

Analysis of forensically used autosomal short tandem repeat markers in Polish and neighboring populations

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Abstract

The purpose of this study was to evaluate the homogeneity of Polish populations with respect to STRs chosen as core markers of the Polish Forensic National DNA Intelligence Database, and to provide reference allele frequencies and to explore the genetic interrelationship between Poland and neighboring countries. The allele frequency distribution of 10 STRs included in the SGMplus kit was analyzed among 2176 unrelated individuals from 6 regional Polish populations and among 4321 individuals from Germany (three samples), Austria, The Netherlands, Sweden, Czech Republic, Slovakia, Belarus, Ukraine and the Russian Federation (six samples). The statistical approach consisted of AMOVA, calculation of pairwise Rst values and analysis by multidimensional scaling. We found homogeneity of present day Poland and consistent differences between Polish and German populations which contrasted with relative similarities between Russian and German populations. These discrepancies between genetic and geographic distances were confirmed by analysis of an independent data set on Y chromosome STRs. Migrations of Goths, Viking

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influences, German settlements in the region of Volga river and/or forced population resettlements and other events related to World War II are the historic events which might have caused these findings.

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1. Introduction

Poland is located in Central Europe and has a population of 38.6 million descending from Western Slavs. The country's history was complicated by repetitious shifts of border-lines and territorial losses mainly to Prussia, Russia and Austria [1,2].

A recent study of Y chromosome STR haplotype distribution showed homogeneity of paternal lineages in the present day Poland and their distinctiveness from lineages from the neighboring countries [2]. A subsequent study based on Y chromosome haplogroups focused on the differences observed between the Polish and German populations were particularly interesting since they contrasted with geographic proximity and known historic interactions between the two nations [3]. Based on Y chromosome data it has been suggested that the pronounced Polish–German differences could be caused by ethnic and linguistic barriers separating the Slavic and Germanic populations of Europe [2] which were significantly enhanced by forced population resettlements which took place after the World War II [3].

Homogeneity of Y chromosome haplotypes in a population does not preclude the stratification of maternal lineages [4] and

consequently the heterogeneity of autosomal gene pool. Since exact knowledge on population stratification is of utmost importance in forensic genetics we decided to evaluate homogeneity of the main regions of Poland with respect to distribution of 10 autosomal STRs which were tentatively chosen as the core markers of the Polish National DNA Intelligence Database. Moreover, we intended to provide reference frequencies for alleles of these markers to be used in forensic routine.

In addition, we were interested whether differences among Polish and neighboring populations can be detected using STRs, and, if so, how they correlate with geography and history of the region. Given the results of Y chromosome studies [2,3] we were particularly interested in the comparison between the Polish and German as well as other Western European populations.

2. Materials and methods

The 2176 unrelated Polish individuals were sampled from six regional populations in Poland designated as Central, Northern, Western, South-Western, Southern and Eastern

Table 1
Characteristics of the studied population samples

Region	Description	N	Method of typing
Central Poland (PoC)	Mazowieckie, Łódzkie, Wielkopolskie	689	SGM plus
Northern Poland (PoN)	Pomorskie, Warmińsko-Mazurskie, Kujawsko-Pomorskie	287	
Western Poland (PoW)	Zachodnio-Pomorskie, Lubuskie	298	
South-Western Poland (PoSW)	Dolnośląskie, Opolskie, Śląskie	298	
Southern Poland (PoS)	Małopolskie, Świętokrzyskie	366	
Eastern Poland (PoE)	Podlaskie, Lubelskie, Podkarpackie	238	
Germany (Ge)	German Caucasians	56	SGM plus
Germany, Berlin (GeB)	German Caucasians, predominantly from Berlin	234	Identifiler
Austria (Au)	Austrian Caucasians	204	SGM plus
Czech Republic (Cz)	(Vanek et al. 2001)	201	SGM plus
Russia (Ru)	(Kornienko et al. 2002) a mixed population including: Northern-Western (22), Central (82), Southern (59), Volga (101), Ural (82), Siberian (45), Far East (11)	402	Profiler Plus
Additional populations included in the expanded data set			
Russia, Krasnodar (RuK)	Russian Caucasian population from region of Krasnodar	58	Identifiler
Russia, Rostov-on-Don (RuR)	Russian Caucasian population from region of Rostov-on-Don	126	Identifiler
Russia, Saratov (RuS)	A population from the southern part of European Russia [16]	51	Identifiler
Russia, Orel (RuO)		72	Identifiler
Russia, Mineralnye Vody (RuMV)		60	Identifiler
Belarus (Be)	A population representative of the country [17]	176	Identifiler
Slovakia (Sl)	Slovak Caucasians from all over the country excluding Roms	119	Identifiler
Ukraine (Ukr)	A population representative of the country		
Germany, Cologne (GeC)	German Caucasians from the region of Cologne	2000	Identifiler
The Netherlands (Ne)	Dutch Caucasians	267	Identifiler
Sweden (Sw)	Swedish Caucasians [18]	295	Identifiler

