

## ORIGINAL ARTICLE

# Charting the Y-chromosome ancestry of present-day Argentinean Mennonites

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Old Order Mennonite communities initially arose in Northern Europe (centered in the Netherlands) and derived from the Anabaptist movement of the 16th century. Mennonites migrated to the New World in the early 18th century, first to North America, and more recently to Mesoamerica and South America. We analyzed Y-chromosome short tandem repeats (STRs) and single nucleotide polymorphisms in males from a community of Mennonites, ‘La Nueva Esperanza’, which arrived to Argentina in 1985 from colonies in Bolivia and Mexico. Molecular diversity indices coupled with demographic simulations show that Mennonites have a reduced variability when compared with local Argentinean populations and 69 European population samples. Mennonite Y-STR haplotypes were mainly observed in Central Europe. In agreement, multidimensional scaling analyses based on  $R_{ST}$  genetic distances indicate that Mennonite Y-chromosomes are closely related to Central/Northern Europeans (the Netherlands, Switzerland and Denmark). In addition, statistical inferences made on the most likely geographic origin of Y-chromosome haplotypes point more specifically to the Netherlands as the populations that best represent the majority of the Mennonite Y-chromosomes. Overall, Y-chromosome variation of Mennonites shows the signatures of moderate reduction of variability when compared with source populations, which is in good agreement with their lifestyle in small endogamous demes. These genetic singularities could also help to understand disease conditions that are more prevalent among Mennonites.

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## INTRODUCTION

Mennonites, also known as ‘Mennist’, are a branch of the Christian church, with roots in the wing of the 16th century Protestant Reformation. The Mennonites derived from the Anabaptist movement of the 1520s and 1530s. They took their denomination from Menno Simons (1596–1561), a Dutch priest who converted to the Anabaptist faith and helped lead it to prominence in Holland by the mid-16th century (1536–1543).<sup>1</sup> He later would carry out evangelization campaigns in Germany, and the Baltic coast. During the period 1561–1786, Anabaptism achieved a strong presence in the Netherlands, and devotees of this movement were particularly persecuted in Belgium. Some Mennonites moved eastwards to Danzig and the Vistula valleys (Supplementary Figure S1). To escape persecution, many Mennonites moved to Chostitza (present-day Ukraine) in 1786–1873 seeking the more accommodating religious climate of Catherine the Great’s Russia. However, the abolition of their privileges in the 1870s and the growing ‘Russification’ policy triggered a wide emigration process to Canada and the United States; many Mennonites migrated to the western states and provinces,

where a large Mennonite community remains to this day. Further colonies of Mennonites moved to Siberia in search for fertile land in 1910, followed by a mass deportation to this area in 1930.

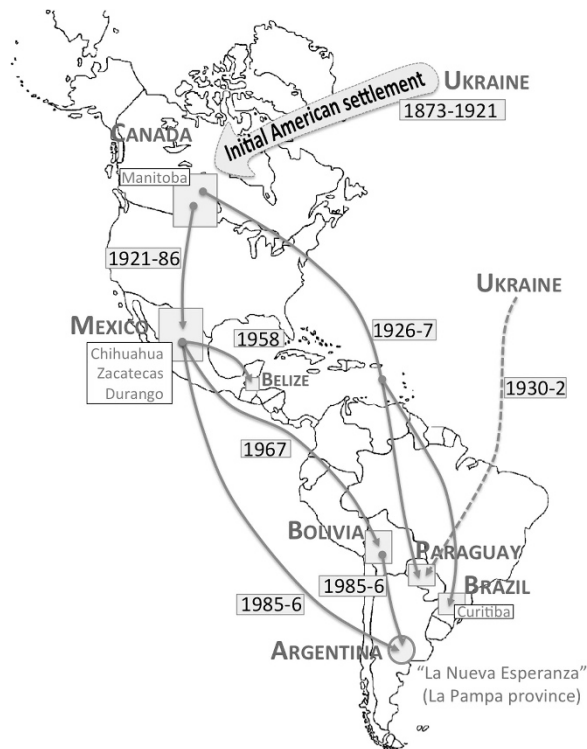
The first migration to America left the Netherlands and Krefeld (West Germany) for New York and German town in the 17th century. Later, in the 19th century, there were mass migrations to North America. Although Prussian Mennonites favored the United States and settled there (mainly in Nebraska, Kansas and the Dakotas), the Chostitza colonies migrated mainly to the Manitoba province in Canada in 1874–1875 (Figure 1). Swiss German Mennonites migrated to North America in the 18th and 19th centuries, settling first in Pennsylvania, then eventually across the Midwestern states. Today, large Mennonite populations can be found in Pennsylvania, Ohio, Indiana and Kansas, although Mennonites live in all parts of the United States. From Canada, Mennonites moved also to Mexico (provinces of Chihuahua first and then to Durango) in 1921–1986, and to Paraguay in 1926–1927. Other destinations in South America include Bolivia, Uruguay and Brazil (Figure 1). The colony established

