The Origin of a Robertsonian Chromosomal Translocation in House Mice Inferred from Linked Microsatellite Markers

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The western European house mouse, *Mus domesticus*, includes many distinct Robertsonian (Rb) chromosomal races. Two competing hypotheses may explain the distribution of Rb translocations found in different populations: they may have arisen independently multiple times, or they may have arisen once and been spread through long-distance dispersal. We investigated the origin of the Rb 5.15 translocation using six microsatellite loci linked to the centromeres of chromosomes 5 and 15 in 84 individuals from three Rb populations and four neighboring standard-karyotype populations. Microsatellite variation on the 5.15 metacentric chromosomes was significantly reduced relative to the amount of variation found on acrocentric chromosomes 5 and 15, suggesting that linked microsatellite loci can track specific mutational events. Phylogenetic analyses resulted in trees which are consistent with multiple origins of the 5.15 metacentric chromosomes found in the three Rb populations. These results suggest that cytologically indistinguishable mutations have arisen independently in natural populations of house mice.

Introduction

A fundamental goal in evolutionary biology is to understand how new species arise. Strongly underdominant mutations, such as chromosomal rearrangements, may act as barriers to gene flow between populations fixed for alternative homozygotes due to the lowered fitness of heterozygous individuals (White 1978; Sites and Moritz 1987; King 1993). An extreme example of multiple chromosomal races is found in the Western European house mouse, *Mus domesticus.* Most *M. domesticus* have acrocentric chromosomes and a diploid number of 2n = 40. However, there are numerous populations with lower diploid numbers as a consequence of Robertsonian (Rb) whole-arm translocations (also called fusions). Approximately 90 different Rb chromosomes are known in *M. domesticus*, comprising over 40 distinct chromosomal races ranging in diploid number from 2n = 22 to 2n = 38 (Nachman and Searle 1995). These Rb populations are typically restricted in range and surrounded by populations of standard-karyotype (i.e., 2n = 40) mice. Most individuals within Rb races are homozygous with respect to their Rb rearrangements, although chromosomal hybrids are found in which either two different Rb races or a Rb race and a 2n = 40 population come into contact (e.g., Hauffe and Searle 1993). Many Rb races have unique combinations of acrocentric chromosome arms involved in translocations, although some Rb translocations are found in multiple geographically separated regions (e.g., Rb 1.3, formed from the fusion of acrocentric arms 1 and 3, is found in Greece and in northern Italy). The origin of metacentric chromosomes in house mice is presumably recent; *M. domesticus* probably entered western Europe within the last 5,500 years, and Rb populations are nearly absent outside of Europe (Auffray, Vanlerberghe, and Britton-Davidian 1990; Boursot et al. 1993). Rb translocations are essentially absent in the sister species *Mus musculus.* Anecdotal evidence suggests that new Rb populations can arise or be lost within decades (Hauffe and Searle 1992; Garagna, Zuccotti, and Redi 1997).

Many demonstrations of reduced fertility in experimental hybrid crosses have led to the conclusion that gene flow between Rb and acrocentric populations is low (Boursot et al. 1993; Searle 1993). However, several recent studies suggest that some gene flow occurs between Rb and 2n = 40 populations. First, some Rb heterozygotes produced from wild-caught mice do not exhibit the high rates of nondisjunction previously observed in crosses between wild Rb mice and 2n = 40 laboratory mice (Britton-Davidian et al. 1990; Viroux and Bauchau 1992; Wallace, Searle, and Everett 1992; Hauffe and Searle 1998). Second, in wild *M. domesticus*, hybrid zones are often found in which the two types of populations come into contact (Said and Britton-Davidian 1991; Searle 1993) and some of the hybrid zones may be quite wide (Searle 1991; Searle, Navarro, and Ganem 1993). Third, neighboring Rb and non-Rb populations typically have similar levels of genetic variability for allozymes (Britton-Davidian et al. 1989) and mtDNA (Nachman et al. 1994), a pattern consistent with gene flow across hybrid zones. Finally, in an experimental study in which Rb mice were released into an island population of 2n = 40 mice, the introduced Rb translocations spread quickly (Berry et al. 1990).

Two hypotheses may explain the evolutionary history of Rb races, although the true history of Rb mice may lie somewhere between these extremes. The traditional view has been that many or most Rb races arose independently of one another, each presumably from a nearby 2n = 40 population (e.g., Sage 1981; Capanna and Redi 1988). The observation that many Rb populations have disjunct geographic distributions and unique sets of translocations is consistent with this view. This hypothesis implies that identical Rb translocations have arisen independently in different populations. Support for this multiple-origin hypothesis comes from studies of allozymes (Britton-Davidian et al. 1990) and

Abbreviation: Rb, Robertsonian.

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Table 1
Genetic Distances of Microsatellite Loci from the Centromeres of Chromosomes 5 and 15 in Two Different Genetic Maps

Locus	Genetic Distance (cM)[a]	Genetic Distance (cM)[b]
D5mit47	0	1.0
D5mit49	0	0
D5mit145	0	0
D15mit12	0	4.7
D15mit13	0	6.7
D15mit174	0	4.3

[a] Whitehead Institute/MIT map. Distances were generated from an (OB × CAST) F_2 intercross (Dietrich et al. 1992, 1996).

