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Similarities and Distinctions in Y Chromosome Gene Pool of Western Slavs

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ABSTRACT Analysis of Y chromosome Y-STRs has proven to be a useful tool in the field of population genetics, especially in the case of closely related populations. We collected DNA samples from 169 males of Czech origin, 80 males of Slovakian origin, and 142 males dwelling Northern Poland. We performed Y-STR analysis of 12 loci in the samples collected (PowerPlex Y system from Promega) and compared the Y chromosome haplotype frequencies between the populations investigated. Also, we used Y-STR data available from the literature for comparison purposes. We observed significant differences between Y chromosome pools of Czechs and Slovaks compared to other Slavic and European populations. At the same time we were able to point to a specific group of Y-STR haplotypes belonging to an R1a haplogroup that seems to be shared by Slavic populations dwelling in Central Europe. The observed Y chromosome diversity may be explained by taking into consideration archeological and historical data regarding early Slav migrations. Am J Phys Anthropol 142:540–548, 2010. ©2010 Wiley-Liss, Inc.

Haplotypes of Y chromosome based on microsatellite (Y-STR) typing have been successfully used for population studies of many human societies, sometimes very closely related ones (Roewer et al., 1996; Derenko et al., 2006; Woźniak et al., 2006). Y-STR data are being rapidly accumulated, especially in the case of European populations, allowing us to make inferences describing the complicated history of the continent in terms of genetic variation (Roewer et al., 2005). Slavs, numbering ~250 million, constitute a significant part of European metapopulation. While many subpopulations of Poland and Russia have already been studied for their Y chromosome genetic diversity, the Czech and Slovakian populations have scarcely been mentioned in scientific papers dealing with Y-STR polymorphism. Given the localization of Czechs and Slovaks on the fringes of Slavdom, a description of their genetic polymorphism, including Y-STRs, may provide interesting insights into the mechanisms which shape the population differentiation of extant Europeans.

It is widely believed that before Slavs entered contemporary Poland, Bohemia, and Slovakia, these lands were inhabited mainly by tribes of Germanic origin represented by the Przeworsk and Wielbark cultures (Barford, 2001; Buko, 2006; Kokowski, 2006). At the end of the fifth century, the archeological record from Poland, Slovakia, and to somewhat lesser extent Bohemia shows a significant decrease in the number of artifacts of these cultures. Various explanations have been given for this phenomenon, the most frequent being migrations in response to the conquest of Roman Empire and marauding hordes of Hun invading Eastern and Central Europe at that time (Barford, 2001). Archeological finds of early Slavic culture (the identity of which was most probably shaped somewhere in the contemporary Ukraine) have been made in the lands of southern Central Europe (along Danube valley). These finds date to the first half of the sixth century, and similar finds dating to the end of the sixth century have been made across the whole territory between the Baltic Sea and the Danube and Elba River (Barford, 2001). Slavic nations have remained in Central Europe since the tenth century, though not always as independent states (Magosci, 2002).

The aim of this study was to assess genetic diversity of Y-STR haplotypes of the populations of the Czech Republic, Slovakia, and Poland and to compare them with other Slavic and Central European populations.

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Using both information on Y-STR variability and the available archeological and historical data regarding Central Europe and Slavic expansion, we aimed to construct a model of the development of Slavic populations which could explain the observed Y-STR differentiation.

MATERIALS AND METHODS

Sample collection

We collected whole blood samples from 169 Czechs and 80 Slovaks and buccal swabs from 142 Poles dwelling in the north of Poland. In accordance with local regulations, appropriate informed consent was obtained from each donor. Of the Czech samples 69 were collected in the Pilsen area and the remaining 100 were collected randomly in different parts of the Czech Republic. From the Slovakian samples, 40 were collected in western Slovakia, the rest in the eastern part of the country. The samples from northern Poland were collected in small towns located near the central part of the Polish coast between Gdansk and Koszalin (Brusy, Hel, Kartuzy, Lębork, Strzepcz, Wejherowo). This region of Poland is called Kaszuby (Kashubia), and a significant part of its subpopulation consists of descendants of the Pomeranian branch of Slavs called Kashubians. In our sample, 64 donors (45%) identified themselves as Kashubians and were considered a subpopulation sample separate from the remaining donors dwelling in the same region of Poland (78 individuals). Additionally, we used 132 anonymous DNA samples obtained from males of Polish origin living in Bydgoszcz and its surrounding areas (referred to as central Poland in the following text), which had been collected by the Institute of Molecular and Forensic Genetics in Bydgoszcz and published earlier (Woźniak et al., 2007).

DNA extraction

DNA from blood samples was extracted using the QIAamp DNA Blood Mini kit (QIAGEN, Hilden, Germany). DNA from buccal swabs was extracted using the standard organic method (Sambrook et al., 1989).