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**Figure 1** Map showing main routes of Mennonite dispersions in America. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

in Argentina moved from Mexico and Bolivia in 1985–1986, and consisted of a relatively small group of 120 families.

Present-day Mennonites number ~1.8 million people worldwide according to the Global Anabaptist Mennonite Encyclopedia (<http://gameo.org>; updated on 2012). In Spanish and Portuguese-speaking countries of South America, they amount to ~80 000 people. The largest numbers are located in Paraguay (15 000), Bolivia (8000) and Brazil (6000). Smaller communities are settled in Argentina, Uruguay and so on; most of them are mainly descendants of Plautdietsch-speaking Mennonites. This branch of the Mennonites is supposed to derive from a population group that inhabited the territories of today's Ukraine. Many people in the older generation of this group continue to speak a Low German Dialect called 'Plautdietsch' and eat traditional food.

A few genetic studies have been carried out for some Anabaptist communities. Pichler *et al.*<sup>2</sup> analyzed Y-chromosome and mitochondrial DNA (mtDNA) control region variation in North American Hutterites. Their results indicated that their genetic pool was closely related to central and eastern European populations, and patterns of variation were similar in males and females, suggesting a similar evolutionary history for both sexes. Several genetic studies have been carried out in a more biomedical context.<sup>3</sup>

Only a few genetic studies have focused on Mennonite communities. Melton *et al.*<sup>4</sup> analyzed mtDNA control region variation in six Mennonite populations living in Kansas and Nebraska (USA). These analyses revealed the European ancestry of their mtDNAs and could not find evidences of admixture with other continental groups. Their data also indicated that the Alexanderwohl congregation appeared to be closest to their founding population in the Netherlands, whereas the other two Mennonite congregations, namely Halderman and Old Order, were more affected by subsequent

demographic events that distinguished them from other European groups.

To the best of our knowledge, Mennonite communities from South America have never before been analyzed from a molecular anthropological angle; furthermore, none of the worldwide communities has been analyzed for the Y-chromosome variation. This is therefore the first attempt at characterizing the Y-chromosome variation in a Mennonite community.

## MATERIALS AND METHODS

### Sample

Samples were collected after written informed consent from 27 individuals belonging to 10 different family groups (Supplementary Figure S2) living in the Mennonite congregation of 'La Nueva Esperanza', located in the Argentinean province of La Pampa (40 km from the city of Guatraché). Twelve males out of the 27 individuals sampled were selected for this study based on their affiliations to different male lineages. According to their internal census, 'La Nueva Esperanza' congregates a few hundred individuals (1278 inhabitants in 2005).<sup>5</sup> This colony belongs to the Anabaptist branch known as Ältkolonier Reinlaender mennoniten Gemeinde (ÄRMG), often referred to as the Old Colony (In Spanish: 'Comunidad de los Menonitas de la Vieja Colonia de Reinland'<sup>5</sup>).

A number of populations from America and Europe and available in the literature were used for population comparisons (Supplementary Table S1).

Genetic analysis of the individuals in the present study was approved by the Comité de Bioética of the Hospital Universitario-Fundación Favaloro (Buenos Aires; Argentina).

### Considerations on Mennonites and other Anabaptist communities

The Mennonites separated from the Amish in the late 1600's; unlike the latter, the Mennonites do not necessarily reject modern technology and conveniences. The Mennonites also differentiate from the Amish in that they do not form an exclusive and tight-knit community. Although certain conservative branches of the Mennonite church still dress simply and require women to wear head coverings, Mennonites are generally not culturally separatist, choosing to incorporate the larger communities outside their church rather than forming a separate community around their own church. Where the Amish believe in keeping themselves spiritually focused by limiting their interaction with modern society, Mennonites believe in practicing Jesus's doctrine of service to others in a broader context (<http://jamesportmissouri.org/index.html><sup>5</sup>).

