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Genetic diversity in *Plantago major* L. populations growing under Conditions of Radioactive and Chemical Contamination

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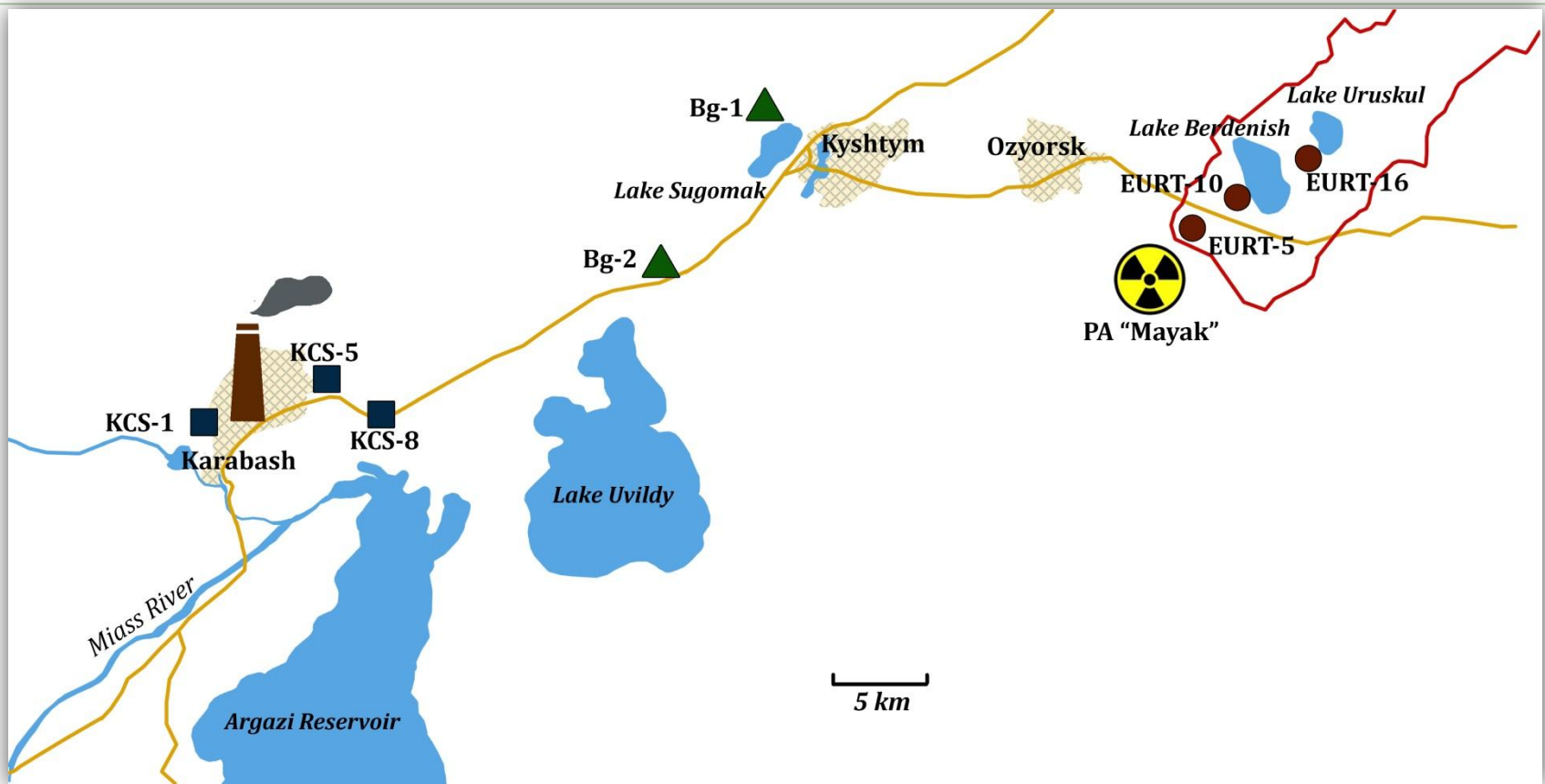
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Research purpose

The purpose of our research is comparison of the genetic diversity in *Plantago major* L. populations growing for a long time under conditions of radioactive or chemical contamination and from background sites.