[b] Mouse Genome Database map. Distances were generated from a composite map based on large multilocus crosses (url: http://www.informatics.jax.org/).

mtDNA (Nachman et al. 1994), which show that Rb populations do not form a monophyletic group within *M. domesticus.*

The alternative view is that many or most Rb races derive from a single lineage of *M. domesticus* which has been spread by long-distance dispersal (Tichy and Vucak 1987; Bauchau 1990). Long-distance dispersal may be quite common in house mice, since they are commensal with humans. This hypothesis implies that mice which gave rise to present-day Rb populations carried either no translocations or few translocations (those that are widely shared), since many Rb populations today harbor unique sets of translocations. Support for a single origin of some Rb populations comes from a phylogenetic analysis of Y chromosome variation in which Rb races from three geographically distant populations in Italy formed a clade (Tucker, Lee, and Eicher 1989). Evidence that geographically proximate Rb populations may have a common origin comes from the observation that such populations often share sets of Rb chromosomes (Capanna and Redi 1988). For example, the metacentrics 5.15, 9.14, 11.13, and 16.17 are shared among several Rhaetian Alps races (Capanna and Redi 1988; Bauchau 1990; Hauffe and Pialek 1997).

Previous efforts to uncover the phylogenetic history of Rb races or of individual Rb chromosomes have been complicated by several factors. First, because of their recent origin, Rb and 2n = 40 races show little genetic differentiation. Second, it may be impossible to identify a single population phylogeny for Rb races, since individual loci may have different genealogies. Third, current or recent gene flow between Rb and 2n = 40 animals may obscure the history of Rb races. Fourth, and perhaps most important, Rb chromosomes may have evolutionary histories that are quite distinct from loci that are not linked to Rb translocations (e.g., mtDNA, most allozymes). In general, linked genomic regions are expected to have correlated evolutionary histories, while recombination (and independent assortment) will create distinct genealogies for different genomic regions.

Here, we investigate the evolutionary history of one widespread Rb chromosome by examining microsatellite loci linked to the translocation. The mouse microsatellite genetic map consists of over 6,000 $(CA)_n$ repeats mapped in a single (OB × CAST) F_2 intercross with an average marker spacing of less than 0.25 cM (Dietrich et al. 1992, 1996). We surveyed six microsatellite loci. Three of these loci cosegregate with the centromere of chromosome 5, and three of the loci cosegregate with the centromere of chromosome 15 in this cross (table 1). Chromosome 5.15 is one of the most widespread Rb fusions and is found in several karyotypically distinct populations (Winking, Dulic, and Bulfield 1988). We surveyed 84 mice from Rb races in northern Italy, central Italy, and Spain containing the Rb 5.15 chromosome and from 2n = 40 populations in each of these geographic regions (table 2). Under a hypothesis of multiple origins, we expect that the alleles found in a given Rb population will be a subset of those found in the neighboring acrocentric population and that a phylogeny of markers linked to the translocation breakpoint will reflect three independent origins. In contrast, under a single-origin hypothesis, markers linked to Rb 5.15 from each of the three Rb populations should form a monophyletic clade. Here, we demonstrate that the evolutionary history of Rb 5.15 is inconsistent with a single-origin hypothesis.

Materials and Methods

Samples, Chromosome Analysis, and DNA Preparation

Eighty-four *M. domesticus* were wild-caught in their native range in Italy and Spain. All mice were kar-

Table 2
Populations and Mice Sampled

Geographic Area	Population	Region	Diploid Number	Rb Chromosomes	Number of Mice	Specimen ID Numbers[a]
Northern Italy	Valley Curone	Piemonte	2n = 40	None	6	1024–1029
	Menconico	Milano	2n = 40	None	8	1030–1034, 1036, 1037, 1039
	Sernio	Lombardia	2n = 24	1.3, 2.8, 4.6, 5.15, 9.14, 10.12, 11.13, 16.17	9	1003–1009, 1011, 1012
Central Italy	Cassino	Lazio	2n = 40	None	22	1102–1119, 1122, 1124–1126
	Bonefro	Molise	2n = 22	1.18, 2.17, 3.13, 4.11, 5.15, 6.7, 8.14, 9.16, 10.12	19	1127, 1128, 1132–1147, 1149
Spain	La Roca del Valles	Barcelona	2n = 40	None	14	1285–1289, 1291–1299
	Avinyonet	Barcelona	2n = 30	4.14, 5.15, 6.10, 9.11, 12.13	6	1306–1311

[a] Specimen numbers refer to collector's numbers. Specimens and collector's notes have been deposited in the Museum of Zoology, University of Michigan.

yotyped as previously described (Nachman et al. 1994), and only individuals with homozygous karyotypes were included in the microsatellite analysis. A total of seven populations are represented in this study; three distinct Rb populations and four standard acrocentric populations, which are geographically proximate to the Rb populations, were sampled (table 2).

Genomic DNA was prepared from frozen liver or kidney tissue following Sambrook, Fritsch, and Maniatis (1989) with modifications described by Kocher et al. (1989). Tissue was macerated in 500 μl extraction buffer (100 mM Tris-HCl [pH 8.0], 10 mM EDTA, 100 mM NaCl, 0.1% SDS, 50 mM DTT, 0.5 mg/ml Proteinase K) and incubated at 55°C for 3–24 h. Then, 0.1 mg RNase was added for the final hour of incubation. The solution was extracted three times with phenol/chloroform, DNA was alcohol precipitated (50 μl 3 M NaOAc and 750 μl 95% isopropyl alcohol, followed by 70% ethanol), dried, and resuspended in pH 8.0 Tris-EDTA to a final concentration of 50 ng/μl DNA.

PCR Amplification of Microsatellite Loci

Six microsatellite loci were chosen for their proximity to the centromeres of chromosomes 5 and 15 (table 1). In some instances, it was not possible to select markers that mapped to the centromere in all crosses, so those available with the smallest distance to the centromere were selected. Primers for these markers were obtained from Research Genetics (Huntsville, Ala.).

