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Empirical demonstration of hybrid chromosomal races in house mice

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Western house mice (*Mus musculus domesticus*) and common shrews (*Sorex araneus*) are important models for study of chromosomal speciation. Both had ancestral karyotypes consisting of telocentric chromosomes, and each is subdivided into numerous chromosomal races many of which have resulted from fixation of new mutations (Robertsonian fusions and whole-arm reciprocal translocations). However, some chromosomal races in both species may alternatively have originated through hybridization, with particular homozygous recombinant products reaching fixation. Here, we demonstrate the process of generation of hybrid chromosomal races for the first time in either species using molecular markers. Analysis of centromeric microsatellite markers show that the Mid Valtellina (IMVA) and Upper Valtellina (IUVA) chromosomal races of the house mouse are recombinant products of hybridization of the Lower Valtellina (ILVA) and Poschiavo (CHPO) chromosomal races, supporting earlier theoretical analysis. IMVA and IUVA occupy a small area of the Italian Alps where ILVA makes contact with CHPO. IUVA and CHPO have previously been shown to be reproductively isolated in one village, emphasizing that hybrid chromosomal races in small mammals, as in plants, have the potential to be part of the speciation process.

KEY WORDS: **Chromosomal speciation, hybridization, *Mus musculus domesticus*, Robertsonian fusion, recombinational speciation, zonal raciation.**

In animals and plants, it is common for closely related species to differ in karyotype, due to the occurrence and fixation of chromosomal rearrangements which alter the morphology and sometimes the number of chromosomes (King 1993). Because chromosome complement is an aspect of species difference, there has been an interest in the role that chromosomal rearrangements may have in the speciation process, including theoretical studies (Kirkpatrick and Barton 2006; Faria and Navarro 2010; Feder et al. 2014). Thus, if distinctive sets of chromosomal rearrangements become fixed in different populations of a species, such that "chromosomal races" are formed, then those races may show partial reproductive isolation on contact with each other or with the ancestral race, due to the properties of the chromosomally heterozygous hybrids between such races (Searle 1993). Heterozygotes for chromosomal rearrangements may suffer reduced fertility, due to meiotic errors associated with the pairing, recombination, and segregation of differentiated chromosomes (leading to death of germ cells and/or zygotes; Searle 1993). Alternatively or additionally, heterozygotes for chromosomal rearrangements may show recombination suppression in the vicinity of the chromosomal breakpoints,

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allowing a buildup of genetic differences between the races that may also contribute to reproductive isolation (Noor et al. 2001; Rieseberg 2001).

There may be progression from partial to total reproductive isolation if there is sufficient accumulation of chromosomal rearrangements in geographic isolation, or through evolutionary change affecting races in contact ("speciation with gene flow": accumulation of incompatibilities or reinforcement: Feder et al. 2012; Abbott et al. 2013).

Whatever reproductive isolation develops in response to chromosomal difference, the first stage of the process is the formation of chromosomal races, and that is what we address in this article. Not only is the process of race formation of interest in speciation, it is also important in interpreting within- and between-species chromosomal phylogenies, of fundamental interest in understanding the development of the "patchwork" of syntenies that characterize comparisons of distantly related genomes (reflecting multiple chromosomal rearrangements; e.g., Zhao et al. 2004).

New chromosomal rearrangements may become fixed because of a selective process (meiotic drive or the bringing together of advantageous combinations of alleles into close linkage: Kirkpatrick and Barton 2006; Chmátal et al. 2014). Genetic drift may also be important in fixation, although that would require small population sizes if chromosomal heterozygotes show unfitness (Lande 1979). In these cases, fixation of the chromosomal rearrangements occurs concurrently with formation of a chromosomal race—it is the fixation of the new rearrangement that leads to a new race.

However, it is also possible for a new chromosomal race to form without fixation of a new chromosomal rearrangement. This occurs when two races come into contact and generate a homozygous recombinant product through hybridization; the bringing together and mixing of rearrangements from the two parental races. The occurrence of such a process has been inferred from phylogenies of chromosomal races (White et al. 2010). It has also been proposed as a mechanism of speciation in plants (recombinational speciation) whereby the hybrid product becomes ecologically differentiated and reproductively isolated (Grant 1981; Abbott et al. 2010).

