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ARTICLE

Drawing the history of the Hutterite population on a genetic landscape: inference from Y-chromosome and mtDNA genotypes

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Although the North American Hutterites trace their origins to South Tyrol, no attempts have been made to examine the genetic migration history of the Hutterites before emigrating to the United States in the 1870s. To investigate this, we studied 9 microsatellite loci and 11 unique event polymorphism (UEP) markers on the Y-chromosome, the hypervariable region I (HVRI) of the mitochondrial DNA (mtDNA), as well as the complete mtDNA genome of Hutterite and South Tyrolean samples. Only 6 out of 14 Y-chromosome UEP+microsatellite haplotypes and 3 out of 11 mitochondrial haplotypes that were present in the Hutterites were also present in the South Tyrolean population. The phylogenetic relationships inferred from Y-chromosome and mtDNA databases show that the Hutterites have a unique genetic background related to a similar extent to central and eastern European populations. An admixture analysis indicates, however, a relatively high genetic contribution of central European populations to the Hutterite gene pool. These results are consistent with historical records on Hutterite migrations and demographic history. In addition, our data reveal similar numbers of Y and mitochondrial haplotypes in Hutterite male and female founders, respectively. The Hutterite male and female gene pools are similar with respect to genetic diversity and genetic distance measures and comparable with respect to their origins, suggesting a similar evolutionary history.
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INTRODUCTION

The Hutterites are a reproductively isolated North American Anabaptist community that originated during the Protestant Reformation in the 1500s in the Tyrolean Alps in Central Europe.[1,2] Ongoing religious persecution resulted in migrations across several European countries, during which time the population experienced multiple bottlenecks followed by expansions. This 350-year journey began in Tyrol, mostly in the Val Pusteria in South Tyrol, where the leader of the Tyrolean Anabaptists, Jakob Huter, was born. After the Tyrolean sovereign ordered the persecution and execution of the Anabaptists, Jakob Huter migrated to Moravia (today's Czech Republic) and established the first Tyrolean community in 1529.[1]

Over the next approximately 100 years, the community dramatically increased in size from 200 to 20 000–25 000 individuals because of the inflow of Anabaptists from Tyrol (South and North Tyrol), Germany, and Switzerland. In 1622, 10 000 community members were expelled to Transylvania (Romania and, what is today, Slovakia) and Hungary, while the remaining community converted to Catholicism. The records from 1622 to 1755 are incomplete, but beginning in 1755, Protestants from Carinthia (Austria) joined the Hutterite community in Transylvania, which had a population census of only 67 individuals at that time.[3] In 1770, they once again escaped persecution and settled in what is today the Ukraine, where the population stabilized and grew from about 116 to >1000 members.[1,4]

Finally, in 1874, about 1265 Hutterites migrated to the United States and settled in what is now South Dakota.[5] Approximately 850 of these immigrants settled as single family farmers; the remaining 443 Hutterites established three communal farms (called 'colonies'), each of which has given rise to one of the three major subdivisions of the Hutterite population: the Schmiedeleut (S-leut), Lehrerleut (L-leut), and Dariusleut (D-leut).[5] Few individuals have joined the community since settling in North America. Nevertheless, the population expanded dramatically, with $>40\,000$ Hutterites now living in >350 colonies in the northern United States and western Canada. The Hutterites retain a Tyrolean dialect as their first language and remain relatively isolated from the outside.[4]

Although there have been no genetic studies comparing the Hutterites to their European ancestral populations, mutation analysis of the cystic fibrosis (CF) gene (*CFTR*) reflects their Tyrolean ancestry. Only two mutations in the *CFTR* gene are present in the Hutterites.[6,7] A 'private' mutation, M1101K, is the more common mutation, whereas ΔF508 is a less frequent mutation in the Hutterites. Recently, however, the M1101K mutation was reported in a single patient among 63 CF families from the Tyrol region,[8] suggesting that at least one founder from the Tyrol introduced this mutation into the Hutterite population. Owing to founder effects and population expansion, this mutation now accounts for 64% of CF mutations among US and Canadian Hutterite CF families[6] and 82% of

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CF mutations in a population-based study in South Dakota Hutterites.[7]

The region of origin of the Hutterite population, South Tyrol (southern part of the Tyrol region), is today the most northern province of Italy, containing a number of valleys and communities separated from one another by mountainous terrain. Unrelated individuals from this region were sampled to represent the German- and Ladin-speaking main valleys of South Tyrol, and including the Val Pusteria valley, where Jakob Huter was born. Here, we used mitochondrial and Y-chromosome polymorphisms in Hutterite and South Tyrolean individuals to estimate the genetic relationship between the two groups, as well as with individuals representing the regions inhabited by the Hutterites during their migrations through Europe.