Amplification and electrophoresis

Twelve Y-STR loci were amplified using the PowerPlex Y system (Promega) and ABI9600 thermocycler, according to the manufacturers' instructions. The amplified samples were electrophoresed using a capillary sequencer ABI3100 equipped with 50-cm capillaries and POP6 polymer, using ILS600 size standard (Promega) and the allelic ladders included in the PowerPlex Y kits. The conditions of electrophoresis were concordant with the PowerPlex Y kit manufacturer's instructions with the exception of electrophoresis time, which was set to 1,800 s to compensate for the slower product migration rate caused by the use of 50-cm capillaries and POP6 instead of the standard 36-cm capillaries and POP4 polymer advised by the manufacturer. The allelic designations were assigned to individual samples using GeneMapper ID ver. 3.2 software. The complete haplotype tables were imported into an Excel file and checked for errors. These data are included in Supporting Information Table 1.

Haplogroup R1 markers M173 (for the whole R1), SRY1532.2 (for R1a), and M17 (for R1a1) were assayed by means of RFLP analysis as described elsewhere (Kharkov et al., 2004, 2005). The Y-SNP haplogroup nomenclature used here complies with the recommendations of the Y Chromosome Consortium (Karafet et al., 2008).

Data used for comparison purposes

To compare the populations under investigation to other European populations, data describing the Y chromosome diversity in the populations of Central Europe were collected from available sources. This data comprised haplotypes made of 12 or 7 Y-STR loci. Twelve loci haplotypes consisted of the following markers: DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385a, DYS385b, DYS437, DYS438, and DYS439. Seven loci haplotypes consisted of the following markers: DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, and DYS393. The order of loci given above has been used in the following text to report haplotypes in the form of a text string where alleles of consecutive Y-STR loci are separated by hyphens. The population samples typed for 12 loci were as follows: Poles from southern Poland (N = 228; designated SPO, Woźniak et al., 2007); Russians from the European part of Russia—one sample (N = 545) designated as RUR (Roewer et al., 2008) and the other (N = 405) designated as RUS. The latter Russian population sample has been partially published by us (Derenko et al., 2006, 2007), but the complete set of Y-STR profiles is included here, in Supporting Information Table 1. All population samples haplotyped for the seven loci were obtained from the study of Roewer et al. (2005). Those population samples comprised the following: Poles from the area of Gdansk (N = 543; GDA), Krakow (N = 107; KRA), Lublin (N = 134; LUB), Warsaw (N = 240; WAR), and Wroclaw (N = 121; WRO); Germans from the area of the following cities: Berlin (N = 549; BER), Cologne (N = 135; COL), Duesseldorf (N = 150; DUE), Freiburg (N = 433; FRE), Greifswald (N = 208; GRE), Hamburg (N = 114; HAM), Leipzig (N = 573; LEI), Magdeburg (N = 283; MAG), Mainz (N = 104; MAI), Munich (N = 250; MUN), Muenster (N = 196; MUE), Rostock (N = 203; ROS), Stuttgart (N = 155; STU), and Tyrol (N = 229; TYR); the populations of Slavic countries such as Belarus (N = 69; BEL), Russia (Moscow; N = 85; MOS), and Ukraine (N = 82; KIE); the populations of the Baltic countries such as Latvia (Riga; N = 145; RIG), Estonia (Tartu; N = 133; TAR), and Latvia (Vilnius; N = 151; VIL); and the populations of other Central European countries such as Romania (N = 145; ROM) and Hungary (Budapest; N = 118; BUD). To maintain the readability of MDS plots the three-letter codes (as given above in parentheses) have been used for population samples. For the populations of the Czech Republic, Slovakia, central Poland, northern Poland, and Kashubians, the following codes were used, respectively: CZE, SLO, BYD, NPO, and KAS.

Statistical and phylogenetic analysis

Statistical analyses such as haplotype diversity assessment, RST distance calculation, and AMOVA were performed using Arlequin ver. 3.11 software (Schneider et al., 2000). The statistical significance tests for RST were performed at 1,000 permutations and for AMOVA at 20,000 permutations. Values were regarded as significant when "P" was lower than 0.05. Arlequin input files were generated in Microsoft Excel using Microsat Toolkit Excel plugin (Park, 2001). The RST distance tables obtained using Arlequin were imported into an Excel file and converted manually into Statistica matrix files by adding four additional rows required by Statistica matrix format. All negative RST values were converted to zero. Resultant files were then imported to Statistica and an MDS plot was generated directly from the matrix. The maximum number of significant dimensions for MDS was assessed based on "scree test" and one to six dimensions. The number of three dimensions was assumed sufficient for the presentation of the analyzed data set. Multidimensional scaling (MDS) was performed using Statistica 8.0 software (StatSoft).

Median-joining (MJ) networks of haplogroup R1a STR-haplotypes were constructed using the Network 4.5.0.0 program (http//www.fluxus-engineering.com). For the network construction, STR loci were weighted according to the average of their variability in the haplogroup (Bandelt et al., 1999). The time to the most recent common ancestor (TMRCA) was estimated within the network from the ρ-statistic, using the mutation rate of 2.5×10^{-3} per 35 years calculated in father–son pairs (Goedbloed et al., 2009) and using the evolutionary effective mutation rate of 6.9×10^{-4} per 25 years based on microsatellite variation within Y chromosome haplogroups in the populations with documented short-term histories (Zhivotovsky et al., 2004). The age of microsatellite variation within the R1a haplogroup was also estimated as the average squared difference in the number of repeats between all current chromosomes and the founder haplotype (formed by the median values of the repeat scores at each microsatellite locus within the haplogroup), averaged over microsatellite loci and divided by means of a mutation rate (Zhivotovsky et al., 2004; Sengupta et al., 2006).