It is not always straightforward to distinguish between the formation of chromosomal races by hybridization and formation by fixation of new rearrangements. The common occurrence of a rearrangement in two races may represent independent mutations or the result of a hybridization event.

In two of the best-studied models of the role of chromosomes in speciation, the common shrew (*Sorex araneus*) and the house mouse (*Mus musculus*), there are strong indications that hybridization has been important in the generation of new chromosomal races (Searle and Wójcik 1998; Piálek et al. 2005). In both these small mammals, the ancestral karyotype consisted of telocentric chromosomes, and chromosomal races are distinguished by different sets of metacentric chromosomes formed by centromeric (Robertsonian) fusion of pairs of autosomal telocentrics, occasionally modified by the swapping of chromosome arms between metacentrics or between metacentrics and telocentrics (whole-arm reciprocal translocation; Searle 1993). For both species, a large number of chromosomal races have been described (over 70 in the common shrew, over 100 in the house mouse), and phylogenies suggest that the karyotypes of particular races include metacentrics that derive from two parental races (White et al. 2010; Hauffe et al. 2012). However, for neither species has it been shown definitively that a race derives from hybridization. Here, we provide that empirical demonstration for a particularly well-studied system of chromosomal races in the house mouse, located in Valtellina (Lombardy, Italy).

The chromosomal variation in the house mouse occurs in Europe and North Africa within the western subspecies (*Mus musculus domesticus*) and represents divergence from the widespread standard 40-chromosome all-telocentric karyotype with presence of metacentrics leading to chromosome numbers in the range $2n = 22$–38 (Piálek et al. 2005). Valtellina is characterized by four metacentric races (Fig. 1, Table 1, and Tables S1 and S2). The karyotypes of these races differ in the arrangement of chromosome arms 2, 7, 8, 10, 12, and 18 (Table 1): the Lower Valtellina race (ILVA; $2n = 22$) carries metacentrics 2.8, 10.12, and 7.18; the Poschiavo race (CHPO; $2n = 26$) has metacentric 8.12 and telocentrics 2, 7, 10, and 18; the Mid Valtellina race (IMVA; $2n = 24$) has metacentrics 8.12, 7.18, and telocentrics 2 and 10; and the Upper Valtellina race (IUVA; $2n = 24$) has metacentrics 2.8, 10.12 and telocentrics 7 and 18 (Hauffe et al. 2004; Piálek et al. 2005). ILVA and CHPO exist as the dominant metacentric races in the villages of Lower Valtellina and Val Poschiavo (Switzerland), respectively, while all four races exist and hybridize in Upper Valtellina (Fig. 1). This distribution, the particular combinations of shared chromosomes, and phylogenetic analysis all indicate that IMVA and IUVA were the products of hybridization of ILVA and CHPO in Upper Valtellina (Hauffe and Searle 1993; Piálek et al. 2001, 2005). More specifically, on initial contact, ILVA and CHPO would have produced an F_1 hybrid characterized by one chain-of-five (2-2.8-8.12-12.10-10) and one chain-of-three (7-7.18-18) configuration at meiosis I, a karyotype that has been found in nature and shown to be associated with reduced fertility but not complete sterility (Hauffe and Searle 1998). It is through the independent segregation of the chain-of-five and chain-of-three configurations in these initially formed F_1s that would have allowed homozygous IMVA and IUVA karyotypes to be produced in F_2 or higher generation hybrids (Hauffe and Searle 1993). These IMVA and IUVA karyotypes could then have increased in frequency in Upper Valtellina through founder events, genetic