MATERIALS AND METHODS

Population samples

The US S-leut Hutterites in our study are descendants of 62 Hutterite ancestors who lived in the early 1700s to mid 1800s in Russia. DNA samples from Hutterites living in 31 South Dakota colonies and representing all four S-leut colony lineages[9,10] were collected as part of other studies.[9–13]

For the Y-chromosome and mitochondrial DNA (mtDNA) studies, we first identified all individuals in the full Hutterite pedigree with missing information on their parents, and referred to these as 'founders'. The number of founders in the S-leut pedigree (N=88) is larger than the number of ancestors of our sample (N=62), referred to above, because not all S-leut founders have living descendants in our sampled population. We next constructed genealogies of all same-sex descendents of each male (N=38) and each female (N=50) founder. Only 12 of the 38 male founders and 15 of the 50 female founders had same-sex descendants related through a same-sex genealogy in the extant population. Within each of the 27 same-sex genealogies, we attempted to sample DNA from multiple individuals in as distant branches of the genealogy as possible. Our final sample included 90 females from the 15 female founder lineages, and 75 males from 11 male founder lineages (we did not have DNA for male descendants of one male founder with same-sex descendants in the same-sex genealogy) (Table 1). For the genealogical inspection, we used the PedViz program.[14]

A total of 292 samples were collected in three German-speaking and the two Ladin-speaking valleys in South Tyrol as part of other studies.[15,16] Participants were unrelated for at least three generations and had at least a three-generation ancestry in the respective valleys. A total of 292 samples were included in the mtDNA analysis (227 males, 65 females) and 227 male samples were included in the Y-chromosome analysis.

Informed consent was obtained from each individual and the study was approved by the Institutional Review Board at the University of Chicago for the Hutterite sample and by the Ethics Committee of the Autonomous Province of Bolzano for the South Tyrolean sample.

We also compiled data for 1668 individuals for the Y-chromosome analysis and 398 individuals for the mtDNA analysis from publicly available databases (Table 2). The sample for the Y-chromosome analysis included 229 individuals from Austrian Tyrol (North Tyrol), 683 from Freiburg and Munich (southern Germany),[17] 231 from Zürich and Bern (Switzerland),[18] 138 from Transylvania,[19] 102 from Romania, 118 from Budapest (Hungary), 82 from Kiev (Ukraine), and 85 from Moscow (Russia).[17] In addition, a pan-European reference dataset composed of 6154 individuals was used.[20] A minimal haplotype of seven microsatellite markers (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, and DYS393) was available for these samples. For the analysis of Y-chromosome unique event polymorphism (UEP) markers, data for 30 populations from Rosser *et al*[21] were used.

The sample for the mtDNA analysis included 347 samples for the hypervariable region I (HVRI) from Austria (N=87), Switzerland (N=60), Germany (N=100), and Russia (N=100)[22] (http://www.hvrbase.de), and 51 samples for the mtDNA coding region from Czech Republic (N=3), Hungary (N=15), Volga-Ural region of Russia (N=4), Ukraine (N=1), and Russia (N=28)[22,23] (http://www.hvrbase.de). In addition, also for the mtDNA analysis, a pan-European reference dataset composed of 1124 individuals was used (http://www.hvrbase.de).

Table 1 Number of Hutterite individuals sampled from 11 male and 15 female founder lineages with same-sex descendants related in same-sex genealogies

Male founder lineage	*Number of sampled individuals*	*Female founder lineage*	*Number of sampled individuals*
1	2	1	2
2	2	2	2
3	3	3	6
4	2	4	1
5	4	5	9
6	5	6	2
7	2	7	10
8	4	8	2
9	16	9	4
10	23	10	8
11	12	11	4
12	0	12	11
		13	18
		14	10
		15	1
	Total=75		Total=90

Twenty-six of the male founders and 35 of the female founders do not have same-sex descendants related in same-sex genealogies in the extant population.

Y-chromosome genotyping

All samples (75 Hutterite and 227 South Tyrolean samples) were genotyped in Bolzano for nine Y-chromosomal microsatellite loci (DYS19, DYS388, DYS390, DYS391, DYS392, DYS393, DYS389I/II, and DYS426) and 11 UEP markers (92R7, M9, M13, M17, M20, SRY465, SRY4064, SRY10831, sY81, Tat, and YAP) as described earlier.[24] Samples were processed on a 3130xl-Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) and genotypes assigned using Genemapper version 3.7. Microsatellite repeat sizes were assigned according to the nomenclature of Kayser *et al*.[25] Y-chromosome haplogroups, defined by the 11 UEP markers, were classified according to the nomenclature proposed by the Y Chromosome Consortium.[26] Additional individuals from two male lineages with more than one Y-chromosome haplotype were genotyped for the discrepant loci in Chicago.

mtDNA sequencing

A sequence of 360 bp within the HVRI region (positions 16 024–16 383 of the revised Cambridge reference sequence rCRS[27]) was obtained by using L15997 and H16401 as sequencing primers. Whole mtDNA was amplified using

Table 2 Samples from publicly available databases (Y-chromosome and mtDNA analyses) included in this study

Y-chromosome		*mtDNA HVRI*		*mtDNA coding region*	
Country	*Number of individuals*	*Country*	*Number of individuals*	*Country*	*Number of individuals*
Austria (Tyrol)	229	Austria	87	Czech Republic	3
Southern Germany	683	Germany	100	Hungary	15
Switzerland	231	Switzerland	60	Volga-Ural	4
Transylvania	138	Russia	100	Ukraine	1
Romania	102			Russia	28
Hungary	118				
Ukraine	82				
Russia	85				

32 partially overlapping fragments of 600–900 bp in length modified from Rieder *et al.*[28] PCR products were purified using the Montage PCR96 Cleanup Kit (Millipore, Billerica, MA, USA) and the sequencing was performed using the BigDye terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Sequencing reactions were purified with Montage SEQ96 Sequencing Reaction Cleanup Kit (Millipore) and samples were analyzed on the ABI Prism 3100-Avant Genetic Analyzer (Applied Biosystems). Each sample was sequenced in both directions. Sequences were visually verified to detect the presence of heteroplasmy; all polymorphic positions were confirmed by re-sequencing. All mtDNA nucleotide positions given are relative to the rCRS.[27] Comparison with rCRS was performed using the sequence alignment software from Fluxus Engineering (http://www.fluxus-engineering.com/align.htm). The mitochondrial sequence studies were performed in Bolzano. DNA from members of one female lineage with two mtDNA haplotypes was re-sequenced in Chicago.

