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Genetic Structure in Contemporary South Tyrolean Isolated Populations Revealed by Analysis of Y-Chromosome, mtDNA, and Alu Polymorphisms

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Abstract Most of the inhabitants of South Tyrol in the eastern Italian Alps can be considered isolated populations because of their physical separation by mountain barriers and their sociocultural heritage. We analyzed the genetic structure of South Tyrolean populations using three types of genetic markers: Y-chromosome, mitochondrial DNA (mtDNA), and autosomal *Alu* markers. Using random samples taken from the populations of Val Venosta, Val Pusteria, Val Isarco, Val Badia, and Val Gardena, we calculated genetic diversity within and among the populations. Microsatellite diversity and unique event polymorphism diversity (on the Y chromosome) were substantially lower in the Ladin-speaking population of Val Badia compared to the neighboring German-speaking populations. In contrast, the genetic diversity of mtDNA haplotypes was lowest for the upper Val Venosta and Val Pusteria. These data suggest a low effective population size, or little admixture, for the gene pool of the Ladin-speaking population from Val Badia. Interestingly, this is more pronounced for Ladin males than for Ladin females. For the pattern of genetic *Alu* variation, both Ladin samples (Val Gardena and Val Badia) are among the samples with the lowest diversity. An admixture analysis of one German-speaking valley (Val Venosta) indicates a relatively high genetic contribution of Ladin origin. The reduced genetic diversity and a high genetic differentiation in the Rhaetoroman-and German-speaking South Tyrolean populations may constitute an important basis for future medical genetic research and gene mapping studies in South Tyrol.

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South Tyrol, in the eastern Italian Alps, is a region with complex ethnic structure and population history. The local populations are geographically separated from each other as a result of the mountainous terrain and are linguistically heterogeneous. Three main languages (German, Italian, and Ladin) are spoken in the region. The total population of South Tyrol is 463,000: 69% are German speakers, 26% are Italian speakers, and 4% are Ladin speakers. The Italian speakers of South Tyrol arrived after the detachment of this region from Austria in 1918, and they live mainly in larger towns or cities (Parteli 1988).

The marriage pattern has been conservative up to recent times and was geographically and/or linguistically confined. Until 1950, 50% of marriages were made within the same village, 40% within the immediate surroundings, and only 10% with other villages.

Isolated populations present many advantages over outbred populations in gene mapping studies because of reduced genetic complexity and high linkage disequilibrium (Peltonen et al. 2000; Heutink and Oostra 2002). They have proven useful for studying monogenic diseases (Hiltunen et al. 1991; Visapaa et al. 2002), but they are also useful for studying complex diseases because a smaller number of susceptibility loci and disease alleles at each locus can be reasonably expected.

Genetic variation can elucidate the history of human populations (Underhill et al. 2000), allow us to better understand genetic and evolutionary relationships that interact with cultural and geographic distances, and determine disease susceptibility (Botstein and Risch 2003). A better understanding of genetic variation can help to guide gene mapping efforts. Before using a population isolate as a resource for complex disease gene mapping, investigators must complete studies of genetic diversity. The investigation of genetic diversity and biodemographic parameters, such as population origin, immigration, expansion, and effective size, are of particular relevance (Risch 2000).

Historical documentation for the South Tyrol region began with the arrival of the Romans 2,000 years ago (Dal Ri 2002). At that time the territory of the eastern Italian Alps was inhabited by a native population known as Rhaetii (Metzger 1992). With the breakup of the Western Roman Empire, German tribes moved in from the north. By the millennium's end (A.D. 1000) a period of intensive Germanization began, and the Rhaetoroman-speaking (Ladin) areas contracted and became increasingly isolated (Loose 1996). Today the Ladin speakers in South Tyrol are restricted to two main valleys, Val Badia and Val Gardena. The distribution of toponyms in the eastern Alps confirms a much broader diffusion of Rhaetoroman language in the early Middle Ages (Kramer 2004).

A few genetic studies on populations in South Tyrol were previously conducted using mtDNA, with a focus on the Ladin speakers (Stenico et al. 1996, 1998; Vernesi et al. 2002). There was no attempt at an area-wide mapping of the other isolated German-speaking valleys in the eastern Italian Alps.

In this study we analyze the genetic diversity within and among the contemporary populations of the main valleys in South Tyrol [Val Venosta (Vinschgau), Val Isarco (Eisacktal), Val Pusteria (Pustertal), and the Ladin valleys Val

Badia (Gadertal) and Val Gardena (Grödnertal)]. We used three types of genetic markers: Y-chromosome, mtDNA loci, and autosomal *Alu* markers. Because of their uniparental inheritance and the absence of recombination, Y-chromosome and mtDNA polymorphisms are particularly useful for tracing the separate history of paternal and maternal lineages. Variation in autosomal *Alu* insertion polymorphisms reflects both the maternal and paternal history of the populations.

Materials and Methods

DNA Samples. A total of 277 blood samples were randomly collected: 126 participants from Val Venosta (taking into account the historical division between the upper and lower Val Venosta), 42 participants from Val Pusteria, 39 participants from Val Isarco, 35 participants from Val Badia, and 35 participants from Val Gardena. Participants from these valleys were recruited by general practitioners and from blood donor centers in the region's hospitals (Figure 1). All study participants, selected for third-generation ancestry in the appropriate valleys, were older than 18 years of age and gave their informed consent for the study, which was approved by the Ethics Committee of the Autonomous Province of Bolzano. In addition, we focused on a small village, Stelvio (STE) (1,500 inhabitants), in an isolated subvalley of the upper part of Val Venosta, where we were able to take DNA samples from two different groups of males, those with Rhaetoroman surnames (STErhae, $N = 20$) and those with German surnames (STEgerm, $N = 17$). Consanguinity up to the third degree among sampled individuals was avoided on the basis of the detailed genealogical records available. Total genomic DNA was extracted from whole blood according to standard procedures (Sambrook et al. 1989).

Y-Chromosome Genotyping. Slowly evolving biallelic unique event polymorphisms (UEP markers, mutation rate of 10^{-8} per generation) on the Y chromosome allow identification of genealogical groups (haplogroups) of chromosomes related by descent, whereas rapidly evolving microsatellites (STRs, mutation rate of 10^{-3} per generation) are used to distinguish more closely related chromosomes within haplogroups. The allelic state of nine microsatellite loci and eleven biallelic polymorphisms (UEPs) was determined using four multiplex PCR kits (Thomas et al. 1999):

Microsatellite kit 1 (MS1): *DYS19*, *DYS388*, *DYS390*, *DYS391*, *DYS392*, *DYS393*
Microsatellite kit 2 (MS2): *DYS388* as internal control, *DYS389I/II*, DYS426
UEP kit 1 (UEP1): 92R7, Tat, sY81, SRY465, SRY4064
UEP kit 2 (UEP2): M9, M13, M17, M20, SRY10831

Multiplex kits UEP1 and UEP2 were digested with a cocktail of restriction enzymes and were subsequently typed for DNA fragments with specific size