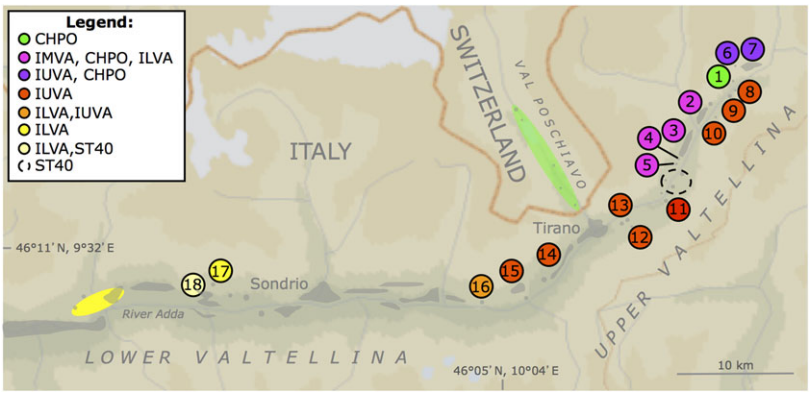

Figure 1. Map of chromosomal races of house mouse in Valtellina based on published data (Hauffe and Searle 1993; Hauffe et al. 2004) and our new results (Table S1), with metacentric races abbreviated as follows: ILVA, IMVA, IUVA = Lower, Mid, and Upper Valtellina races, respectively; CHPO = Poschiavo race. The populations sampled for this study are numbered according to Table S2. Lower Valtellina is dominated by a single race (ILVA) as is the adjacent Val Poschiavo (CHPO). We propose that it was the secondary contact of these two races that led to the production, through hybridization, of IMVA and IUVA in Upper Valtellina where all four races are found. IMVA is predominant in the well-connected group of villages 2–5 (about 80% of haploid sets of chromosomes were IMVA among the individuals sampled; Table S2), although the parental races are also present. IUVA is predominant in the villages 8–15, and is found together at approximately equal frequency to one of the parental races in villages 6, 7, and 16. The 40-chromosome standard race (ST40) is found sporadically in the valley, and is thought to have been introduced following the establishment of the metacentric races (Hauffe and Searle 1993).

Table 1. The four chromosomal races of house mouse in Valtellina.

CHPO	1.3	2	4.6	5.15	7	8.12	9.14	10	11.13	16.17	18	19
IMVA	1.3	2	4.6	5.15	**7.18**	8.12	9.14	10	11.13	16.17		19
IUVA	1.3	**2.8**	4.6	5.15	7		9.14	**10.12**	11.13	16.17	18	19
ILVA	1.3	**2.8**	4.6	5.15	**7.18**		9.14	**10.12**	11.13	16.17		19

On grounds of distribution and chromosomal phylogeny, CHPO and ILVA are believed to be the ancestral races, with IMVA and IUVA homozygous recombinant hybrid forms, having autosomes that derive from each of the ancestral races. Race-specific chromosomes for CHPO and ILVA are shown underlined and in bold indicating the proposed origin of chromosomes in IMVA and IUVA.

drift, or by selection operating on chromosome combinations, as previously modeled by Piálek et al. (2001).

Here, we demonstrate using molecular markers (centromeric microsatellites) that the IMVA and IUVA races are the product of hybridization, and validate this process by which chromosomal races are formed, for the first time in a small mammal system.

Materials and Methods

The full set of 154 Valtellina house mice typed with centromeric microsatellites (Table S2) included individuals either previously karyotyped (Hauffe and Searle 1993; Hauffe et al. 2004) or newly karyotyped by the same methods (Table S1). The 12 microsatellite loci were selected for their close proximity to the centromere (0–5.5 cM) according to Dietrich et al. (1996) and scored in all 154 individuals. In the following list, the centi-Morgan distance from the centromere given in parentheses accords with the most up-to-date mouse genome assembly released by the Genome Reference Consortium (GRCm38/mm10; http://www.informatics.jax.org/): chromosome 7: D7Mit178 (2.02), D7Mit306 (6.42), D7Mit143 (7.27); chromosome 10: D10Mit75 (unknown), D10Mit246 (4.23); chromosome 12: D12Mit145 (unknown), D12Mit182 (5.52), D12Mit11 (8.23); chromosome 18: D18Mit166 (unknown), D18Mit19 (3.02), D18Mit219 (4.46), D18Mit167 (4.59). Although the cM distance for D10Mit75, D12Mit145, and D18Mit166 are categorized as unknown in the current listing, they were classed as tightly centromeric by Dietrich et al. (1996), the source informing our study design. The molecular methods were as previously described in Panithanarak et al. (2004). Also some of the data used in this study for D10Mit75, D10Mit246, D12Mit145, D12Mit182, and D12Mit11 were collected by Panithanarak et al. (2004) for a different purpose; those relating to populations 1–14 (Table S2).

