

The Gene Pool of Belgorod Oblast Population: Study of Biochemical Gene Markers in Populations of Ukraine and Belarus and the Position of the Belgorod Population in the Eastern Slavic Gene Pool System

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Abstract—The characteristics of the gene pools of indigenous populations of Ukraine and Belarus have been studied using 28 alleles of 10 loci of biochemical gene markers (HP, GC, TF, PI, C'3, ACP1, GLO1, PGM1, ESD, and 6-PGD). The gene pools of the Russian and Ukrainian indigenous populations of Belgorod oblast (Russia) and the indigenous populations of Ukraine and Belarus have been compared. Cluster analysis, multi-dimensional scaling, and factor analysis of the obtained data have been used to determine the position of the Belgorod population gene pool in the Eastern Slavic gene pool system.

INTRODUCTION

Study of the differentiation of modern populations and estimation of their genetic similarity are among the key goals of population genetics [1]. However, the genetic relationships between the most numerous eastern European ethnic groups—Russians, Ukrainians, and Belarussians—have been poorly studied to date. Although many Russian populations have been studied with respect to various immunological and biochemical polymorphic systems, a unified range of eight to ten classical gene markers has been analyzed in only 10–11 populations within the area historically populated by Russians [2–8]. The gene pools of other Eastern Slavs have been studied even more poorly. According to the most comprehensive review [1, 2], Ukrainian and Belorussian populations have been studied mostly with respect to five classical gene markers (AB0, RH, HP, TF, and GC).

To determine the position of the Belgorod population in the system of Eastern Slavic gene pools, we studied the genetic structures of the indigenous populations of Ukraine and Belarus with the use of a unified, large set of the same biochemical gene markers that had been studied in the population of Belgorod oblast (region) of Russia as described in our previous article [9]. In this study, we collected data that served as a basis for comparison of the gene pools of Russian and Ukrainian indigenous residents of Belgorod oblast,

Ukrainians from Ukraine, and Belarussians from Belarus and determined the position of the Belgorod population gene pool in the Eastern Slavic gene pool system.

MATERIALS AND METHODS

Populations. We studied the distribution of biochemical gene markers in the indigenous populations from Ukraine (samples of 50 subjects from Khmel'nitskii and Lviv oblasts) and Belarus (samples of 50 subjects from Vitebsk and Brest oblasts) (Fig. 1). Although Khmel'nitskii and Lviv oblasts are geographically close to each other, their populations are classified with different anthropological variants (the western Ukrainian and central Ukrainian variants, respectively). Thus, the total Ukrainian sample studied consisted of approximately equal proportions of all the main groups of Ukrainians, namely, the western (Lviv), central (Khmel'nitskii), and eastern (Belgorod) ones; hence, it representatively reflected Ukrainians as an ethnic group.

The sample from Vitebsk oblast (the northern variant of the Belorussian anthropological type) comprised the rural indigenous Belorussian population of Dokshitsy raion (district) located in western Vitebsk oblast. The Belorussian sample from Brest oblast was the population of western Polesye. Thus, the total Belorussian

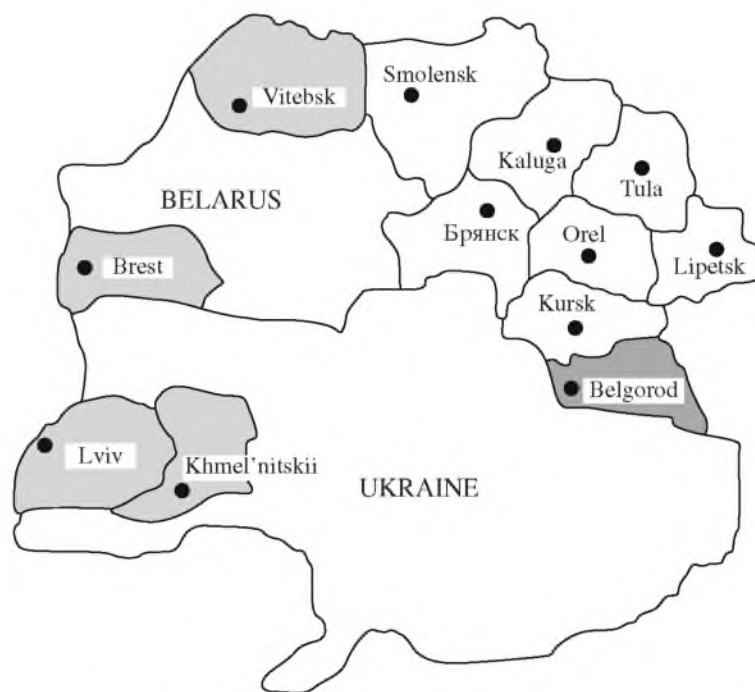


Fig. 1. The map of southern central Russia, Ukraine, and Belarus. The regions where the samples were collected are shaded.

sample studied consisted of approximately equal proportions of both groups of Belarussians, namely, the northern and Polesye ones; hence, it representatively reflected Belarussians as an ethnic group.

The sample from Belgorod oblast consisted of indigenous Russian (Prokhorovka and Krasnoe raions) and Ukrainian (Graivoron and Krasnogvardeiskoe raions) populations. Prokhorovka raion is located in northern Belgorod oblast; its population is 22878 people. Krasnoe raion is located in northwestern Belgorod oblast; its population is 12737 people. We performed the study in five rural municipalities, geographically remote from one another, of each raion. The population samples from all municipalities were approximately equal (30 subjects). The Russian population of Prokhorovka and Krasnoe raions formed in the period from the 17th to the 18th centuries. Its founders were mostly migrants from central Russia (the Moscow region) and Ryazan'. The population belongs to the upper Desna variant of the upper Oka anthropological type.