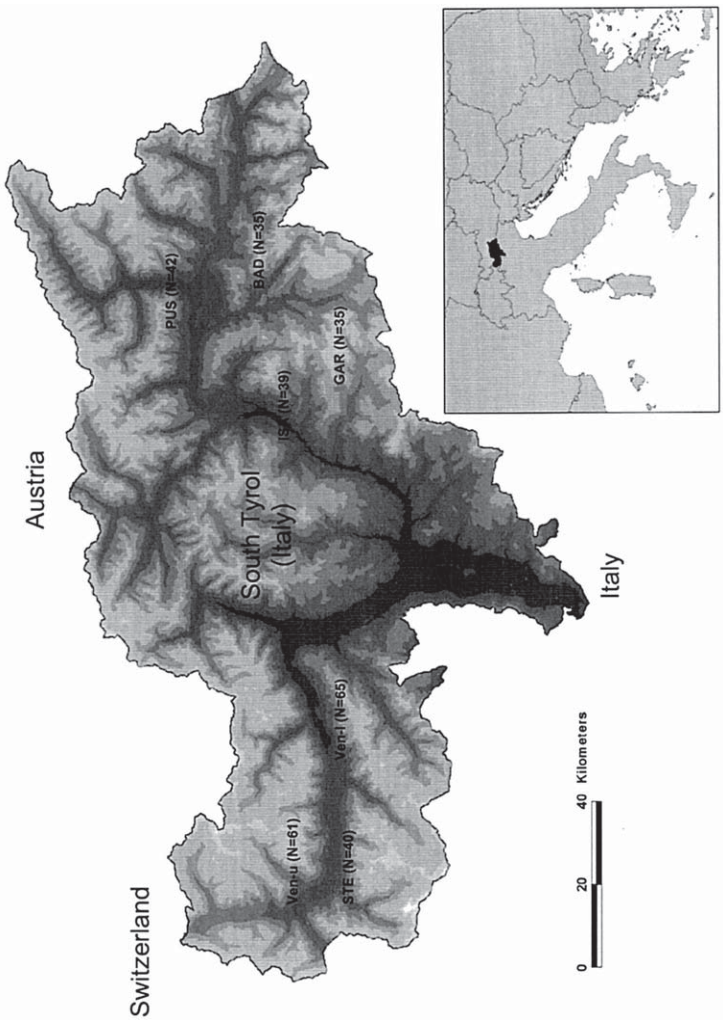

Figure 1. Map of South Tyrol indicating the valleys (PUS, Val Pusteria; ISA, Val Isarco; BAD, Val Badia; VEN-u, upper Val Venosta; VEN-l, lower Val Venosta; GAR, Val Gardena) and the small villageStelvio (STE) sampled for this study.

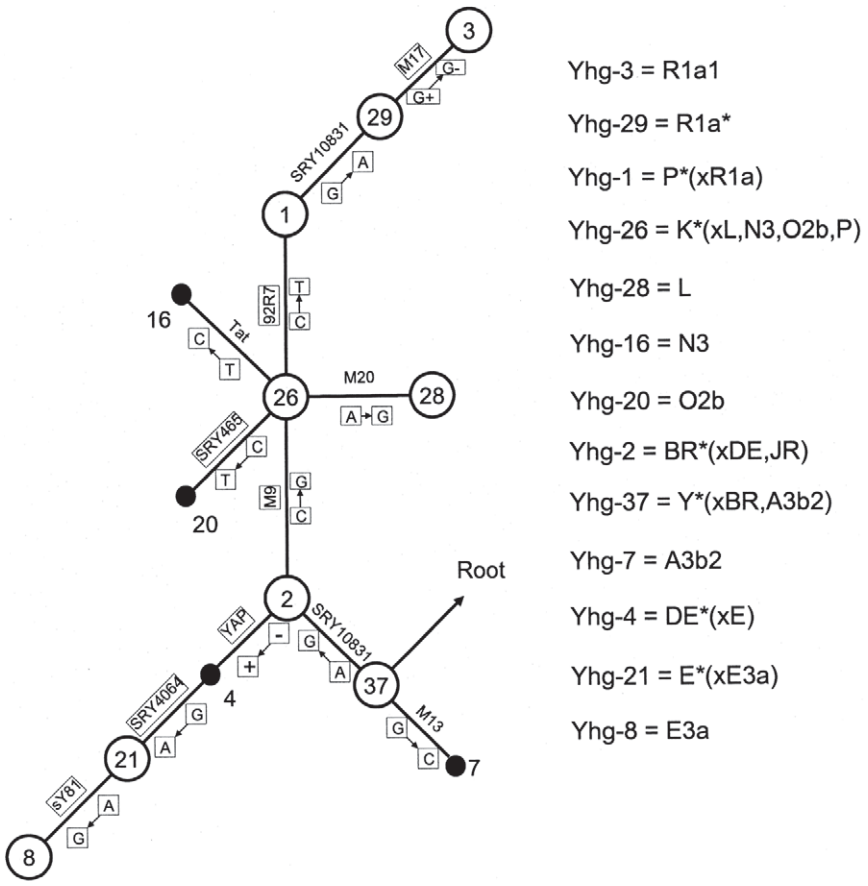

Figure 2. Y-chromosome haplogroup network defined by 11 UEP markers. Haplogroups indicated with open circles were found in the South Tyrolean populations; those with filled circles were not found.

and dye label. Samples were processed on the ABI Prism 3100-Avant Genetic Analyzer (Applied Biosystems, Foster City, California). In addition, we performed a single PCR for the YAP *Alu* insertion polymorphism, and products were visualized on 1% agarose gel. Microsatellite repeat sizes were assigned according to the nomenclature of Kayser et al. (1997). UEP-defined haplogroups were assigned using a nomenclature modified from Rosser et al. (2000) and Weale et al. (2001) (Figure 2). The correspondence between this nomenclature and that proposed by the Y Chromosome Consortium (2002) is as follows: Yhg1 = P* (xR1a), Yhg2 = BR* (xDE, JR), Yhg3 = R1a1, Yhg4 = DE* (xE), Yhg7 = A3b2, Yhg8 = E3a, Yhg16 = N3, Yhg20 = O2b, Yhg21 = E* (xE3a), Yhg26 = K* (xL, N3, O2b, P), Yhg28 = L, Yhg29 = R1a*, Yhg37 = Y* (xBR, A3b2).

mtDNA Sequencing. mtDNA variation was assessed in the hypervariable region I (HVRI) of the relatively fast evolving control region. mtDNA was amplified using the primers L15926 and H16498, and PCR products were purified using the Montage PCR96 Cleanup Kit (Millipore, Billerica, Massachusetts). A sequence of 360 bp within the HVRI region [positions 16024–16383 of the Cambridge Reference Sequence (CRS) (Anderson et al. 1981)] was obtained by using L15997 and/or H16401 as sequencing primers (Vigilant et al. 1991) and the ABI BigDye terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Sequencing reactions were purified with the Montage SEQ96 Sequencing Reaction Cleanup Kit (Millipore). Samples were analyzed on the ABI Prism 3100-Avant Genetic Analyzer (Applied Biosystems).

mtDNA haplotypes were assigned to haplogroups by identifying key combinations of HVRI mutations according to Macaulay et al. (1999), Richards et al. (2000), and Maca-Meyer et al. (2001), as follows:

6069T, 16126C = Mhg-J
16069T, 16126C, 16145A, 16261T = Mhg-J1
16069T, 16126C, 16193T = Mhg-J2
16224C, 16311C = Mhg-K
16126C, 16294T = Mhg-T
16126C, 16163G, 16186T, 16189C, 16294T = Mhg-T1
16126C, 16294T, 16304C = Mhg-T2
16051G = Mhg-U2
16343G = Mhg-U3
16356C = Mhg-U4
16270T = Mhg-U5
16172C, 16219G = Mhg-U6
16298C = Mhg-V 16223T, 16292T = Mhg-W

For the remaining haplotypes those with a T at position 16223 were assigned to Mhg-MNL and those with a C at position 16223 were assigned to Mhg-HVR.