RESULTS

General polymorphism of Y-STR haplotypes in the populations of the Czech Republic, Slovakia, and northern Poland (with Kashubians treated separately) was measured by calculating the number of distinct haplotypes and haplotype diversity for each population (Table 1). The percentage of distinct haplotypes was slightly higher in the Slovakian population sample, but this observation can be explained by the relatively small number of chromosomes investigated. The observed haplotype diversity, on the other hand, was very similar in all four populations and in accordance with other published populations of Europe (Schmidt et al., 2003; Veselinovic et al., 2008).

TABLE 1. Number of distinct haplotypes and haplotype diversity of populations under investigation

	Number of chromosomes	Number of distinct haplotypes	Haplotype diversity	SD
Northern Poland	78	61 (78.2%)	0.9927	±0.0034
Kashubians	64	54 (84.4%)	0.9841	±0.0098
Czechs	169	140 (82.8%)	0.9963	±0.0015
Slovaks	80	75 (93.8%)	0.9978	±0.0026

SD, standard deviation.

Genetic distance calculations between the populations under investigation and other populations of Europe were performed in two tiers. The first set of calculations embraced eight Slavic populations haplotyped in 12 Y-STR loci. These populations included Kashubians, two Russian samples, samples from Czech Republic, Slovakia, and northern, southern, and central Poland. The genetic distances between these populations, expressed in terms of RST values, are given in Table 2. Figure 1 presents the plot of the first two dimensions of the MDS data obtained from the eight population samples haplotyped in 12 loci. Clearly visible in the first dimension is the separation of Czechs and Slovaks from Poles and Russians while all the population samples of Polish origin are clustered together and separated from the Russian samples in the second dimension.

The AMOVA analysis of the seven Slavic populations indicated that the lowest and insignificant value of variance among populations within groups was achieved when Czechs and Slovaks were analyzed as one group. This group is separated from the two other groups, one of which contains populations of Polish origin and the other contains populations of Russian origin (Table 3). All other combinations of grouping for the eight Slavic populations gave insignificant values of variation among groups and significant values of variation among populations within groups.

The other tier of samples taken into consideration for the purpose of this publication comprised 35 population samples originating from Central and Eastern Europe, including eight Slavic populations studied in the first tier, as well as the population samples from countries neighboring the Czech Republic, Slovakia, and Poland (i.e., Germany, Ukraine, Belarus, Latvia, Lithuania, Estonia, Hungary, Austria, and Romania). For those population samples, the data for seven Y-STR loci were available. Genetic distances were obtained in the form of RST values and subjected to MDS analysis at the three dimension levels, as described above. Figure 2 presents the MDS plot obtained. The central position of the Czech and Slovakian samples among Germanic and Slavic

TABLE 2. RST calculations for 12 Y-STR loci available for eight Slavic populations

	NPL	SPL	BYD	KAS	SLO	CZE	RUS	RUR
NPL		−	−	−	+	+	−	−
SPL	0.00299		−	−	+	+	+	+
BYD	−0.00261	−0.00247		−	+	+	+	+
KAS	0.0068	0.00809	0.0051		+	+	+	+
SLO	**0.03119**	**0.03047**	**0.03883**	**0.04952**		+	+	+
CZE	**0.03284**	**0.01491**	**0.02696**	**0.03764**	**0.01101**		+	+
RUS	0.00366	**0.01466**	**0.01067**	**0.02969**	**0.01943**	**0.02805**		−
RUR	0.00422	**0.0112**	**0.00845**	**0.02263**	**0.02735**	**0.02708**	0.00114	

Above the diagonal, significant and nonsignificant differences are indicated as "+" and "−" signs, respectively.
RST distances given in bold are significant.
Population designations as described in Material and Methods.

populations is presented clearly on this plot. It is worth noting that Czechs and Slovaks were split in the second dimension in this analysis. The same plot and related RST values (see Supporting Information Table 3) suggested a closer relation between the Y chromosomes of Russians and Czechs/Slovaks than between Poles and Czechs/Slovaks. This phenomenon is best illustrated by the relatively high value of RST between Czechs/Slovaks and the neighboring population of southern Poland.

AMOVA analysis of the abovementioned 35 populations of Central and Eastern Europe was performed. Groups were divided according to linguistic as well as geographic criteria (Table 4). The lowest variance amongst the populations within the groups was achieved when Czechs and Slovaks were put together in a separate group and the rest of the populations were divided according to linguistic criteria. Geographical grouping gave diversity values similar to those of the linguistic grouping, with Czechs and Slovaks included in the Eastern Slavic group. Grouping Czechs and Slovaks with Western Slavs gave the highest diversity levels among the populations within groups and the lowest diversity among groups (Table 4).

