

Mitochondrial DNA diversity in six Mennonite communities

from Kansas and Nebraska

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Introduction:

Mitochondrial DNA (mtDNA) has been used extensively to study the genetic history and structure of worldwide indigenous populations (Schurr and Sherry 2004, Richards et al., 2002, Salas et al., 2002, Macaulay et al., 1999). However, less research has focussed on the European religious diasporas that have occurred over the last 500 years. Populations belonging to the Anabaptist movement have an intriguing history and a unique genetic structure (Crawford 2000) due to the evolutionary population dynamics that have shaped their biological makeup. Briefly, the Anabaptist movement began during the Reformation in the 16th century and can be divided into three contemporary religious denominations: 1) Mennonites – originating in the Netherlands and northern Germany; 2) Hutterites – starting in Austria; and 3) Amish – beginning in Switzerland and southern Germany (Rogers and Rogers 2000). Each of these groups went through a number of persecutions and demographic events that eventually led to immigration to the Americas. This study examined mtDNA diversity in six Mennonite communities from Kansas (Goessel, Lone Tree, Garden View, Meridian, Old Order) and Nebraska (Henderson) in order to further elucidate the genetic structure and population history of these groups.

Objectives:

1. Determine the maternal genetic relationship between the six study populations using mtDNA.
2. Investigate the genetic relationship among three different congregations of Mennonites.
3. Establish whether population subdivision within these groups demonstrates patterns of fission-fusion as seen in classical genetic markers.