Graivoron raion is located in southwestern Belgorod oblast; its population is 20606 people. Krasnogvardeiskoe raion is located in eastern Belgorod oblast; its population is 35027 people. The first Ukrainian settlements in these areas appeared in the second half of the 16th century. Most migrants were from Right-bank Ukraine and the neighboring Slobodian Ukraine [10, 11]. Ukrainians from Belgorod oblast are classified with the eastern Ukrainian anthropological type.

The total sample from Belgorod oblast comprised 382 subjects, including 298 Russians from Prokhorovka

and Krasnoe raions (146 and 152 subjects, respectively) and 84 Ukrainians from Krasnogvardeiskoe and Graivoron raions (42 subjects from each).

All samples were collected and genotyped according to a unified protocol. The samples included only subjects that were not related within the third degree of relationship all of whose ancestors during three generations originated from the given population. Blood was sampled according to international standards, after an informed written consent of the subjects, and under the supervision of the Committee for Ethics of the Medical Genetic Research Center of the Russian Academy of Medical Sciences. The ethnic and population affiliations of the subjects were determined on the basis of the ethnicities and birthplaces of three generations of their ancestors in both parental lines.

Methods of genotyping genetic biochemical markers. The material for laboratory study was venous blood separated into the serum and erythrocyte fractions by centrifuging at 3000 rpm. The obtained material was stored at a temperature of -20°C . The subjects were genotyped with respect to ten polymorphic erythrocyte and serum proteins: TF, GC, HP, C'3, PI, ESD, GLO1, ACP1, PGM1, and 6-PGD.

The HP, C'3, and 6-PGD biochemical loci were identified by the standard method of vertical electrophoresis in 7.5% polyacrylamide gel (PAAG); the GLO1 locus was identified by the same method in 5% PAAG [1, 3]. We used a Protean II xi 2-D electrophoretic cell (Bio-Rad, United States). The TF, GC, ESD, PGM1, PI, and ACP1 loci were typed by means

of isoelectrofocusing (IEF) in PAAG or agarose [1, 3]. A Multiphor device (LKB, Sweden) was used for IEF. The results of the electrophoretic separation of serum proteins were visualized using a Gs-710 densitometer (Bio-Rad, United States). The ESD and ACP1 phenotypes were identified in a dark box with the use of a transilluminator from UVP.

The ABO and RH blood groups were determined under field conditions by means of individual interviews.

Statistical analysis. The phenotypic and gene frequencies were calculated by standard methods [12]. The genetic distances between the populations were calculated according to Nei [13, 14] with the use of the DJ Genetic software (version 0.03 beta) developed by Seregin and Balanovskaya (Medical Genetic Research Center, Russian Academy of Medical Sciences).

The obtained results were treated using multivariate statistical methods (multidimensional scaling (MDS) and factor analysis) by means of the Statistica 5 software. Matrices of genetic distances were used for MDS in order to show graphically, in a two- or three-dimensional space, the mutual positions of the studied populations according to the indices of their genetic relationship. To assess the quality of the MDS results, we used the stress index (S_0) and the alienation coefficient (K_0). The results were considered acceptable if the stress did not exceed the level $S_0 \leq 0.10$ [15]. A correlation matrix was used for factor analysis by the principal factor method. The result of the factor analysis was a plot showing the positions of the studied populations in a two- or three-dimensional space of the identified significant principal factors [15].

RESULTS AND DISCUSSION

Table 1 shows the data on the distribution of phenotypes and gene frequencies, the observed and expected heterozygosities, and fixation index of blood polymorphic systems in the indigenous populations of Ukraine and Belarus. For comparison, we used published data on the distribution of the studied biochemical gene markers in Russian, Ukrainians, and Belorussians [1, 7, 8]. Table 2 shows the mean frequencies of gene markers in the populations studied.

Distribution of Biochemical Gene Markers in the Ukrainian and Belorussian Populations

The haptoglobin (HP) system. The Ukrainian and Belorussian populations studied here significantly differed from each other in the distribution of the HP^*1 allele. The HP^*1 allele frequency in the indigenous population of Ukraine (0.33) was about the same as in Russians (0.33) and Ukrainians from Belgorod oblast (0.32). This parameter was below the lower limit of variation of the HP^*1 allele frequency in the population of Ukraine according to data reported in the review [1], 0.35–0.48 (the mean frequency is 0.442). Note that most data on the Ukrainian population reported in [1]

were obtained by the same research team using samples from middle Right-bank Ukraine and the middle Dnieper region. In the Belorussian sample studied, the HP^*1 allele frequency (0.42) was significantly higher ($p < 0.05$) than in Ukrainians and Russians from Belgorod oblast (0.32 and 0.33, respectively) and populations from Ukraine (0.33). Our data on Belorussian populations agreed with literature data [1], according to which the HP^*1 allele frequency varies from 0.35 to 0.46, the mean value being 0.41.

The third component of complement (C'3) system. The populations from Ukraine and the Russian and Ukrainian populations of Belgorod oblast studied here did not differ from one another in the $C'3^*F$ allele frequency (0.12, 0.12, and 0.14, respectively) and agree with literature data on the total Russian gene pool (where the $C'3^*F$ allele frequency varies from 0.07 to 0.29, the mean frequency being 0.159) [1–4]. Note that the $C'3^*F$ frequency in the Belorussian populations studied (0.07–0.08) was significantly lower ($p < 0.01$) than in the total Russian gene pool.