***Alu* Genotyping.** Eight autosomal *Alu* insertion polymorphisms (*D1*, *A25*, *TPA25*, *PV92*, *F13B*, *ACE*, *B65*, and *APO*) on six different chromosomes were genotyped. These polymorphisms are widely used in genotyping different world populations (Stoneking et al. 1997; Nasidze et al. 2001). Primers and PCR amplification conditions have been described previously (Arcot et al. 1995a, 1995b). Products were visualized on 1% agarose gel.

Statistical Analysis. We used several measures of the within-population diversity. Average gene diversities over all *Alu* loci and Y-chromosome markers were calculated for each population sample with the software package Arlequin (available at http://lgb.unige.ch/arlequin). Average nucleotide diversities of the mtDNA sequence data were similarly computed. Unbiased genetic diversity esti-

mates of mtDNA and Y-chromosome haplotypes were calculated according to Nei (1987).

Population differentiation was tested by permutation tests (10,000 permutations) based on *F* statistics as implemented by Arlequin. Population pairwise F_{ST} values were transformed according to Reynolds et al. (1983) in order to linearize them with population divergence time. Although the assumption of equal demographic history may be wrong, Reynolds distances are more appropriate for a genetic drift model than raw F_{ST} values are. The Reynolds distances were used in a standard multidimensional scaling algorithm (The R-Project for Statistical Computing) to visualize the genetic relationship among the population samples. Mantel tests with 100,000 permutations were performed to test for correlations between genetic and geographic distance matrices. Geographic distances were measured along the valleys, which were the main migration routes.

The relative genetic (Y-chromosome) contributions of German or Ladin origin to the Val Venosta population was tested by a likelihood-based approach with the software LEA (Chikhi et al. 2001). In addition, samples from the small village Stelvio in the upper Val Venosta were separated according to the German and Rhaetoroman surnames, in order to test for differential genetic traces in these different surname groups. The admixture model assumes two parental populations that mix and create a hybrid population some time in the past. After that event the three populations evolve under independent genetic drift without migration and mutation (Chikhi et al. 2001). We used our Val Badia sample data and published data of Germans (Rosser et al. 2000) as potential parental populations of Ladin and German origin, respectively. We pooled the UEP haplogroup 9 with haplotype 2, haplogroup 22 with haplotype 1, haplogroups 28 and 20 with haplotype 26, haplogroup 29 with haplotype 3, and haplogroup 37 with haplotype 7 because not exactly the same set of UEP markers were genotyped by Rosser et al. (2000) and in our study.

Results

The 11 biallelic UEP markers on the Y chromosome defined 9 haplogroups in the population samples under study (Table 1; Figure 2), and the UEP + microsatellite markers defined 120 haplotypes (Table 2). The Val Badia sample showed the most differentiated haplogroup distribution. Haplotypes Yhg1 and Yhg28 are overrepresented compared to the German population samples. The Val Badia and Val Pusteria populations share a frequent haplotype, which is rare in all other populations sampled. This Yhg1 haplotype (haplotype 2) is found in the Val Badia sample at a frequency of 25% and in the Val Pusteria sample at a frequency of 17% (Table 2). Homoplasy detected by microsatellite haplotypes nested within UEP haplogroups was low (6/120).

Eighty different mtDNA haplotypes were observed in the 219 individuals sequenced (Table 3). Overall, 77 sites were polymorphic. The haplotypes were clustered in 14 haplogroups based on the occurrence of key mutations (see

Table 1. Frequencies of Y-Chromosome Haplogroups (UEP-Defined) in South Tyrolean Populations

Yhg Haplogroup	*Val Pusteria* ($N = 35$)	*Val Isarco* ($N = 34$)	*Val Badia* ($N = 24$)	*Lower Val Venosta* ($N = 32$)	*Upper Val Venosta* ($N = 32$)	*Stelvio* ($N = 37$)	*Total* ($N = 194$)
hg1	16 (0.457)	16 (0.471)	14 (0.584)	15 (0.469)	18 (0.562)	13 (0.351)	92 (0.474)
hg2	12 (0.343)	10 (0.294)	5 (0.208)	11 (0.344)	10 (0.312)	12 (0.324)	60 (0.309)
hg29	2 (0.057)	5 (0.147)	3 (0.125)	–	–	–	10 (0.052)
hg21	3 (0.086)	1 (0.029)	–	1 (0.031)	3 (0.094)	5 (0.135)	13 (0.067)
hg26	–	1 (0.029)	–	1 (0.031)	–	5 (0.135)	7 (0.036)
hg3	–	–	–	4 (0.125)	1 (0.031)	1 (0.027)	6 (0.031)
hg28	1 (0.029)	–	2 (0.083)	–	–	–	3 (0.015)
hg8	–	1 (0.029)	–	–	–	1 (0.027)	2 (0.010)
hg37	1 (0.029)	–	–	–	–	–	1 (0.005)

Materials and Methods section). Table 4 summarizes the frequency of mtDNA haplogroups in our sample. All haplogroups considered typical of Europe were also observed in our populations. The most frequent haplogroup was Mhg-HVRI, followed by Mhg-U, J, and K. Haplogroup T, which has been described elsewhere as common among Ladin speakers (Stenico et al. 1996, 1998), was found in our Ladin sample from Val Badia at a frequency of 8.5% (T1 + T2); the maximum frequency in all populations sampled was 12.7% for lower Val Venosta. A maximum frequency difference of 23.5% for Mhg-U5 was observed.

Table 5 indicates the observed frequencies of the *Alu* insertions at the eight loci typed in this study. All loci were polymorphic in all populations.

The largest population differences in terms of within-population genetic diversity appear for the Y-chromosome markers (Table 6). In particular, the Val Badia sample sticks out with a substantially lower genetic diversity than all other population samples. The difference of the Val Badia diversity is especially pronounced for the microsatellite markers, indicating a relatively recent population bottleneck and/or genetic isolation for the male gene pool of this Ladin valley. mtDNA variation, which is shaped by female-specific genetic history, shows a different pattern (Table 6). Relatively low diversity values appear for the upper Val Venosta and Val Pusteria samples but not for the Val Vadia sample. The autosomal *Alu* markers represent the male and female effects, and again the Ladin populations (Val Badia and Val Gardena) are among the samples with lowest genetic diversities. The independence of the different marker types is also obvious from the lack of significant correlations among the genetic distance matrices of the different marker types. Particularly contrasting patterns between Y-chromosome and mtDNA markers are indicated for the between-population structure. The Val Badia sample deviates significantly ($p < 0.05$) from the Val Venosta samples (upper Val Venosta, lower Val Venosta, Stelvio) for the microsatellite markers on the Y chromosome (Figure 3A). This corroborates the previously mentioned

Table 2. Frequencies of Observed Y-Chromosome UEP + Microsatellite Haplotypes in South Tyrolean Populations[a]