The Mennonite community strongly recommends marriages between members of the ÄRMG (not necessarily from the same colony), and the prescriptive minimum biological distance in mates is second cousins. It is also common that two (or three) brothers marry two (or three) non-related sisters; from a biological point of view, these marriages facilitate a broadening of the universe of potential mates for the next generations.

### DNA extraction and genotyping

DNA extraction was performed from ethylenediaminetetraacetic acid-collected blood samples using the QIAamp DNA Blood Mini Kit (Qiagen, Redwood City, CA, USA).

Y-short tandem repeat (STR) typing for the PPY23 system was performed according to manufacturer's instructions (PowerPlex Y23 System Technical Manual, Promega Corp., Madison, WI, USA) reducing the PCR final volume to 10 µl and accordingly adjusting the proportions of reagents in the reaction mix. A volume of 1 µl of each DNA extract containing ~0.5–1 ng was used as template. Separation and detection of amplified products were carried out in an ABI PRISM 3130 genetic analyzer. Allele calling was carried out by comparison with the reference ladder provided with the kit using GeneMapper v3.2 software (Applied Biosystems, Foster City, CA, USA). Y-chromosome haplotypes are shown in Supplementary Table S2.

A total of 27 Y-single nucleotide polymorphisms (SNPs) were genotyped in each of the samples (Supplementary Table S2), following the same protocol as in Cárdenas *et al.*<sup>6</sup> Haplotypes were classified into haplogroups following both the Phylotree (<http://www.phylotree.org/Y/>; version 30 November 2014) and

the Y-Chromosome Consortium (<http://www.isogg.org>; version 10.47 from September 2015) nomenclatures.

### Statistical analyses

Some phylogeographic considerations were carried out based on searches performed on the Y-Chromosome STR Haplotype Reference Database (YHRD; <https://yhrd.org/>). This database contains information on 143 044 minimal haplotypes (MHT) from 940 populations of 128 countries, and 25 499 full Y23 haplotypes from 179 populations of 55 countries (Release 49—17 Feb 2015, accessed on 20 June 2015). The Y23 Y-STR haplotypes of Mennonites were searched for in the YHRD in worldwide populations and using MTH.

The most likely geographic origin of a Y-STR profile was computed using the procedure originally described in Egeland *et al.*<sup>7</sup> In brief, the procedure involves two main steps, (i) the dimensions of the data are reduced by means of principal component analysis and (ii) quadratic discriminant analysis is used to perform classification.

Although haplogroups could be directly inferred from Y-SNP data, haplogroup inference from Y-chromosome haplotypes was additionally carried out using the tool available in <http://predictor.ydna.ru/>, considering the Y23 markers that are allowed by the tool: DYS456, DYS389I, DYS390, DYS389II, DYS458, DYS19, DYS393, DYS391, DYS439, DYS635, DYS392, GATAH4, DYS437, DYS438 and DYS448.

Haplotype diversity (HD), discrimination capacity (DC) and matching probability (MP), were estimated as in Purps *et al.*<sup>8</sup> taking into considerations the issues regarding DYS385 and DYS389 loci. The average number of pairwise differences was also computed. For most of the analyses, the Arlequin software v3.5.1.2 was used.<sup>9</sup> All the indices were calculated using the full PPY23 haplotypes as well as collapsing these haplotypes to their corresponding MHT. Computation of indices was also carried out considering different national and regional grouping schemes.

A computational simulation was performed in order to determine whether the haplotype diversity observed in the Mennonites was significantly different to the values expected in an alleged European source population (using the Dutch and the German data set as surrogates) and the residence population (Argentina). A total of 10 000 re-samples containing 12 Y-STR profiles of the Argentinean, Dutch or the German data set were taken at random and with replacement. For each sub-sample, HD values were computed. *P*-values were obtained as the percentage of simulations with a lower HD than our Mennonite sample set.

$R_{ST}$  genetic distances on Y-STR profiles between Mennonite profiles and other reference populations were computed using the Arlequin software v3.5.1.2. DYS385ab marker was excluded from these analyses, as well as samples containing duplications, and null or intermediate alleles. The number of repeats in DYS389I was subtracted from DYS389II.

Multidimensional scaling analyses (MDS) based on the  $R_{ST}$  distance matrices were performed as a way to depict the relationships between the Mennonite samples and others, considering for the analyses the genetic distances obtained from the full PPY23 profiles and the MHT analyses. The package *stats* (function: *cmdscale*) of the statistical package R (<http://www.r-project.org>) was used for this purpose.