The group-specific component (GC) system. The GC^*2 allele frequency in the populations from Ukraine studied (0.27) was lower than in Ukrainians from Belgorod oblast (0.34) but agreed with literature data on Ukrainians (0.29; the variation range is 0.27–0.33) [1]. The GC^*1S allele frequency in populations from Ukraine estimated in our study (0.68) was significantly higher ($p < 0.01$ –0.05) than in Ukrainians from Belgorod oblast (0.57) and the published value for the Russian gene pool (0.57). The populations from Ukraine studied significantly differed from the Russian population of Belgorod oblast and the total Russian gene pool ($p < 0.001$ –0.01) in the GC^*1F gene frequency (0.05, 0.10, and 0.14, respectively) [1]. The Belorussian populations studied significantly differed from the total Russian gene pool in the frequencies of alleles GC^*1S and GC^*1F ($p < 0.05$).

The transferrin (TF) system. The populations from Ukraine significantly differed in the TF^*C1 allele frequency (0.86) from Ukrainians (0.72) and Russians (0.77) living in Belgorod oblast and the total Russian gene pool (0.778) [3] ($p < 0.01$ –0.05). We also found a significant difference between the populations from Ukraine studied (0.11) and Ukrainians living in Belgorod oblast (0.18) in the TF^*C2 allele frequency ($p < 0.01$). In the Belorussian populations studied, the TF^*C1 allele frequency (0.84) was considerably higher, and the TF^*C2 frequency was lower, than in Russians (0.77 and 0.15, respectively) and Ukrainians (0.72 and 0.18, respectively) from Belgorod oblast ($p < 0.01$). The TF^*C2 frequency in Belorussians (0.10) significantly differed from that in the total Russian gene pool (0.15) [7] ($p < 0.05$).

The $\alpha 1$ antitrypsin (PI) system. The distribution of alleles of the PI system in Ukrainian populations was rather homogeneous. Rare alleles were found only in Lviv oblast at a frequency of 0.02. A significant differ-

Table 1. Distribution of the phenotypes, gene frequencies, observed (H_O) and expected (H_E) heterozygosities, and fixation indices (D) of polymorphic blood group systems in Belorussians and Ukrainians

System and alleles	Belorussians			Ukrainians		
	Vitebsk oblast	Brest oblast	Total of Belorussians	Lviv oblast	Khmel'nitskii oblast	Total of Ukrainians
HP ΣN	47	46	93	48	49	97
N_O 1-1	3	5	8	7	5	12
1-2	32	30	62	16	24	40
2-2	12	11	23	25	20	45
N_E 1-1	7.68	8.70	16.35	4.69	5.90	10.56
1-2	22.64	22.61	45.29	20.63	22.20	42.89
2-2	16.68	14.70	31.35	22.69	20.90	43.56
$\chi^2_{(HWE)}$	8.04**	4.92*	12.66***	2.41	0.32	0.44
H_O	0.68	0.65	0.67	0.33	0.49	0.41
H_E	0.48	0.49	0.49	0.43	0.45	0.44
D	+0.41	+0.33	+0.37	-0.22	+0.08	-0.07
t	2.80	2.34	3.57	1.25	0.47	0.54
$HP*1$	0.40	0.43	0.42	0.31	0.35	0.33
$HP*2$	0.60	0.57	0.58	0.69	0.65	0.67
C'3 ΣN	48	49	97	49	48	97
N_O SS	41	42	83	35	39	74
FS	7	6	13	14	8	22
FF	0	1	1	0	1	1
N_E SS	41.26	41.33	82.58	36.00	38.52	74.48
FS	6.49	7.35	13.84	12.00	8.96	21.03
FF	0.26	0.33	0.58	1.00	0.52	1.48
$\chi^2_{(HWE)}$	0.30	1.65	0.36	1.36	0.55	0.21
H_O	0.15	0.12	0.13	0.29	0.17	0.23
H_E	0.14	0.15	0.14	0.24	0.19	0.22
D	+0.08	-0.18	-0.06	+0.17	-0.11	+0.05
t	0.16	0.42	0.18	0.50	0.27	0.18
$C'3*S$	0.93	0.92	0.92	0.86	0.90	0.88
$C'3*F$	0.07	0.08	0.08	0.14	0.10	0.12
GC ΣN	49	42	91	48	47	95
N_O 2-2	2	3	5	6	2	8
2-1S	21	17	38	21	13	34
2-1F	2	0	2	0	1	1
1S-1S	19	16	35	17	26	43
1S-1F	5	4	9	4	5	9
1F-1F	0	2	2	0	0	0
N_E 2-2	3.72	3.15	6.87	5.67	1.72	6.84
2-1S	17.63	14.51	32.14	20.28	13.40	34.63
2-1F	1.93	2.19	4.12	1.38	1.15	2.68
1S-1S	20.90	16.72	37.61	18.13	26.06	43.79
1S-1F	4.57	5.05	9.64	2.46	4.47	6.79
1F-1F	0.25	0.38	0.62	0.08	0.19	0.26
$\chi^2_{(HWE)}$	1.90	9.75*	5.98	2.54	0.33	2.26
H_O	0.57	0.50	0.54	0.52	0.40	0.46
H_E	0.49	0.52	0.50	0.50	0.40	0.46
D	+0.16	-0.04	+0.07	+0.04	-0.00	-0.00
t	0.96	0.20	0.56	0.23	0.01	0.02
$GC*2$	0.28	0.27	0.27	0.34	0.19	0.27
$GC*1S$	0.65	0.63	0.64	0.61	0.75	0.68
$GC*1F$	0.07	0.10	0.09	0.05	0.06	0.05

Table 1. (Contd.)