Despite the clear differences between Y-STR profiles in Western Slavic populations, one group of haplotypes connects all Western Slavs. Haplogroup R1a is very frequent (50% on average) in various Western as well as Eastern Slavic populations, but within this haplogroup there is a group of lineages characteristic mainly in Western Slavs. Haplotype 17-13-30-25-10-11-13-10,14 is one of the most frequent haplotypes among Czechs, Slovaks and Poles. Analysis of the YHRD showed that the distribution of this haplotype is limited, as it is more often found in Poland (4.8%),

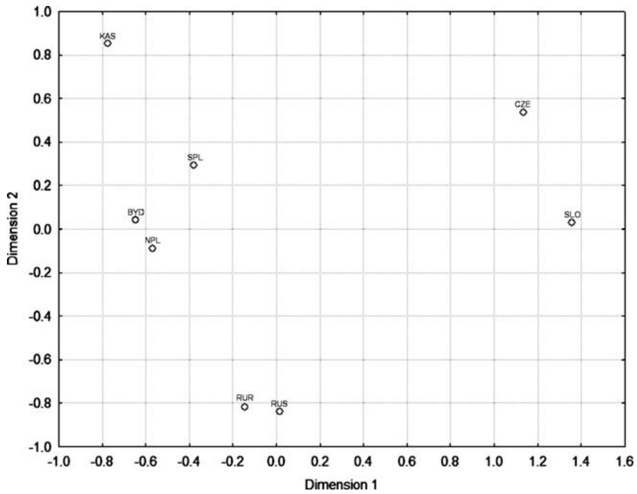

Fig. 1. MDS plot of RST distances for 12 YSTR haplotypes shows that in the first dimension Czechs and Slovaks separate from both Russians and Poles while in the second dimension Slovaks seem to be separated from Czechs and Poles are separated from Russians. Stress value for three-dimensional MDS = 0.0000049.

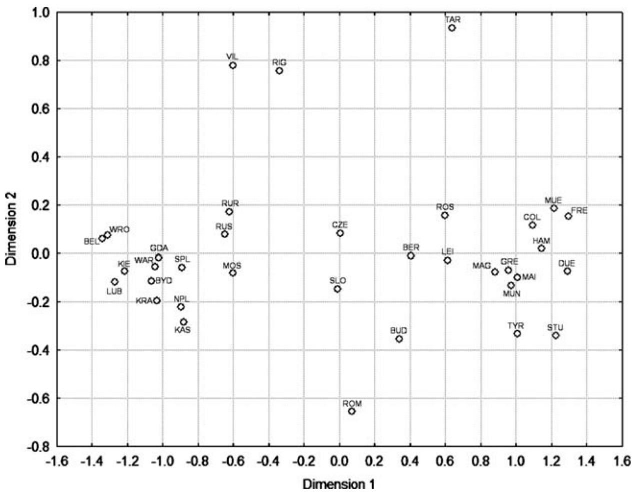

Fig. 2. MDS plot of RST values calculated for Central and Western European population samples. Sample names are presented as three-letter codes explained in the "Materials and Methods" section. Distinct position of Czech and Slovakian population is indicated. Stress value for three-dimensional MDS = 0.0256856.

TABLE 3. AMOVA calculation results for 11 Y-STRs in eight Slavic population samples

Groups analyzed	Among groups	Among populations within groups	Within populations
1. Poles 2. Russians 3. Czechs and Slovaks	1.57 (P = 0.00584)	0.26 (P = 0.07099)	98.17 (P = 0.00000)
1. Northern Poland, Kashubians, and Bydgoszcz area 2. Czechs, Slovaks, and Southern Poland 3. Russians	0.95 (P = 0.02752)	0.72 (P = 0.00119)	98.33 (P = 0.00000)
1. Poles and Czechs 2. Russians and Slovaks	0.69 (P = 0.08950)	1.01 (P = 0.00000)	98.30 (P = 0.00000)
1. Poles, Czechs, and Slovaks 2. Russians	0.57 (P = 0.14802)	1.07 (P = 0.00000)	98.36 (P = 0.00000)
1. Poles 2. Russians, Czechs, and Slovaks	0.65 (P = 0.08238)	1.08 (P = 0.00000)	98.27 (P = 0.00000)
1. Poles and Slovaks 2. Russians and Czechs	0.15 (P = 0.28848)	1.34 (P = 0.00000)	98.51 (P = 0.00000)

Each row of the table presents values of variation and "P" values obtained for the grouping scheme presented in the first column of the table.