At last, the databases of Mennonite Y-chromosome profiles and/or haplogroups available in The Mennonite DNA Project (<http://www.mennonitedna.com/>) and 23andMe (<http://mennodna.remotewebaccess.com/23andme/index.html>) were explored for further information. These databases do not contain open information on geographic origin or relationships between donors, and therefore these data have important limitations for population-based studies. For instance, the Mennonite DNA Project database contains >800 Y-chromosome profiles grouped in ~195 different surnames; these donors not always share the same known genealogical ancestors. Moreover, these databases contain information on names and surnames of donors but genealogical information is intricate. In this regards, it is important to highlight that genetic and clinical studies rarely reveal information that might drive to the identification of donors, and this is the procedure we followed with our sample cohort, where the samples were anonymized at the time of collection.

## RESULTS

### Phylogeography of Y-chromosome haplotypes

All the Mennonite Y-chromosome profiles were searched for the YHRD database in order to investigate the geographic locations where these profiles have been observed previously. First, Y23 haplotypes were collapsed to their MHT in order to detect global patterns of variation (given that Y23 haplotypes are singletons and therefore uninformative when searching for exact matches in YHRD). Supplementary Figure S3 shows worldwide maps of Mennonite MHT-haplotype frequencies. Only haplotype #H6 is ubiquitous; it appeared 2976 times worldwide in YHRD, most of them in Europe and America. All Mennonite haplotypes were observed in Central Europe; with the Netherlands and Germany being the countries showing the largest proportion of haplotype matches (83% and 75%, respectively). Haplotypes H1 and H5 were observed exclusively in Central, whereas the haplotype represented by #H8 and #H11 is particularly frequent in Germany (~20% of the total matches were observed there). Most of the haplotypes were also observed in America, and although some of them appeared to be widely spread in this continent (for example, #H7, #H9), other haplotypes were found only in a few sub-continental locations (for example, #H2, #H3, #H10 and so on).

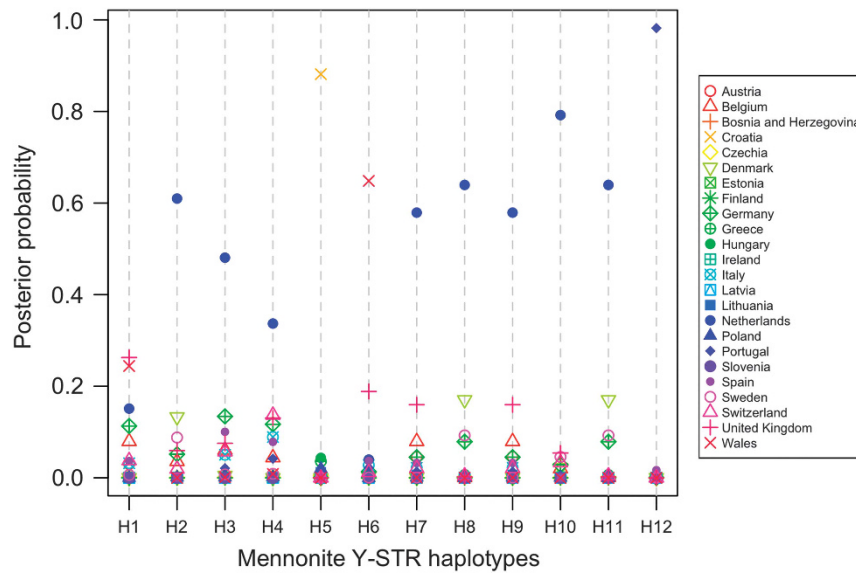
Haplogroups were obtained by means of genotyping 27 Y-SNPs representing the main European branches of the Y-chromosome phylogeny (Supplementary Table S2). In addition, inference of haplogroups was also obtained using Y-STR profiles. The prediction of haplogroups fits well with the assignment based on SNPs; the main difference lies on the precision of sub-haplogroup assignment. Haplogroup assignment indicates that 50% of the haplotypes belong to haplogroup R1b1, and 42% to haplogroup I2b1. One haplotype was allocated to haplogroup E1b1b1. In agreement with previous observations on the geographic location of the Mennonite STR profiles, all haplogroups observed in our sample were of typical European ancestry. Only E1b1b1 is originally from Northeast Africa (Ethiopia and Somalia); this haplogroup represents the last major direct migration from Africa into Europe, and it is therefore also present in contemporary European populations.