MtDNA haplotypes were assigned to haplogroups by identifying key combinations of HVRI mutations according to Macaulay *et al*,[29] Richards *et al*,[30] Maca-Meyer *et al*,[31] and Brandstatter *et al*[32] as follows: 16069T, 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; 16126C, 16292T, 16294T=Mhg-T3; 16249C=Mhg-U1; 16051G=Mhg-U2; 16343G=Mhg-U3; 16356C=Mhg-U4; 16270T=Mhg-U5; 16172C, 16219G=Mhg-U6; 16318T=Mhg-U7; 16298C=Mhg-V; 16223T, 16292T=Mhg-W; 16189C, 16278T=Mhg-X for the remaining haplotypes, those with a T at position 16223 were assigned to Mhg-N and those with a C at position 16223 were assigned to Mhg-H. For samples in which the entire mtDNA was sequenced, haplogroups were assigned following van Oven and Kayser[33] (http://www.phylotree.org/).

Data analyses

Within-population diversity and population differentiation. Average within-population genetic diversity (h) was calculated using the formulae given by Nei.[34] Populations were compared for differences in mtDNA sequence data using pairwise F_{ST} values and for differences in Y-chromosomal microsatellite data using R_{ST} values. These analyses were performed using ARLEQUIN software (http://lgb.unige.ch/arlequin).

Network analysis. Median-joining networks were constructed by use of the Network 4.1 program[35,36] (http://www.fluxus-engineering.com), based on Y-chromosomal haplotype data and on nucleotide variation in the HVRI region (positions from 16048 to 16383) and in the coding region of the mtDNA. The repeat sizes of the Y-chromosomal microsatellite markers were weighted according to the mutation rates of the different loci[37] and the ε value was set to 0. HVRI-nucleotide positions were weighted according to Roostalu *et al*[38] and an ε value of 0 was used.

Multidimensional scaling. Patterns of genetic differentiation were visualized using multidimensional scaling plots, based on R_{ST} values for Y-chromosomal microsatellite data and F_{ST} values for UEP-based haplogroup and mtDNA HVRI data using the statistical package 'R' (http://ww.R-project.org/).

Admixture analysis. Admixture analyses were carried out using LEA (likelihood-based estimation of admixture) software, using the method described by Chikhi *et al*[39] (http://www.cnrs-gif.fr/pge/bioinfo/lea), based on Y-chromosome microsatellite data and mtDNA haplogroup data.

RESULTS

Studies of Y-chromosome DNA

The allelic states of 9 Y-chromosome microsatellite loci and 11 UEP markers were determined in 227 men from South Tyrol and 75 Hutterite men. The UEP markers defined nine haplogroups in total (Supplementary Figure S1), four of which were present in the Hutterites. The 11 UEP and 9 microsatellite markers defined 125 haplotypes (111 in the South Tyroleans only, 8 in the Hutterites only, and 6 in both). Three instances of homoplasy of microsatellite haplotypes across UEP haplogroups were observed in the South Tyroleans: microsatellite haplotype 14 12 31 24 11 13 13 12 10 was found on two R1a* and one P*(xR1a) chromosomes, haplotype 14 13 30 24 11 13 13 12 10 was found on three P*(xR1a) and two R1a* chromosomes, and haplotype 16 12 28 25 11 11 12 15 9 was found on three BR*(xDE,JR) and one Y*(xBR,A3b2) chromosomes. None of these haplotypes were present in the Hutterites. The 14 Y-chromosome haplotypes, defined by 11 UEP and 9 microsatellite markers, that are present in the Hutterites are shown in Table 3 (South Tyrolean haplotypes are shown in Supplementary Table S1). Interestingly, two lineages corresponding to two different Hutterite surnames had identical Y-chromosome haplotypes (haplotype #1 in lineages 2 and 10; Table 3), and two lineages each had three haplotypes (haplotypes #10, 11, 12 in lineage 6 and haplotypes #6, 7, 8 in lineage 11; Table 3). The multiple haplotypes within lineages 6 and 11 differed from each other by one repeat unit at two markers, and were validated by typing additional individuals within each lineage. Moreover, pedigree analysis indicated that haplotypes 6 and 11 or 12 are the likely ancestral haplotypes in lineages 11 and 6, respectively (Figure 1). Thus, 10 ancestral Y-haplotypes were present in the 11 Hutterite male founders. The original sample of 75 individuals represented 673 independent meioses. The average mutation rate at the nine microsatellite loci in this sample was 6.6×10^{-4} per generation (95% CI 1.5×10^{-4}, 1.3×10^{-3}). In the literature, a discrepancy of average mutation rates at Y-chromosome markers is reported, depending on whether the estimate is pedigree based or phylogeny based. By direct count in deep-rooted pedigrees, a mutation rate of 2×10^{-3} per generation has been estimated,[40] and by studying father/son pairs, a similar average mutation rate of 3.17×10^{-3} per locus per generation was estimated.[37] A recent study analyzed 17 microsatellite markers for 18 earlier published studies in combination with a new dataset of father/son pairs (135 212 meiotic transfers in total) and found a median mutation rate of 2.2×10^{-3}.[41] Phylogeny-based studies such as Zhivotovsky *et al*[42] and Forster *et al*[43] estimated a lower mutation rate of 6.9×10^{-4} per locus per 25 years and 2.6×10^{-4} per 20 years, respectively. The low mutation rate estimated in the Hutterites may reflect their history of recurrent bottlenecks, because such processes can affect estimates of mutation rates in our deep-rooted pedigree-based approach, as it has already been shown for phylogeny-based approaches.[44,45]