Test sites

The Southern Urals (Russia) has territories with different types of industrial contamination. East Ural Radioactive Trace (EURT) was formed in 1957 after the serious accident at the Mayak Production Association, where a tank with radioactive waste exploded. Chemically contaminated area is the zone of influence of the Karabash Copper Smelter (KCS). These contaminated territories are unique natural ecological test sites for comparative studies (Fig. 1).



Detailed description of test sites is presented in our papers:

Shimalina N.S. et al., Assessment of Biological Effects in *Plantago major* L. Seed Progeny in the Zone of Impact from a Copper Smelter // Russian Journal of Ecology. 2017. V. 48. № 6. P. 513–523.

<https://doi.org/10.1134/S1067413617060108>

Shimalina N.S. et al. Features of Prooxidant and Antioxidant Systems of Greater Plantain *Plantago major* Growing for a Long Time under Conditions of Radioactive Contamination // Russian Journal of Ecology. 2018. V. 49. № 5. P. 375-383.

<https://doi.org/10.1134/S1067413618050120>

Figure 1

Plot	Specific radioactivity in the soil, Bq/kg (average and min-max)			Concentration ratios			Absorbed dose rate, $\mu\text{Gy/h}^*$
	^{90}Sr	^{137}Cs	$^{239, 240}\text{Pu}$	^{90}Sr	^{137}Cs	$^{239, 240}\text{Pu}$	
Background	6.92 (2.0-12.95)	9.89 (3.65-6.5)	n.o.	0.66	0.054	0.0014	0.108
EURT-16	24000 (4040-55200)	1010 (176-3740)	103 (60-192)				19.1
EURT-10	90900 (155-271000)	4400 (138-18500)	235 (47-399)				73.1
EURT-5	195000 (88300-292000)	9860 (3990-17000)	514 (60-1350)				157.1

*Absorbed dose rate calculated including background radiation which equal to 0.1 $\mu\text{Gy/h}$ for the Ural region

Karimullina E., Mikhailovskaya L.N., Pozolotina V., Antonova E. Radionuclide uptake and dose assessment of 14 herbaceous species from the East-Ural Radioactive Trace area using the ERICA Tool // Environmental Science and Pollution Research. 2018.

<http://dx.doi.org/10.1007/s11356-018-1544-y>

Toxic loads in the KCS zone and in background plots

Plot	Heavy metal contents, µg/g soil				K _i
	Cu	Cd	Pb	Zn	
Background	40.0 ± 5.7	0.56 ± 0.15	27.8 ± 10.7	43.1 ± 7.9	1.0
KCS-8	914.7 ± 94.0	5.2 ± 0.32	214.4 ± 37.8	525.7 ± 51.1	13.0
KCS-5	695.0 ± 1.1	4.9 ± 1.1	239.6 ± 109.8	879.1 ± 480.7	13.8
KCS-1	2569 ± 506	11.4 ± 2.4	586.6 ± 37.4	2655.4 ± 169.5	41.8

$$K_i = \frac{1}{n} \sum_{j=1}^n \frac{C_{ji}}{C_{jf}}$$

K_i is the index of pollution at the *i*th point,

C_{ji} is the concentration of the *j*th element at this point,

C_{jf} is the concentration of the *j*th element in the background zone,

n is the number of elements included in analysis.

Hypotheses

- 1) genetic diversity in the *P. major* populations in the zone of radioactive contamination (EURT) is increased, since ionizing radiation is a mutagenic factor;
- 2) genetic diversity in *P. major* populations in the chemical contamination zone is reduced due to the selection of metal-resistant organisms.

Material and methods

Leaves of 25 plants (growing at least 10 m away from each other) were picked in each population. Under field conditions collected leaves were frozen in liquid nitrogen. In the laboratory samples were stored at -20°C .