### Inferring the most likely geographic origin of Y-chromosome profiles

The most likely geographic origin of a Y-chromosome profile was estimated using principal component analysis combined with quadratic discriminant analysis (Figure 2). Classification was carried out assuming 69 European source populations. This analysis indicates that the Netherlands is the population that best explains 75% of the Mennonite haplotypes, 58% of them with probabilities higher than 0.5 (a very high value when compared with those calculated for the remaining countries). #H6 receives a higher probability in the Wales and the UK data sets, but this haplotype, unlike to the rest, is ubiquitous in Europe. Haplotype #H5 shows a higher probability in Croatia, whereas #H1 has a more ambiguous ascription according to this analysis. Note, however, that the 23-STR haplotype #H1 appeared only three times in the database, all of them in the Netherlands, whereas the 23-STR haplotype #H5 appeared exclusively in Poland.

### Molecular diversity

Different diversity indices were computed (Table 1). Mennonites have very low diversity indices compared with other Argentinean and European populations; compare also with values in Table 1 in Toscanini *et al.*<sup>10</sup> Supplementary Table S3 also shows that Mennonite



**Figure 2** Most likely geographic origin of Mennonite Y23 haplotypes in 69 European population samples.

**Table 1** Diversity and forensic parameters for full PPY23 and MHT haplotypes

Population	n	PPY23 haplotypes							MHT (9 markers)						
		K	S	S (%)	HD	M	DC	MP	K	S	S (%)	HD	M	DC	MP
Argentina North <sup>a</sup>	180	169	159	88	0.9993±0.0007	15.19±6.82	0.8833	159	151	125	69	0.9980±0.0009	5.35±2.59	0.6944	132
Argentina Center <sup>a</sup>	311	303	296	95	0.9998±0.0003	14.67±6.59	0.9518	294	247	214	69	0.9971±0.0008	4.99±2.43	0.6881	163
Argentina South <sup>a</sup>	130	128	126	97	0.9998±0.0010	14.17±6.40	0.9692	126	112	102	78	0.9951±0.0026	4.94±2.42	0.7846	80
Europe <sup>a</sup>	2168	2133	2098	97	1.0000±0.0000	14.52±6.51	0.9677	2100	1255	979	45	0.9956±0.0005	4.98±2.43	0.4516	205
Mennonites	12	10	8	67	0.9697±0.0443	14.94±7.20	0.6667	9	9	6	50	0.9545±0.0467	5.32±2.76	0.5000	8

Abbreviations, DC, discrimination capacity ( $S/n$ ); HD, haplotype diversity; K, number of different haplotypes; M, average number of pairwise differences; MP, matching probability;  $MP = (1/\sum p_i^2)$ ; n, Sample size; S, singletons; S(%),  $S/n$ .

<sup>a</sup>This sample sets were analyzed in Toscanini *et al.*<sup>10</sup>

haplotypes have the lowest HD values compared with 69 European samples.

Given that values of molecular diversity were lower than those of European and neighboring Argentinean populations, we conceived a computational simulation that aimed to investigate if HD observed in Mennonites was significantly different from the values expected in the alleged European source population and the residence (non-Mennonite) Argentinean population. As seen in Figure 3, the amount of variation observed in Mennonites is significantly lower than that expected if we take random samples of size 12 (our sample size of Mennonites) from the Netherlands (Y23:  $P$ -value=0.001; MHT:  $P$ -value 0.065), Germany (Y23:  $P$ -value<0.001; MHT:  $P$ -value=0.015) or Argentina (Y23:  $P$ -value=0.021; MHT:  $P$ -value=0.005).