Yhg Haplo-group	*Microsatellite Haplotype*	*Number*	*Val Pusteria* (*N* = 35)	*Val Isarco* (*N* = 34)	*Val Badia* (*N* = 24)	*Lower Val Venosta* (*N* = 32)	*Upper Val Venosta* (*N* = 32)	*STEgerm* (*N* = 17)[b]	*STErhae* (*N* = 20)[b]	*Total* (*N* = 194)
hg1	14 12 24 10 12 13 10 12 29	1	0	0	0	0	0	1	0	1
hg1	14 12 24 11 13 13 10 12 28	2	6	1	6	0	1	1	2	17
hg1	14 12 25 10 13 12 10 12 28	3	0	0	0	0	0	1	0	1
hg1	14 12 23 11 13 13 10 12 28	4	0	2	0	0	0	0	0	2
hg1	14 12 24 11 13 13 10 11 30	5	0	1	0	0	0	0	0	1
hg1	14 12 24 11 13 13 10 12 29	6	0	2	1	0	0	0	0	3
hg1	14 12 24 11 13 13 10 13 29	7	0	0	0	1	0	0	0	1
hg1	15 12 23 10 15 11 09 13 29	8	0	0	0	0	0	1	0	1
hg1	13 12 24 11 13 13 10 12 28	9	0	0	0	0	1	0	1	2
hg1	14 12 22 11 13 12 10 13 30	10	0	0	0	0	0	0	1	1
hg1	14 12 23 10 13 13 10 12 28	11	1	1	0	1	1	0	3	7
hg1	14 12 25 11 13 13 10 12 28	12	0	2	0	0	0	0	1	3
hg1	15 11 24 11 13 13 10 13 29	13	0	0	0	0	0	0	1	1
hg1	14 12 24 11 13 13 10 11 29	14	1	0	0	0	0	0	0	1
hg1	14 13 23 11 13 13 10 11 26	15	1	0	0	0	0	0	0	1
hg1	15 12 23 11 13 11 10 13 29	16	1	0	0	0	0	0	0	1
hg1	14 12 23 10 13 13 10 13 29	17	1	0	0	0	0	0	0	1
hg1	14 12 24 11 13 13 11 12 28	18	1	1	0	0	0	0	0	2
hg1	14 12 23 10 12 13 10 12 28	19	1	0	0	0	0	0	0	1
hg1	15 12 23 11 13 13 10 12 29	20	1	0	0	0	0	0	0	1
hg1	14 12 25 11 13 15 10 13 29	21	1	0	0	0	0	0	0	1
hg1	14 12 24 10 13 13 10 12 28	22	1	0	0	1	0	0	0	2
hg1	14 12 24 11 13 13 10 12 30	23	0	0	2	0	0	0	0	2
hg1	14 12 24 10 13 13 11 12 29	24	0	2	1	0	0	0	0	3
hg1	14 12 24 10 13 13 10 13 30	25	0	0	1	0	0	0	0	1
hg1	15 12 24 11 13 13 10 12 29	26	0	0	1	0	0	0	0	1

Table 2. (*continued*)

Yhg Haplo-group	*Microsatellite Haplotype*	*Number*	*Val Pusteria* (*N* = 35)	*Val Isarco* (*N* = 34)	*Val Badia* (*N* = 24)	*Lower Val Venosta* (*N* = 32)	*Upper Val Venosta* (*N* = 32)	*STEgerm* (*N* = 17)[b]	*STErhae* (*N* = 20)[b]	*Total* (*N* = 194)
hg1	14 12 24 10 13 13 11 12 30	27	0	0	1	0	0	0	0	1
hg1	14 12 24 11 13 14 10 12 28	28	0	0	1	1	0	0	0	2
hg1	14 12 24 10 14 12 10 13 30	29	0	1	0	0	0	0	0	1
hg1	14 12 25 11 13 13 10 12 29	30	0	1	0	0	0	0	0	1
hg1	14 12 25 10 13 13 10 12 28	31	0	1	0	0	0	0	0	1
hg1	15 12 23 12 13 13 10 12 29	32	0	1	0	0	0	0	0	1
hg1	13 12 24 10 16 13 10 12 27	33	0	0	0	1	0	0	0	1
hg1	14 12 23 11 13 13 10 11 27	34	0	0	0	2	0	0	0	2
hg1	14 12 24 10 13 14 10 12 28	35	0	0	0	2	0	0	0	2
hg1	14 12 24 11 12 14 10 12 28	36	0	0	0	1	0	0	0	1
hg1	14 12 24 11 13 12 10 13 30	37	0	0	0	1	0	0	0	1
hg1	14 12 24 11 13 13 11 13 28	38	0	0	0	1	0	0	0	1
hg1	14 12 24 11 14 13 10 12 28	39	0	0	0	1	0	0	0	1
hg1	15 12 24 10 14 12 10 12 28	40	0	0	0	1	0	0	0	1
hg1	15 13 24 11 13 13 10 12 27	41	0	0	0	1	0	0	0	1
hg1	14 10 23 11 13 13 10 11 27	42	0	0	0	0	1	0	0	1
hg1	14 12 23 11 15 13 10 12 28	43	0	0	0	0	2	0	0	2
hg1	14 12 24 10 13 13 11 12 28	44	0	0	0	0	2	0	0	2
hg1	14 12 24 10 14 12 10 12 28	45	0	0	0	0	1	0	0	1
hg1	14 12 24 11 13 10 10 12 28	46	0	0	0	0	1	0	0	1
hg1	14 12 24 11 13 13 09 12 27	47	0	0	0	0	2	0	0	2
hg1	14 12 25 10 14 13 10 12 28	48	0	0	0	0	1	0	0	1
hg1	14 12 25 11 13 13 10 11 27	49	0	0	0	0	1	0	0	1
hg1	14 12 25 11 13 14 10 12 28	50	0	0	0	0	1	0	0	1
hg1	15 12 24 11 13 13 10 12 28	51	0	0	0	0	1	0	0	1
hg1	16 12 25 10 13 12 09 12 28	52	0	0	0	0	2	0	0	2