* For the Polish populations the administrative districts (Voievodships) constituting each region of sampling are given, for the mixed Russian population names of the Federal Districts (numbers of individuals in parentheses) of Russia are listed.

Poland. The criterion used was the administrative district (Voivodeship) of current residence. Genotyping was carried out at Biology Department of Central Forensic Laboratory of Polish Police and Department of Forensic Medicine of Medical University of Warsaw. The numbers of individuals and constituting each subpopulation are given in Table 1. The numbers of individuals selected from each subpopulation approximately correspond to the regional population density.

The first stage of the analysis of neighboring populations was based on samples from The Russian Federation and The Czech Republic which were subject of previous studies [5,6] as well as two subpopulations from Germany and one sample from Austrian population. Conclusions from this part of study were verified in the second stage of analysis which was based on independently collected sample from German population, five samples from Russian population as well as samples from Ukrainian, Belarusian, Slovak, Swedish and Dutch populations. The geographic location of all studied populations is shown in Fig. 1 whereas their description is given in Table 1.

The samples were typed using commercially available kits standardized for forensic purposes (Table 1). Ten STR loci (D8S1179, D21S11, D18S51, D3S1358, vWA, D16S539, D2S1338, D19S433, TH01, FGA) were analyzed except for the first Russian sample for which only six loci (D3S1358, vWA, D8S1179, D21S11, D18S51 and FGA) were available.

Analysis of molecular variance (AMOVA), calculation of Rst values [7] and tests for Hardy–Weinberg equilibrium were performed with *Arlequin* [8]. The probability (P) values were estimated from 10,000 permutations. The analysis of statistical

significance was not based on Bonferroni correction but on replication of initial findings using population samples collected in the second stage of the study. Multidimensional scaling analysis (MDS) was performed with *Statistica* (Statsoft) software package. To assess the impact of imperfect repeats commonly found in the TH01 locus (allele 9.3) all analyses were repeated after exclusion of this locus but the results were not changed.

3. Results

3.1. Homogeneity of Polish population

In order to evaluate whether the main regions of the present day Poland are homogenous with respect to the studied STRs the analysis of molecular variance (AMOVA) on grouped Polish populations was performed. We were unable to detect statistically significant differences between the subregions of Poland ($P = 0.5$) which indicates genetic homogeneity among the analyzed Polish populations. Analysis of individual STRs by locus-by-locus AMOVA revealed that none of the loci was associated with statistically significant variation caused by differences among populations ($P > 0.1$ for all loci, data not shown). Further, no statistically significant Rst values were found among Polish populations in any of the pairwise analyses (Table 2).

The frequencies of the alleles of the studied loci derived from the group of pooled Polish individuals ($N = 2176$) are shown in Appendix A. All loci were in Hardy–Weinberg equilibrium (Appendix A).

