

Recent Levels of Radionuclide Contamination in the Eastern Ural Radioactive Trace and Biological Effects in Local Populations of *Plantago major* L.

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Abstract—The results of the study have shown that the stocks of ^{90}Sr and ^{137}Cs in the frontal part of the EURT are 6700–15000 and 200–400 kBq/m², respectively. The coefficients of their accumulation in different herbaceous plant species in this impact zone are lower than in other areas. The accumulative capability of plants has probably decreased in the course of selection as an adaptation to the effect of radiation. The viability and growth parameters of *Plantago major* L. seeds from the EURT zone are similar to those of seeds from the background sample. Cenopopulations chronically exposed to radiation are characterized by a higher mutation rate in the progeny and an unstable response to additional acute irradiation in most characters studied. A high level of inbreeding is observed in all *P. major* cenopopulations of plantain. The cenopopulation of the impact zone is characterized by a lower variation of allozyme loci, which may be a result of stringent selection for individuals better adapted to the complex of conditions existing in its habitat.

Key words: Eastern Ural Radioactive Trace, radionuclides, cenopopulations, *Plantago major* L., viability, radiosensitivity, mutability of seed progeny, allozyme structure.

The Kyshtym accident at the Mayak Production Association in 1957 resulted in radionuclide discharge that formed the Eastern Ural Radioactive Trace (EURT). Nine years later, the Eastern Ural State Reserve (EUSR) was established in the frontal part of this trace and actually became the test ground for experiments in nature, which had no analogs in the world. For reasons of secrecy, only fragmentary data were included in accessible publications, usually without indicating the place and conditions of its contamination. General data were published only in the 1990s (*Itogi...*, 1990; *Ekologicheskie posledstviya...*, 1993; *Posledstviya...*, 2002; *Opytnaya nauchno-issledovatel'skaya...*, 2003). The RF State Program on Radiation Rehabilitation of the Ural Region (1993) initiated open studies in the EURT zone, in which the authors of this article took an active part. Their results were published (Aarkrog *et al.*, 1997; Molchanova and Karavaeva, 2001; Pozolotina, 2003).

The purpose of this work was to evaluate recent levels and the pattern of distribution of radionuclides in the soil and plant cover along the contamination gradient, to study specific features of common plantain (*Plantago major* L.) cenopopulations from the EURT zone, and to estimate the ecological-genetic pathways of their adaptation to chronic radiation exposure.

MATERIAL AND METHODS

The EURT area was provisionally divided into two zones: the impact zone that covered the territory of the EUSR and the buffer zone extending along the EURT central axis. The background plot was beyond the contaminated zone.

The impact plot was 13 km away from the accident epicenter (55°46' N, 60°53' E) on the Lake Berdyanish shore and on the territory of a village abandoned after the accident. Background gamma radiation varied from 14 to 75 $\mu\text{R}/\text{h}$, with single measurements reaching 154 $\mu\text{R}/\text{h}$; background beta radiation varied from 83 to 475 cpm/cm².

Subaquatic primitive, poorly sodded soils of light texture prevailed on the lake shore. A drop in elevation between the shore and catchment area reached 2 m in some places. The catchment area was open, 2–2.5 km² in size, and bordered a birch forest. The soil cover in the village consisted of native and anthropogenically disturbed old-arable gray and dark gray forest soils. The type of plant community was classified as a secondary upland herb–grass meadow with a stratified herbaceous cover (20 cm to 1.5 m tall) and 100% coverage. Bearded couch (*Elytrigia canina* (L.) Drob.) and awnless brome (*Bromopsis inermis* (Leyss.) Holub.) were dominant, but large areas were also covered by great nettle (*Urtica dioica* L.) and ruderal

hemp (*Cannabis ruderalis* Janisch). Common plantain did not form a continuous cover.

To evaluate the levels of soil contamination with radionuclides, soil pits were dug in duplicate or triplicate on the lake shore, at different distances from the water edge, as well as in the central part of the treeless site, in the forest, and on the forest margin. The samples were taken layer-by-layer to a depth of 50–60 cm, with regard to the area and boundaries of genetic soil horizons. The samples of herbage and some species of herbaceous plants were taken in the immediate vicinity of soil pits. The sampling procedure in other plots was the same.