System and alleles	Belorussians			Ukrainians		
	Vitebsk oblast	Brest oblast	Total of Belorussians	Lviv oblast	Khmel'nitskii oblast	Total of Ukrainians
TF ΣN	49	48	97	49	49	98
N_O C1C1	37	32	69	34	38	72
C1C2	5	10	15	12	7	19
C1C3	4	5	9	2	3	5
C2C2	1	1	2	0	0	0
C2C3	1	0	1	1	1	2
C3C3	1	0	1	0	0	0
N_E C1C1	35.15	32.51	67.64	34.31	37.73	72.00
C1C2	6.78	9.88	16.70	10.88	7.02	18.00
C1C3	5.93	4.11	10.02	2.51	3.51	6.00
C2C2	0.33	0.75	1.03	0.86	0.33	1.13
C2C3	0.57	0.63	1.24	0.40	0.33	0.75
C3C3	0.25	0.13	0.37	0.05	0.08	0.13
$\chi^2_{(HWE)}$	5.15	1.03	2.33	2.04	1.87	3.56
H_O	0.20	0.31	0.26	0.31	0.23	0.27
H_E	0.27	0.30	0.29	0.28	0.22	0.25
D	-0.25	+0.03	-0.11	+0.09	+0.01	+0.05
t	0.84	0.09	0.51	0.29	0.04	0.22
$TF*C1$	0.85	0.82	0.84	0.84	0.88	0.86
$TF*C2$	0.08	0.13	0.10	0.13	0.08	0.11
$TF*C3$	0.07	0.05	0.06	0.03	0.04	0.03
GLO1 ΣN	49	50	99	50	50	100
N_O 1-1	6	2	8	4	7	11
1-2	19	22	41	22	20	42
2-2	24	26	50	24	23	47
N_E 1-1	4.90	3.38	8.20	4.50	5.78	10.24
1-2	21.19	19.24	40.59	21.00	22.44	43.52
2-2	22.90	27.38	50.20	24.50	21.78	46.24
$\chi^2_{(HWE)}$	0.53	1.03	0.01	0.11	0.59	0.12
H_O	0.39	0.44	0.41	0.44	0.40	0.42
H_E	0.43	0.38	0.41	0.42	0.45	0.44
D	-0.10	+0.14	+0.01	+0.05	-0.11	-0.04
t	0.58	0.67	0.07	0.25	0.64	0.28
$GLO1*1$	0.32	0.26	0.29	0.30	0.34	0.32
$GLO1*2$	0.68	0.74	0.71	0.70	0.66	0.68
ESD ΣN	50	50	100	50	50	100
N_O 1-1	43	35	78	46	43	89
1-2	6	14	20	4	2	6
1-5	0	1	1	0	3	3
2-2	1	0	1	0	2	2
N_E 1-1	42.32	36.13	78.32	46.08	41.41	87.42
1-2	7.36	11.90	19.47	3.84	5.46	9.35
1-5	0	0.85	0.88	0	2.73	2.80
2-2	0.32	0.98	1.21	0.08	0.18	0.25
$\chi^2_{(HWE)}$	1.70	1.56	0.17	0.09	20.91*	13.66*
H_O	0.12	0.30	0.21	0.08	0.10	0.09
H_E	0.15	0.26	0.20	0.08	0.17	0.12
D	-0.19	+0.17	+0.03	+0.04	-0.40	-0.27
t	0.42	0.51	0.10	0.06	1.04	0.78
$ESD*1$	0.92	0.85	0.89	0.96	0.91	0.94
$ESD*2$	0.08	0.14	0.10	0.04	0.06	0.05
$ESD*5$	0	0.01	0.01	0	0.03	0.01

Table 1. (Contd.)

System and alleles	Belorussians			Ukrainians		
	Vitebsk oblast	Brest oblast	Total of Belorussians	Lviv oblast	Khmel'nitskii oblast	Total of Ukrainians
6-PGD ΣN	50	50	100	50	50	100
N_O AA	45	47	92	46	47	93
AC	4	3	7	3	3	6
CC	1	0	1	1	0	1
N_E AA	44.18	47.05	91.20	45.13	47.05	92.16
AC	5.64	2.91	8.60	4.75	2.91	7.68
CC	0.18	0.04	0.20	0.13	0.04	0.16
$\chi^2_{(HWE)}$	4.22	0.05	3.44	6.79*	0.05	4.79*
H_O	0.08	0.06	0.07	0.06	0.06	0.06
H_E	0.11	0.06	0.09	0.10	0.06	0.08
D	-0.29	+0.03	-0.19	-0.37	+0.03	-0.22
t	0.58	0.04	0.43	0.68	0.04	0.48
6-PGD*A	0.94	0.97	0.96	0.95	0.97	0.96
6-PGD*C	0.06	0.03	0.04	0.05	0.03	0.04
ACPI ΣN	44	49	93	46	49	95
N_O AA	7	6	13	5	6	11
AB	20	16	36	17	14	31
AC	1	3	4	4	1	5
BB	12	18	30	15	19	34
BC	4	6	10	5	9	14
CC	0	0	0	0	0	0
N_E AA	6.96	4.90	11.71	5.22	3.72	8.85
AB	19.09	18.35	37.61	17.52	16.81	34.49
AC	1.99	2.85	4.97	3.03	2.76	5.80
BB	13.09	17.16	30.20	14.70	18.98	33.60
BC	2.73	5.33	7.98	5.09	6.22	11.30
CC	0.14	0.41	0.53	0.44	0.51	0.95
$\chi^2_{(HWE)}$	1.36	1.09	1.44	0.78	4.73	2.59
H_O	0.57	0.51	0.54	0.57	0.49	0.53
H_E	0.54	0.54	0.54	0.56	0.53	0.54
D	+0.05	-0.06	-0.01	+0.01	-0.07	-0.03
t	0.34	0.39	0.11	0.10	0.45	0.29
ACPI*A	0.40	0.32	0.35	0.34	0.28	0.31
ACPI*B	0.54	0.59	0.57	0.56	0.62	0.59
ACPI*C	0.06	0.09	0.08	0.10	0.10	0.10
PGMI ΣN	44	44	88	This system was not studied		
N_O 1S1S	20	21	41			
1S1F	5	2	7			
1S2S	12	16	28			
1S2F	3	1	4			
1F1F	2	0	2			
1F2S	0	0	0			
1F2F	0	0	0			
2S2S	1	3	4			
2S2F	1	1	2			
2F2F	0	0	0			

Table 1. (Contd.)