TABLE 4. AMOVA results for Central European and Eastern Europe

	Among groups	Among populations within groups	Within populations
Linguistic grouping; Czechs and Slovaks as a separate group	8.02*	0.56*	91.42*
Linguistic grouping; Czechs and Slovaks in Eastern Slavic group	7.96*	0.69*	91.35*
Linguistic grouping; Czechs and Slovaks in Germanic group	8.12*	0.76*	91.12*
Linguistic grouping; Czechs and Slovaks in Western Slavic group	7.83*	0.82*	91.35*
Geographical grouping: Germans, Poles, Czechs and Slovaks, Baltic countries, Eastern Europe, and Southern Europe	7.81*	0.73*	91.46*

For linguistic grouping the following groups were constructed: Western Slavs including Polish population samples; Eastern Slavs including Russians, Belarussians, and Ukrainians; Baltic including Latvians and Lithuanians; Ugro-finnic including Estonians and Hungarians; Germanic including population samples from Germany and Austria; and Romance including Romanians. Czechs and Slovaks were consecutively assigned to each linguistic group or treated as a separate group and AMOVA was calculated. The last row of the table shows geographical groups used for AMOVA calculations. "Eastern Europe" designates all population samples collected eastward from Poland, and "Southern Europe" designates all population samples collected southward from Slovakia and Czech Republic.

$^*P = 0.00000$.

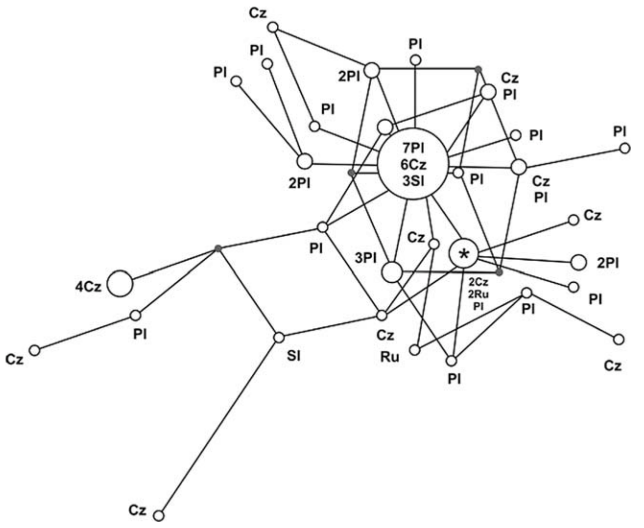

Fig. 3. Median-joining network of subhaplogroup R1a1-WSL based on 12 STR loci. The network includes 61 R1a1-chromosomes (according to data presented in Supporting Information Table 2). Each circle represents a haplotype, defined by a combination of STR markers. Circle size is shown proportional to haplotype frequency. The node marked with * (haplotype 16-10,14-13-30-25-10-11-13-14-11-10 for loci DYS19-DYS385a, b-DYS389I-DYS389II-DYS390-DYS391-DYS392-DYS393-DYS437-DYS438-DYS439, respectively) is used as ancestral for the TMRCA estimates. Haplotypes are labeled as follows: Cz, Czechs; Sl, Slovaks; Pl, Poles; Ru, Russians.

the Czech Republic (2%), Slovakia (1.8%), Ukraine (2.2%), and Hungary (1.7%). In neighboring populations, this haplotype was detected at the frequency of 0.9% in Germans and 0.5% in Belarussians, Russians, and Southern Slavs (such as Bulgarians, Slovenians, Macedonians, Serbians, Bosnians, and Croats). This haplotype belongs to the R1a1-subhaplogroup of lineages (designated here as R1a1-WSL) characterized by a remarkable allele combination 10,14 and 9,14 in DYS385ab (Supporting Information Table 2). In the populations studied, subhaplogroup R1a1-WSL was frequently found in Czech (13%), Polish (11.5%), and Slovak (5%) populations, but Russian populations almost entirely avoided the influence of carriers of this Y-STR subcluster (Supporting Information Table 2). Only northwestern Russians from the region of Novgorod are characterized by an appreciable frequency (7.9%) of the R1a1-WSL subgroup.

Figure 3 demonstrates the MJ-network of the 12-locus Y-STR haplotypes belonging to R1a1-WSL subhaplogroup in Poles, Czechs, Slovaks, and Russians. Analysis of this network showed that 61 males who were investigated carried Y-chromosomes having a TMRCA (based on the ρ-statistic within the network equal to 1.9 ± 0.8) of 3.4 ± 1.3 thousand years (ky) for the pedigree mutation rate and 8.0 ± 3.2 ky for the evolutionary mutation rate. The age of the accumulated STR variation within the subgroup R1a1-WSL [calculated using the Zhivotovsky et al. (2004) method] indicated that the estimated ages of this subcluster were 2.3 ± 0.9 ky or 5.5 ± 2.0 ky for the pedigree and evolutionary mutation rates, respectively.

DISCUSSION

As the populations of Poland, the Czech Republic, and Slovakia share common Slavic origin and are classified linguistically as Western Slavs, it could be expected that they share many of the common Y-STR haplotypes as well. However, RST distances obtained in this study suggest the existence of a split between the northern and southern branches of Western Slavs. Czechs and Slovaks differed significantly from Poles and Russians, and from each other, although the value of P was close to 0.05 in the case of the RST distance between Czechs and Slovaks. Similarly, when the abovementioned Slavic populations were analyzed in the context of the whole of Eastern and Central Europe, the distinctiveness of the Czech and Slovakian samples was clearly visible, with possible differentiation between Czechs and Slovaks suggested by the second MDS dimension.