DNA was amplified using PCR (Saiki et al. 1988) with *Taq* polymerase (Amersham) in 10-μl-volume reactions overlaid with mineral oil. The forward primer was end-labeled with γ-^{33}P ATP (Amersham) using T4 kinase (GibcoBRL) following the manufacturer's instructions. DNA was amplified in 35 cycles of 94°C for 45 s, 55°C or 57°C for 45 s, and 72°C for 1 min. Each primer pair was optimized for pH, $MgCl_2$, and KCl concentrations.

All PCR products were run on an 8% acrylamide gel with an end-labeled 10 bp DNA ladder (GibcoBRL). Gels were dried and exposed to film overnight. A subset of PCR products from each gel were electrophoresed together to verify fragment size. A second PCR reaction was attempted for individuals which had no apparent PCR products. Five individuals did not PCR amplify at one locus (MWN 1122: D15mit12; 1127: D5mit145; 1293: D5mit145; 1296: D5mit145; 1306: D15mit12); these five individuals were excluded from analysis only for the locus which did not amplify.

Data Analysis

Allele frequencies for each locus and population were tabulated. Two series of Monte Carlo row-by-column permutation tests were run, each with 10,000 trials. First, allele frequencies of all populations were permuted to determine whether there was significant population structure in the distributions of alleles. To check whether the two northern Italian acrocentric populations could be combined as one, a separate Monte Carlo permutation test was run for significant population structure between these two populations only.

The most common allele size, mean allele size, variance in repeat number, expected heterozygosity, and observed heterozygosity were calculated for each locus-population combination. To test for deviations between observed and expected heterozygosity, an exact test of Hardy-Weinberg equilibrium (Guo and Thompson 1992) using ARLEQUIN (Schneider et al. 1997) was performed for each locus-population combination. Variance in repeat number and expected heterozygosity in individual Rb populations were compared with those of their neighboring standard karyotypic populations by means of a Wilcoxon signed-ranks test (Sokal and Rohlf 1995) across all loci. Since two northern Italian 2n = 40 populations were included in the study, the average values of these two populations (Curone and Menconico) were compared with the values of their Rb neighbor, Sernio. For each locus, average variance and heterozygosity were calculated by weighting each population by the number of individuals sampled. The weighted locus averages of variance and heterozygosity in Rb and non-Rb populations were compared with a Mann-Whitney *U*-test (Sokal and Rohlf 1995).

A variety of distance measures can be used with microsatellite data; some distance measures have been formulated without assumptions about the underlying model of mutation and are not specific to properties of microsatellites. Several distance measures, such as D_{sw} (Shriver et al. 1995), $(\delta\mu)^2$ (Goldstein et al. 1995*b*), and R_{st} (Slatkin 1995) have been formulated under a stepwise model of mutation which attempts to account for the specific mutational properties of microsatellite repeats. Some traditional distance measures have been shown to outperform those based on a stepwise mutation model with empirical data (Paetkau et al. 1997) and simulations (Takezaki and Nei 1996) under conditions of recent divergence. In particular, D_c and D_a have higher probabilities of obtaining the correct tree topology under a variety of different conditions and underlying mutational models (Takezaki and Nei 1996). A further consideration is that measures of divergence between populations that include a component of within-population diversity, such as D_s, D_m, D_{sw}, and R_{st}, can be strongly influenced by reduced within-population variability (Charlesworth 1998). The appropriateness of different distance measures in different situations is an area of active research (Goldstein and Pollock 1997).

We used several distance measures to examine this data set. D_m (Nei 1973) and D_a (Nei, Tajima, and Tateno 1983) were calculated using GeneDist (Paetkau et al. 1997), D_{sw} (Shriver et al. 1995), $(\delta\mu)^2$ (Goldstein et al. 1995*b*), and R_{st} (Slatkin 1995 following Goodman 1997) were calculated by MICROSAT (url: http://lotka.stanford.edu/microsat.html), and D_s (Nei 1972), D_c (Cavalli-Sforza and Edwards 1967), and D_r (Reynolds, Weir, and Cockerham 1983) were calculated in the Gendist subprogram of PHYLIP (Felsenstein 1993). Neighbor-joining trees (Saitou and Nei 1987) were constructed from the eight distance matrices using Neighbor in PHYLIP.

There was broad concordance among trees obtained with different distance measures. Subsequent analyses

focused on Cavalli-Sforza and Edwards' (1967) chord distance. This measure does not include a component of within-population diversity and is therefore more likely to recover the correct topology when divergence is recent, as is the case with populations in this study. Cavalli-Sforza and Edwards' (1967) distance is based on the chord distance between two populations represented on the surface of a multidimensional hypersphere, their positions determined by allele frequencies, where D_c (Felsenstein 1993) is calculated for multiple loci as

$$D_c^2 = \frac{4 \sum_m \left(1 - \sum_{i=1}^{a} p_{1mi}^{1/2} p_{2mi}^{1/2}\right)}{\sum_m (a - 1)}, \quad (1)$$

where p_{1mi} is the frequency of the ith allele at the mth locus in population 1, p_{2mi} is the frequency of the ith allele at the mth locus in population 2, and a is the number of alleles at the mth locus. Bootstrap replicates were performed using PHYLIP (Felsenstein 1993) for the whole data set (six loci) and separately for the loci on chromosomes 5 and 15 (three loci each). To verify that observed relationships did not rely on a single locus, jackknifing was performed by individually dropping each locus and constructing a neighbor-joining tree based on D_c (six jackknifes constructed from five loci each).