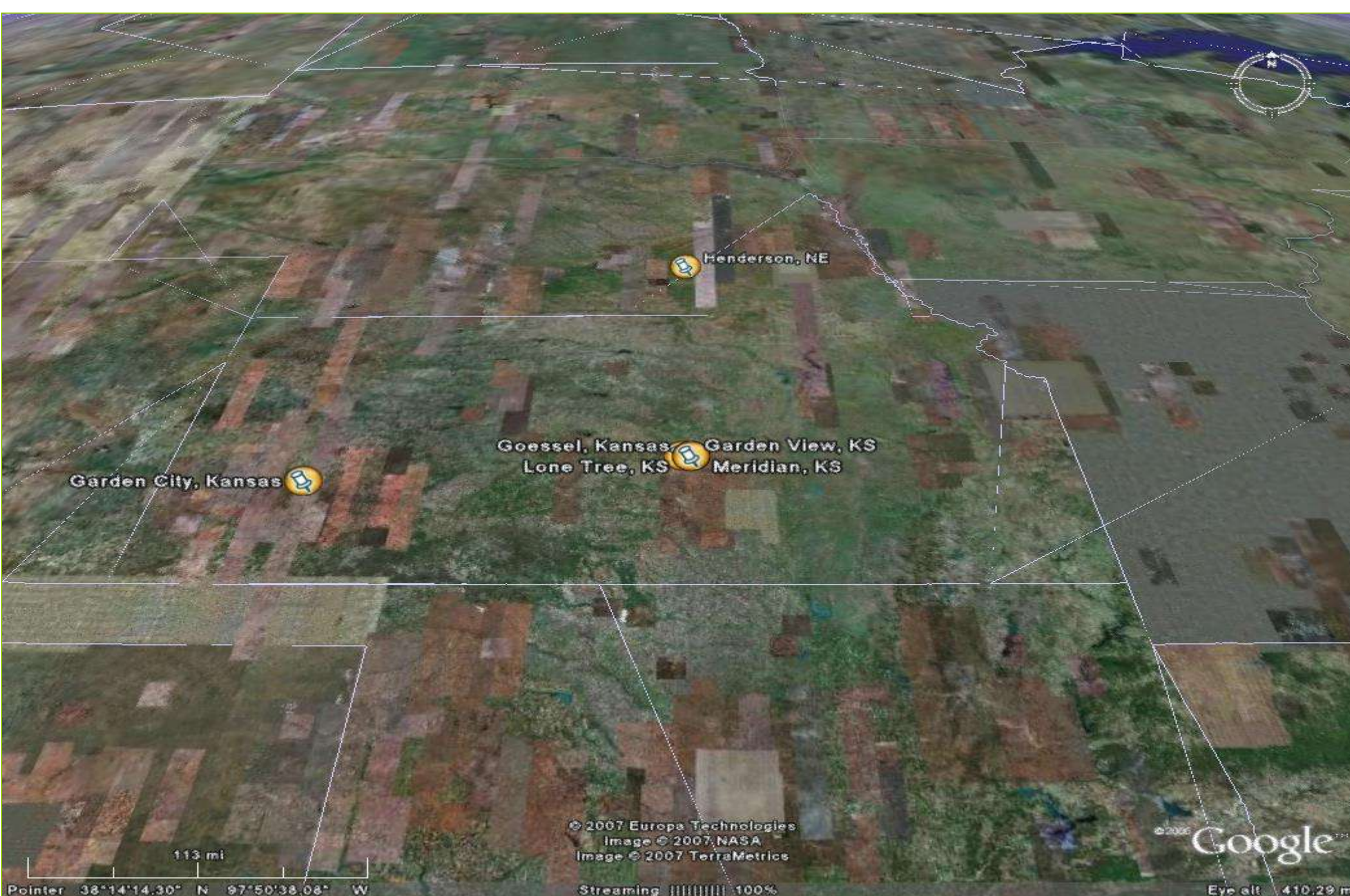


Figure 1: Geographic location of six Mennonite Communities in Kansas and Nebraska

Mennonite Population Background:

The Mennonite communities of Kansas and Nebraska may be divided into three religious congregations: 1) Alexanderwhol, which includes the communities of Goessel, KS and Henderson, NE who immigrated to the United States from Russia in the 1870s (Crawford, 2000); 2) Holderman, which includes the communities of Meridian, Garden View, and Lone Tree KS and are considered a heterogeneous population of Pennsylvania Dutch and German population mixed with Mennonite Russian immigrants (Crawford et al., 1989); and 3) Old Order from Garden City, KS who have recently immigrated to the US from Chihuahua, Mexico but originated in Canada (Allen and Redkopp 1987).

This study included biological samples from 117 individuals (Goessel =21, Henderson=19, Meridian=17, Garden View =21, Lone Tree=23, Old Order=16) collected as part of a long term multidisciplinary study of Midwestern Mennonite communities.

Materials and Methods:

HVS-I sequencing and RFLP analysis

- HVS-I sequences (n=117) were generated on an ABI 370 capillary system sequencer through automated fluorescence using the ABI ver. 3.0 Big Dye sequencing cycle kit and primers 15976F and 16422R. All sequencing was performed at the DNA Sequencing Laboratory located in the University of Kansas Natural History Museum. In order to distinguish between haplogroups U and H all samples were analyzed for the characteristic absence of RFLP cut site at mtDNA np 7025. All sequences were evaluated between mtDNA HVS-I base pairs 16090-16365.

Multidimensional Scaling Plot (MDS):

- A MDS plot (Kruskal 1964) was constructed in order to visualize the biological relationship between the study populations utilizing a Tamura-Nei model of substitution (Tamura and Nei 1993) and a γ -value of 0.26 (Meyer et al., 1999). The resulting MDS plot was generated using NTSYS (Applied Biostatistics) and the matrix was computed using Arlequin 3.0 (Excoffier et al., 2005).

Median Joining Network (MJ):

- A MJ network was constructed in order to determine genetic relationships between haplotypes found within the studied groups (Bandelt et al. 1995, 1999). Networks were constructed using the program Network 4.0 (www.fluxusengineering.com).