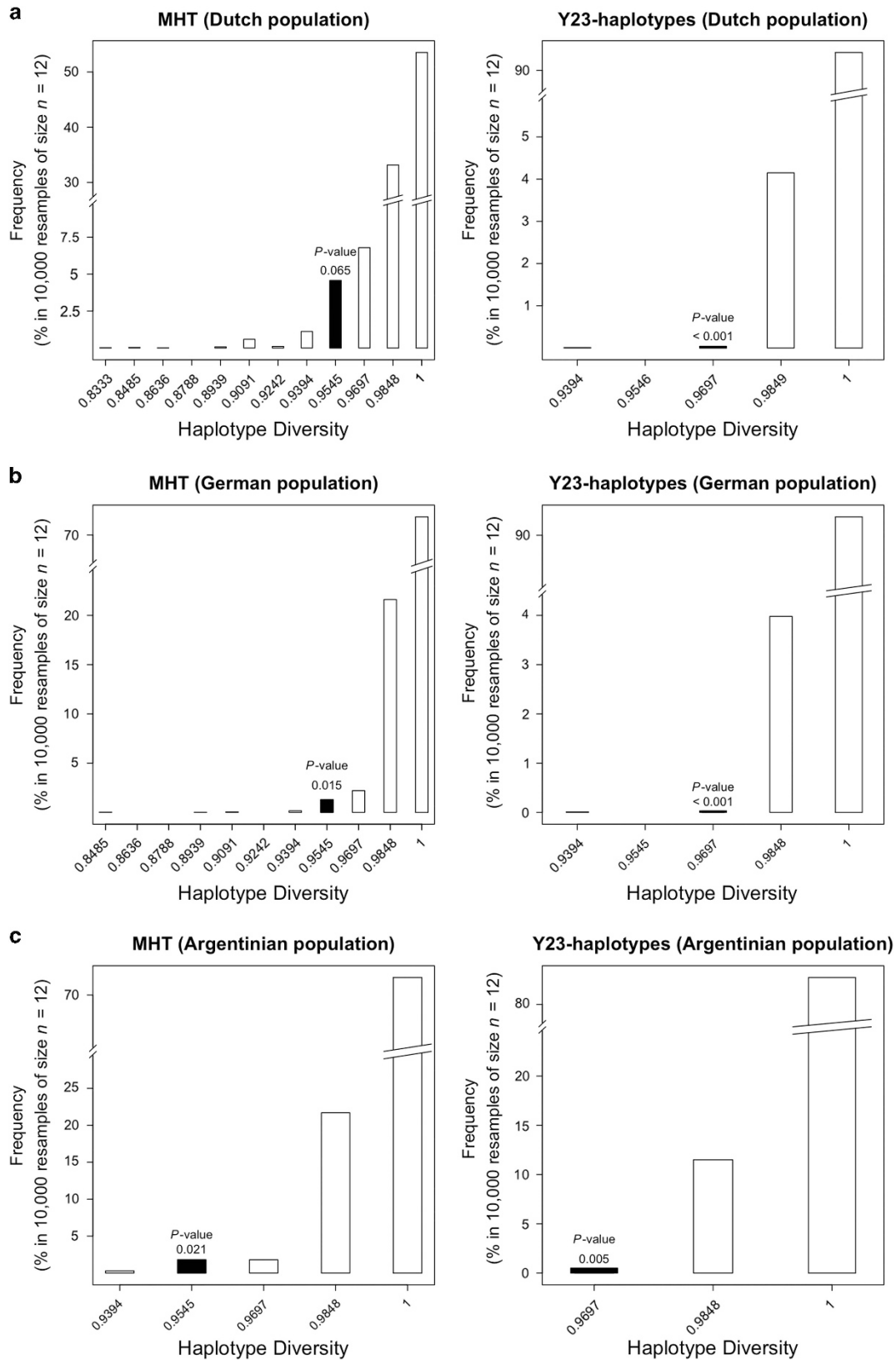
### MDS of Y-chromosome profiles

$R_{ST}$  distances were computed between genetic profiles in the Mennonites and other reference populations. These distances were visualized by means of a MDS analysis. First, a preliminary MDS was carried out in order to investigate the genetic relationship of Mennonite Y-chromosomes and the European populations (Figure 4). This analysis involved a total of 69 European samples,

and it showed the close relationship of Mennonite Y-chromosomes to other Central European populations (in both dimensions 1 and 2), including Zürich and Basel (Switzerland), a local (Friesland) and a general population from the Netherlands, Copenhagen (Denmark) and so on. In this MDS analysis, some structure in the European samples was revealed; for instance, Nordic populations appear clustered in one pole of the MDS; and Sweden is the country more closely related to Mennonite haplotypes (Skaraborg and Östergötland/Jönköping). A further elaboration on this MDS plot encompassed another analysis collapsing the 69 European samples in 23 groups representing the different European countries (Figure 5). This analysis showed again that the Netherlands, Denmark and Switzerland are the countries that are most closely related to our sample of Mennonite Y-chromosome haplotypes.