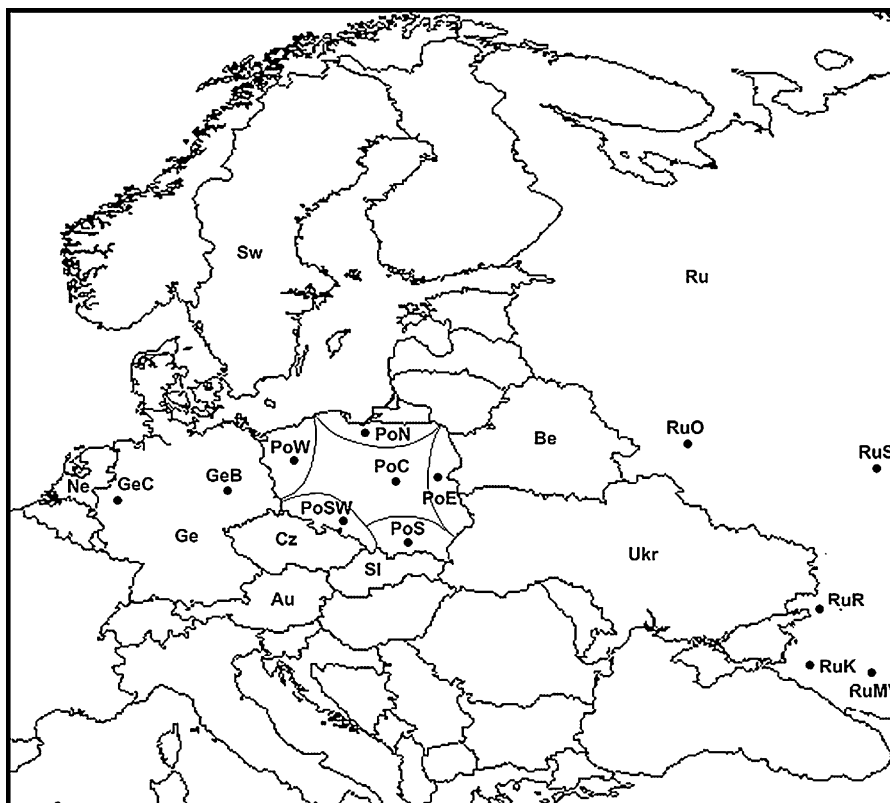


Fig. 1. Map showing geographic locations of studied populations.

Table 2
Pairwise Rst values (below diagonal) and their associated P values (above the diagonal)