Mismatch and Intermatch Distribution:

- Mismatch distributions (Harpending et al., 1993; Rogers and Harpending, 1992) were constructed by counting diversity between each pair of subjects and using histograms to display the frequencies of nucleotide variant sites (Rogers et al., 1996). Intermatch distributions were calculated with IWAVE (Sherry et al., 1994) in order to determine potential population divergence.

Analysis of Molecular Variance (AMOVA)

- AMOVA was used to determine the amount of population subdivision among the six study populations based on HVS-I sequence variation. The six populations were grouped into three congregations: 1) Alexanderwhol (Goessel, Henderson); 2) Holderman (Meridian, Lone Tree, Garden View), and 3) Old Order (Garden City). Significance values were obtained by bootstrapping data 1000 times and calculated using Arlequin 3.0 (Excoffier et al. 2005).

Results: Mennonite Haplogroup Distribution

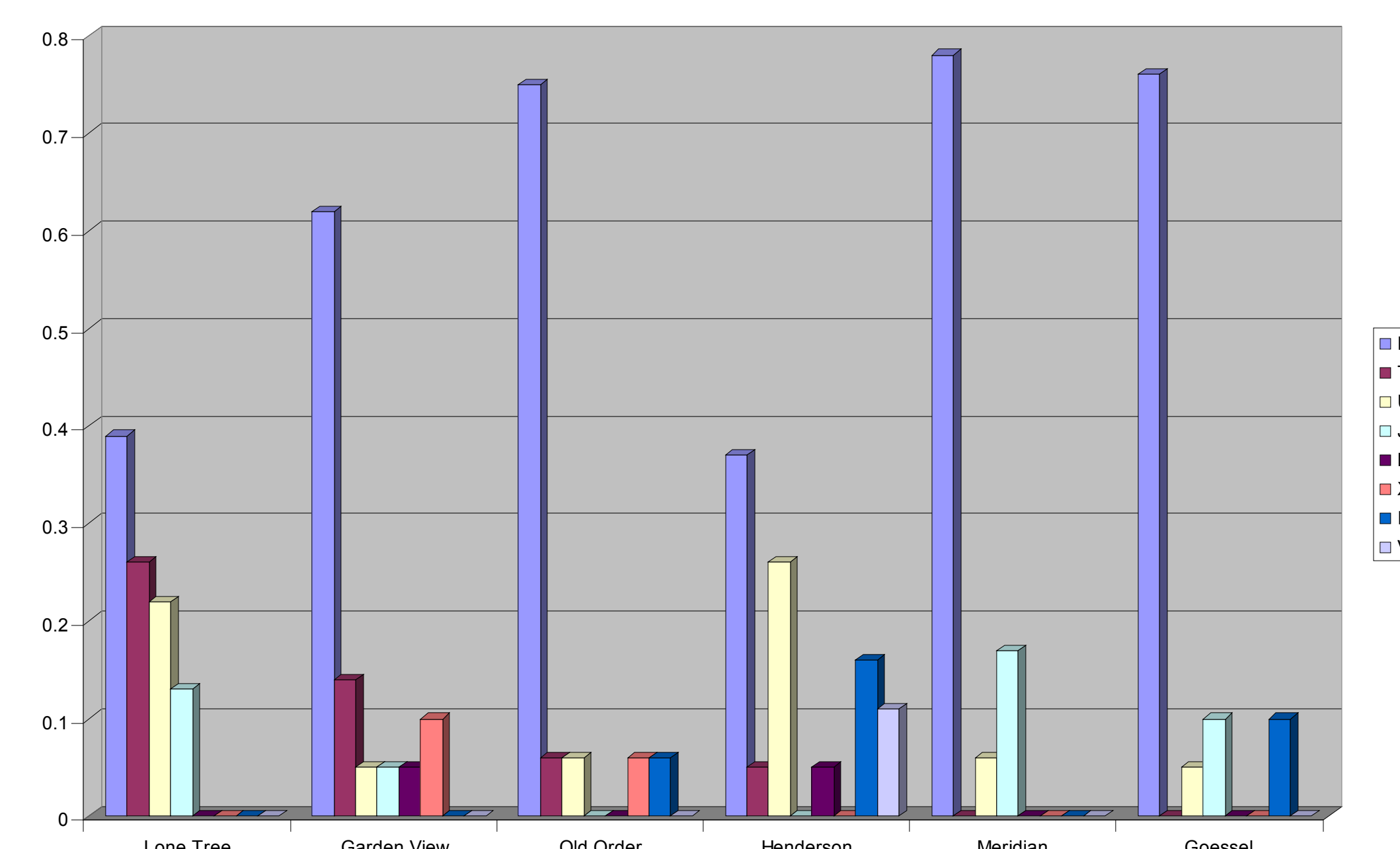


Figure 2: Results demonstrate that eight (H, T, U, J, K, X, I, and V) of the ten common European haplogroups were present in these communities. Haplogroup H was the most frequent in all the study populations and ranged from 78% in Meridian to 37% in Henderson. Henderson ($h=0.78$) and Garden View ($h=0.68$) demonstrated the largest amount of haplogroup diversity while Meridian ($h=0.38$) demonstrated the lowest. The unequal distribution of mtDNA haplogroups may be indicative of population fission based on the migration of familial groups during the Mennonite diaspora.

