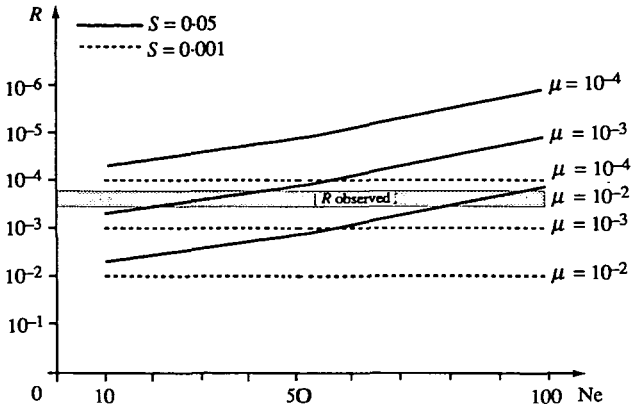

Fig. 5. Chromosomal fixation rates for varying bottleneck sizes, selective disadvantage coefficients and mutation rates. The range of the observed rate of fixation was computed considering that nine fusions are fixed in 5500 to 10000 years and 3–4 generations of mice/year. Theoretical fixation rates (R) were calculated according to the formulae provided by Lande (1979) for three bottleneck sizes ($Ne = 10, 50, 100$), three mutation rates ($u = 10^{-2}, 10^{-3}, 10^{-4}$) and two selection coefficients ($s = 0{\cdot}001, 0{\cdot}05$), the latter being within the range provided by the data on hybrid sterility (Winking, 1986).

fixation of chromosomal variants in large bottlenecks is compatible with even a relatively conservative range of mutation rates (see Lande, 1979, for discussion of spontaneous mutation rates for chromosomal variants).

(2) The bottleneck events during which fixation of chromosomal rearrangements and decrease of genetic variability occur, were followed by periods of extensive gene flow. As suggested by Capanna *et al.* (1977) and White (1978), chromosomal differentiation in mice occurs through the accumulation of Rb translocations which are acquired successively by the same population. Therefore, at each step in the process of accumulation, the populations in contact differ by only a small number of translocations resulting in a minimal loss of fertility as shown previously. Thus gene flow is only slightly reduced suggesting that variability which may have been diminished by genetic drift, can be restored to its previous level (see Spirito *et al.* 1983 for theoretical support). However, for genic variability to be recovered, genetic recombination must be maintained between metacentrics and their acrocentric homologs. Although suppression of recombination has been documented for certain fusions in laboratory crosses (Cattanach, 1978), support for continued recombinational events has been found in the Rb populations from Tunisia (Said *et al.* 1986). Furthermore, if fixation is taking place in small bottlenecks, this implies that the demes in which centric fusions are being fixed do not remain isolated for extended periods of time. In this model, reproductive isolation becomes effective only when the most chromosomally differentiated populations come into contact.

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That fixation of Rb translocations may have occurred through genetic drift during moderate or extreme bottlenecks is suggested by preliminary studies of mt DNA variability and H-2 polymorphisms. In an analysis of mt DNA variation within *M. m. domesticus*, Ferris *et al.* (1983) indicate that Rb populations may be less variable than all-acrocentric mice. However, more extensive sampling is necessary to confirm these results. Additionally, estimates of H-2 variability (H-2K and H-2D) show that Rb populations have suffered a 24% loss in mean heterozygosity and a 42% loss in mean number of alleles as compared to all-acrocentric mice (computed from data in Nadeau *et al.* 1981). These results suggest then that the size of the bottlenecks was sufficiently small to produce a loss of variability at these loci. However, both of these genetic systems are more sensitive to founder effects than are allozyme polymorphisms, the mt DNA molecule because the effective population size is $\frac{1}{4}$ of that for nuclear genes (Wilson *et al.* 1985) and H-2 genes because they are multiallelic loci (up to 13 alleles were recorded in one population). Thus, the mtDNA and H-2 results could be consistent with either model, although caution must be expressed in their interpretation, for H-2, mt DNA and allozyme polymorphisms could be subject to very different selective pressures (cf. Nadeau *et al.* 1988).

The data presented here indicate that chromosomal divergence has occurred without leading to genetic impoverishment, although the accumulation of slightly deleterious chromosomal rearrangements can lead to a substantial amount of reproductive isolation (see Walsh, 1982 for theoretical support). Results suggest that Rb systems have originated independently through fixation of centric fusions within local all-acrocentric mice from which they are only slightly differentiated owing to the very recent acquisition of reproductive isolation. The processes of karyotype differentiation in mice described herein are compatible with both the cumulative fusion model (Capanna *et al.* 1977 and White, 1978) and the model of speciation by monobrachial homology (Baker & Bickham, 1986) as discussed by Sites & Moritz (1987). Both models consider chromosomal differences as post-mating barriers to gene flow, but differentiation between monobrachially homologous populations would be predicted to proceed faster to speciation owing to the higher heterozygote disadvantage it entails. Such a situation has been documented in Lombardy where premating barriers isolate two Rb populations showing monobrachial homology (Capanna *et al.* 1985*b*), whereas no evidence for such isolation has yet been observed between Rb and all-acrocentric mice.

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