To compare our data with the data from publicly available databases,[17–19] the minimal haplotype of seven common microsatellite markers was used (Table 3). The South Tyrolean, Austrian (North Tyrol), and German samples shared six or eight haplotypes with the Hutterites, whereas the other populations shared five (Transylvania, Hungary) or fewer (Swiss, Romania, Ukraine, Russia) haplotypes with the Hutterites. Two haplotypes (#1 and #14, Table 3), including the one haplotype that was present in two Hutterite founders, were each shared among all but one population (Transylvania and Switzerland, respectively). The frequency of the four Hutterite haplotypes not shared with any of the investigated reference populations was between 0.007 and 0.01% in the Y-STR haplotype reference database (YHRD).[46]

The Hutterite sample stands out with a substantially lower Y-chromosome h-value at the level of microsatellite haplotype frequencies than all other population samples, reflecting the recurrent population bottlenecks and genetic isolation for the male gene pool of the Hutterites (Supplementary Table S3). A similar pattern was observed using the genetic distance measure R_{ST} that is also consistent with the Hutterite isolation (Supplementary Table S4; Supplementary Figure S2).

A phylogenetic network (Supplementary Figure S3) was constructed incorporating publicly available Y-microsatellite data from Austria

Table 3 Fourteen Y-chromosome UEP + microsatellite haplotypes present in the Hutterites, their frequency in South Tyrol, and the frequency of the microsatellite haplotypes in European populations

Haplotype #	Male founder lineage	Haplogroup	Microsatellite haplotype	Hutterites[a] N=75	South Tyrol N=227	Austria N=229	Germany N=683	Switzerland N=231	Transylvania N=138	Romania N=102	Hungary N=118	Ukraine N=82	Russia N=85
1	2 and 10	BR*(xDE,JR)	14 12 28 23 10 11 13 [14] [9]	25	1 (0.004)	4 (0.017)	13 (0.019)	1 (0.004)	0	1 (0.009)	3 (0.025)	3 (0.036)	2 (0.023)
2	5	K*(xL,N3,O2b,P)	14 13 28 24 11 13 13 [12] [10]	4	0	3 (0.013)	2 (0.002)	0	0	0	0	0	0
3	1	P*(xR1a)	14 13 29 24 10 13 13 [12] [10]	2	5 (0.022)	2 (0.008)	24 (0.035)	11 (0.047)	2 (0.014)	0	1 (0.008)	0	0
4	9	P*(xR1a)	14 13 29 24 11 13 13 [12] [10]	16	17 (0.075)	10 (0.04)	50 (0.073)	16 (0.069)	1 (0.007)	1 (0.009)	3 (0.025)	0	0
5	8	BR*(xDE,JR)	15 12 28 22 10 11 13 [15] [9]	4	0	0	7 (0.010)	0	0	0	1 (0.008)	0	0
6	11	BR*(xDE,JR)	15 12 29 22 10 11 14 [13] [9]	10	1 (0.004)	1 (0.004)	10 (0.014)	1 (0.004)	4 (0.028)	0	0	0	0
7	11	BR*(xDE,JR)	15 12 29 23 10 11 14 [13] [9]	1	1 (0.004)	2 (0.008)	0	0	1 (0.007)	0	0	0	0
8	11	BR*(xDE,JR)	15 12 30 22 10 11 14 [13] [9]	1	0	2 (0.008)	2 (0.002)	0	0	0	0	0	0
9	3	R1a1	15 13 30 25 10 11 12 [12] [10]	3	0	0	0	0	0	0	0	0	0
10	6	BR*(xDE,JR)	16 12 28 21 10 11 13 [12] [9]	1	0	0	0	0	0	0	0	0	0
11	6	BR*(xDE,JR)	16 12 28 21 10 11 13 [13] [9]	3	0	0	0	0	0	0	0	0	0
12	6	BR*(xDE,JR)	16 12 28 21 10 11 14 [12] [9]	1	0	0	0	0	0	0	0	0	0
13	4	BR*(xDE,JR)	16 13 30 24 10 11 12 [14] [9]	2	0	0	0	0	0	0	0	0	0
14	7	R1a1	16 13 30 25 11 11 13 [12] [10]	2	1 (0.004)	1 (0.004)	10 (0.014)	0	1 (0.007)	1 (0.009)	2 (0.016)	5 (0.060)	10 (0.117)

[a]For the Hutterites, the number of individuals is shown because the sampling was not population based (see Materials and Methods). The frequencies in the Hutterites and South Tyroleans are based on nine microsatellite markers. Microsatellite repeat sizes are given in the order DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS388, and DYS426. The two microsatellites typed only in the Hutterite and South Tyrolean samples are shown in brackets. Shaded rows show multiple haplotypes in two lineages (Figure 1).