Total DNA was isolated using the CTAB method (Doyle, 1991). 9 microsatellite loci derived from *P. major* and its closely related *P. intermedia* were included in the analysis (Squirrell, Wolff, 2001; Wolff et al., 2009).

The polymerase chain reaction (PCR) conditions were determined according to the protocols (Squirrell, Wolff, 2001; Wolff et al., 2009) and carried out using Thermal Cycler 2720 (Applied Biosystems). The 5'-end of the F primer was modified with a fluorescent label (FAM, ROX, TAMRA, R6G). Capillary electrophoresis was performed on an ABI3130 automatic genetic analyzer (Applied Biosystems) in the presence of marker S-550 (Gordiz). Chromatograms were interpreted in the GeneMapper v.3.7 program (Applied Biosystems).

Genetic parameters were calculated using the GenAlex 6.501 program (Peakall, Smouse, 2012).

RESULTS

The parameters characterizing the genetic variability of *P. major* populations are listed in the [table 3](#) . 65 alleles were found in 9 microsatellite loci (one of them is monomorphic), the number of alleles in the locus ranged from 1 to 25.

In all plantain populations high level of inbreeding was revealed as a result of high rate of self-pollination in *P. major*. Despite the high level of inbreeding, the genetic diversity within the *P. major* populations is high enough. According to values of the mean (N_a) and effective (N_e) number of alleles per locus and the number of private alleles (PA), genetic diversity in populations from the zones of chemical and radioactive contamination was lower than in background populations. The lowest genetic diversity was in population of the most contaminated plot from KCS zone.

Mantel test for isolation by distance between KCS and background populations showed that the roads are an important factor in the migration of plantain seeds (Fig.2). Populations of the EURT are isolated because this area is strictly limited for the movement of people.