The data comparing pairs of the races CHPO, ILVA, IMVA, and IUVA in different combinations were subjected to an analysis

Table 2. AMOVA partitioning variance at three hierarchical levels for pairs of metacentric races (out of ILVA, CHPO, IMVA, and IUVA), using data for (A) loci on chromosomes 10 and 12 and (B) loci on chromosomes 7 and 18.

Microsatellite locus	% Variance Among pairs of races	Among populations within pairs of races	Within populations
A			
CHPO + IMVA versus ILVA + IUVA:			
D10Mit75	16.99**	29.10	53.91
D10Mit246	39.96**	23.38	36.67
D12Mit145	64.69**	14.44	20.87
D12Mit182	28.47**	28.17	43.35
D12Mit11	31.23*	22.64	46.13
All 10 and 12 centromeric loci	**36.38*** **	**23.65**	**39.97**
ILVA + IMVA versus CHPO + IUVA:			
D10Mit75	6.81	40.07	53.13
D10Mit246	−8.20	65.51	42.69
D12Mit145	2.88	65.21	31.91
D12Mit182	−4.55	56.13	48.42
D12Mit11	−3.14	49.19	53.95
All 10 and 12 centromeric loci	**−1.24**	**54.77**	**46.46**
B			
CHPO + IMVA versus ILVA + IUVA:			
D7Mit178	2.21	36.64	61.14
D7Mit306	1.07	42.85	56.08
D7Mit143	2.62	39.50	57.88
D18Mit166	10.20*	34.04	55.76
D18Mit19	−2.59	46.30	56.29
D18Mit219	4.82	38.03	57.14
D18Mit167	6.06	35.57	58.37
All 7 and 18 centromeric loci	**3.67***	**38.79**	**57.54**
ILVA + IMVA versus CHPO + IUVA:			
D7Mit178	26.56**	22.63	50.80
D7Mit306	29.92**	27.42	42.66
D7Mit143	4.05	35.71	60.24
D18Mit166	4.93	41.93	53.15
D18Mit19	10.19*	40.95	48.86
D18Mit219	11.48*	36.13	52.39
D18Mit167	8.24*	37.90	53.86
All 7 and 18 centromeric loci	**13.29*** **	**34.80**	**51.92**

Tests of significance for individual and overall loci: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

of molecular variance (AMOVA) using ARLEQUIN 3.11 (Excoffier et al. 2005) to test whether the IMVA and IUVA races were products of hybridization (Table 2). A nonparametric permutation method in ARLEQUIN (16,000 permutations) was applied to partition total genetic variance over three levels of genetic structure: among the two pairs of races, among populations within the two pairs of races being compared, and within populations. Standard datasets were used to compute a separated AMOVA for each locus and an overall AMOVA.