(North Tyrol), southern Germany, Switzerland, and eastern European populations (Transylvania, Romania, Hungary, Ukraine, and Russia).[17–19] The repeat sizes of seven microsatellite loci (out of nine genotyped in the South Tyrolean and Hutterite samples) were available for all populations (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, and DYS393); only haplotypes with frequency of at least two occurrences were included in the analysis. The 10 Hutterite haplotypes defined by seven microsatellite markers and with at least two occurrences fall into three population clusters. Most Hutterite haplotypes (N=7) are present in clusters A and B and are grouped together not only with the central European populations (South Tyrol, Austria (North Tyrol), southern Germany, Switzerland), but also with haplotypes from Transylvania and Hungary. This clustering is consistent with historical records documenting Hutterite settlement in Transylvania and Hungary during the second stage of their migration.[1] Interestingly, three Hutterite haplotypes fall into cluster C, in which most haplotypes from Romania, Ukraine, and Russia are present, suggesting contributions from these populations as well.

Multidimensional scaling plots based on R_{ST} values calculated using microsatellite data revealed that the Hutterites are very distant to all European populations; they show a similar distance to the populations from Transylvania and Hungary and to the populations from Austria (North Tyrol), southern Germany, Switzerland, and South Tyrol. The populations from Romania, Ukraine, and Russia are more distant (Supplementary Figure S2), which is consistent with the results of the phylogenetic network analysis. Including a pan-European dataset, the Hutterites are positioned at a similar distance to central, western, and eastern European populations, except Ukraine and Russia (Figure 2). A similar pattern was obtained when using the distribution of Y-chromosomal haplogroups, defined by UEP markers, for 30 European populations[21] including the Hutterites and South Tyroleans from this study (data not shown).

Using an admixture-based approach of Chikhi *et al*,[39] we tested the relative genetic contributions of two putative source populations to the Hutterites: Central Europe (South Tyrol, Austria, Switzerland, and southern Germany) and Eastern Europe (Hungary, Transylvania, Romania, Ukraine, and Russia). This analysis reveals a relatively high genetic contribution (82%) of central European populations to the male gene pool of the Hutterites.

Studies of mtDNA

Mitochondrial HVRI haplotypes were evaluated in 292 individuals from South Tyrol and 90 Hutterites. Eighty-nine HVRI sites were polymorphic in these samples, defining 99 unique haplotypes (89 were present only in the South Tyroleans, 8 were present only in the Hutterites, and 3 were present in both). The 11 HVRI haplotypes that are present in the Hutterites are shown in Table 4 (South Tyrolean haplotypes are shown in Supplementary Table S2). Two female lineages had the same mtDNA haplotype (haplotype #6 in lineages 4 and 15; Table 4), and two sets of three lineages each had the same haplotype (haplotype #7 in lineages 7, 10, and 12, and haplotype #8 in lineages 2, 6, and 14; Table 4). None of the 'shared' Hutterite haplotypes were present in any of the European populations, except for one occurrence of haplotype #8 in the Austrian sample. In addition, one lineage had two haplotypes (haplotypes #7 and #11 in lineage 10; Table 4). Haplotype #7 is also present in lineages 7 and 12, whereas haplotype #11 is unique to lineage 10. These two haplotypes differ at 13 positions, and their co-occurrence in lineage 10 was confirmed by re-sequencing of DNA from individuals in this lineage in a second laboratory. Interestingly, the two individuals with haplotype #11 are in a distant branch of the genealogy with the most recent

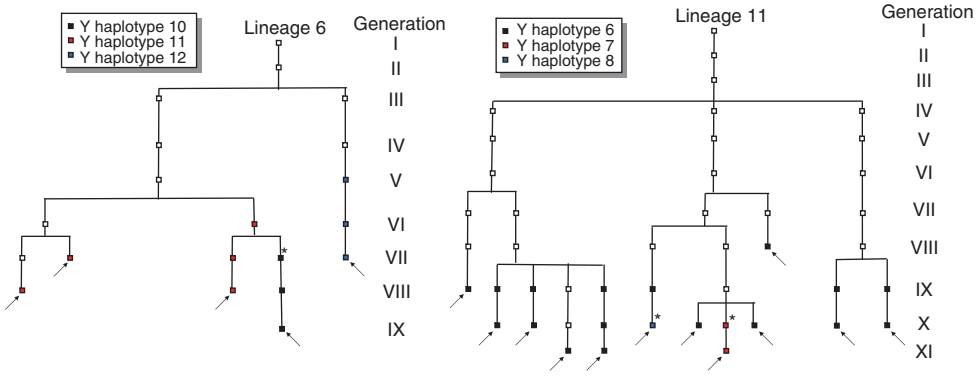

Figure 1 Male lineages 6 and 11. Filled symbols show males who were genotyped. Arrows indicate males who were included in the original sample (Table 1) and asterisks indicate sons of fathers in whom mutations arose at DYS388 (lineage 6, individual VII.4), DYS389II (lineage 11, individual X.5), and DYS390 (lineage 11, individual X.7). Haplotype 6 (29 repeats at DYS398II and 22 repeats at DYS390) is likely the ancestral haplotype in lineage 11 and haplotypes 11 (13 repeats at DYS393 and at DYS388) or 12 (14 repeats at DYS393 and 12 repeats at DYS388) are the likely haplotypes in lineage 6. The allelic composition of each haplotype is shown in Table 3.