However, it should be noted that Rębała et al. (2007) have observed the genetic homogeneity of Western and Eastern Slavic populations extending from Slovakia and the Ukraine to Belarus and Russia, and also involving some Southern Slavic populations (such as Slovenians and western Croatians); that research however contains no data on the Czech Y-STR variation. Meanwhile, another study has shown the existence of significant differences between Poles and Czechs as far as Y-SNP polymorphism is concerned (Luca et al., 2007). Moreover, the same study reports that Czech populations display frequencies of Y chromosome haplogroups intermediate to those of Poles and Germans, mainly due to the lower frequency of R1a characteristic among Slavs and higher frequency of R1b characteristic among Germans and other populations of Western Europe (Luca et al., 2007). Thus, in the case of Poles, Czechs, and Germans, our study of Y-STR polymorphism replicates the results obtained earlier for Y-SNPs. On the other hand, in contrast to the study based on 9 Y-STRs by Rębała et al. (2007), our study detected significant differentiation of Y-STR haplotypes between Poles and Slovaks in RST calculation for seven Y-STRs. The reasons for this discrepancy are unclear, although it is not impossible that the differences in Y-STR sets used for the comparison (7 vs. 9), coupled with relatively small size of the Slovakian sample used in this study, could cause such an effect. Also, no information is available on the exact place of origin of the Slovakian men from whom DNA samples were taken for the study by Rębała et al. (2007), and so the possibility that the observed discrepancy is the result of yet to be uncovered regional differences in Y-STR haplotype frequencies in Slovakia cannot be excluded.

We believe that the significant differences in Y-STR haplotype frequencies between Western Slavs, observed in this and in the previous study (Luca et al., 2007), can be explained using historical and archeological records regarding the early history of Slavic settlements in Central and Eastern Europe. It is generally assumed that the spread of Slavic culture to the West between late sixth and ninth century took two alternative routes along the Carpathian Mountains, on their northern and southern sides. It is worth noting that artifacts from Germanic culture, dating to the first half of the sixth century, have been convincingly reported in the vicinity of present-day Prague; then, Slavic artifacts dating to the second half of the century have been reported in the same area (Barford, 2001). On the other hand, traces of Germanic settlements in the sixth century are scarce on the northern side of Carpathian mountains and are confined mainly to the area of present-day western Poland (Barford, 2001; Kokowski, 2006). In the light of archeological evidence, it would thus not seem unreasonable to assume that Slavic culture, spreading on the southern side of Carpathian Mountains, quickly made contact with Germanic tribes. This branch of Slavs may have given rise to the Prague culture, which later spread North between the Elbe and Oder Rivers, and may also have given rise to Polabian Slavs. In the late sixth century, Slavic Sukov culture is present on the southern shores of the Baltic Sea. Artifacts of this culture share many resemblances to those of the Prague culture, although there is insufficient evidence to prove that the Sukov culture derived directly from the Prague culture. At the end of the sixth century, the spread of Slavic culture on the northern side of the Carpathian Mountains is represented by Korchak type cultures present in the territories of present-day southeastern Poland, relatively distant from the territories where Germanic artifacts from this period have been found. The ages of Slavic expansion, which followed in Central Europe, are characterized by the formation of better organized tribal societies and, finally, Slavic states. In the same time period, the Carolingian Empire extended its borders to the east and came into contact with Slavs mainly of Polabians and Bohemian cultures (Barford, 2001). In tenth-century Central Europe, it is possible to differentiate four distinct groups of Slavs: the Polabians (between the Oder and Elba Rivers), the Pomeranians (on the south shores of the Baltic Sea), the Bohemians (on the south side of the Carpathian Mountains), and Poles (in the Warta and Vistula River valleys). Of these groups, the Polabians and Bohemians shared relatively long borders with the Ottonian Empire (i.e. Germanic populations), while the Pomeranians and Poles were generally separated from the Empire by the other two groups, and the regions of direct contact were relatively small and few (Barford, 2001).

The cultural and ethnographic landscape of Central Europe described above is based mainly on archeological findings, which are sometimes regarded as insufficient to draw definite conclusions. However, we believe that the general picture emerging from archeological findings in this region shares some resemblance with the Y-STR data from Western Slavs and could begin to explain the phenomena we have observed in our study. Biological data on haplotype frequency, which are general in their very nature, can be superimposed on the similar general historical picture of interactions between Slavic and Germanic tribes and thus creating a new research perspective that will allow a better understanding of the long-term interpopulation interactions.