System and alleles	Belorussians			Ukrainians		
	Vitebsk oblast	Brest oblast	Total of Belorussians	Lviv oblast	Khmel'nitskii oblast	Total of Ukrainians
N_E 1S1S	20.45	21.14	41.59			
1S1F	6.14	1.39	7.56			
1S2S	10.23	15.94	26.13			
1S2F	2.73	1.39	4.13			
1F1F	1.53	0.02	0.34			
1F2S	0.41	0.52	2.38			
1F2F	1.28	0.05	0.38			
2S2S	0.68	3.01	4.10			
2S2F	0.09	0.52	1.30			
2F2F	0.52	0.02	0.10			
$\chi^2_{(HWE)}$	7.95	1.43	11.41			
H_O	0.48	0.45	0.47			
H_E	0.49	0.45	0.48			
D	-0.03	+0.01	-0.02			
t	0.17	0.05	0.15			
$PGMI*1S$	0.68	0.69	0.69			
$PGMI*1F$	0.10	0.03	0.06			
$PGMI*2S$	0.17	0.26	0.22			
$PGMI*2F$	0.05	0.02	0.03			
$PI \Sigma N$	This system was not studied			49	42	91
N_O M1M1				30	27	57
M1M2				10	8	18
M1M3				4	6	10
M1R				1	0	1
M2M2				1	1	2
M2M3				2	0	2
M2R				1	0	1
N_E M1M1				28.70	27.52	56.18
M1M2				11.48	8.10	19.64
M1M3				4.59	4.86	9.43
M1R				1.53	0	1.57
M2M2				1.15	0.60	1.72
M2M3				0.92	0.71	1.65
M2R				0.31	0	0.27
$\chi^2_{(HWE)}$				3.70	1.48	2.97
H_O				0.37	0.33	0.35
H_E				0.39	0.33	0.36
D				-0.05	+0.02	-0.02
t				0.22	0.08	0.12
$PI*M1$				0.77	0.81	0.78
$PI*M2$				0.15	0.12	0.14
$PI*M3$				0.06	0.07	0.07
$PI*R$				0.02	0	0.01

Note: ΣN , the size of the population studied; N_O , the observed number of genotypes; N_E , the expected number of genotypes; H_O , the observed heterozygosity; H_E , the expected heterozygosity; D , the coefficient of deviation of the observed heterozygosity from the expected one.
 * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 2. Distribution of classical gene markers in Russians, Ukrainians, and Belorussians

Allele	This study				Literature data		
	Russians from Belgorod oblast	Ukrainians from Belgorod oblast	Populations from Ukraine	Populations from Belarus	Belorussians [1]	Ukrainians [1]	Russians [6, 7]
<i>ACPI*A</i>	0.31	0.29	0.31	0.35	–	–	0.312 (32)
<i>ACPI*B</i>	0.59	0.62	0.59	0.57	–	–	0.648 (32)
<i>ACPI*C</i>	0.10	0.09	0.10	0.08	–	–	0.040 (32)
<i>C3*F</i>	0.12	0.14	0.12	0.08	0.131 (1)	–	0.159 (18)
<i>ESD*I</i>	0.93	0.94	0.94	0.89	–	–	0.903 (32)
<i>GC*1F</i>	0.10	0.09	0.05	0.09	–	–	0.141 (22)
<i>GC*1S</i>	0.67	0.57	0.68	0.64	–	–	0.568 (22)
<i>GC*2</i>	0.23	0.34	0.27	0.27	–	0.286 (10(12))	0.292 (22)
<i>GLO1*I</i>	0.31	0.33	0.32	0.29	–	–	0.374 (18)
<i>HP*I</i>	0.33	0.32	0.33	0.42	0.409 (7)	0.442 (18)	0.365 (51)
<i>PGM1*1F</i>	0.08	0.09	–	0.06	–	–	0.107 (27)
<i>PGM1*1S</i>	0.63	0.66	–	0.69	–	–	0.604 (27)
<i>PGM1*2F</i>	0.07	0.04	–	0.03	–	–	0.057 (27)
<i>PGM1*2S</i>	0.22	0.21	–	0.22	–	–	0.232 (27)
<i>PI*M1</i>	0.84	0.83	0.78	–	–	–	0.787 (19)
<i>PI*M2</i>	0.11	0.11	0.14	–	–	–	0.145 (19)
<i>PI*M3</i>	0.04	0.04	0.07	–	–	–	0.057 (19)
<i>PI*R</i>	0.01	0.02	0.01	–	–	–	0.011 (19)
<i>TF*C1</i>	0.77	0.72	0.86	0.84	–	–	0.778 (20)
<i>TF*C2</i>	0.15	0.18	0.11	0.10	–	–	0.149 (20)
<i>TF*C3</i>	0.07	0.06	0.03	0.06	–	–	0.051 (20)
<i>6-PGD*C</i>	0.04	0.04	0.04	0.04	–	–	0.039 (29)

Note: The number of populations studied with respect to each locus is indicated in parentheses.