hg2	15 13 22 10 11 13 09 11 29	53	0	0	0	0	0	3	0	3
hg2	15 13 22 10 11 13 09 11 27	54	0	0	0	0	0	1	0	1
hg2	15 15 23 10 11 13 08 11 28	55	0	2	0	1	1	1	0	4
hg2	15 15 24 10 11 12 09 11 27	56	0	2	0	1	1	1	0	4
hg2	14 14 22 11 11 13 09 12 29	57	0	0	0	0	1	1	0	2
hg2	14 15 23 11 11 12 09 12 28	58	0	0	0	0	0	1	0	1
hg2	14 14 22 10 11 13 09 11 27	59	2	0	1	0	0	0	2	5
hg2	14 15 22 10 11 13 09 12 29	60	0	0	0	0	0	0	1	1
hg2	17 15 24 10 11 12 09 11 28	61	0	0	0	0	0	0	1	1
hg2	16 15 25 11 11 12 09 11 27	62	3	0	0	0	0	0	0	3
hg2	15 13 25 10 12 15 09 12 28	63	1	0	0	0	0	0	0	1
hg2	15 12 21 10 11 14 08 11 28	64	1	0	0	0	0	0	0	1
hg2	15 15 24 10 11 12 09 12 29	65	2	0	0	0	0	0	0	2
hg2	14 12 21 10 11 14 08 11 27	66	1	0	0	0	0	0	0	1
hg2	14 13 23 10 11 13 09 12 29	67	1	0	0	0	0	0	0	1
hg2	15 14 23 11 11 13 09 11 28	68	1	0	1	0	0	0	0	2
hg2	15 12 23 10 11 13 09 11 29	69	0	0	2	0	0	0	0	2
hg2	14 16 24 10 11 12 09 11 27	70	0	0	1	0	0	0	0	1
hg2	15 10 22 10 11 12 09 12 28	71	0	1	0	0	0	0	0	1
hg2	17 13 26 11 11 13 09 12 28	72	0	1	0	0	0	0	0	1
hg2	14 14 22 11 11 13 09 12 28	73	0	1	0	0	0	0	0	1
hg2	14 15 22 11 11 12 09 12 30	74	0	1	0	0	0	0	0	1
hg2	15 13 23 10 12 16 09 12 28	75	0	1	0	0	0	0	0	1
hg2	14 14 22 11 11 14 09 11 27	76	0	1	0	0	0	0	0	1
hg2	14 14 23 10 11 13 09 12 27	77	0	0	0	1	0	0	0	1
hg2	14 15 24 11 11 12 09 12 28	78	0	0	0	1	0	0	0	1
hg2	15 13 22 10 11 13 09 11 28	79	0	0	0	2	0	0	0	2
hg2	15 13 22 10 11 13 09 12 29	80	0	0	0	1	0	0	0	1
hg2	15 13 22 10 11 14 09 11 28	81	0	0	0	1	0	0	0	1
hg2	15 13 23 10 11 14 09 11 28	82	0	0	0	1	0	0	0	1
hg2	15 14 22 10 11 13 09 11 27	83	0	0	0	1	0	0	0	1
hg2	15 14 22 10 11 13 09 11 27	84	0	0	0	1	0	0	0	1

Table 2. (*continued*)

Yhg Haplo-group	*Microsatellite Haplotype*	*Number*	*Val Pusteria* (*N* = 35)	*Val Isarco* (*N* = 34)	*Val Badia* (*N* = 24)	*Lower Val Venosta* (*N* = 32)	*Upper Val Venosta* (*N* = 32)	*STEgerm* (*N* = 17)[b]	*STErhae* (*N* = 20)[b]	*Total* (*N* = 194)
hg2	17 15 23 10 11 12 09 11 27	85	0	0	0	1	0	0	0	1
hg2	14 13 23 10 11 14 09 12 28	86	0	0	0	0	1	0	0	1
hg2	14 14 23 10 11 13 09 11 27	87	0	0	0	0	1	0	0	1
hg2	14 16 22 10 11 14 09 11 27	88	0	0	0	0	1	0	0	1
hg2	15 13 22 10 13 15 09 12 28	89	0	0	0	0	2	0	0	2
hg2	15 15 25 10 11 12 09 11 27	90	0	0	0	0	3	0	0	3
hg29	16 12 24 10 11 13 10 12 29	91	1	1	0	0	0	0	0	2
hg29	15 12 24 11 13 13 10 12 27	92	1	0	0	0	0	0	0	1
hg29	14 12 24 11 13 13 10 12 29	93	0	1	1	0	0	0	0	2
hg29	14 12 24 10 13 13 10 12 28	94	0	0	2	0	0	0	0	2
hg29	14 12 24 11 13 13 10 11 30	95	0	2	0	0	0	0	0	2
hg29	16 12 25 11 11 13 10 12 29	96	0	1	0	0	0	0	0	1
hg21	13 12 24 10 11 13 09 12 29	97	1	1	0	0	0	0	3	5
hg21	13 12 24 10 11 13 09 13 39	98	0	0	0	0	0	0	1	1
hg21	13 12 24 10 11 13 09 11 29	99	0	0	0	0	0	0	1	1
hg21	13 12 24 10 15 13 09 11 28	100	1	0	0	0	0	0	0	1
hg21	13 13 25 10 11 13 09 12 31	101	1	0	0	0	0	0	0	1

hg21	13 12 23 09 11 15 09 12 29	102	0	0	0	1	0	0	0	1
hg21	13 12 24 09 11 13 09 13 30	103	0	0	0	0	2	0	0	2
hg21	13 12 24 11 11 13 08 12 29	104	0	0	0	0	1	0	0	1
hg26	14 12 23 11 13 13 11 13 28	105	0	0	0	0	0	1	0	1
hg26	14 12 25 10 14 13 10 11 27	106	0	0	0	0	0	1	0	1
hg26	15 11 24 10 13 13 10 13 29	107	0	0	0	0	0	0	1	1
hg26	15 12 23 10 14 11 09 13 29	108	0	1	0	0	0	0	0	1
hg26	14 12 24 11 13 12 10 12 29	109	0	0	0	1	0	0	0	1
hg26	14 12 23 11 13 13 10 12 28	110	0	0	0	0	0	1	0	1
hg26	14 12 24 11 13 13 10 13 29	111	0	0	0	0	0	1	0	1
hg3	15 12 24 11 11 14 10 12 29	112	0	0	0	0	0	1	0	1
hg3	17 12 25 10 11 13 10 12 29	113	0	0	0	1	0	0	0	1
hg3	15 12 25 11 11 14 10 12 29	114	0	0	0	1	0	0	0	1
hg3	15 14 24 10 11 13 10 13 30	115	0	0	0	1	0	0	0	1
hg3	16 12 24 10 11 13 10 12 28	116	0	0	0	1	0	0	0	1
hg3	16 12 26 11 11 14 10 12 29	117	0	0	0	0	1	0	0	1
hg28	14 12 22 10 14 11 09 11 26	118	1	0	2	0	0	0	0	3
hg8	15 12 21 11 11 13 09 13 32	119	0	1	0	0	0	0	1	2
hg37	16 15 25 11 11 12 09 11 27	120	1	0	0	0	0	0	0	1

a. Microsatellites are given in the order *DYS19, DYS388, DYS390, DYS391, DYS392, DYS393, DYS426, DYS389I, DYS389II*. The Atlantic modal haplotype (AMH) is highlighted in gray.

b. STEgerm = Stelvio inhabitants with German surnames; STErhae = Stelvio inhabitants with Rhaetoroman surnames.

Table 3. Distribution of mtDNA Haplogroups in South Tyrolean Populations[a]