In the first and third sets of tests reported in Table 2 (CHPO + IMVA vs. ILVA + IUVA), villages were classified as either CHPO and/or IMVA populations or ILVA and/or IUVA populations on the basis of the predominant karyotypes (Fig. 1). For Sommacologna and Sondalo, the villages were each treated as two separate populations, because of their mixed characteristics (Table S2): Sommacologna (CHPO) consisting of the two definitive CHPO individuals; Sommacologna (IUVA)—three definitive IUVA; Sondalo (CHPO)—two definitive CHPO; Sondalo (IUVA)—four definitive IUVA. Thus, the CHPO and/or IMVA populations were Sommacologna (CHPO individuals), Sondalo (CHPO individuals), Migiondo, and the interconnected group of populations (overall predominantly IMVA): Grosio, Grosotto, Prada, and

Vione. The ILVA and/or IUVA populations were Sommacologna (IUVA individuals), Sondalo (IUVA individuals), Sontiolo, Tiolo, Lago, Lovero, Biolo, Sernio, Villa di Tirano, Tresenda, San Giacomo, Polaggia, and Berbenno. Thus, all individuals within each of these villages were treated as belonging to the specified races, even if a small minority of the individual karyotypes were of a different race (Table S2). We took the predominant race or races as defining the village genetically; this is consistent with genetic exchange even at centromeric loci among individuals of different races in the same village (Förster et al. 2016). Only in Sondalo and Sommacologna was it not possible to define a predominant race.

In the second and fourth sets of tests reported in Table 2 (ILVA + IMVA vs. CHPO + IUVA), villages were classified as either ILVA and/or IMVA populations or CHPO and/or IUVA populations on the basis of the predominant karyotypes (Table S2), except for San Giacomo. Again, this village was treated as two populations because of its mixed characteristics: San Giacomo (ILVA) consisting of the 13 definitive ILVA individuals; San Giacomo (IUVA)—10 definitive IUVA. The ILVA and/or IMVA populations were San Giacomo (ILVA individuals), Polaggia, Berbenno, and the interconnected group of populations (overall predominantly IMVA): Grosio, Grosotto, Prada, and Vione. The CHPO and/or IUVA populations were San Giacomo (IUVA individuals), Migiondo, Sommacologna, Sondalo, Sontiolo, Tiolo, Lago, Lovero, Biolo, Sernio, Villa di Tirano, and Tresenda. Again, all individuals within these villages were treated as belonging to the specified races, even if a few individual karyotypes were of a different race (Table S2).

Multilocus data of mice from the 18 populations sampled were used to assess genetic structure using a Bayesian clustering analysis performed in STRUCTURE (Falush et al. 2003). A probabilistic assignment of individuals to the population of origin, or to two or more populations if they show admixed genotypes, was made. A Monte Carlo Markov Chain (MCMC) procedure was used to estimate the posterior probability values for K clusters [Pr(X|K)] and proportions of membership for each individual to each cluster. Multilocus genotypes were tested under the linkage ancestry model, which assumes each individual has a mixed ancestry in more than one K population and linked loci coming from the same population. A correlated allele frequency model among populations was assumed because allele frequencies in different populations can be correlated due to common ancestry or immigration. STRUCTURE analysis was applied to the microsatellite data from all 18 populations integrating the geographical localities of individuals as prior information (LOCPRIOR). Three separate tests were run: with prior $K = 2$ when testing for a particular separation of the races into two pairs on the basis of the microsatellite loci being tested (five loci on chromosomes 10 and 12; and seven loci on chromosomes 7 and 18), and prior $K = 4$ when data from all 12 microsatellite loci on chromosomes 10, 12, 7, and 18 should separate individuals precisely into the four races—CHPO, ILVA, IMVA, and IUVA. Priors $K = 2$ and $K = 4$ were simulated for 200,000 MCMCs replicates after a burn-in period of 100,000 iterations. Ten independent runs for each value of K were performed to corroborate that the estimates of the posterior probabilities of K were consistent across runs.