### DISCUSSION

Historical and oral documentation indicates that the Mennonites in America come from Ukraine, and ultimately, from Central Europe, with the Netherlands being the main original homeland of present-day Mennonites. Migration patterns of Mennonites were however complex; the congregation established in Argentina followed this general



**Figure 3** Results from a simulation indicating that haplotype diversity observed in Mennonites is significantly lower than expected when compared with (a) present-day Dutch, (b) present-day Germans and (c) non-Mennonite Argentinians (representing the residence population of the Mennonites). A total of 10 000 re-samples with replacement of size 12 (the same sample size as Mennonites) of the Dutch, German and Argentinian data sets were obtained; haplotype diversity was obtained for each resample and represented in the figures.

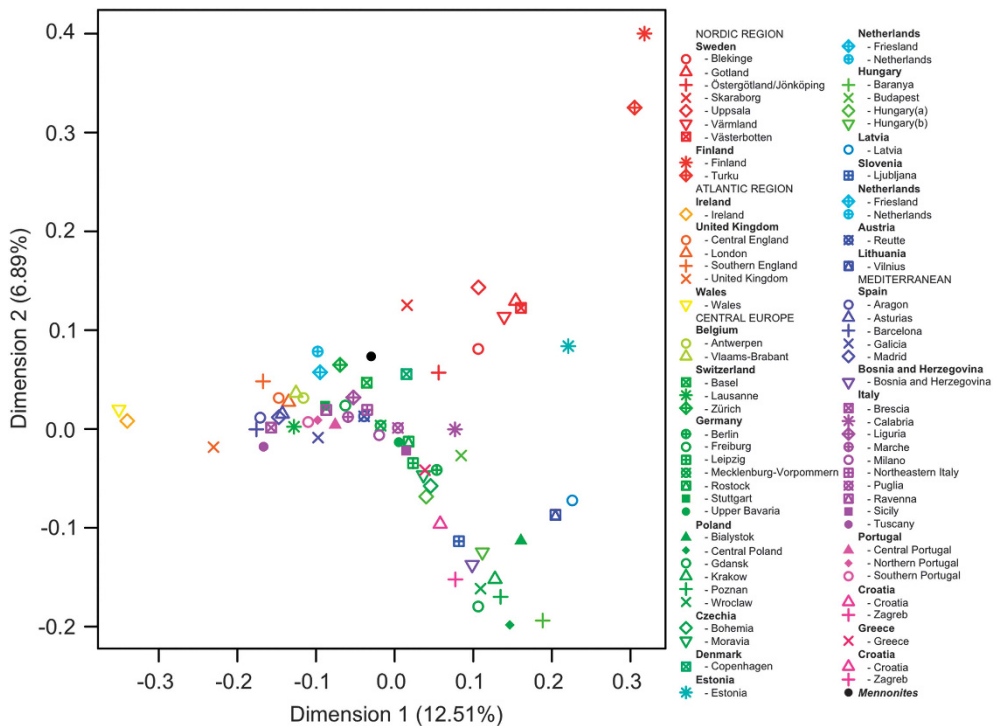


Figure 4 MDS plot based on  $R_{ST}$  distances based on Y23 haplotypes and 69 European population samples.