In addition to examining the relationships between populations, it is possible to look at the relationships between the individuals sampled from those populations. For example, Bowcock (1994) used distances between human individuals scored for microsatellite polymorphisms to create a phylogeny that is highly concordant with geography. This allele-sharing method performs well in simulations in which divergence is recent (Bowcock et al. 1994; Goldstein et al. 1995*a*). We calculated distance between individuals as $1 - P_s$, where P_s is the proportion of shared alleles, and the resultant distance matrix was used to generate a tree using neighbor-joining (Saitou and Nei 1987). Finally, we analyzed the data in a cladistic framework by coding all alleles as presence/absence irreversible characters (Buth 1984). Trees were constructed under general parsimony and also with constrained monophyly of all Rb individuals using PAUP*, version 4.0b2 (Swofford 1998). The lengths of the two parsimony trees were compared by the winning-sites method, which compares the number of characters at which two trees differ with a binomial distribution (Prager and Wilson 1988; Swofford et al. 1996).

Results

Allele Distributions, Population Structure, and Levels of Variability

The distribution of alleles for each locus is shown in table 3. The number of alleles, the variance in repeat number, and the observed and expected heterozygosities for each locus in each population are summarized in table 4. An exact test for Hardy-Weinberg equilibrium (Guo and Thompson 1992) revealed a significant deficiency of heterozygotes in the Cassino population (central Italy, 2n = 40) at locus D5mit49 ($P < 0.0001$, corrected for multiple tests). None of the other 41 population-locus combinations deviated significantly from Hardy-Weinberg expectations. The power of these tests is low, but in most cases, there was good agreement between observed and expected levels of heterozygosity (table 4).

Monte Carlo permutation tests for population structure across all populations were highly significant for each locus ($P < 0.001$, corrected for multiple tests), indicating that these samples do not derive from a single panmictic population. In addition, significant differences between the two northern Italian acrocentric populations, Curone and Menconico, were detected at D5mit145 and D15mit12 ($P < 0.05$, corrected for multiple tests). As a consequence of detectable population structure between these two populations, they are treated separately in all subsequent analyses.

The number of alleles per locus ranged from 4 at D5mit47 to 13 at D5mit49. One Rb population, Sernio, is notable for its low number of alleles at each locus compared with other populations. At three loci (D5mit47, D5mit145, D15mit174), the Sernio population is fixed for one allele, and at three loci (D5mit49, D15mit12, D15mit13), the population is nearly fixed, with only a single occurrence of a second allele (table 3). The most common alleles found in the Rb populations of Bonefro (central Italy) and Avinyonet (Spain) are generally found in their nearby acrocentric populations, Cassino (central Italy) and La Roca (Spain) (table 3). In contrast, Sernio (northern Italy) has a greater affinity to another Rb population, Bonefro (central Italy), than to the geographically proximate acrocentric populations of Curone and Menconico, both in northern Italy. For each of the three loci on chromosome 5, the most common allele in Sernio (northern Italy) and Bonefro (central Italy) is the same, and for loci D15mit13 and D15mit174, the most common alleles are different in size by one repeat unit. The affinity between Sernio and Bonefro is most striking for allele 156 at locus D15mit12; allele 156 is found in both Bonefro and Sernio but is completely lacking in Curone and Menconico (both in northern Italy). These relationships are also reflected in the phylogenetic analyses (below).

Heterozygosity and variance were compared between Rb and 2n = 40 populations in two ways, and both revealed significantly more variation in 2n = 40 populations than in Rb populations. First, for each locus, we compared each Rb population with its adjacent 2n = 40 population. There were three such population pairs for each of six loci, representing a total of 18 comparisons (table 4). In a significant majority of these comparisons, variance and expected heterozygosity were lower in the Rb population than in the adjacent 2n = 40 population (Wilcoxon signed-ranks test, variance $P = 0.0053$; heterozygosity $P = 0.0003$). In the second method, we compared mean values (of variance and heterozygosity) for Rb populations with mean values for 2n = 40 populations (table 5). The reductions in ex-

Table 3
Distribution of Alleles in Each Population

		Northern Italy			Central Italy		Spain	
Locus	Alleles	Curone (2n = 40)	Menconico (2n = 40)	Sernio (Rb)	Cassino (2n = 40)	Bonefro (Rb)	La Roca (2n = 40)	Avinyonet (Rb)
D5mit47	112	0	0	0	0	0	8	4
	120	1	0	0	0	0	0	0
	124	11	16	18	25	38	13	3
	128	0	0	0	19	0	7	5
D5mit49	120	5	5	0	0	0	0	0
	122	0	0	0	0	0	1	0
	124	2	2	0	0	0	0	0
	126	0	0	0	14	0	0	0
	128	3	0	0	1	2	1	0
	130	0	0	0	4	0	1	1
	132	0	0	0	10	1	7	6
	134	1	3	17	7	29	2	5
	136	0	0	0	5	0	4	0
	138	1	2	1	3	1	4	0
	140	0	4	0	0	0	4	0
	142	0	0	0	0	5	0	0
	144	0	0	0	0	0	4	0
D5mit145	112	0	0	0	0	0	4	0
	118	3	0	0	1	0	6	5
	120	2	0	0	5	9	0	0
	122	1	2	0	6	1	6	0
	124	4	2	18	21	25	6	1
	126	2	5	0	2	1	0	0
	128	0	7	0	6	0	0	5
	130	0	0	0	1	0	0	0
	132	0	0	0	1	0	0	1
	134	0	0	0	0	0	2	0
	136	0	0	0	1	0	0	0
D15mit12	140	0	0	0	0	1	0	0
	142	2	12	0	4	0	0	0
	144	1	0	0	0	0	0	0
	146	2	3	0	12	2	0	0
	148	2	0	0	16	1	1	0
	150	0	0	0	5	9	9	0
	152	4	1	0	0	1	1	1
	154	0	0	0	3	7	6	4
	156	0	0	17	0	7	7	4
	158	0	0	1	0	0	0	1
	162	1	0	0	0	0	4	0
D15mit13	130	0	0	0	1	0	0	0
	134	0	0	0	2	0	0	0
	136	2	1	0	0	0	0	0
	138	7	12	0	3	0	5	11
	140	1	3	1	21	1	9	1
	142	0	0	0	2	23	10	0
	144	1	0	17	0	5	1	0
	146	1	0	0	3	8	3	0
	148	0	0	0	1	1	0	0
	152	0	0	0	11	0	0	0
D15mit174	116	0	2	0	1	0	0	0
	118	0	0	0	8	0	8	0
	120	1	0	0	13	6	1	0
	122	2	2	0	16	0	1	0
	124	2	0	0	3	21	0	0
	126	5	11	18	3	0	12	11
	128	1	1	0	0	11	0	1
	134	0	0	0	0	0	1	0
	136	0	0	0	0	0	5	0
	148	1	0	0	0	0	0	0