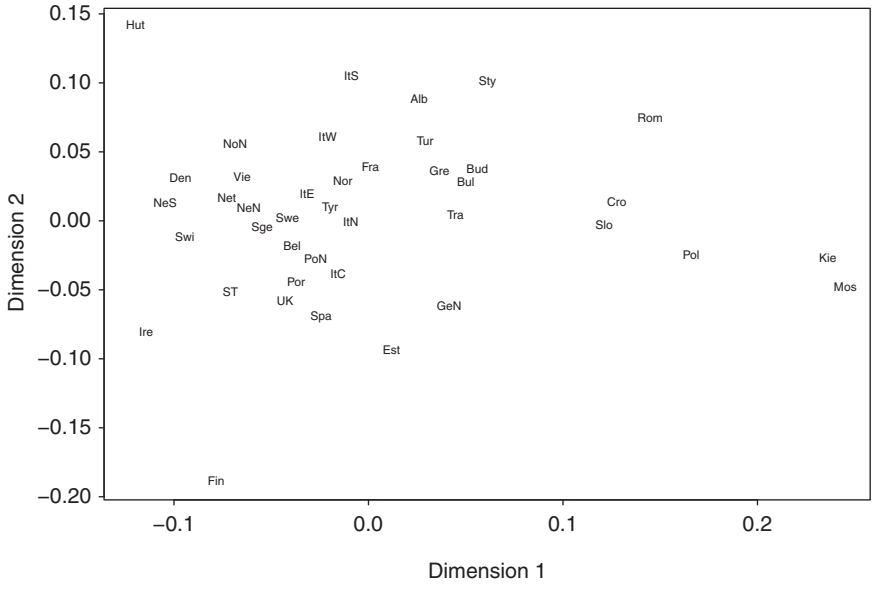

Figure 2 Multidimensional scaling plot, based on linearized R_{ST} distances among the Hutterites and a pan-European reference dataset composed of 6154 individuals (Roewer *et al*, 2005), calculated from microsatellite data. The stress value is 0.16. Mos, Moskow; Kie, Kiev; Pol, Poland; Slo, Slovenia; Cro, Croatia; Rom, Romania; GeN, Northern Germany; Sge, Southern Germany; Vie, Vienna; Tra, Transylvania; Bul, Bulgaria; Bud, Budapest; Sty, Styria; Gre, Greek; Tur, Turkey; Alb, Albania; ItS, Southern Italy; ItW, Western Italy; Fra, France; Est, Estonia; Nor, Norway; NoN, Northern Norway; ItN, Northern Italy; ItE, Eastern Italy; ItC, Central Italy; Spa, Spain; Por, Portugal; PoN, Northern Portugal; Bel, Belgium; Swe, Sweden; UK, United Kingdom; Tyr, Tyrol; ST, South Tyrol; Swi, Switzerland; Net, Netherlands; NeN, Northern Netherlands; NeS, Southern Netherlands; Den, Denmark; Fin, Finland; Ire; Ireland; Hut, Hutterites.

common Hutterite ancestor born in 1785 (Supplementary Figure S4). Lastly, we observed heteroplasmy in two individuals (see footnote in Table 4).

Five of the Hutterite mitochondrial haplotypes are present in other populations: three overlap exclusively with a haplotype from either South Tyrol, Austria, or Germany, one is present in both South Tyrol and Austria, and one is present in South Tyrol, Austria, and Switzerland (Table 4). The frequency of the six Hutterite haplotypes not shared with any population was between 0.01 and 1.2% in the pan-European dataset used (http://www.hvrbase.de).

The complete mtDNA was sequenced in a subset of 55 South Tyroleans and 20 Hutterites, who represent the frequencies of the haplogroups defined by the HVRI sequence. The coding region of these 75 samples contained 266 sequence changes, defining 57 haplotypes (47 were present only in the South Tyroleans, 10 were present only in the Hutterites, and none were shared between the two groups). Among the 10 Hutterite mitochondrial sequences, three unique sequences each corresponded to HVRI haplotypes #3 and #7, and two unique sequences each corresponded to HVRI haplotypes #8 and #10 (data not shown). Accession numbers for the sequences of the entire mtDNA are FJ348151 to FJ348170 for the Hutterite sequences and FJ348171 to FJ348225 for the South Tyrolean sequences.