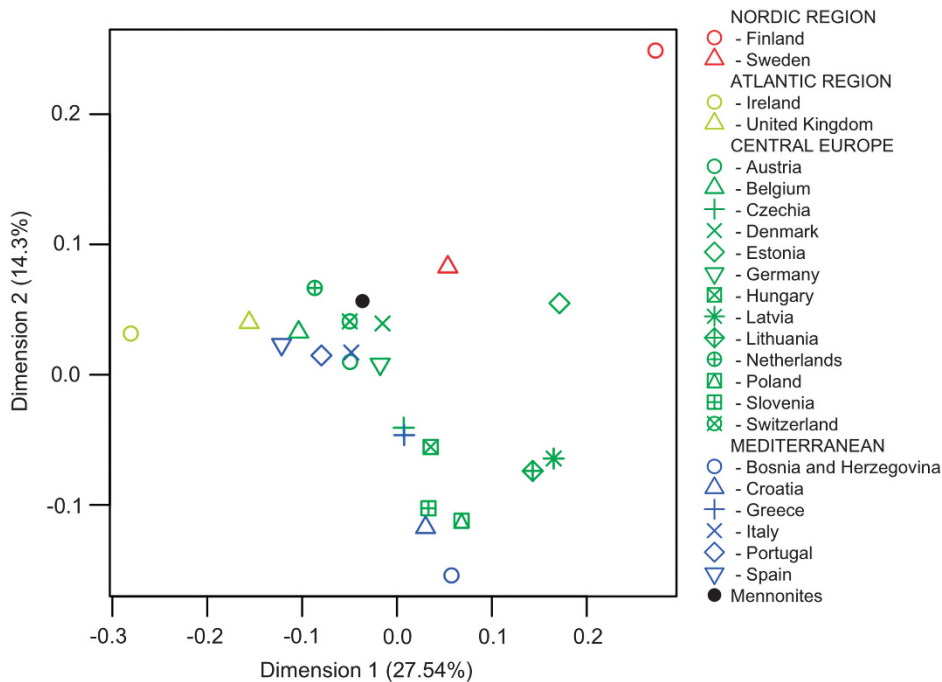


Figure 5 MDS plot based on  $R_{ST}$  distances based on Y23 haplotypes and 23 sample sets that represent European countries (the result of collapsing the 69 different sample sets used in Figure 4).

path: Netherlands > Germany > Danzig/Vistula valleys > Chostitza (Ukraine) > Canada > Mexico (> Bolivia) > 'La Nueva Esperanza'. Given the different migrations undergone by the Mennonites during the last few 100 years, some level of admixture with local populations could be expected, especially if we take into account that the Mennonites, unlike other Anabaptist groups (for example, Amish)

are culturally permeable to local neighboring populations. In America, this admixture process could have involved Native American and 'Afro-American' individuals.

The data however indicate that all the Y-chromosome haplotypes observed in Argentinean Mennonites are of European ancestry. This is observed in (i) their Y-chromosome haplogroup composition and

(ii) their Y-chromosome phylogeographic/phylogenetic patterns. The data contained in public databases of 23andMe and The Mennonite DNA Project (see Methods) indicate an almost lack of profiles of Native and recent African ancestry. There are only a few profiles that could be assigned to the Native American haplogroup Q. Therefore, these data are in agreement with a virtually lack of admixture between Mennonites and local American Natives or other American residents with Native or African ancestry.

Interestingly, the results from the present study point to the Netherlands as the population in Europe that has the closest Y-chromosome affinity to present-day Mennonite Y-chromosomes. Thus,  $R_{ST}$  distances indicate that Mennonites haplotypes are genetically related to haplotypes from populations living in Central Europe, especially in Switzerland and the Netherlands, whereas analysis of the most likely geographical origin of Y-STR profiles points to the Netherlands as the population that best explains the composition observed in the Mennonites.

On the other hand, analysis of molecular diversity indicates that the Mennonites show a much lower molecular diversity than neighboring populations from Argentina or European populations. This might be indicative of a moderate level of endogamy in Argentinean Mennonites. Supportive evidence comes from simulation experiments indicating that this level of HD is unlikely to be explained by chance.

This study suffers from several limitations. Given the moderate sample size analyzed and the fact that the European component is also the major ancestral component in the Y-chromosome pool of Argentina<sup>10,11</sup> (as it also occurs in other non-Native South American populations<sup>6,12</sup>), our sample might lack statistical power to detect other genetic components (if any) different from the European one; although 12 lineages seems quite representative of 'La Nueva Esperanza' congregation (with a census of only 1278 inhabitants in 2005). Further analyses are needed in order to confirm if Argentinean Mennonites were biologically permeable to other neighboring populations. The mtDNA could be particularly useful given that the Native American component in the mtDNA pool of admixed Americans is significantly higher than the one observed on the Y-chromosomes.<sup>13–15</sup> At last, Y-chromosome haplogroups of the individuals analyzed in the present study were determined directly using Y-SNPs but also inferred using predictive software that uses Y-STR profiles. Although we agree that caution is mandatory when using these predictive tools,<sup>16</sup> it seems that the use of a reasonable number of Y-STR markers (for example, >17) provides a good estimate of haplogroup status.<sup>17</sup> The results from the present study support this claim, at least for the basal nodes of the Y-chromosome tree. The minor nomenclature differences observed in the present study could be related to: (i) the lower resolution of the Y-SNPs employed compared with the inference obtained by means of software, (ii) predictive errors and/or (iii) the use of different nomenclatures.

In conclusion, although Mennonite migration routes were complex since their foundation in the Netherlands to the present, the results of the present study indicate that Argentinean Mennonites have European Y-chromosome ancestry without Native American or African introgression. Moreover, the statistical analyses carried out in the present study suggest that their Y-chromosome pool has been most likely inherited from their original homeland in Europe. They

have reduced molecular variability owing to their relative isolation from neighboring populations. This could at least in part explain some clinical conditions that are typically more prevalent in these young isolated populations settled with a small number of founders.<sup>3</sup>

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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