Whether the spread of Slavic culture was based mainly on demography or cultural exchange is still a matter of debate; but nevertheless it seems plausible that whatever the dominant force behind the spread of Slavic culture was, the members of the culture were more likely to meet and then perhaps mix with representatives of Germanic cultures to the South of the Carpathian Mountains and to the West of the River Oder. Thus, late sixth-century Prague culture and its derivatives (the Bohemians and possibly Polabians) should be considered the earliest meeting point between Slavic and Germanic cultures. Such a contact, in the very beginning of Slavdom, when population sizes were not particularly large compared to later periods, could be a cornerstone of the similarities between the populations of Czechs, Slovaks, and Germans observed today. Similarly, the relatively great distance between the Germanic tribes and the first settlements of Proto-Poles, together with the "buffer zone" of Polabians, could explain the relatively lower similarity between Y-STR haplotypes of contemporary Germans and Poles. The above-mentioned primary distinctions between the tribes that were to become Western Slavs could have been retained, and possibly even enhanced, in the course of history due to numerous interpopulation contacts in different historical contexts. Nevertheless, as population growth starting from the tenth century led to a substantial increase in the number of individuals dwelling in particular parts of Europe (Russel, 1987), it is difficult to identify any other period in history when a significant Germanic component admixture could have altered the common Slavic background of Western Slavs' Y chromosomes, even though the ten centuries following the creation of the first Slavic states were characterized by frequent ruler and border changes.

We realize however that the line of reasoning described above is partly speculative and based on two main assumptions: of Slavic population migration and of genetic isolation. Regarding the first assumption, to generate the Y-STR diversity observed, a significant part of the spread of Slavic culture should result from real population movements. If Slavic culture was spread by cultural exchange only (or in very large part) then there would be no chance of any significant mixing between peoples of Slavic and Germanic origin. We assume that migration was indeed a significant factor in the success of Slavdom in Central Europe as this adequately explains the shift from the technologically advanced artifacts of the Germanic tribes inhabiting this region until the dawn of the Roman Empire to the less technologically demanding artifacts of the Early Slavs. Moreover, archeological evidence supports the suggestion that the first period of Slavic migrations through Central Europe, and Poland especially, was characterized, at least in part, by nomadic behavior (Buko, 2006). As to the second assumption, to maintain the differences in Y-STR haplotype diversity of Germans, Czechs, Slovaks, and Poles for more than 1,000 years of mutual contact, genetic borders should exist between those populations. Such genetic borders, between Poles and Germans, and between Poles and Czechs, have been detected in earlier studies (Kayser et al., 2005; Luca et al., 2007). However, the nature of those borders remains unclear. From a historical perspective, the area of the Carpathian Mountains, which is currently shared by the Czech Republic, Slovakia, and Poland, is a region characterized by many border shifts, where different states and rulers claimed various parts of the area and ruled them for shorter or longer periods. For instance, throughout the second half of the nineteenth century until the end of the First World War the area of the present Czech Republic, Slovakia, and Southern Poland belonged to one state, namely the Austro-Hungarian Empire (Barford, 2001; Magosci, 2002). Additionally, as Poles, Czechs, and Slovaks speak very similar languages and share at least some features of the common Slavic cultural heritage, no strong cultural antagonisms should be expected in the investigated area. Under such conditions, the existence of a genetic barrier between Poles and Czechs/Slovaks, observed in our study as well as in previous studies (Luca et al., 2007), is difficult to explain. One could argue that the differences in Y-STR haplotype frequencies between the population samples of Polish, German, Czech, and Slovakian origin, detected in our study and other studies (Kayser et al., 2005; Luca et al., 2007), could be of recent origin, due to the massive migrations that took place in Poland after World War II. But our previous study proved that the population of the Southern Poland has not changed significantly during the last 100 years as far as Y chromosome polymorphism is concerned (Woźniak et al., 2007). On the other hand, if the detected genetic borders date back to the beginning of Slavdom, then they might be explained by a relatively low rate of interpopulation gene exchange at this early stage of population growth, due to geographic and cultural reasons. Although the Carpathian Mountains are not significant physical barrier between southern and northern parts of Central Europe, it seems reasonable to assume, given the archeological records (Barford, 2001), that the Prague culture migrated mainly along the Danube River and north, to the territories of contemporary eastern Germany. If the abovementioned assumption of migratory spread of Slavic culture is true, then most population movements of the Prague culture would follow the abovementioned path, thus rendering any potential interpopulation contacts across the mountains relatively rare. Additionally, if the Polabians were descendants of the Prague culture, then they could bear Y-STR haplotypes derived from the putative mixed population of Germanic and Slavic origin, thus creating the observed genetic border on the Oder River. Such a border could have been retained, and maybe even strengthened by Germanic influences in the region between the Elbe and Oder Rivers in the centuries following the first Slavic settlements in this area.