Table 4
Numbers of Alleles, Allele Sizes, Variances in Repeat Number, and Expected and Observed Heterozygosities for Six Microsatellite Loci in Each of Seven Populations

Locus	Population	No. of Alleles	Most Common Allele	Mean Allele Size	Variance	Expected Heterozygosity	Observed Heterozygosity
D5mit47.....	Northern Italy, 2n = 40 (Curone)	2	124	123.7	0.333	0.153	0.167
	Northern Italy, 2n = 40 (Menconico)	1	124	124.0	0.000	0.000	0.000
	Northern Italy, Rb	1	124	124.0	0.000	0.000	0.000
	Central Italy, 2n = 40	2	124	125.7	1.004	0.491	0.500
	Central Italy, Rb	1	124	124.0	0.000	0.000	0.000
	Spain, 2n = 40	3	124	121.6	10.175	0.640	0.643
	Spain, Rb	3	128	121.7	13.424	0.653	1.000
	All individuals	4	124	123.9	3.286	0.416	0.321
D5mit49.....	Northern Italy, 2n = 40 (Curone)	5	120	125.3	9.152	0.722	0.833
	Northern Italy, 2n = 40 (Menconico)	5	120	130.4	19.096	0.773	0.750
	Northern Italy, Rb	2	134	134.2	0.222	0.105	0.111
	Central Italy, 2n = 40	7	126	131.0	4.116	0.795	0.500
	Central Italy, Rb	5	134	134.8	2.624	0.604	0.316
	Spain, 2n = 40	9	132	135.9	6.958	0.884	0.643
	Spain, Rb	3	132	132.7	0.424	0.569	1.000
	All individuals	13	134	132.7	7.158	0.809	0.524
D5mit145.....	Northern Italy, 2n = 40 (Curone)	5	124	122.0	2.364	0.764	0.667
	Northern Italy, 2n = 40 (Menconico)	4	128	126.1	1.129	0.680	0.500
	Northern Italy, Rb	1	124	124.0	0.000	0.000	0.000
	Central Italy, 2n = 40	9	124	124.4	2.353	0.718	0.773
	Central Italy, Rb	4	124	123.0	0.829	0.458	0.556
	Spain, 2n = 40	5	122, 124	120.8	8.428	0.778	0.500
	Spain, Rb	4	118, 128	123.8	7.356	0.639	1.000
	All individuals	11	124	123.5	3.323	0.729	0.580
D15mit12.....	Northern Italy, 2n = 40 (Curone)	6	152	148.8	7.902	0.792	1.000
	Northern Italy, 2n = 40 (Menconico)	3	142	143.3	1.963	0.398	0.375
	Northern Italy, Rb	2	156	156.1	0.056	0.105	0.111
	Central Italy, 2n = 40	5	148	147.5	1.987	0.719	0.750
	Central Italy, Rb	7	150	151.9	3.772	0.763	0.929
	Spain, 2n = 40	6	150	154.1	4.332	0.765	0.929
	Spain, Rb	4	154, 156	155.0	0.722	0.660	1.000
	All individuals	11	156	150.3	6.719	0.856	0.737
D15mit13.....	Northern Italy, 2n = 40 (Curone)	5	138	139.0	2.273	0.611	0.333
	Northern Italy, 2n = 40 (Menconico)	3	138	138.3	0.250	0.398	0.375
	Northern Italy, Rb	2	144	143.8	0.222	0.105	0.111
	Central Italy, 2n = 40	8	140	143.0	8.672	0.695	0.652
	Central Italy, Rb	5	142	143.2	0.894	0.571	0.737
	Spain, 2n = 40	5	142	141.1	1.365	0.724	0.571
	Spain, Rb	2	138	138.2	0.083	0.153	0.167
	All individuals	10	138	141.8	3.996	0.752	0.524
D15mit174....	Northern Italy, 2n = 40 (Curone)	6	126	126.5	12.750	0.750	0.833
	Northern Italy, 2n = 40 (Menconico)	4	126	124.4	3.229	0.492	0.375
	Northern Italy, Rb	1	126	126.0	0.000	0.000	0.000
	Central Italy, 2n = 40	6	122	121.0	1.325	0.738	0.818
	Central Italy, Rb	3	124	124.5	1.767	0.586	0.684
	Spain, 2n = 40	6	126	125.4	10.360	0.699	0.786
	Spain, Rb	2	126	126.2	0.083	0.153	0.167
	All individuals	10	126	124.1	4.558	0.800	0.607

pected heterozygosity and variance were significant (Mann-Whitney *U*-test, variance $P = 0.045$; heterozygosity $P = 0.0104$). Reduced variability at microsatellite loci linked to Rb chromosomes is consistent with the notion that these translocations have a recent origin (see *Discussion*).