Similar to the Hutterite male gene pool, the female gene pool shows the lowest genetic diversity values (h) (Supplementary Table S3), and

Table 4 Frequencies of the mitochondrial HVRI-haplotypes present in the Hutterites, and their frequency in European populations

Haplotype #	*Female founder lineage*	*HVRI-haplotypes*	*Haplogroup*	*Hutterites*[a] *N=90*	*Complete mtDNA sequenced N=20*	*South Tyrol N=292*	*Austria N=87*	*Germany N=100*	*Switzerland N=60*	*Russia N=100*
1	3	16093C, 16129A, 16258C, 16316G	H	6	0	6 (2.1)	0	0	0	0
2	8	16248T	H	2	0	2 (0.7)	2 (2.3)	0	1 (1.7)	0
3	13	16070G	H22[b]	18	7	0	0	0	0	0
4	1	16183C, 16189C, 16261T, 16278T	H	2	0	0	0	0	0	0
5	11	16235G, 16291T, 16293G	H	4[c]	0	0	0	1 (1.0)	0	0
6	4, 15	16209C	H	2	0	0	0	0	0	0
7	7, 10, 12	16108T, 16182C, 16183C, 16189C, 16223T, 16232T, 16255A, 16278T	X2c1[b]	27	4	0	0	0	0	0
8	2, 6, 14	16069T, 16092C, 16126C, 16261T	J1c7[b]	14[d]	5	0	1 (1.1)	0	0	0
9	5	16069T, 16126C, 16183C, 16189C, 16245T	J	9	0	0	0	0	0	0
10	9	16069T, 16126C, 16145A, 16188insC, 16261T	J1[b]	4	3	0	0	0	0	0
11	10	16069T, 16126C, 16145A, 16231C, 16261T	J2a1a1[b]	2	1	2 (0.7)	3 (3.4)	0	0	0

[a]For the Hutterites, the number of individuals is shown because the sampling was not population based (see Materials and Methods).
[b]Haplogroup assignment based on complete mtDNA data.
[c]One individual with heteroplasmy (16234 C/T).
[d]One individual with heteroplasmy (16311 C/T).
Differences from the revised Cambridge Reference Sequence[25] are shown.
Hutterite haplogroups were assigned based on HVRI-sequences as described and, where possible, on complete mtDNA sequence data.

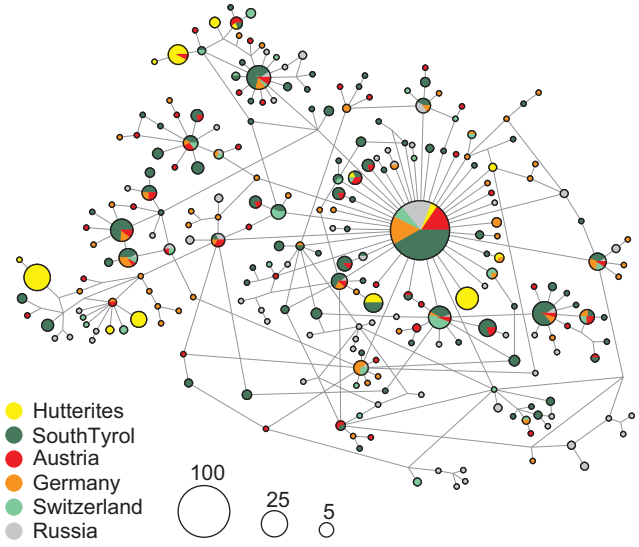

Figure 3 Network analysis of mitochondrial HVRI haplotypes. The size of the circles indicates the number of individuals for a specific haplotype.

the between-population structure of the Hutterites deviates from all other investigated populations (Supplementary Table S4; Supplementary Figure S5).

Median-joining networks for both the HVRI and the coding regions were constructed using the Hutterite and South Tyrolean data along with published HVRI sequences from Austria, Germany, Switzerland, and Russia,[22] and coding region sequences from Czech Republic, Hungary, Volga-Ural, Ukraine, and Russia.[22,23] In the HVRI network (Figure 3), the Hutterite sequences cluster fairly tightly together with the samples from South Tyrol, Austria, Germany, and Switzerland, and to a lower extent with the Russian samples. In the network for the mitochondrial coding region (Supplementary Figure S6), the Hutterite haplotypes are tightly connected to the samples from South Tyrol, but cluster also with samples from Czech Republic and Hungary, two populations that were not represented in the sample of the HVRI analysis. Similar to the network with the HVRI-data, there is no connection between the Hutterites and Russians (including Volga-Ural and Ukraine).

Lastly, the genetic relationships among the populations in the HVRI dataset are visualized in a multidimensional scaling plot (Supplementary Figure S5). This analysis shows again the high genetic distance of the Hutterites to all European populations; they are nearly the same distance to the central European populations compared with the Russian sample. A similar pattern was observed when adding a pan-European reference dataset (data not shown).

Using the haplogroup frequencies for the central and eastern European populations as source populations, the extent of admixture from the central European populations in the female Hutterites is 89%.

DISCUSSION

We investigated the genetic relationship between the Y- and mitochondrial chromosomes from Hutterites and South Tyroleans (from the valleys in which the Hutterites originated[2]), as well as the Austrian, German, and Swiss populations (in which many early Hutterites came from), and in samples from regions representing the major settlements of the Hutterites as they migrated throughout Europe between 1529 and the 1870s. During that period, the Hutterite population experienced a series of expansions followed by bottlenecks.[2] For example, a dramatic reduction in population size to only 67 individuals occurred from 1622 to 1755 when they lived in Transylvania. Only 116 Hutterites settled in Russia in 1770, but the population grew to 1265 before emigrating to the United States in the 1870s. Finally, of the 1265 individuals that moved to the United States, only 443 settled on communal farms (colonies). There has been negligible immigration into the Hutterite population since settling in North America and currently there are $>40\,000$ Hutterites living on colonies in the United States and Canada.[1]