Results: Multidimensional Scaling Plot

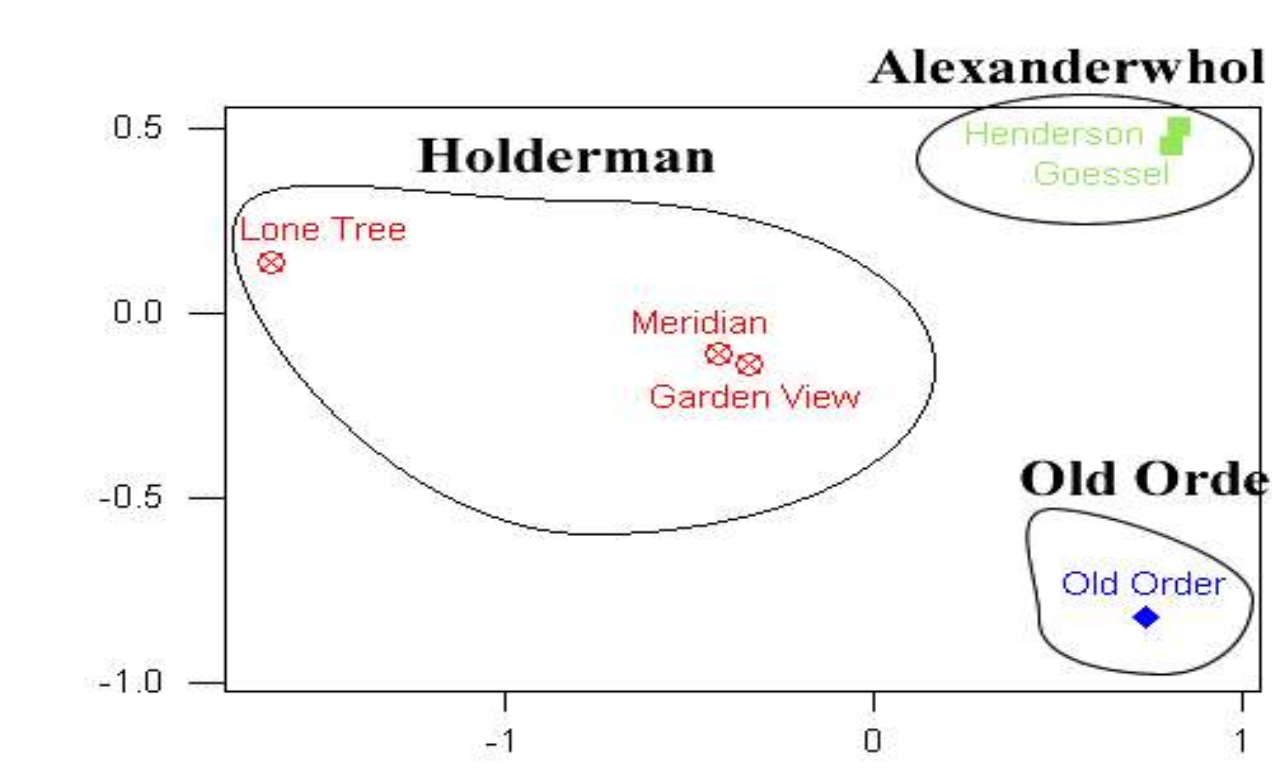


Figure 3: MDS Plot of six Mennonite Communities (Stress Value =0.02, Goodness of Fit =0.98) based on mtDNA HVS-I Sequence Data. This plot separates out the six communities into their associated congregations. This is demonstrative of the shared evolutionary and migratory histories of these populations. The Alexanderwhol congregation immigrated to the US in 1874 from Russia whereas the Holderman congregation is a more heterogeneous population and these relationships are clearly denoted in this figure (Crawford et al., 1989). The Old Order Mennonites immigrated to Mexico from Canada in the 1920s (Allen and Redkopp 1987) and have recently immigrated to the US and are clearly distinguished from the other two Mennonite congregations.