Relationships Between Populations

Neighbor-joining trees based on different distance measures consistently revealed several important patterns. Trees were constructed using all six loci, the three loci from chromosome 5 and the three loci from chromosome 15. None of these trees revealed a single origin of the Rb 5.15 chromosome. Figure 1 shows the phylogenetic tree produced by Cavalli-Sforza and Edwards' (1967) chord distance, with bootstrap values given for all six loci and for the loci on each chromosome separately. With only six loci from which to sample, high bootstrap scores are not expected, given the large effects of individual loci. It is possible that the loci on chromosome 15, which are situated several map units from the centromere in some genetic maps (table 1), have been more dissociated from the Rb translocation by recombination than the loci on chromosome 5, which map close to the centromere. While the loci from each chromosome yield the same tree, bootstrap support from loci on chromosome 5 is higher than that from loci on chromosome 15 or from the total data set (fig. 1). In 1,000

Table 5
Variability at Microsatellite Loci in 2n = 40 and Rb Populations[a]

Locus	Populations	Variance	Expected Heterozygosity	Observed Heterozygosity
D5mit47	2n = 40	3.331	0.414	0.420
	Rb	2.369	0.115	0.176
D5mit49	2n = 40	5.469	0.571	0.509
	Rb	1.600	0.466	0.382
D5mit145	2n = 40	3.875	0.732	0.646
	Rb	1.789	0.366	0.485
D15mit12	2n = 40	3.406	0.688	0.771
	Rb	2.033	0.533	0.679
D15mit13	2n = 40	4.511	0.646	0.547
	Rb	0.573	0.374	0.471
D15mit174	2n = 40	5.530	0.689	0.740
	Rb	1.002	0.354	0.412

[a] For each locus, the given values are means for the three Rb populations and means for the four 2n = 40 populations. Mean values are weighted by individual population sizes.

bootstrap replicates of all six loci, a monophyletic clade of the three Rb populations was present in only 4% of the trees and was never present when bootstrapping was restricted to either chromosome 5 or chromosome 15. None of the jackknifed trees support monophyly of Rb 5.15. Furthermore, none of the loci individually support monophyly of the Rb 5.15 chromosome.

While the data strongly reject a single origin for Rb 5.15, there is less statistical support for specific phylogenetic relationships among the chromosomes sampled. In figure 1, the Spanish populations of La Roca (2n = 40) and Avinyonet (Rb) appear as sister groups, consistent with the hypothesis that the Avinyonet Rb chromosomes arose from nearby acrocentric chromosomes. In contrast, the Italian Rb populations of Sernio and Bonefro form a clade, consistent with a single origin of Rb 5.15 in Italy. These two clades (La Roca + Avinyonet, Sernio + Bonefro) were observed in most of the analyses based on different distance measures. Jackknifing by dropping a single locus at a time also always recovers these clades, although bootstrap values are not high (fig. 1).

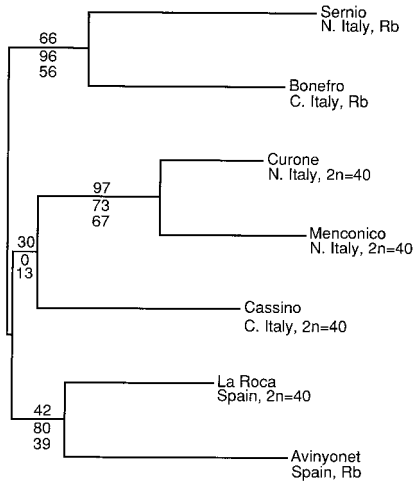

FIG. 1.—Unrooted neighbor-joining tree based on D_c values between populations. Bootstrap values (%) for 1,000 bootstrap replicates are indicated; percentages based on the total data set (six loci) are above branches, with percentages based on chromosome 5 loci and chromosome 15 loci below (see text for details). Karyotype and geographic location are indicated.

A neighbor-joining tree based on a distance matrix among individuals is shown in figure 2. A nearly identical tree was obtained from the parsimony analysis (length, 298 steps). Neither tree supports a single origin of Rb 5.15. When the parsimony analysis is constrained to require monophyly of Rb 5.15, the resulting tree is seven steps longer and provides a significantly worse fit to the data (winning-sites method of Prager and Wilson 1988; $P = 0.03$). The tree in figure 2 also reveals some of the relationships seen in figure 1: there are close associations between the two Italian Rb populations (Sernio and Bonefro) and between the Spanish Rb population (Avinyonet) and the Spanish standard karyotype population (La Roca). In figure 2, each of the Rb populations defines a monophyletic or nearly monophyletic group, while individuals from acrocentric populations are widely dispersed on the tree.

Discussion

Genetic Variability in Rb and 2n = 40 Populations

New underdominant mutations such as Rb translocations are generally expected to be lost from populations. Theoretical work suggests that underdominant mutations can be fixed either by strong deterministic processes (e.g., selection or meiotic drive) or as a consequence of small population size (e.g., due to inbreeding or small populations in isolation) (Lande 1979; Hedrick 1981; Walsh 1982). Deterministic forces are expected to lead to a reduction in variability at linked neutral loci through the action of genetic hitchhiking (Maynard Smith and Haigh 1974), while population bottlenecks are expected to reduce variability at all loci. Thus, the fixation of underdominant Rb rearrangements should be associated with a reduction in variation at linked loci. The reduction in variation (variance and heterozygosity) observed in our data is consistent with theorectical expectations but does not allow us to address whether the reduction was caused by deterministic or stochastic forces.