Differences from the Cambridge Reference Sequence (Anderson et al. 1981)	*Val Pusteria* (*N* = 37)	*Val Isarco* (*N* = 34)	*Val Badia* (*N* = 35)	*Lower Val Venosta* (*N* = 55)	*Upper Val Venosta* (*N* = 58)	*Total* (*N* = 219)	*mtDNA Haplo-group*
Cambridge Reference Sequence	7	3	6	10	13	39	HVR
126C, 355T, 362C	1	0	0	0	0	1	HVR
266T, 311C	8	0	0	0	0	8	HVR
261T, 291T, 311C	3	1	0	0	0	4	HVR
189C, 192T	2	0	0	0	2	4	HVR
354T	2	2	0	0	0	4	HVR
183delA	2	0	0	0	0	2	HVR
70T, 129A	0	1	0	0	0	1	HVR
129A	0	2	1	0	0	3	HVR
172C, 362C	0	1	4	0	0	5	HVR
235G	0	1	0	0	0	1	HVR
287T, 304C, 311C	0	1	0	0	0	1	HVR
304C, 311C	0	1	0	2	1	4	HVR
42A, 288C	0	1	0	0	0	1	HVR
261T	0	0	3	0	0	3	HVR
352C, 354T	0	0	3	0	0	3	HVR
300G, 325C, 362C	0	0	1	0	0	1	HVR
181G	0	0	1	0	0	1	HVR
92C	0	0	1	0	0	1	HVR
93C, 293G, 311C	0	0	1	0	0	1	HVR
311C	0	0	0	2	5	2	HVR
362C	0	0	0	0	1	1	HVR
126C, 145A, 231C, 261	0	0	0	0	1	1	HVR
155G, 181T, 185G	0	0	0	0	1	1	HVR
248T	0	0	0	0	1	1	HVR
146G, 342C	0	0	0	3	1	1	HVR
67T	0	0	0	0	1	1	HVR
85A, 311C	0	0	0	0	1	1	HVR
193T	0	0	0	0	2	2	HVR
148T	0	0	0	0	1	1	HVR
126C, 261T	0	0	0	0	1	1	HVR
184T, 362C	0	0	0	2	1	3	HVR
181G, 354T	0	0	0	2	1	3	HVR
260T, 304C	0	0	0	1	0	1	HVR
93C, 169T, 265G	0	0	0	1	0	1	HVR
93C, 169T, 263C	0	0	0	1	0	1	HVR
274A	0	0	0	1	0	1	HVR
192T, 311C	0	0	0	1	0	1	HVR
126C, 176T, 265G, 319A	0	0	0	1	0	1	HVR
69T, 126C, 251C	1	0	0	0	0	1	J
69T, 126C	2	1	1	3	6	13	J
69T, 126C, 169T	0	0	1	0	0	1	J
69T, 126C, 189C	0	0	0	1	0	1	J
69T, 126C, 224C	0	0	0	2	0	2	J
69T, 126C, 261T	0	0	0	0	1	1	J1

Table 3. (*continued*)

Differences from the Cambridge Reference Sequence (Anderson et al. 1981)	*Val Pusteria* (*N* = 37)	*Val Isarco* (*N* = 34)	*Val Badia* (*N* = 35)	*Lower Val Venosta* (*N* = 55)	*Upper Val Venosta* (*N* = 58)	*Total* (*N* = 219)	*mtDNA Haplo-group*
69T, 126C, 193T, 265G, 278T	0	0	2	0	0	2	J2
69T, 126C, 193T, 290T	0	0	0	0	1	1	J2
69T, 126C, 145A, 231C, 261T	0	0	0	1	0	1	J1
224C, 311C	2	3	3	4	1	13	K
93C, 189A, 224C, 274A, 311C, 362C	0	0	0	0	1	1	K
129A, 224C, 311C	0	0	0	0	1	1	K
93C, 153A, 224C, 274A, 311C	0	0	0	1	0	1	K
224C, 311C, 320T	0	0	0	1	0	1	K
93C, 167T, 224C, 311C	0	0	0	0	1	1	K
192T, 223T, 325C	0	2	0	0	0	2	MNL
129A, 148T, 192T, 223T, 294T	0	0	2	0	0	2	MNL
129A, 223T	0	0	0	0	1	1	MNL
126C, 163G, 186T, 189C, 294T	2	0	1	1	0	4	T1
126C, 163G, 186T, 189C, 261T, 294T	0	0	0	2	1	3	T1
126C, 147T, 224C, 294T, 296T, 297C, 304C, 362C	0	0	2	0	0	2	T2
126C, 294T, 296T, 304C	0	0	0	4	1	5	T2
51G, 162G, 266T	0	1	0	0	0	1	U2
51G, 129C, 183C, 209C, 260T, 362C	0	0	0	0	1	1	U2
233G, 256T, 311C, 343G	0	0	1	0	0	1	U3
343G	0	0	0	0	2	2	U3
179T, 356C	0	3	0	0	0	3	U4
356C	0	0	0	0	3	3	U4
176T, 319A, 356C, 362C	0	0	0	1	0	1	U4
192T, 249C, 256T, 270T	1	4	0	0	0	5	U5
168T, 192T, 256T, 270T	1	1	0	2	0	4	U5
192T, 256T, 270T	0	3	0	0	0	3	U5
192T, 256T, 270T, 320T	0	0	0	0	1	1	U5
192T, 256T, 270T, 294T	0	0	0	4	0	4	U5
298C	2	2	0	0	0	4	V
162G, 298C	0	0	1	0	0	1	V
192T, 223T, 292T	1	0	0	0	1	2	W
223T, 234T, 292T	0	0	0	0	1	1	W
223T, 249C, 292T	0	0	0	1	0	1	W

a. Only differences from the Cambridge Reference Sequence (Anderson et al. 1981) between positions 16024 and 16383 are shown.

Table 4. Frequencies of mtDNA Haplogroups in South Tyrolean Populations

mtDNA Haplogroup	*Val Pusteria* ($N = 37$)	*Val Isarco* ($N = 34$)	*Val Badia* ($N = 35$)	*Lower Val Venosta* ($N = 55$)	*Upper Val Venosta* ($N = 58$)	*Total* ($N = 219$)
HVR	25 (0.676)	14 (0.412)	21 (0.600)	27 (0.491)	34 (0.586)	121 (0.553)
J	3 (0.081)	1 (0.029)	2 (0.057)	5 (0.091)	7 (0.121)	18 (0.082
J1	–	–	–	1 (0.018)	1 (0.017)	2 (0.009)
J2	–	–	2 (0.057)	1 (0.018)	–	3 (0.014)
K	2 (0.054)	3 (0.088)	3 (0.086)	6 (0.109)	4 (0.069)	18 (0.082)
MNL	–	2 (0.059)	2 (0.057)	–	1 (0.017)	5 (0.023)
T1	2 (0.054)	–	1 (0.029)	3 (0.055)	1 (0.017)	7 (0.032)
T2	–	–	2 (0.057)	4 (0.072)	1 (0.017)	7 (0.032)
U2	–	1 (0.029)	–	–	1 (0.017)	2 (0.009)
U3	–	–	1 (0.029)	–	2 (0.034)	3 (0.014)
U4	–	3 (0.088)	–	1 (0.018)	3 (0.052)	7 (0.032)
U5	2 (0.054)	8 (0.235)	–	6 (0.109)	1 (0.017)	17 (0.078)
V	2 (0.054)	2 (0.059)	1 (0.029)	–	–	5 (0.023)
W	1 (0.027)	–	–	1 (0.018)	2 (0.034)	4 (0.018)

hypothesis that Ladin males experienced a past population bottleneck and/or isolation. Similarly, the male gene pool of the upper Val Venosta sample with a high proportion of Ladin admixture is differentiated from all other populations. There is an overall positive correlation between geographic distances and genetic distances calculated from Y-chromosome microsatellite data ($p = 0.021$; $r^2 = 0.084$), indicating a general isolation-by-distance effect for the male gene pool. A similar differentiation pattern is obvious from Y-chromosome UEP markers (Figure 3B), although it was not significant in the Mantel test.