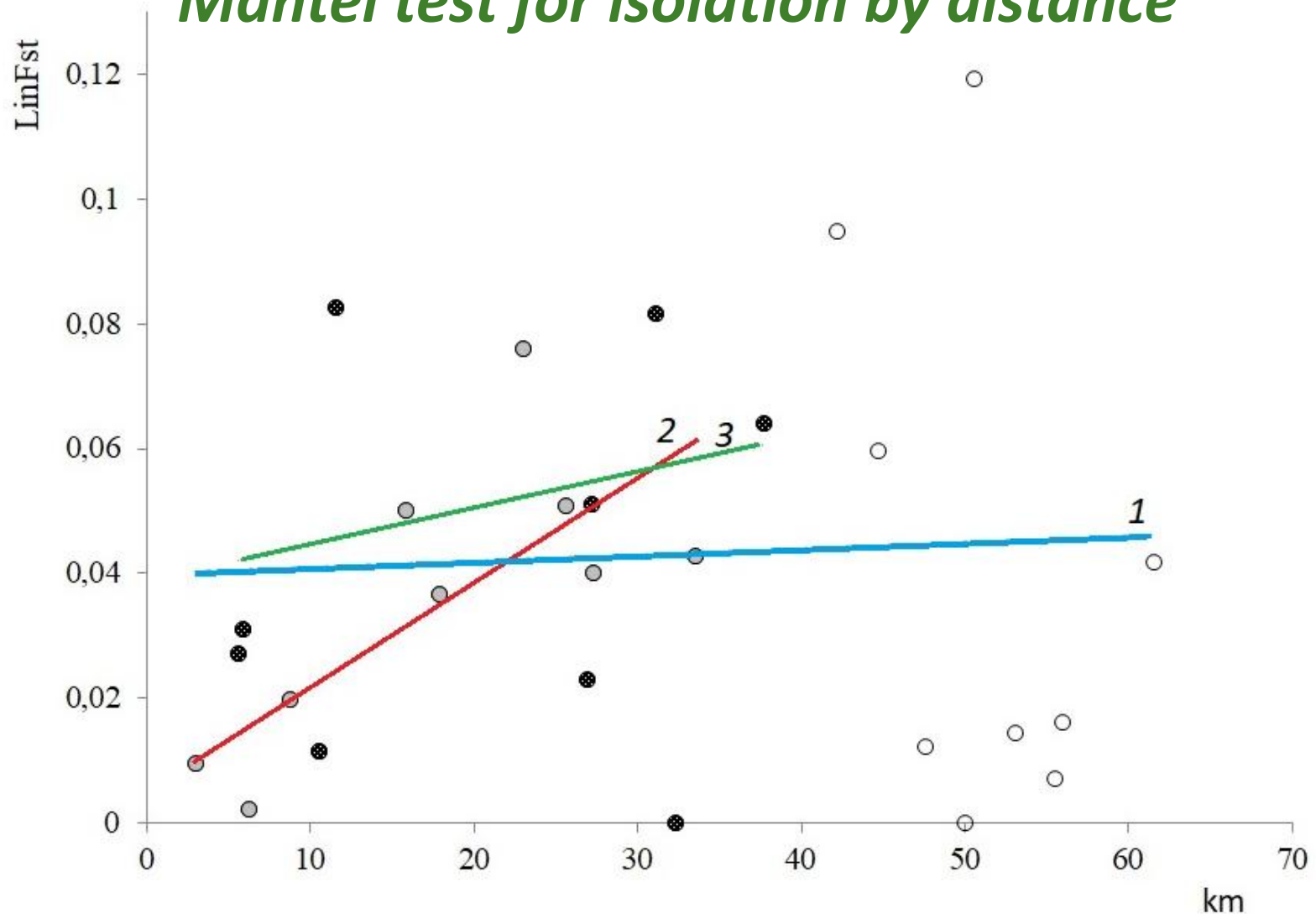
RESULTS

P. major genetic diversity parameters from different zones

Site	Na	Ne	Ho	He	F	PA
Background-1	5.44	3.09	0.16	0.45	0.66	5
Background-2	4.67	2.84	0.23	0.5	0.56	3
Mean±SE	5.06±0.39	2.97±0.13	0.20±0.04	0.48±0.03	0.61±0.05	4.00±1.00
KCS-8	4.11	2.88	0.2	0.50	0.59	2
KCS-5	4.33	2.87	0.21	0.51	0.59	2
KCS-1	3.78	2.33	0.1	0.45	0.77	0
Mean±SE	4.07±0.2	2.69±0.18	0.17±0.04	0.49±0.02	0.65±0.06	1.33±0.67
EURT-16	4.00	2.54	0.23	0.52	0.55	1
EURT-10	4.11	2.59	0.28	0.50	0.44	0
EURT-5	4.11	2.71	0.22	0.48	0.54	2
Mean±SE	4.07±0.04	2.61±0.05	0.24±0.02	0.50±0.01	0.51±0.04	1.00±0.58

Na – mean number of alleles, Ne – effective number of alleles,
Ho – observed heterozygosity, He – expected heterozygosity,
F – inbreeding coefficient, PA – number of private alleles.

Mantel test for isolation by distance



- 1) all populations: $R^2 = 0.0026$, $p=0.361$;
- 2) background and KCS zone, gray dots: $R^2 = 0.5463$; $p=0.032$;
- 3) background and EURT, black dots: $R^2 = 0.027$; $p=0.313$

Conclusions

The first tested hypothesis was not confirmed: the P. major genetic diversity in the EURT zone was lower than in the background populations, which possibly results from the reduced migration of seeds (genes) in EURT populations due to limited access of people to this territory. Probably, the frequency of occurrence of radiation-induced mutations in plants with existing dose loads is not sufficient to compensate for the loss of genetic diversity resulting from isolation.

The second tested hypothesis was confirmed: in the KCS zone P. major genetic diversity was decreased, especially at the most polluted site. Despite the constant flow of seeds (genes) into the population within this plot, not all migrants are able to survive in conditions of high soil contamination.

Thus, the reduction of genetic diversity in the P. major populations in zones of radioactive and chemical contamination compared to background plots occurs due to various reasons.

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Thank you for your attention!