Given the observed location of Czechs and Slovaks on the MDS plot, between Germans and Russians, and farther from Ukrainians, Belarussians, and especially Poles, and taking into consideration the proposed routes of the Early Neolithic migrations to Europe, one could also argue that the Y chromosomes of Czechs and Slovaks could have preserved a similarity to the early population of Europe (Luca et al., 2007). This line of argument could be supported by a phylogenetic analysis of the Y-STR haplotypes belonging to haplogroup R1a1 that allowed us to recognize subcluster R1a1-WSL, characteristic mainly among Western Slavs. It is worth noting that in a recent work by Underhill et al. (2009) a new Y chromosome haplogroup R1a1a7 (defined by SNP marker M458) is described that is most frequent in Central Europe and especially in Poland. Interestingly, ~33% of chromosomes belonging to this haplogroup bear the DYS385 10;14 haplotype, compared to ~3% of chromosomes belonging to the R1a1a*(xM458) haplogroup and bearing the same haplotype. Similarly, 50% of R1a1a7 chromosomes bear DYS385 11;14 haplotype compared to ~75% of R1a1a*(xM458) chromosomes. Those frequency differences are statistically significant ($P = 0.0000$ and $P = 0.0003$, respectively). Additionally, almost 15% of R1a1a7 chromosomes published by Underhill et al. belong to the R1a1-WSL haplotype (17-13-30-25-10-11-13-10,14), and this haplotype is not found outside the haplogroup. Based on this information, it seems reasonable to argue that a large part (and possibly all) of R1a1-WSL chromosomes described by us belong to the R1a1a7 haplogroup. As the coalescence time for this haplogroup is estimated to be 7.9 ± 2.6 ky based on evolutionary mutation rate, with a maximum of 10.7 ± 4.1 ky among Polish R1a1a7 carriers (Underhill et al., 2009), the R1a1-WSL haplotypes age should be less.

Age calculations based on evolutionary and pedigree mutation rates gave significantly different date estimates, 5.5–8.0 and 2.3–3.4 ky, respectively. In our opinion, the age calculations of the subcluster R1a1-WSL based on the pedigree mutation rate appear to be more consistent with the archeological record, as well as with the limited distribution of this Y-STR subcluster in Europe. Although it is suggested that pedigree rates do not consider the evolutionary consequences of population dynamics (Zhivotovsky et al., 2006), it seems that pedigree rates could be used for age estimations of some subclades of the phylogeny, specific to particular regions and ethnic groups. We think that younger age is more suitable for subcluster R1a1-WSL, because the pattern of its distribution in Europe seems to be in agreement with the distribution of some archeological cultures existing at about the same time. The Lusatian culture (3.3–2.5 ky ago) spread over a region that reached from the central basin of the Oder river (eastern Germany) and the Bohemian/Slovakian mountain ridge to the east as far as the Ukraine and in the north to the shores of the Baltic Sea (Sedov, 1979; Šavli, 1996). In the north, the Lusatian culture was succeeded by the Pomeranian culture extending over the Baltic coastal region between the mouths of the Oder and Vistula rivers. It is sometimes suggested that present-day Pomeranians or Kashubians represent the descendants of the bearers of the Pomeranian culture; however, our study did not show significant differences between them and other Poles as far as Y chromosome polymorphism is regarded. This may be explained however by a relatively close contact between Pomeranians and Proto-Poles at the time of early Slavic migrations, as mentioned earlier. Southern parts of present Poland were under Celtic influence. In the second century B.C., the Celts arrived in southern Poland via the Moravia and Bohemia regions, where they prevailed with their La Tène culture from the fifth century B.C. Therefore, it is probable that the R1a/R1b proportion varied in those regions according to the degree of influence of one population or another (i.e., Slavic or Celtic). Thus the study of Y-STRs of haplogroup R1a may suggest that the genetic history of Slavic people in the territory of Slavdom is much longer than its cultural history, i.e., Slavic culture was acquired by Proto-Slavic tribes that were previously cohabitating the territories of Germanic cultures, and left scant physical evidence of their existence until the disappearance of those materially dominating groups. After the fall of the Germanic cultures of Central Europe, those previously unknown tribes could have accepted a new cultural domination of Slavic origin. It is worth noting however that the theory of the cultural domination of Slavs over the tribes dwelling in formerly Germanic lands does not exclude the possibility of the massive Slavic tribal migrations described above. Additionally, the presence of a subhaplogroup that seems to be Western Slav-specific (R1a1-WSL) may alternatively be explained by the assumption that the specific Y-STR haplotypes (DYS385 10,14 and 9,14) had been present for some time among early Slavic tribes, before the spread of Slavdom that that took place in the sixth century. The relatively high frequency of R1a1-WSL among extant Western Slavs could be related to the founder effect due to the relatively small number of individuals taking part in the original Westward Slavic migrations. Thus, the most important question to be resolved remains: to what extent was the spread of Slavdom through Europe affected cultural exchange and to what extent by migration?

We believe that possible explanations of the phenomena described in this study should take into consideration the unique geographical and historical placement of the region under investigation in terms of genetic, archeological, linguistic, and historical data. The distinctiveness of both Czech and Slovak populations, compared to other neighboring populations, and to other Slavs in particular, requires further study, involving more samples from different parts of both countries and from other parts of Europe as well as the analysis of more archeological sites. Unfortunately, we were unable to analyze haplogroups other than R1a1 in our samples. Although meta-analysis of different published data sets seems to be a useful tool in such situations, complete haplogroup information could possibly produce data of greater complexity and could perhaps be more informative. As more Y chromosome data from Europe becomes available, together with new archeological data, a better understanding of the processes shaping the Y chromosome diversity of Central Europe should be expected in the near future.

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