Table 3. The matrix of genetic distances between the Russian and Ukrainian populations of Belgorod oblast, Belorussians, and Ukrainians

Population	Russians from Belgorod oblast	Ukrainians from Belgorod oblast	Belorussians from Vitebsk oblast	Belorussians from Brest oblast	Ukrainians from Lviv oblast	Ukrainians from Khmel'nitskii oblast	"Average Russian" population
Russians from Belgorod oblast	0	0.003	0.004	0.005	0.002	0.004	0.003
Ukrainians from Belgorod oblast	0.003	0	0.008	0.008	0.007	0.004	0.002
Belorussians from Vitebsk oblast	0.004	0.008	0	0.003	0.006	0.003	0.006
Belorussians from Brest oblast	0.005	0.008	0.003	0	0.007	0.006	0.006
Ukrainians from Lviv oblast	0.002	0.007	0.006	0.007	0	0.006	0.006
Ukrainians from Khmel'nitskii oblast	0.004	0.004	0.003	0.006	0.006	0	0.005
"Average Russian" population	0.003	0.002	0.006	0.006	0.006	0.005	0
Mean genetic distance	0.004	0.005	0.004	0.005	0.005	0.004	0.004

Note: The matrix is based on the frequencies of 21 alleles of eight loci of biochemical markers.

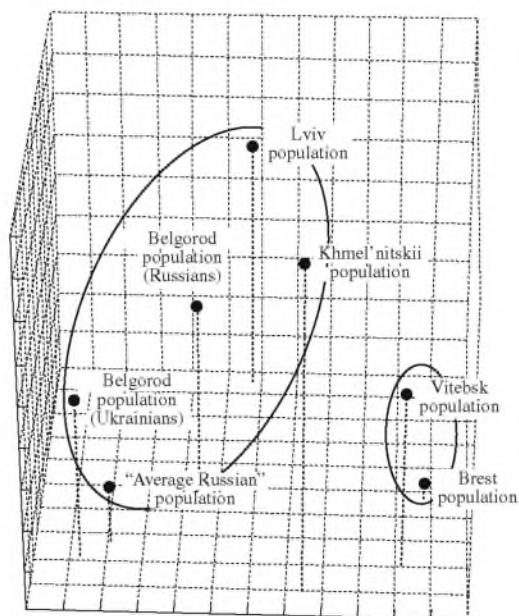


Fig. 2. The MDS plot showing the spatial positions of the analyzed populations from Belgorod oblast, Ukraine, and Belarus and the "average Russian" population as determined by the frequencies of 21 alleles of eight biochemical marker genes.

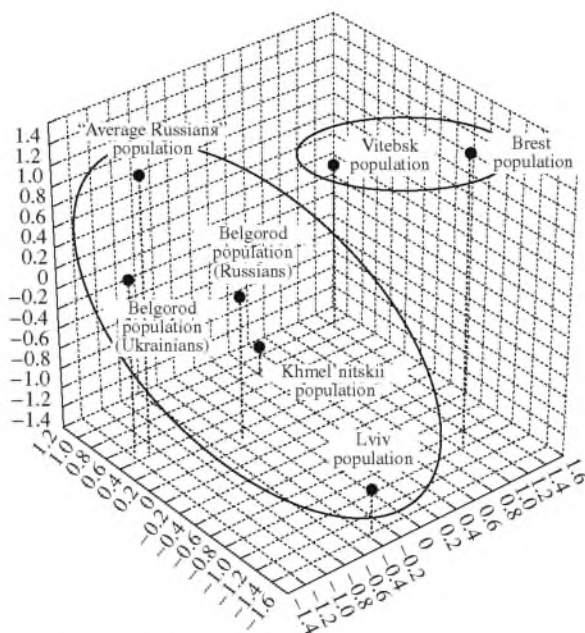


Fig. 3. The positions of the analyzed populations from Belgorod oblast, Ukraine, and Belarus and the "average Russian" population in the space of three principal factors as determined by the frequencies of 21 alleles of eight biochemical marker genes.

ences in the *PI*M3* allele frequency between the populations from Ukraine studied (0.07) and the total Russian gene pool (0.04) [1] ($p < 0.05$) is noteworthy.

The glyoxalase 1 (GLO1) system. The *GLO1*1* allele frequency in the indigenous Ukrainian populations (0.32) was lower than the mean frequency in Russians (0.37) but considerably higher than in Central Asian and Far Eastern ethnic groups, where its frequency was no higher than 20% [3, 8]. According to our data, the *GLO1*1* allele frequency in Belorussians (0.29) significantly differed from the mean frequency in Russians (0.37) [3] ($p < 0.05$).

The erythrocyte esterase D (ESD) system. The populations from Ukraine significantly differed from the Belorussian populations studied and the total Russian gene pool in the *ESD*1* and *ESD*2* allele frequencies ($p < 0.01$ – 0.05). The *ESD*1* frequency in Belorussians (0.89) was significantly lower ($p < 0.05$) than in Russians from Belgorod oblast (0.93) and was almost the same as its frequency in the total Russian gene pool (0.90). In all populations analyzed in this study, the frequency of the "Asian" *ESD*2* allele (0.05–0.11) was substantially lower than in indigenous Far Eastern and Central Asian populations, in some of which its frequency is as high as 30%, and was beyond the variation range of this allele frequency in European populations (11–15%) [3, 8].