	PI C	PI N	PI W	PI SW	PI S	PI E	<u>Ru*</u>	<u>RuR</u>	<u>RuK</u>	<u>RuS</u>	<u>RuO</u>	<u>RuM</u>	Be	Ukr	Cz	Sl	Ge	GeB	GeC	Au	Ni	Sw
PI C		0.07593	0.86863	0.38313	0.28948	0.35729	0.04505	0.44491	0.1582	0.00366	0.1188	0.15721	0.1476	0.0321	0.49658	0.0878	0.2371	0.00683	0.0001	0.0004	0.0003	0.0015
PI N	0.00092		0.1093	0.73478	0.58004	0.08415	<u>0.30997</u>	0.82853	0.50817	0.14702	0.63608	0.62033	0.2433	0.6886	0.40788	0.7552	0.1581	0.01515	0.0125	0.0629	0.0004	0.1205
PI W	-0.00073	0.00115		0.68954	0.72537	0.70399	<u>0.07197</u>	0.5745	0.23612	0.00802	0.11662	0.3271	0.7541	0.0863	0.68043	0.1096	0.1706	0.02554	0.001	0.0008	0.00059	0.0401
PI SW	-0.00007	-0.0007	-0.0006		0.8809	0.43105	<u>0.08494</u>	0.69696	0.40739	0.05227	0.38808	0.53797	0.6433	0.4724	0.48758	0.3318	0.2075	0.02297	0.0036	0.0088	0.0002	0.0621
PI S	0.00009	-0.0003	-0.0006	-0.00085		0.66835	<u>0.08564</u>	0.56628	0.27215	0.02614	0.30037	0.68399	0.3562	0.3021	0.81358	0.2011	0.0851	0.00436	0.0002	0.0014	0.0001	0.0306
PI E	0.00005	0.00163	-0.0007	-0.00005	-0.0005		0.02732	0.15919	0.12098	0.01287	0.13464	0.28769	0.1159	0.1398	0.24988	0.0371	0.0751	0.0005	0.0002	0.0003	0.0002	0.0777
<u>Ru*</u>	0.00156	0.00006	0.00181	0.00164	0.00122	0.0039		0.71726	0.38828	0.44055	0.31997	0.16196	0.0535	0.5051	0.4254	0.3391	0.0504	0.34502	0.0035	0.053	0.05059	0.0699
<u>RuR</u>	-0.00012	-0.0014	-0.0006	-0.00096	-0.0005	0.00164	-0.0016		0.94565	0.14385	0.62766	0.58034	0.7562	0.5466	0.81824	0.9855	0.5159	0.51698	0.4219	0.5737	0.10553	0.2667
<u>RuK</u>	0.00224	-0.0006	0.0016	-0.00003	0.00094	0.00371	-0.0008	-0.0041		0.44491	0.74428	0.5738	0.4389	0.5695	0.40402	0.981	0.4559	0.37244	0.477	0.9163	0.17444	0.4012
<u>RuS</u>	0.01226	0.00317	0.01184	0.00595	0.00776	0.01137	-0.0015	0.00402	-0.00036		0.18384	0.20156	0.023	0.7197	0.02148	0.3361	0.0185	0.00762	0.0619	0.2264	0.00099	0.2773
<u>RuO</u>	0.0024	-0.0012	0.00282	0.00005	0.00055	0.00289	0.00009	-0.0014	-0.00326	0.00366		0.84378	0.1299	0.7084	0.26631	0.7281	0.3252	0.11652	0.3613	0.3147	0.05039	0.5679
<u>RuM</u>	0.00214	-0.0013	0.00065	-0.00087	-0.0015	0.00125	0.00347	-0.0012	-0.00182	0.00378	-0.0039		0.4373	0.7124	0.4551	0.5824	0.0716	0.06673	0.0829	0.0622	0.00931	0.5176
Be	0.0009	0.00061	-0.0009	-0.00063	0.00009	0.00168	0.00358	-0.0013	-0.00024	0.00906	0.00269	-0.0002		0.2643	0.31027	0.2513	0.1225	0.08623	0.0068	0.0096	0.00287	0.2495
Ukr	0.0038	-0.001	0.00291	-0.00021	0.00054	0.00238	-0.0011	-0.0004	-0.00081	-0.002	-0.0018	-0.0019	0.0012		0.21493	0.425	0.2135	0.02455	0.0719	0.1308	0.00158	0.5544
Cz	-0.0002	0.00002	-0.0007	-0.00023	-0.0009	0.00065	-0.0004	-0.0015	0.00014	0.00916	0.00117	-0.0002	0.0004	0.0016		0.4317	0.3968	0.21414	0.0518	0.0359	0.01277	0.1161
Sl	0.00188	-0.0012	0.00198	0.00026	0.00093	0.00413	-0.0001	-0.0031	-0.00485	0.00073	-0.0022	-0.0014	0.0009	0.0002	-0.0001		0.37	0.31284	0.4127	0.8799	0.04584	0.2335
Ge	0.00125	0.00246	0.0025	0.00176	0.00371	0.00493	0.00254	-0.0008	-0.00079	0.01282	0.0008	0.00719	0.0034	0.0031	0.00015	0.0002		0.90476	0.8817	0.4546	0.96386	0.327
GeB	0.00259	0.00311	0.00242	0.00254	0.00374	0.00631	-0.0003	-0.0005	0.00016	0.01145	0.00279	0.00467	0.0018	0.005	0.00073	0.0004	-0.003		0.2753	0.0747	0.54232	0.0694
GeC	0.00414	0.0019	0.00371	0.00248	0.00348	0.00539	0.00286	0.00005	-0.00017	0.00641	0.00047	0.00439	0.0034	0.0028	0.00163	0.00002	-0.003	0.00033		0.2844	0.03643	0.0003
Au	0.00546	0.0019	0.00605	0.00395	0.005	0.00809	0.00275	-0.0007	-0.00334	0.00175	0.00055	0.00479	0.0047	0.0023	0.00304	-0.002	-0.0005	0.00174	0.0004		0.01782	0.1159
Ni	0.00478	0.00706	0.0055	0.00659	0.00778	0.00929	0.00235	0.00228	0.00265	0.0188	0.00498	0.0105	0.0061	0.0101	0.00406	0.0036	-0.004	-0.0003	0.0015	0.0034		0.0542
Sw	0.01111	0.00523	0.00759	0.00713	0.00698	0.00685	0.0017	0.00371	0.00178	0.00239	0.0007	0.00094	0.0047	0.0017	0.00571	0.0041	0.0023	0.00719	0.0127	0.0054	0.00778	