Table 5. Frequencies of *Alu* Insertions at Eight *Alu* Loci

Alu	*Val Pusteria* ($N = 42$)	*Val Isarco* ($N = 39$)	*Val Badia* ($N = 30$)	*Lower Val Venosta* ($N = 65$)	*Upper Val Venosta* ($N = 61$)	*Val Stelvio* ($N = 40$)	*Total* ($N = 35$)
D1	31 (0.369)	32 (0.410)	22 (0.367)	43 (0.330)	41 (0.336)	20 (0.250)	28 (0.400)
A25	9 (0.107)	7 (0.090)	8 (0.133)	11 (0.084)	21 (0.172)	8 (0.100)	6 (0.086)
TPA25	49 (0.583)	45 (0.577)	40 (0.667)	71 (0.546)	77 (0.631)	47 (0.588)	29 (0.414)
PV92	13 (0.155)	12 (0.154)	6 (0.100)	23 (0.176)	19 (0.156)	19 (0.238)	6 (0.086)
F13B	57 (0.679)	41 (0.526)	27 (0.450)	42 (0.323)	30 (0.246)	37 (0.463)	33 (0.471)
ACE	32 (0.381)	31 (0.397)	24 (0.400)	63 (0.484)	69 (0.566)	35 (0.438)	24 (0.343)
B65	43 (0.512)	44 (0.564)	36 (0.600)	67 (0.515)	51 (0.418)	42 (0.525)	35 (0.500)
APO	83 (0.988)	75 (0.962)	57 (0.950)	124 (0.953)	117 (0.959)	73 (0.913)	68 (0.971)

Table 6. Average Gene Diversity and Haplotype Diversity for All Analyzed Populations

	ValPusteria (*N* = 35)	*Val Isarco* (*N* = 34)	*Val Badia* (*N* = 24)	*Lower Val Venosta* (*N* = 32)	*Upper Val Venosta* (*N* = 32)	*Stelvio* (*N* = 37)[a]	
Y chromosome							
UEP gene diversity	0.142 ± 0.096	0.136 ± 0.093	0.116 ± 0.084	0.141 ± 0.096	0.134 ± 0.093	0.156 ± 0.104	
UEP haplogroup diversity	0.681	0.688	0.666	0.665	0.596	0.754	
STR gene diversity	0.594 ± 0.326	0.556 ± 0.308	0.443 ± 0.256	0.599 ± 0.330	0.585 ± 0.323	0.576 ± 0.317	
STR haplotype diversity	0.961	0.982	0.909	0.994	0.982	0.978 (STEgerm) 0.958 (STErhae)	
	ValPusteria (*N* = 37)	*Val Isarco* (*N* = 34)	*Val Badia* (*N* = 35)	*Lower Val Venosta* (*N* = 55)	*Upper Val Venosta* (*N* = 58)		
mtDNA HVRI (360 bp)							
Nucleotide diversity	0.009 ± 0.005	0.011 ± 0.0006	0.014 ± 0.008	0.013 ± 0.007	0.009 ± 0.005		
Haplotype diversity	0.911	0.961	0.945	0.949	0.935		
	ValPusteria (*N* = 42)	*Val Isarco* (*N* = 39)	*Val Badia* (*N* = 30)	*Lower Val Venosta* (*N* = 65)	*Upper Val Venosta* (*N* = 61)	*Stelvio* (*N* = 40)	*Val Gardena* (*N* = 35)
Alu insertions							
gene diversity	0.359 ± 0.211	0.372 ± 0.218	0.365 ± 0.215	0.370 ± 0.216	0.370 ± 0.216	0.386 ± 0.225	0.353 ± 0.209

a. STEgerm = Stelvio inhabitants with German surnames; STErhae = Stelvio inhabitants with Rhaetoroman surnames.

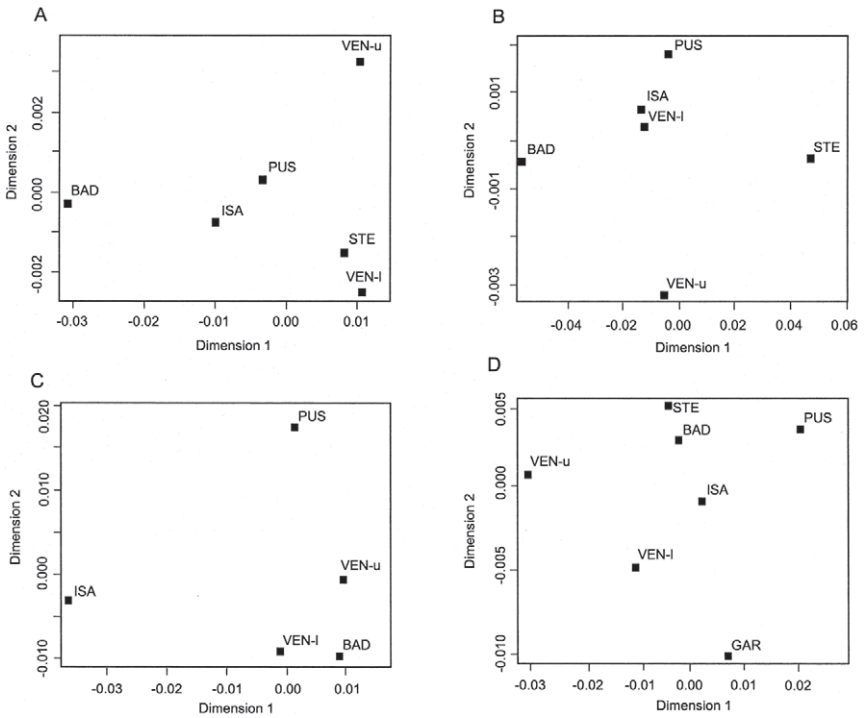

Figure 3. Multidimensional scaling plots based on Reynolds distances among samples calculated from (A) Y-chromosome microsatellite and (B) UEP data, (C) mtDNA data, and (D) *Alu* data. PUS, Val Pusteria; ISA, Val Isarco; BAD, Val Badia; VEN-u, upper Val Venosta; VEN-1, lower Val Venosta; GAR, Val Gardena.

For the mtDNA (Figure 3C) only the Val Isarco and Val Pusteria valleys are significantly differentiated ($p < 0.05$) from all other populations, and no overall isolation-by-distance effect can be observed.

The *Alu* variation (Figure 3D) is significant among the following population samples: upper Val Venosta with all other populations except lower Val Venosta; Val Gardena with Stelvio; and lower Val Venosta with Val Pusteria. It also indicates a relatively high differentiation between the Val Badia and Val Gardena Ladin valleys and a general correlation between genetic and geographic distances ($p = 0.030$; $r^2 = 0.178$).

Using the admixture-based approach of Chikhi et al. (2001), we tested the relative genetic contributions of putative source populations (German and Ladin) to the contemporary Val Venosta population, a German-speaking valley with a broad diffusion of the Rhaetoroman language until the late Middle Ages. We estimated the admixture proportions using Y-chromosome haplogroup frequencies. Putative source populations were our Ladin sample from Val Badia and published

Table 7. Posterior Estimates of Ladin Admixture Proportions (p1 in the LEA Software) for the Val Venosta and Stelvio Samples[a]

Sample	*p1 Median*	*p1 25th Percentile*	*p1 75th*
Val Venosta	0.57	0.35	0.82
STEgerm[b]	0.44	0.23	0.73
STErhae[b]	0.61	0.38	0.81

a. Admixture was tested for the parental populations Val Badia and Germans (Rosser et al. 2000) based on Y-chromosome haplogroups (UEP markers).

b. STEgerm = Stelvio inhabitants with German surnames; STErhae = Stelvio inhabitants with Rhaetoroman surnames.

data for a German population (Rosser et al. 2000). The analysis of Y-chromosome haplogroup frequency data indicates a relatively high genetic contribution of Ladins in the Val Venosta population (Table 7), which is also seen in the high number of Rhaetoroman surnames in this valley. In addition, we tested samples from one isolated village (Stelvio) in upper Val Venosta, separated according to the German and Rhaetoroman surnames. Data indicate differential genetic traces in these different surname groups and verify that the language affiliation of present surnames still bears genetic traces. Individuals with Rhaetoroman surnames have a higher probability of Ladin origin than individuals with German surnames (see Table 7).