The paucity of mitochondrial and Y-chromosome haplotypes in the US S-leut Hutterites and the high degree of differentiation between

the S-leut-Hutterites and European samples reflects their history of recurrent bottlenecks followed by reproductive isolation. We studied DNA from female descendants of 15 of the 50 S-leut female founders, which included all lineages with female descendents in the extant S-leut population who are related through female (mitochondrial) lines. We identified 11 distinct mitochondrial haplotypes belonging to only 4 haplogroups among the 15 female founder lineages. Two lineages share one haplotype (haplotype #6 in lineages 4 and 15) and two sets of three lineages each share the same haplotype (haplotype #7 in lineages 7, 10, and 12, and haplotype #8 in lineages 2, 6, and 14; Table 4). These haplotypes are either absent or very rare in the other populations (Table 4), suggesting that the lineages sharing haplotypes may have inherited them identical-by-descent from a recent common (female) ancestor. In addition, two divergent haplotypes were detected in female descendents of female lineage 10, which likely represents either an early adoption into a Hutterite family (between 1818 and 1894) or an error in the pedigree in one of these early generations (Supplementary Figure S4). Regardless of which explanation is correct, these results indicate the presence of an additional female founder in the Hutterites, who carried a mitochondrial haplotype that is currently also present in South Tyrol and Austria.

Of the 11 mitochondrial haplotypes in the Hutterites, only 5 overlap with one or more other populations: haplotypes #1, #2, and #11 are present in the South Tyrolean population, haplotypes #2, #8, and #11 in Austrians, haplotype #5 in Germans, and haplotype #2 in Swiss. These data suggest that the female Hutterite founders are largely derived from Central Europe, although there are no samples from Transylvania and Hungary in the HVRI dataset, populations that showed an affinity with the Hutterites for the male lineages. Moreover, it is possible that one or more of the 35 female founders not represented in our sample joined the community during their tenure in Eastern Europe.

The Hutterites included in this study represented 11 of the 38 S-leut male founders, including all but one lineage with male descendents in the extant S-leut population who are related through male (Y-chromosome) lines. There were 10 distinct ancestral Y-chromosome haplotypes among the 11 male founder lineages. Y-chromosome haplotype #1 was present in male descendants of two different founders (founders 2 and 10), suggesting that these two lineages may be related through the male line (Table 3), even though the two founders of these lineages did not have the same surname. On the other hand, this is one of the more common haplotypes present in all of the surveyed samples except one. Therefore, it might not be surprising that this haplotype is shared by ancestors with different surnames. Alternatively, these haplotypes may differ at loci other than these nine markers surveyed here.

Two additional Y-chromosome haplotypes were present in male descendents of each of two founders (founders 6 and 11). These haplotypes arose as a result of recent mutations at microsatellite loci, and we were able to determine in whom the mutations arose for three of the four observed mutations (Figure 1). Interestingly, all 3 of the lineage 11 haplotypes were present in multiple other sampled populations (Table 3), suggesting that these haplotypes may have an unusually high mutation rate. A recent study also showed relatively high mutation rates (2.2×10^{-3} and 3.1×10^{-3}, respectively) for DYS390 and DYS389II, the two markers showing mutations in lineage 11.[41] In contrast, none of the haplotypes present in lineage 6 was present in other European populations.

Network analyses of the Y-chromosomal and mitochondrial haplotypes show contributions to both the male and female founders from all central European populations investigated (South Tyrol, Austria, southern Germany, and Switzerland). The Hutterite Y-chromosome haplotypes also show affinities to Transylvania and Hungary, and fewer and more distant relations to Russia and the Ukraine. The mitochondrial haplotypes show lesser affinities to Czech Republic and Hungary, and fewer relations with Russia and the Ukraine. The multidimensional scaling plots reflect the high genetic distance of the Hutterites to all European populations and a similar distance of the Hutterites to central and eastern European populations. However, the admixture analyses indicate a higher genetic contribution from central European populations compared with eastern European populations to the Hutterite gene pool.

Taken together, the high genetic distance of the Hutterites to European populations, their low genetic variability, and the difficulty of identifying a single population as the major contributor to the Hutterite gene pool reflect the effect of their demographic history in shaping their genetic background. We hypothesize that recurrent bottlenecks and partial gene flow events led to the formation of a unique genetic background with small genetic contributions from several populations and periodic reduction in genetic variability.

The number of mtDNA and Y-chromosomes is small and similar for both male and female founders (10 ancestral Y-haplotypes and 11 mitochondrial HVRI haplotypes among 12 male and 15 female founders, respectively), and both are derived from Central Europe, with somewhat smaller contributions from Eastern Europe. Thus, unlike studies of Y and mitochondrial chromosomes in many other human populations,[47–50] the Hutterite male and female gene pools are similar with respect to genetic diversity and comparable with respect to their origins, suggesting a similar evolutionary history.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Electronic database information:

http://www.hvrbase.de (compilation of human mtDNA HVRI-sequences)
http://www.yhrd.org/ (Y-STR haplotype reference database (YHRD))
http://www.phylotree.org/ (for mtDNA haplogroup assignment using complete mtDNA data)
http://www.fluxus-engineering.com (for Network 4.1: software for network construction)
http://www.fluxus-engineering.com/align.htm (for DNA Alignment 1.3.0.1: software for sequence alignment)
http://lgb.unige.ch/arlequin (for population genetic analyses)
http://ww.R-project.org/) (for MDS plots)
http://www.cnrs-gif.fr/pge/bioinfo/lea (for admixture analyses)

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