Part of the table containing Polish populations is marked with bold line. Significant P values ($P < 0.05$) and their associated Rst values are boldfaced. Additional populations included in the expanded data set are underlined. Abbreviations are explained in Table 1.

*Analysis based on D3, vWA, D8, D21, D18, FGA.

3.2. Comparison between Polish and neighboring populations (the first data set)

Analysis of pairwise Rst values among Polish and neighboring populations collected at the first stage of analysis indicated differences between Polish and both German and Austrian samples. As can be seen from Table 2 Rst values associated with $P < 0.05$ were found in all but one comparison between the Polish populations and populations of Austria and Germany (Berlin).

To further study the differences between the investigated populations we analyzed the pairwise Rst values multidimensional scaling (MDS). A two-dimensional plot from the MDS analysis is shown in Fig. 2. As can be seen Polish samples were relatively well separated from both and German and Austrian samples. Interestingly, we noted that the distribution of populations on the MDS plot did not fully correlate with geography. In particular, Russian population which is geographically located eastwards from Poland, Germany and Austria (Fig. 2) was placed between Germanic and Polish populations on the plot (Fig. 1). This unexpected configuration was also supported by lack of statistically significant differences between German and Russian populations in pairwise analyses (Table 3) even when the German populations were pooled for the comparison purpose ($Rst = 0.00228$, $P > 0.05$).

To further study genetic distances among the populations of Germany, Poland and Russia we reanalyzed an independently collected data on Y-STR haplotypes in Polish and neighboring populations [2]. Because no significant within-country differences were found among individual Polish or German populations [2] the respective populations were pooled yield-

ing groups of 919 Polish and 439 German Y-STR profiles which were then compared with each other, as well as the sample from Russia. We found that the Rst value reflecting distance between Russia and Germany was approximately three times smaller than that the distance between Poland and Germany, and similar to the Polish-Russian distance (Table 3). The Rst values obtained in all three comparisons were statistically significant (Table 3).

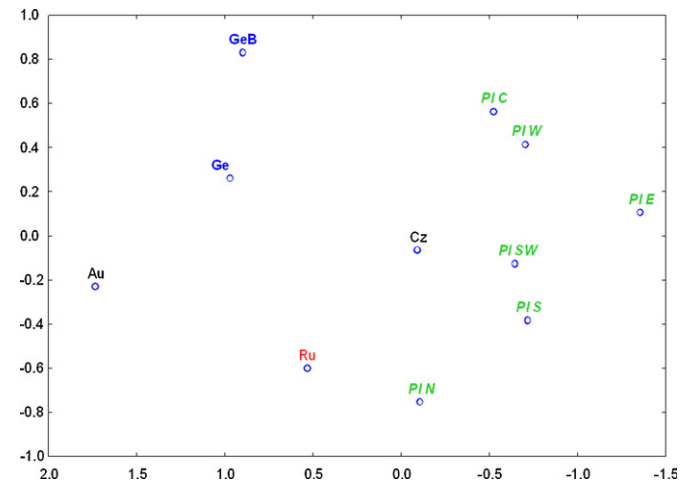


Fig. 2. Two-dimensional plot from multidimensional scaling (MDS) analysis of pairwise Rst values calculated for set of populations investigated at the first stage of study. The designations of the Polish populations are in italics and boldface (green) and those of the German populations are boldface (blue). Stress = 0.08. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Table 3

Pairwise Rst values calculated among Polish, German and Russian populations based on Y-STR polymorphism (reanalysis of data from [2])

	Poland	Russia
Russia	0.03409 0.0003	–
Germany	0.06442 <0.00001	0.02385 0.00337

Respective *P* values are shown at the bottom of each cell.