The erythrocyte 6-phosphogluconate dehydrogenase (6-PGD) system. The populations of Ukraine, Belarus, and Belgorod oblast of Russia did not differ in the *6-PGD*C* allele frequency (0.04 in all populations

studied). The variation of the *6-PGD*C* frequency in these populations fell within the range of its variation in the total Russian gene pool (2–8%; the mean frequency is 4%) [1, 2].

The phosphoglucomutase (PGM1) system. The *PGM1*1S* allele frequency in the Belorussian populations studied (0.69) was higher than in both Russians from Belgorod oblast (0.63) and the total Russian gene pool (the mean value is 0.60; the variation range is 0.475–0.700) ($p < 0.05$) [2, 3]. The *PGM1*1F* and *PGM1*2F* allele frequencies in Belorussians (0.06 and 0.03, respectively) were significantly lower ($p < 0.05$) than its mean values in 27 Russian populations (0.11, with a variation range of 0–0.196 and 0.057, with a variation range of 0–0.16, respectively) [2, 8].

The erythrocyte acid phosphatase (ACPI) system. The frequency distributions of ACPI alleles in the analyzed populations from Belarus and Ukraine were similar, the allele frequencies falling within their variation ranges in the total Russian gene pool. However, the populations from Ukraine studied significantly ($p < 0.001$) differed from the total Russian population [8] in the *ACPI*C* frequency (0.10 and 0.04, respectively).

Thus, we found distinctive features in the gene pools of the indigenous populations of Ukraine and Belarus with respect to many biochemical gene markers.

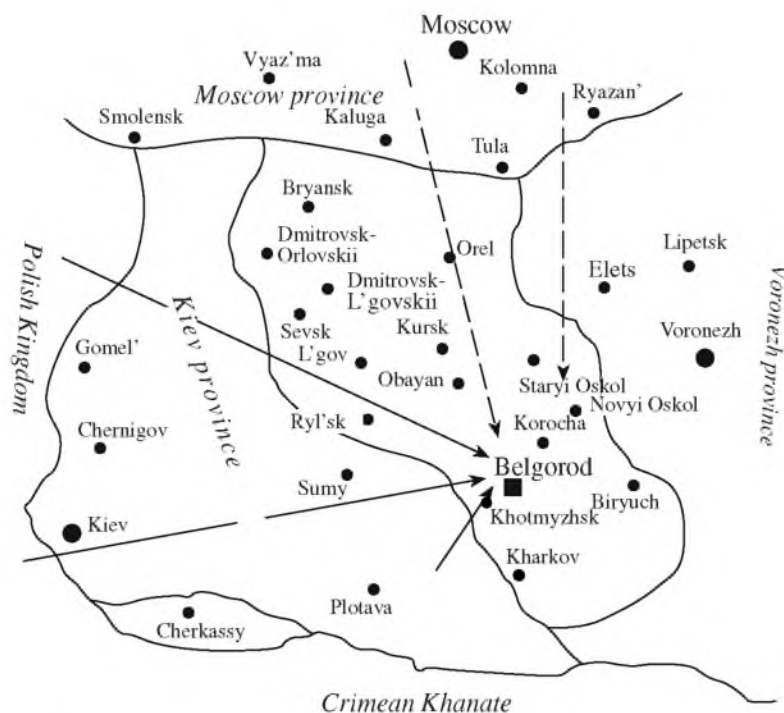


Fig. 4. Russian (dashed arrows) and Ukrainian (solid arrows) migration flows to Belgorod province in the 17th century.

Genetic Relationships between the Populations of Belgorod Oblast, Ukraine, and Belarus and the Total Russian Population

We studied the genetic relationships between the Russian and Ukrainian populations of Belgorod oblast, populations from Ukraine and Belarus, and the "average Russian" population with respect to the frequencies of 21 alleles of eight biochemical gene markers. To characterize the "average Russian" population, we used the mean frequencies of classical gene markers in the Russian gene pool published earlier [1, 2, 7, 8] (Table 2). Note that the genetic distance between Russians from Belgorod oblast and Ukrainians from Lviv oblast was the smallest (0.002) among all estimated interpopulation distances.

The matrix of genetic distances was used for MDS (Fig. 2). The stress coefficient of this plot is $S_0 = 0.01$, which allows us to consider the results of MDS satisfactory. The populations studied formed two clusters in the three-dimensional space.

The large first cluster comprised Russians and Ukrainians from Belgorod oblast, the two populations from Ukraine, and the "average Russian" population. The two populations from Belarus formed the second cluster. Note that Russians from Belgorod oblast were intermediate between the "average Russian" population, Ukrainians from Belgorod oblast, and Ukrainians from Lviv and Khmel' nitskii oblasts.

Factor analysis (Fig. 3) yielded similar results: in the space of three principal factors, Russians and Ukrai-

nians from Belgorod oblast were clustered together with indigenous Ukrainians and the "average Russian" population, and Belorussians formed a separate cluster. As in the MDS plot, Russians from Belgorod oblast were intermediate between the "average Russian" population and Ukrainians from Belgorod, Lviv, and Khmel' nitskii oblasts.

Thus, analysis of the position of the Belgorod population gene pool in the system of the Eastern Slavic gene pool has shown that the Russian and Ukrainian Belgorod populations cluster together with the total Russian population and Ukrainian populations of Khmel' nitskii and Lviv oblasts. Together they form a large "southern" cluster in MDS and factor analysis plots. Note that Russians from Belgorod oblast occupy an intermediate position between the "average Russian" population, Ukrainians from Belgorod oblast, and the two populations from Ukraine, although, geographically, these two Ukrainian populations are 700 and 900 km away from the Belgorod population.

Belorussians from Brest and Vitebsk oblasts forming a separate "northern" cluster of Eastern Slavs are more remote genetically from both the Belgorod oblast population and the indigenous population of Ukraine.