Discussion

On the basis of historical data it has been suggested that the South Tyrolean populations of the different valleys have been strongly affected by the entrance of the Romans and of several German tribes during the historically reported migration events. For addressing genetic research in a given population, it is important to determine these components and evaluate their prevalence in the populations. Within-population diversity and population differentiation measures help us to understand how population demography and ethnicity can influence the distribution of genetic disease alleles.

The Y chromosome is particularly useful to unveil past reductions in population size because of its effective population size being four times lower than that of autosomal loci. Because of its paternal mode of inheritance and lack of recombination, the Y chromosome is extremely sensitive to founder effects and genetic drift, as seen by the isolation-by-distance pattern. The substantially lower genetic diversity in the Ladin Val Badia sample and the high degree of Y-chromosome differentiation among this Ladin sample and the neighboring populations suggest a past population bottleneck and/or genetic isolation together with a small effective population size, leading to genetic drift in the male gene pool of this Ladin valley. A high degree of haplotype sharing within populations or of population-specific haplotypes or both indicates small and/or isolated populations (Kayser et al. 2001). Y-chromosome haplotype 2 (within Yhg1) is frequently observed in

only two populations, Val Badia and Val Pusteria. UEP markers also indicate a differentiated Y-chromosome haplogroup distribution for Val Badia. The most common haplogroup (Yhg1) and the Atlantic modal haplotype AMH (defined as 14,12,24,11,13,13 for the markers *DYS19*, *DYS388*, *DYS390*, *DYS391*, *DYS392*, *DYS393*) have been suggested to be general (Semino et al. 2000) and specific (Wilson et al. 2001) markers for Paleolithic origin in Europe, respectively. Yhg1 occurs in the contemporary Ladin sample from Val Badia and in the German-speaking upper Val Venosta sample, which is known to have a high Ladin contribution, at a frequency of 58.3% and 56.3%, respectively (highest frequencies in our samples). In the Ladins haplotype AMH was found at a frequency of 41.7%, whereas in Val Pusteria, Val Isarco, Val Venosta, and Stelvio the frequencies were markedly lower. This suggests a predominantly Paleolithic rather than Neolithic origin for the Ladin Val Badia sample. The observed haplotype diversity values for the studied populations can be considered low compared to the values obtained for two other Northern Italian samples (Cerri et al. 2005; Turrina et al. 2006) and one Austrian sample (Berger et al. 2005). Studying 47 European populations, Rosser et al. (2000) observed a high degree of geographic differentiation of Y-chromosome haplogroups. Haplogroups Yhg1 and Yhg2 were shown to be the most frequent haplogroups (37% and 22%), which is consistent with the South Tyrolean data.

mtDNA analysis can be important to reveal female migrations and demographic history that shape the current genetic structure of a population. For the mtDNA markers the Ladin Val Badia sample does not show such a reduction in gene diversity values relative to the other German-speaking populations as observed on the Y chromosome. This could be interpreted as genetic drift and isolation being more pronounced for Ladin males than for Ladin females. Admixture as a result of female-mediated gene flow provides one possible explanation. Females migrate for longer distances than males in communities where sons inherit the farms (Videsott 2000).

mtDNA haplogroups were inferred from HVRI data; these data are not always sufficient to dissect Mhg HVRI from Mhg-U, which constitutes a possible bias in haplogroup assignment. Previous studies on mtDNA variation in Ladin populations (Stenico et al. 1996, 1998) showed a high frequency of Mhg-T, which is rare in Europe, and high levels of mtDNA diversity, both within Ladin populations and with regard to other European populations. More recently, the data of Stenico et al. (1996) were attributed to systematic sequencing errors (Bandelt et al. 2001). Vernesi et al. (2002), in an additional study, still observed high intrapopulation diversity with an extensive differentiation from other European populations in a sample of 20 Ladin-speakers. These patterns have been interpreted as reflecting the absence of severe bottlenecks in Ladin population history. In our Ladin sample from Val Badia we could not replicate the unusually high frequency of Mhg-T (our data: 8.5% T1 +T2), which was previously reported to be 24.9% in 13 individuals from the same Ladin valley (Stenico et al. 1998). Stenico et al. (1996) found high amounts of internal diversity in seven samples from the Alps

(each of 10 individuals, belonging to the three main linguistic groups). Genetic diversity in our samples is in the same range in all populations and lower than in neighboring populations (Austria, Germany, Switzerland) (Arnason 2003; Simoni et al. 2000).

Alu insertion polymorphisms are stable markers that reflect unique evolutionary events, namely, the insertion of an *Alu* element into a new chromosomal location. The pattern of genetic *Alu* variation resembles the geographic location of the samples, indicating weak but old migrational relationships among all study populations (Leitner et al. 1985). Geographic boundaries might explain the relatively high differentiation between the Val Badia and Val Gardena Ladin valleys.

The latest historical theories, which deal with linguistic and cultural transfer during the Middle Ages in current German-speaking South Tyrolean populations (Kramer 2004; Loose 1996), suggest that there is not a strict correspondence between the genetic and linguistic boundaries. We applied the admixture-based approach of Chikhi et al. (2001) to investigate this hypothesis, estimating the relative contributions of Ladin and German origin (Rosser et al. 2000) to the Val Venosta population and a sample from one isolated village (Stelvio in upper Val Venosta. The Val Venosta is a German-speaking valley with a broad diffusion of the Rhaetoroman language and culture until the late Middle Ages, where the process of Germanization was accompanied by immigration and settling of non-Rhaetian people (German tribes such as Alemanns) (Haider 1985). Our results suggest a relatively high genetic component of the Rhaetoroman Ladins in the Val Venosta population. The high number of Rhaetoroman surnames in Val Venosta shows additional evidence of the presence of a genetic contribution from Rhaetoromans to this population. Because Y chromosomes tend to cosegregate with surnames, we separated male samples from the village of Stelvio according to their Rhaetoroman and German surnames. We then tested for a differential genetic contribution of the Ladin source population to the samples from those males with Rhaetoroman surnames compared to those with German surnames. The results reflect the higher Rhaetoroman ancestry component in males with Rhaetoroman surnames.

Each genetically isolated population has its own demographic history with its own advantages and disadvantages. Simplified genetic diversity caused by genetic drift might aid genetic studies. The main limiting problems are smaller sample sizes and lower marker heterozygosity (Lonjou et al. 1999). In addition, findings in isolated populations might not be valid in the general population. On the other hand, these findings are important for our understanding of the underlying biological processes leading to disease (Heutink and Oostra 2002).

Our results, using three types of markers, show reduced genetic diversity and a high genetic differentiation in the Rhaetoroman- and German-speaking populations. The South Tyrol region shows two genetically distinct subpopulations ("younger" German-speaking and "older" Rhaetoroman-speaking isolates) sharing a similar environment. In the younger isolates, linkage disequilibrium over long genomic regions can be expected, whereas older populations could be more useful for fine-scale mapping (Reich et al. 2001). In addition, these subpopulations

could allow investigators to gain insight into the contribution of genetic heterogeneity of complex diseases.

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