Results and Discussion

To demonstrate that IMVA was a hybrid product, it is necessary to show that this race inherited metacentric 7.18 from ILVA and metacentric 8.12 and telocentrics 2 and 10 from CHPO, while a hybrid IUVA should have inherited metacentrics 2.8 and 10.12 from ILVA and telocentrics 7 and 18 from CHPO (Table 1). To this end, centromeric microsatellite loci (five for chromosomes 10 and 12 and seven for chromosomes 7 and 18) were selected to best represent a particular telocentric or a chromosome arm of a particular metacentric (Riginos and Nachman 1999; Piálek et al. 2001; Panithanarak et al. 2004). If, as predicted, the chromosome 10 and 12 alleles in IMVA came from CHPO, and those in IUVA came from ILVA, we expected there to be significant among-group variance in an AMOVA when grouping CHPO + IMVA individuals (characterized by chromosomes 8.12, 2, 10) in comparison with ILVA + IUVA individuals (characterized by 2.8, 10.12). Therefore, we would not expect significant among-group variance when comparing ILVA + IMVA and CHPO + IUVA. Indeed, these expectations held for all five loci (Table 2). Similarly, if, as predicted, the alleles for chromosomes 7 and 18 in IMVA originated from ILVA, and those in IUVA from CHPO, then significant among-group variance would be expected when grouping ILVA + IMVA (characterized by 7.18) compared to CHPO + IUVA (characterized by 7, 18), but not when comparing CHPO + IMVA vs. ILVA + IUVA: these expectations also held for five and six of seven loci, respectively (Table 2). These conclusions are supported by Bayesian analysis using STRUCTURE (Fig. 2A and B). Furthermore, considering the full set of 12 centromeric microsatellite loci, the four chromosomal races appear as four separate entities in the STRUCTURE analysis (Fig. 2C). Clearly, these four races are distinct in terms of centromeric loci as well as chromosomes.

In our AMOVA tests, we grouped the four races into the two predicted combinations of pairs and examined among-group variance between the pairs. This provided a rigorous test of our hybrid raciation hypothesis, which was strongly supported. There were, however, unexpected results obtained for D7Mit143 and D18Mit166 (Table 2), perhaps reflecting that, from the most up-to-date mouse genome assembly, D7Mit143 is now known to be not particularly closely linked to the centromere and D18Mit166 is a locus that is now classified as of unknown position. Anomalous