In conclusion, we would like to emphasize that the Lviv population is genetically closer to the geographically remote Russian population of Belgorod oblast (0.002) than to the neighboring Ukrainian population of Khmel' nitskii oblast (the genetic distance is 0.006, i.e., three times longer) and even to the Ukrainian population of Belgorod oblast (0.007). We are planning further

study of these populations with the use of molecular genetic markers to test the obtained results.

In general, our data agree with the history of the Belgorod population. Available historical data [10] indicate that Ukrainians have made a greater contribution to the formation of the Belgorod population than Belorussians have. For example, large-scale migration of Ukrainians to the area of the modern Belgorod oblast began in the second half of the 16th century (Fig. 4). In the 1670s, Ukrainians founded the Graivorony settlement (later, the town of Graivoron). Most migrants were from Right-bank Ukraine (Zhabotin, Belaya Tserkov', Gadyach, Korsun', and Uman'). There were also many migrants from the neighboring Sumy, Akhtyrka, Vorozhba, Sudzha, Bogodukhov, and Bakaleyka. Between the late 17th and early 18th centuries, a large cluster of Ukrainian settlements appeared in the basins of the Valui, Aidar, and Tikhaya Sosna rivers in southeastern Belgorod oblast (the modern Valuiki, Roven'ki, and Krasnogvardeiskoe raions) [11].

Thus, the Belgorod oblast population forms, together with the indigenous population of Ukraine and the "average Russian" population, a large "southern" cluster, in which it occupies an intermediate position between Russians and Ukrainians. Belorussian populations form a separate northern branch of Eastern Slavs and are genetically remote from both the Belgorod population and the indigenous population of Ukraine.

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REFERENCES

1. *Genofond i genogeografiya narodonaseleniya* (Population Gene Pool and Gene Geography), vol. 1: *Genofond naseleniya Rossii i sopredel'nykh stran* (The Gene Pool of the Population of Russia and Neighboring Countries), Rychkov, Yu.G., Ed., St. Petersburg: Nauka, 2000.
2. *Genofond i genogeografiya narodonaseleniya* (Population Gene Pool and Gene Geography), vol. 2: *Genogeograficheskii atlas naseleniya Rossii i sopredel'nykh stran* (The Gene Geographical Atlas of the Population of Russia and Neighboring Countries), Rychkov, Yu.G., Ed., St. Petersburg: Nauka, 2003.
3. Spitsyn, V.A., Kukhkoizer, V., Makarov, S.V., et al., The Russian Gene Pool: Frequencies of Genetic Markers, *Russ. J. Genet.*, 2001, vol. 37, no. 3, pp. 308–314.
4. Shneider, Yu.V., Tikhomirova, E.V., and Shil'nikova, I.N., Materials on Investigation of the Gene Pool of Russia and Adjacent Countries: The Russian Population of Tver' Oblast, *Russ. J. Genet.*, 1994, vol. 30, no. 3, pp. 372–380.
5. Shneider, Yu.V., Tikhomirova, E.V., and Shil'nikova, I.N., Materials on the Study of the Gene Pool of Russia and Adjacent Countries: The Russian Population of Vologda Oblast', *Russ. J. Genet.*, 1994, vol. 30, no. 4, pp. 488–493.
6. Shneider, Yu.V., Shil'nikova, I.N., and Zhukova, O.V., Materials on the Study of the Gene Pool of Russia and Adjacent Countries: The Russian Population of the Pskov Oblast', *Russ. J. Genet.*, 2002, vol. 38, no. 11, pp. 1322–1326.
7. Balanovskaya, E.V., Balanovsky, O.P., Spitsyn, V.A., et al., The Russian Gene Pool: Gene Geography of Serum Gene Markers (HP, GS, PI, and TF), *Russ. J. Genet.*, 2001, vol. 37, no. 8, pp. 939–950.
8. Balanovskaya, E.V., Balanovsky, O.P., Spitsyn, V.A., et al., The Russian Gene Pool: Gene Geography of Erythrocytic Gene Markers (ACP1, RGM1, ESD, GLO1, and 6-PGD), *Russ. J. Genet.*, 2001, vol. 37, no. 8, pp. 951–963.
9. Lependina, I.N., Balanovskaya, E.V., and Churnosov, M.I., Study of Population Genetic Structure of the Population of Belgorod Oblast': Distribution of Immune Biochemical Markers, *Russ. J. Genet.*, 2008, vol. 44, pp. 551–565.
10. *Belgorodovedenie: Uchebnik dlya obshcheobrazovatel'nykh uchrezhdenii* (Study on Belgorod Region: A Textbook for Secondary Education Institutions), Shapovalov V.A., Ed., Belgorod, 2002.
11. *Belgorodskaya oblast'* (Belgorod Oblast) Vorotnikov, R.V., Ed., Voronezh, 1974.
12. Zhivotovsky, L.A., Statistical Methods for Gene Frequencies Analysis in Natural Populations, *Itogi Nauki Tekh., Ser. Obshch. Genet.*, 1983, pp. 76–104.
13. Nei, M., Analysis of Gene Diversity in Subdivided Populations, *Proc. Natl Acad. Sci. USA*, 1973, vol. 70, pp. 3321–3323.
14. Nei, M., F-Statistics and Analysis of Gene Diversity in Subdivided Populations, *Ann. Hum. Genet.*, 1977, vol. 41, pp. 225–233.
15. Deryabin, V.E., *Mnogomernye biometricheskie metody dlya antropologov* (Multivariate Biometrics for Anthropologists), Moscow: VINITI, 2001, pp. 105–265.