Polish population includes 919 individuals from 6 pooled samples (Bydgoszcz, Krakow, Gdansk, Wroclaw, Warsaw and Lublin). Russian population includes 85 individuals from Moscow area. German population includes 439 individuals from pooled samples from Berlin and Leipzig.

3.3. Comparison between Polish and neighboring populations (the extended data set)

Given the limited data available from Russian population (a single sample typed for six loci) in order to further study the disproportion between Polish–German vs. Russian–German genetic distances we collected more population samples from the region (Table 1). In particular we analyzed five independent samples from western part of Russia typed for 10 loci, a large sample from German population (Cologne, $N = 2000$) and samples from Belarus, Ukraine, Slovakia, The Netherlands and Sweden (Table 1). The results of pairwise comparisons among all the available populations as well as the appropriate MDS plot are shown in Table 2 and Fig. 3, respectively. As can be seen using the novel dataset from Germany (Cologne) we were able to reproduce the statistically significant ($P < 0.05$) differences with all Polish populations samples. In contrast, among 15 comparisons between newly collected Russian samples and the German samples there were only two marginally significant differences ($P \sim 0.01$, Table 3). The disproportion between Polish–German vs. Russian–German genetic distances was also confirmed by the MDS plot based on extended set of populations (Fig. 3). As can be seen, three out of five additionally collected Russian samples clustered close to originally analyzed sample localizing between the Polish and German populations (Fig. 3). From the two remaining Russian samples one (Mineralnye Vody) was intermingled among Polish populations and the other (Saratov) localized relatively far from other samples being closest to Sweden (Fig. 3).

4. Discussion

In the present paper the distribution of a set of 10 autosomal STRs which is routinely used in forensic casework was analyzed among six Polish populations (representatively covering the country's territory) as well as in the extensive set of neighboring populations. We found that, with respect to the studied markers, present day Poland is homogenous whereas significant differences exist between Polish and German populations which are in contrast to relatively short genetic distance between the Russian and German populations.

The lack of correlation between geographic locations and genetic distances among Germany, Poland and Russia is

interesting. The fact that conclusions from the initial as well as the expanded analysis of autosomal STRs were consistent with each other as well as with the results based on distribution of Y-STR haplotypes makes it unlikely that a sampling bias or typing error could be the explanation. Further, we noted that similarities between Russian and German populations were also found in studies of mtDNA polymorphism: Orekhov et al. observed that the Russian and German populations were located next to each other in an UPGMA tree based on sequences of hypervariable region I [9] whereas Maliarchuk and Derenko concluded that the T4336C variant of mtDNA might be a common component of the Russian and German gene pool [10].

However, taking into account that Russian populations analyzed here have been represented mostly by Russians from southern and central part of European Russia, the obtained results should be treated with caution. One should bear in mind that Russian maternal and paternal gene pool is considerably heterogeneous, being represented by two major clusters: Russian populations from the southern and western parts of European Russia are separated from those living on East and North [11,12]. Therefore, it is unclear whether autosomal STR variation within Russian populations from northern European Russia should demonstrate a pattern similar to those analyzed here coming from southern and central European Russia.

Notwithstanding the discussed limitations, it is interesting to speculate on the historical explanation of these findings. One reason for genetic similarities between Russia and Germany may be associated with the migrations of the Goths–Germanic people inhabiting territories of southern Scandinavia in the late Bronze Age [13]. In 2nd century BC the Goths started a migration in the South-Eastern direction to eventually reach the Black Sea in 2nd century AD. Although the Goths were defeated by the Huns in 375 there is an evidence of their physical presence in the region until 15–16th century AD. The influence