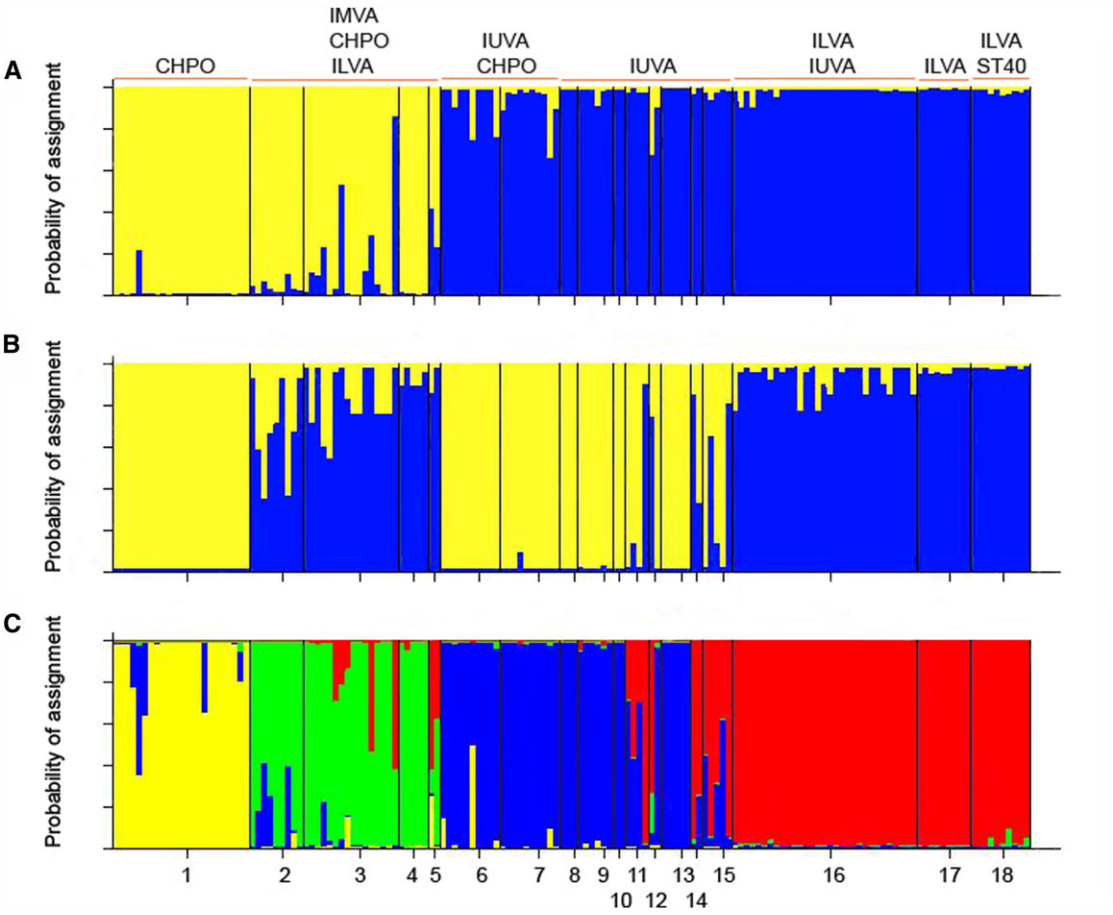

Figure 2. **Assignment of Valtellina mice to clusters on the basis of microsatellite genotype. Individuals (vertical lines) are ordered within 18 populations of defined karyotype as described in Figure 1 and Table S2. The races are listed in order of prevalence within groups of villages. Assignment to *K* clusters (different colors) follows analyses using STRUCTURE, based on data from: (A) all microsatellite loci on chromosomes 10 and 12, $K = 2$; (B) all microsatellite loci on chromosomes 7 and 18, $K = 2$; (C) all 12 microsatellite loci, $K = 4$. The results fit the expectation that IMVA and IUVA are hybrid products; that is, for loci on chromosomes 10 and 12, IMVA and CHPO individuals form one cluster, and IUVA and ILVA individuals another cluster (A); while for loci on chromosomes 7 and 18, the clusters are formed by IMVA and ILVA individuals and by IUVA and CHPO individuals (B); and when all loci are included in the analysis, individuals of the different races each form a separate cluster (C). For each of the analyses in (A), (B), and (C), there were 10 independent STRUCTURE runs. All 10 runs supported the partitioning shown in (A) and (B), and five of 10 runs supported the partitioning in (C).**

results due to homoplasy are another possibility (Estoup et al. 2002) but the allele size range at these loci is not particularly restricted or unusual (Table S3).

Our molecular analysis of hybrid raciation is important given previous findings from purely cytogenetic studies of common shrews and house mice. In the common shrew, Fedyk et al. (1991) provided evidence that selection favors a particular homozygous recombinant product in the hybrid zone between the Drnholec and Łęgucki Młyn chromosomal races in Poland (see also Wójcik et al. 2002). Another example of a homozygous, apparently hybrid, chromosomal form limited to a hybrid zone has been found in the house mouse in the John o'Groats-standard hybrid zone in Scotland (Searle et al. 1993). White et al. (2010) found for both common shrews and house mice that chromosomal races originating as novel homozygous (including hybrid) forms in hybrid zones shorten chromosomal phylogenies. Thus, generation of homozygous recombinant forms has long been recognized as part of a wider process whereby chromosomal races may arise in hybrid zones, termed "zonal raciation" (Searle 1984, 1993). Thus, molecular confirmation of hybrid race formation in house mice is notable.

Considering further the Valtellina hybrid zone, in our previous work we discussed the possible role of founder events, genetic drift and selection in the generation of hybrid races (Piálek et al. 2001; Hauffe et al. 2004). The distribution of mice and chromosomal races is patchy in the valley, reflecting discontinuity of suitable habitat (human dwellings, livestock facilities, and foodstores for humans and livestock), extinction events (e.g., flooding,