Results: Median Joining Network

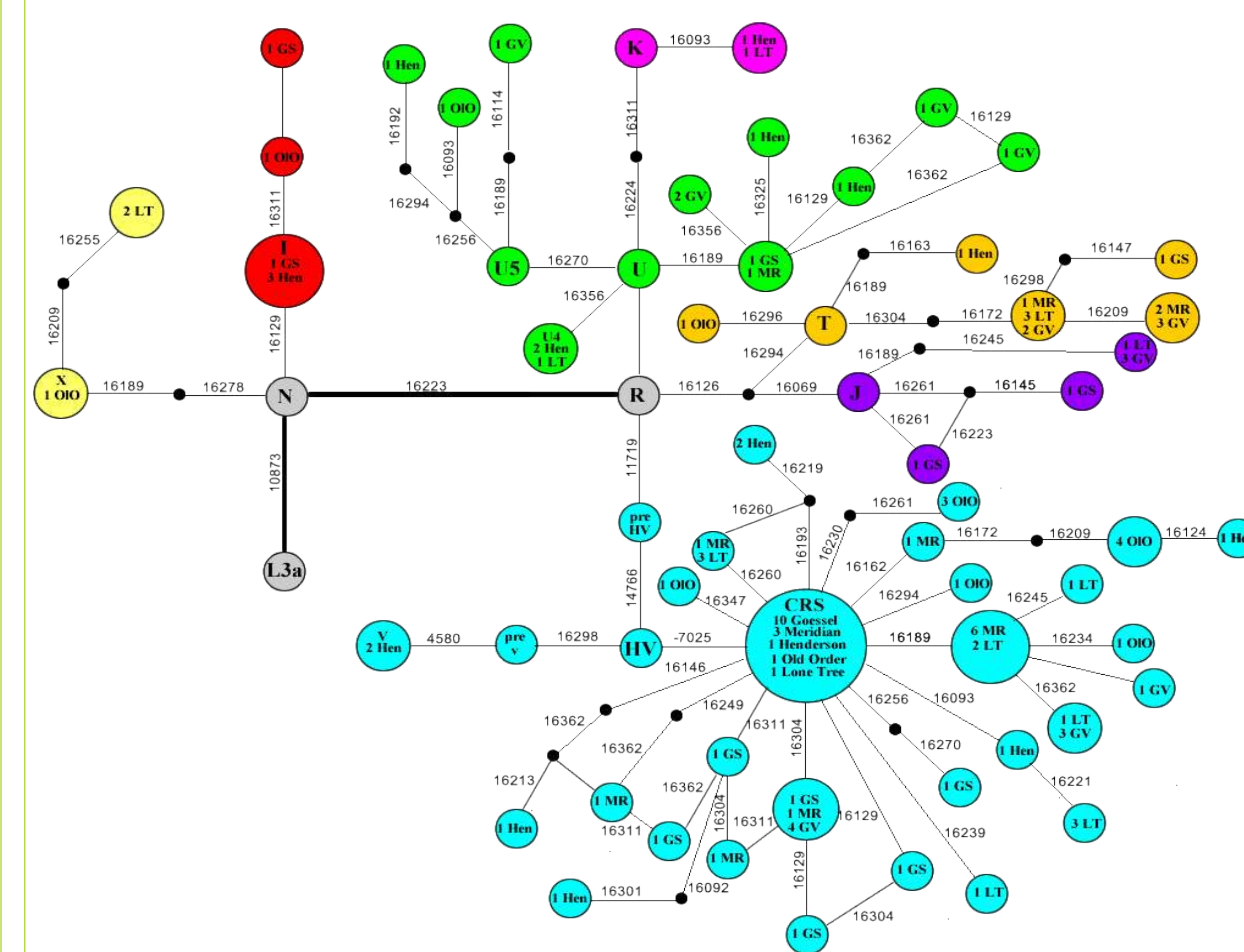


Figure 4: MJ Network for the six study populations. Of the sixty haplotypes found in the 117 samples only 12 of these are shared between the six communities and only the CRS haplotype of Haplogroup H is shared by the majority of the these populations. This supports the assumption that population fission occurred in these communities and even within congregations there is population substructure.

Results: Analysis of Molecular Variance

Sources of Variation	D.F.	Sum of Squares	Variance Components	% of Variation	Φ statistic	p value
Among Groups	2	11.909	0.106	5.66	0.056	0.02
Among populations within group	3	6.846	0.026	1.42	0.015	<0.001
Within populations	112	195.253	1.743	92.93	0.070	<0.001
Total	117	214.008	1.876			

Table 1: AMOVA table for 3 congregations. The majority of variation is found within populations while the lowest amount of genetic information is shared among populations within groups. This is indicative of different demographic histories of these groups. Upon arrival in the US, the Alexanderwhol Mennonite congregation split into two communities and previous research on these communities has demonstrated that this division was not random and occurred along familial lines (Crawford et al., 1989).

Results: Mismatch and Intermatch Distributions:

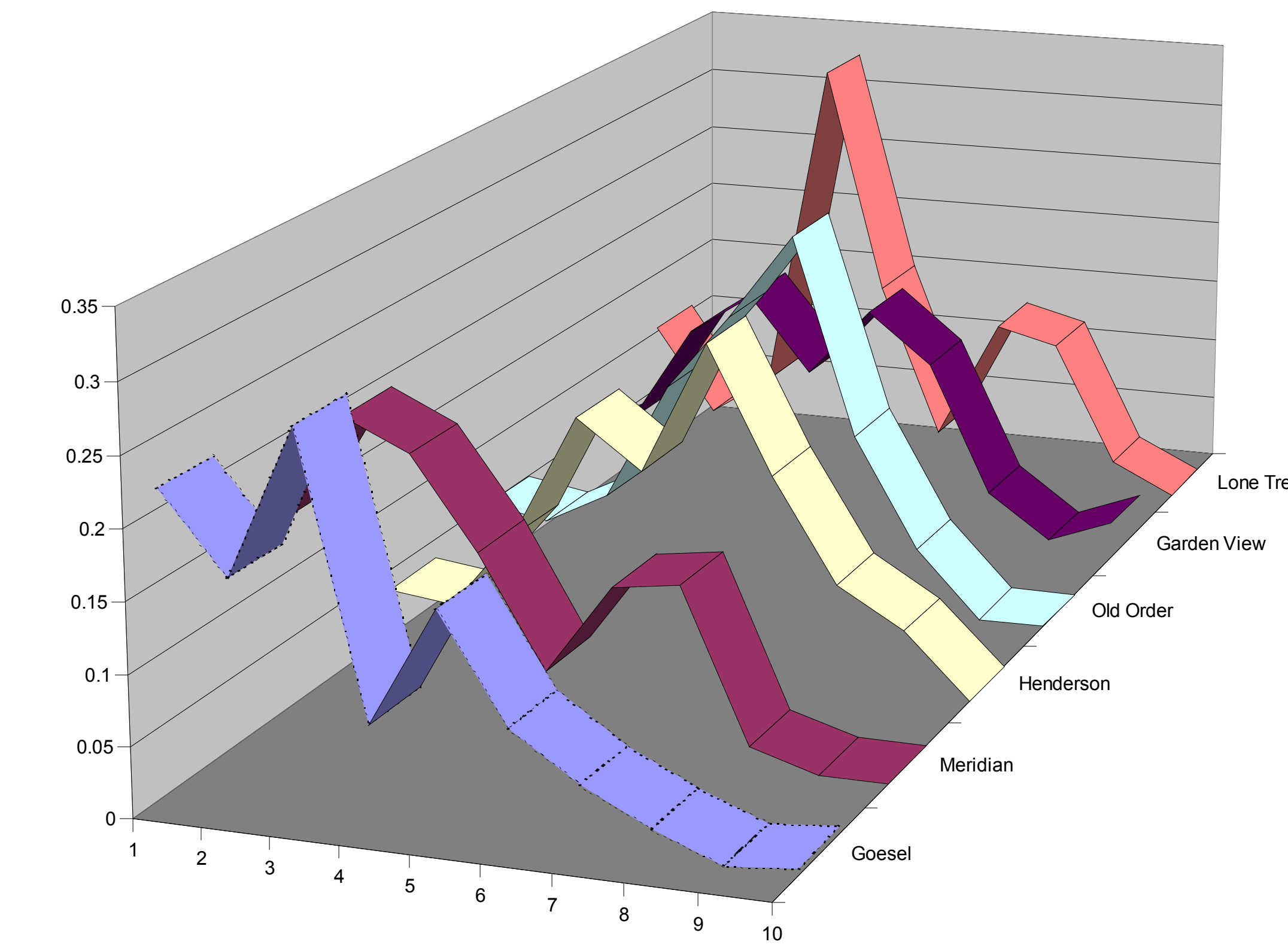


Figure 5: Mismatch distributions for all six study communities. All of the distributions are unimodal but highly ragged (Harpending's raggedness index ranged from 0.42 in Lone Tree to 0.78 in Garden View). Two peaks are clearly visual and may indicate a shared genetic history for these population with the initial peak representing coalescent events that occurred in Europe and the dip may indicate a potential bottleneck that the majority of Anabaptist populations went through prior to immigrating to the Americas with subsequent expansion represented by the 2nd peak.