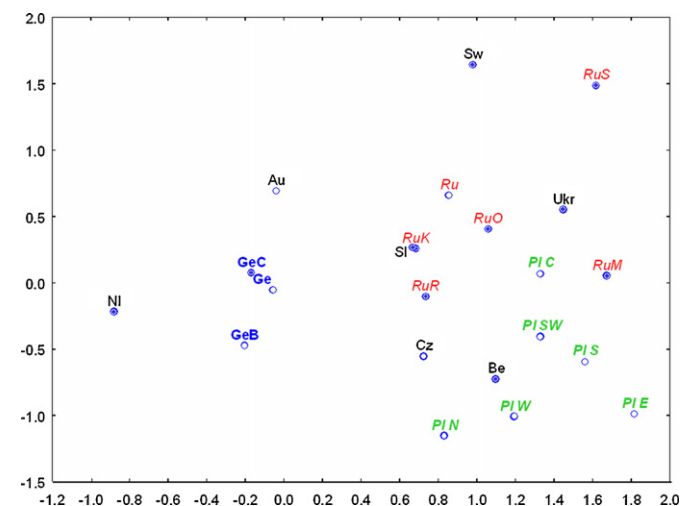


Fig. 3. Two-dimensional plot from multidimensional scaling (MDS) analysis of pairwise Rst values calculated for all of populations investigated. The designations of the Polish populations are in italics and boldface (green), the German populations are boldface (blue), the Russian populations are in italics (red). Populations added in second stage of analysis are marked with filled circles. Stress = 0.16. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Appendix A (Continued)

	D3	vWA	D16	D2	D8	D21	D18	D19	TH01	FGA
21	0.02	0.14	–	3.81	–	–	0.69	–	–	18.45
21.2	–	–	–	–	–	–	–	–	–	0.28
22	–	–	–	2.41	–	–	0.62	–	–	19.23
22.2	–	–	–	–	–	–	–	–	–	1.42
23	–	–	–	9.67	–	–	0.25	–	–	11.70
23.2	–	–	–	–	–	–	–	–	–	0.94
24	–	–	–	10.62	–	–	0.07	–	–	11.81
24.2	–	–	–	–	–	0.02	–	–	–	0.25
25	–	–	–	11.90	–	0.02	0.02	–	–	6.82
25.2	–	–	–	–	–	–	–	–	–	0.05
26	–	–	–	2.21	–	0.16	0.02	–	–	2.62
27	–	–	–	0.28	–	2.92	–	–	–	0.34
28	–	–	–	0.02	–	18.61	–	–	–	0.05
28.2	–	–	–	–	–	0.02	–	–	–	–
29	–	–	–	–	–	20.34	–	–	–	–
29.2	–	–	–	–	–	0.05	–	–	–	–
30	–	–	–	–	–	23.00	–	–	–	–
30.2	–	–	–	–	–	5.15	–	–	–	–
31	–	–	–	–	–	6.57	–	–	–	–
31.2	–	–	–	–	–	8.62	–	–	–	–
32	–	–	–	–	–	1.26	–	–	–	–
32.2	–	–	–	–	–	9.31	–	–	–	–
33	–	–	–	–	–	0.18	–	–	–	–
33.1	–	–	–	–	–	0.02	–	–	–	–
33.2	–	–	–	–	–	3.49	–	–	–	–
34.2	–	–	–	–	–	0.25	–	–	–	–
Obs. Het.	0.796	0.802	0.732	0.882	0.793	0.836	0.877	0.791	0.767	0.871
Exp. Het.	0.795	0.805	0.749	0.879	0.791	0.846	0.875	0.787	0.774	0.865
P	0.133	0.244	0.470	0.882	0.851	0.412	0.621	0.712	0.175	0.374
PD	0.926	0.955	0.896	0.973	0.928	0.958	0.971	0.928	0.913	0.967
PE	0.591	0.683	0.523	0.756	0.600	0.694	0.746	0.599	0.559	0.727

Obs. Het., observed heterozygosity; Exp. Het., expected heterozygosity (under assumption of Hardy–Weinberg equilibrium); P, probability value for the rejection of the null hypothesis assuming Hardy–Weinberg equilibrium; PD, power of discrimination; PE, power of exclusion. PD and PE for all STRs was 0.999999999999837 and 0.999978, respectively.

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