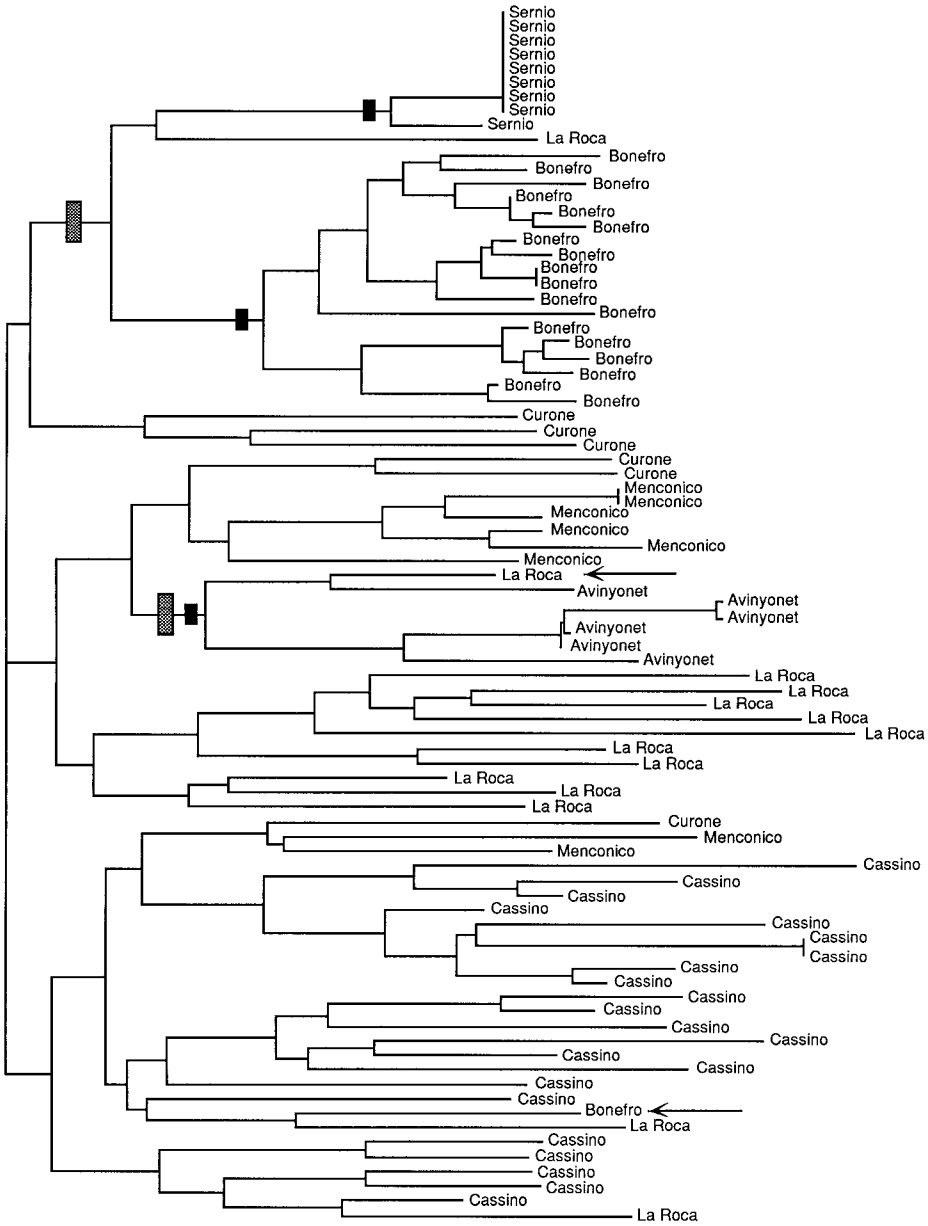

FIG. 2.—Unrooted neighbor-joining tree of individuals constructed from the proportion of alleles shared. Each terminal lineage represents one individual, and collecting localities correspond to tables 1 and 3. Black bars indicate nodes that define the Rb populations, and gray bars indicate the two putative origins of the 5.15 fusion. Arrows point to individuals whose locations on the tree are best explained by one or more recombination events between the translocation breakpoint and microsatellite markers in individuals heterozygous for Rb 5.15.

Following a fixation event, variability is expected to increase gradually as a result of new mutations and the introduction of new alleles from other populations. Microsatellites in mice are known to have relatively high mutation rates (from 10^{-5} to 10^{-3}; Dallas 1992; Dietrich et al. 1992), and thus may recover variability rather quickly following a bottleneck or a selective sweep. The introgression of new alleles at microsatellite loci linked to an Rb chromosome requires (1) gene flow from a neighboring acrocentric population into the Rb population and (2) recombination between the translocation breakpoint and the microsatellite locus in an individual heterozygous for an Rb translocation. The latter is unlikely for closely linked markers, because recombination is known to be significantly suppressed in the centromeric regions of Rb heterozygotes (Davisson and Akeson 1993).

In nearly all cases, variance and heterozygosity at centromeric microsatellite loci of chromosomes 5 and 15 were reduced in Rb 5.15 populations relative to neighboring acrocentric populations (table 4). Variance was reduced in 15 out of 18 pairwise comparisons ($P = 0.0053$), and heterozygosity was lower in 16 out of 18 pairwise comparisons ($P = 0.0003$). The same pattern emerges across geographic areas; the mean variance and the mean expected heterozygosity are lower in Rb populations than in 2n = 40 populations for all six loci (table 5).