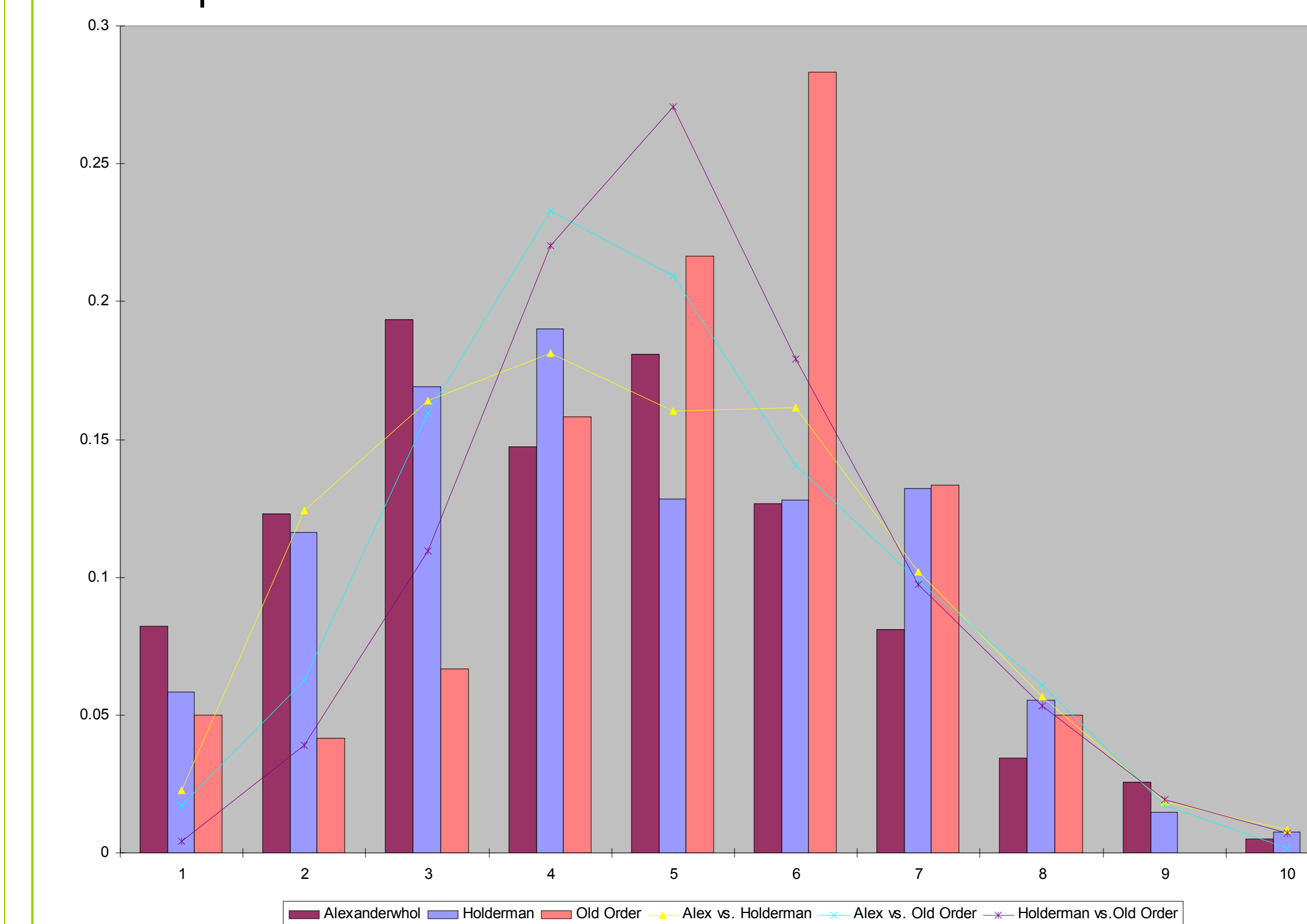


Figure 6: Mismatch (boxes) and Intermatch (lines) distributions for the three studied congregations. Intermatch distributions for all three congregations overlap and indicate a shared genetic history for all Mennonite communities. However, as coalescent events are better in elucidating early demographic events these results are more indicative of the origin of European populations and may be shared with other continental populations.

Key Points:

1. There is a shared biological relationship between Mennonite populations that is delineated through the affiliation within the studied congregational communities.
2. Investigation of these populations at the mtDNA haplotype level indicate that there is genetic differentiation of these populations among communities within congregations.
3. Population subdivision of these communities into congregations support previously published results regarding known patterns of fission-fusion and offer further clarification of the relationship between genetic characteristics and historical events.

Acknowledgements:

This research was sponsored by grants from the National Institute of Aging (#AG01646) and Kansas Attorney General Settlement Grant (#KUCR KAN 30471). We would like to thank all individuals who participated in this study.



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