seasonality of habitat, and food availability due to transhumance) and the nature of colonization (Hauffe et al. 2004). Because humans are involved in the transport of house mice, the founding of populations may have represented erratic long- or short-distance dispersal events, and the populations would have shown boom-bust dynamics thereafter. Thus, within the hybrid zone, populations could, by chance, have become founded by homozygous recombinant mice (either IMVA or IUVA) or become dominated by such individuals shortly thereafter. Also, if a combination of ILVA and CHPO individuals founded a population, then that could also have favored one of the homozygous recombinant forms (either IMVA or IUVA) because, unlike the parental races, such recombinants do not generate hybrids that have BOTH a chain-of-five configuration (CV; 2-2.8-8.12-12.10-10) AND a chain-of-three configuration (CIII; 7-7.18-18) at meiosis I. Instead, they produce hybrids with EITHER a CV (when hybridizing one parental race) OR a CIII (when hybridizing the other parental race). If the unfitness of the CV + CIII hybrids is greater than the additive unfitness of CV hybrids and CIII hybrids, then selection should favor homozygous recombinants, promoting fixation of either IMVA or IUVA karyotype within populations (Piálek et al. 2001).

In flowering plants, there has been a particular interest in the generation of hybrid forms because such forms may become species, through recombinational speciation (Grant 1981; Abbott et al. 2010). Recombinational speciation occurs when the F_1 hybrids between the parental forms are unfit but not completely sterile and which, through further generations of hybridization, produce a novel, fully fertile chromosomally hybrid and chromosomally homozygous form, that increases to high frequency in the zone of hybridization. On chromosomal grounds the new hybrid form will be partially reproductively isolated from the parental forms, and genic changes may complete the speciation process. Among examples of recombinational speciation in plants, *Helianthus* has been particularly well-studied (Rieseberg 1997), including reconstruction of the speciation process through simulation modeling (Buerkle et al. 2000; Buerkle and Rieseberg 2008). The speciation process may involve the evolution of ecological differences, for example, the new hybrid species in *Helianthus* are more tolerant of xeric or marshy conditions than their parental species (Rieseberg 1997). There has also been an interest in the recombinational speciation process in insects, including generation of a population of a recombinant form in the laboratory (Harini and Ramachandra 2003).

Although the hybrid races in Valtellina have not followed precisely the same path in speciation as described in flowering plants, reproductive isolation has developed in this system also. It is noteworthy that the Valtellina chromosomal races are distinct in terms of centromeric loci as well as the chromosomes because it is genetic differentiation in the vicinity of the centromeres that might be expected to lead to reproductive isolation (Piálek et al. 2001; Panithanarak et al. 2004; Giménez et al. 2013). Such differentiation, at loci associated with mating preference, may explain the reproductive isolation (lack of hybridization) of CHPO and IUVA in the village of Migiondo recorded several decades ago (Capanna and Corti 1982; Hauffe and Searle 1992; Piálek et al. 2001). This observation demonstrates that one of the hybrid races (IUVA) has, at least once, developed traits over an extremely short period of time that prevented it interbreeding with one of the parental races (CHPO). In this way, hybrid raciation in the house mouse may lead to processes associated with speciation. It should, however, be emphasized that the selective process proposed to favor homozygous recombinants in hybrid zones locally reduces the occurrence of highly unfit F_1 hybrids between the parental races, and in this respect increases gene flow and reduces the opportunity for speciation, rather than enhancing it.

Whatever the extent to which homozygous recombinant forms in small mammals may or may not be involved in speciation does not detract from our demonstration that such forms exist. Our use of molecular markers to do that provides substance to previous studies based solely on chromosomal analysis. Thus, it can now be argued strongly that the diversity of chromosomal races in house mice and common shrews has increased through hybridization as well as through fixation of new chromosomal rearrangements, and that it is realistic to include this process of race formation in chromosomal phylogenies (White et al. 2010).

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DATA ARCHIVING

The doi for our data is http://dx.doi.org/10.5061/dryad.54g7g.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. List of all new populations and their karyotypes obtained for this study during 2005.
Table S2. Numbers of individuals with different karyotypes in each of the populations being studied.
Table S3. Range of microsatellite allele lengths for each locus for each race.