Table 6
Mean Variances in Repeat Number and Mean Expected Heterozygosities Across Six Microsatellite Loci in Three Rb Populations

Population	Variance	Expected Heterozygosity
Avinyonet	3.682	0.471
Bonefro	1.648	0.497
Sernio	0.083	0.053

The reduced variability at centromeric microsatellite loci of chromosomes 5 and 15 in Rb 5.15 individuals indicates that linked microsatellite markers are not assorting independently from the Rb translocation. Thus, these microsatellite markers generally reflect the evolutionary history of the translocation to which they are linked. This stands in contrast to results from most previous studies, in which markers unlinked to Rb fusions were used to make inferences about the evolution of chromosomal rearrangements. Surveys of multiple Rb and acrocentric populations utilizing allozymes (Britton-Davidian et al. 1989) and mtDNA (Nachman et al. 1994) detected no significant reduction in variation in Rb populations. There are, however, at least two cases in which a population bottleneck and absence of subsequent gene flow may have resulted in reduced variability at some allozyme loci in Rb populations (Said and Britton-Davidian 1991; Said et al. 1993; Fraguedakis-Tsolis, Hauffe, and Searle 1997).

While the absence of variability at the microsatellite loci in this study suggests that recombination between these loci and the Rb 5.15 translocation has been infrequent, the phylogenetic positions of two individuals are probably best explained by gene flow and recombination (fig. 2). In one case, a 2n = 40 individual from La Roca clusters within a clade consisting of all individuals from Avinyonet, the adjacent Rb population. In another case, a single Rb individual from Bonefro falls within a clade consisting largely of individuals from the adjacent 2n = 40 population, that of Cassino.

The amount of variation in each of the Rb populations may give some indication of their relative ages. The average variance across all loci is highest for the Avinyonet population in Spain and lowest for the northern Italian population of Sernio (table 6), suggesting that the Spanish Rb population is the oldest and the northern Italian Rb population is the youngest. A similar pattern is evident in the average heterozygosity of each population (table 6) and in the relative depths of the clades containing these individuals (fig. 2). Alternatively, the low variation observed in Sernio may reflect a population bottleneck subsequent to the fixation of the 5.15 translocation.

Phylogenetic Relationships

The central result of the phylogenetic analyses is that the Rb 5.15 chromosome did not originate once. Neither the analysis of populations (fig. 1) nor the analysis of individuals (fig. 2) support monophyly of Rb 5.15. A parsimony tree constrained to Rb monophyly provides a significantly worse fit to the data than the tree in figure 2.

How many times did Rb 5.15 arise? While we can confidently reject a single origin, there is only weak statistical support for some of the nodes in figure 1. Our results suggest that Rb 5.15 may have arisen twice, once in Italy and once in Spain, although we cannot rule out the possibility of additional origins.

Interestingly, some of the phylogenetic relationships suggested by this study are supported by previous work. The independent origins of the Italian and Spanish 5.15 metacentric chromosomes are concordant with a phylogeny of Rb populations based on the translocations themselves as characters (Bauchau 1990). The close relationship between the central and northern Italian Rb populations is not evident in a phylogeny based on mtDNA (Nachman et al. 1994), but it is supported by limited Y chromosome data (Tucker, Lee, and Eicher 1989). In the Y chromosome data, one northern Italian population carrying Rb 5.15 clusters with a central Italian Rb population, but two other populations from the northern Italy/Switzerland region which also carry Rb 5.15 show no affinity to the central Italian Rb population. The association between the central and northern Italian Rb 5.15 chromosomes raises the possibility that this chromosome was carried from one population to another by migration of males. Our results are also consistent with the presumed range expansion of *M. domesticus* into Europe. Fossil (Auffray, Vanlerberghe, and Britton-Davidian 1990), allozyme (Britton-Davidian et al. 1990), and mtDNA (Sage et al. 1990) evidence indicates that *M. domesticus* expanded westward from the Middle East along the Mediterranean basin before expanding northward into central and western Europe. Northward range expansion of central Italian *M. domesticus* may have carried the 5.15 metacentric chromosome into central Europe, where it became fixed in northern Italian, Swiss, and German populations.

Implications for Speciation

Robertsonian chromosomal evolution in house mice has served as a classic model of chromosomal speciation in vertebrates (e.g., White 1978; King 1993), although the degree of reproductive isolation caused by Rb translocations is certainly open to debate (e.g., Hauffe and Searle 1998). Nonetheless, it is clear that in mice many Rb translocation heterozygotes are associated with some reduction in fitness, and in the case of complex heterozygotes (formed when two different Rb races with monobrachial homology come into contact), this reduction in fitness can be substantial (Searle 1993; Hauffe and Searle 1998). The rate at which Rb translocations arise in natural populations of mice thus informs us about the rate at which populations of house mice may become reproductively isolated as a consequence of chromosomal rearrangements.

It is still somewhat of a mystery why Rb races are so common in *M. domesticus* yet essentially absent from the sister species *M. musculus.* Possibilities include a higher mutation rate for Rb translocations, higher levels of meiotic drive or selection, or a population structure

that facilitates the fixation of Rb translocations in one species and not in the other (Nachman and Searle 1995). Our results demonstrate that cytologically indistinguishable translocations have arisen independently at least twice, raising the possibility that other Rb chromosomes shared among populations did not result from a single mutation and gene flow, but from convergent mutations in different populations. Evidence of convergent mutations suggests that the formation of Rb translocations is frequent and may help explain the widespread distribution of some Rb chromosomes in *M. domesticus.*

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