The buffer plot was 86 km away from the accident epicenter, near Lake Tygish (56°22' N, 61°35' E). The soil cover was represented by gray forest soils. The plant community was classified as a secondary upland herb–grass meadow. The herbaceous cover was stratified and thinned. Dominant species were awnless brome (*Bromopsis inermis* (Leys.) Holub.), coach (*Elytrigia repens* L.), European wood strawberry (*Fragaria vesca* L.), dandelion (*Taraxacum officinale* s.l.), and common yarrow (*Achillea millefolium* L.). Common plantain did not form a dense cover. Gamma radiation in this plot was 15–21 μ R/h.

The background plot was in an old arable field (56°41' N, 61°02' E). Soil was soddy podzolic. The plant community was classified as a secondary upland herb–grass meadow. The herbaceous cover was stratified, with 95% coverage. Stickseed (*Lappula squarrosa* (Retz.) Dumort.), mugwort wormwood (*Artemisia vulgaris* L.), bay willow (*Chamaenerion angustifolium* (L.) Rafin.), and dandelion (*Taraxacum officinale* s.l.) were dominant. Common plantain grew in clumps. Gamma radiation in the plot was 5–9 μ R/h.

Concentrations of ^{90}Sr in all samples were determined radiochemically, from the content of daughter ^{90}Y , and ^{137}Cs was determined using a Canberra multi-channel gamma-analyzer with a semiconductor detector. The statistical error of measurements did not exceed 15%, and the radionuclide detection limit was 1 Bq/kg.

Common plantain has been chosen as a test object because it grows in all the plots, is fairly sensitive to irradiation, and has not been involved in previous radioecological research. It is a perennial diploid species ($n = 6$) with sexual reproduction, mainly through self-pollination (Van Dijk *et al.*, 1988). The dose loads on meristematic tissues were calculated from data on radionuclide concentrations in the upper 5-cm soil layer.

To study variation in *P. major* seed viability, the seeds were individually collected from 10 plants in each cenopopulation and placed in filter paper rolls, in which they germinated for 30 days. The following parameters were recorded: seed vigor, germination rate, survival of seedlings, number of plants with a true leaf, and root length and the frequency of morphological disturbances in seedlings. To estimate radioresis-

tance of the seed progeny of plants, the seeds from each cenopopulation were additionally irradiated at doses of 100, 200, and 300 Gy and analyzed as described above. The results were processed statistically using the Excel and STATISTICA 6.0 programs.

To study the allozyme structure of cenopopulations, the seeds were collected from the plants growing 3–14 m apart along 500- to 800-m transects, 50–70 plants per cenopopulation. An analysis of ADH (EC 1.1.1.1), EST (EC 3.1.1.1), GDH (EC 1.4.1.2), GOT (EC 2.6.1.1), 6-PGDH (EC 1.1.1.44), PGI (EC 5.3.1.9), SDH (EC 1.1.1.25), DIA (EC 1.8.1.4), IDH (EC 1.1.1.42), and PGM (EC 5.4.2.2) allozymes in seedlings was performed using electrophoresis in 6.4% PAAG in a Tris–EDTA–borate system (Peacock *et al.*, 1965), with histochemical staining by conventional methods (Harris and Hopkinson, 1976). The following parameters were calculated for the loci studied: allele frequencies (p); average number of alleles per locus (N_A); average heterozygosity, observed (H_O) and expected (H_E); and the number of loci with a significant deviation of genotype frequencies from the expected distribution (N_{HW}). Interpopulation differences were evaluated using Wright's F-statistics (Wright, 1951), coefficients of genetic distances (Nei, 1973), and contingency tables. Statistical data processing was performed with the BIOSYS-1 program (Swofford and Selander, 1981).

RESULTS AND DISCUSSION

Levels of soil and plant contamination. The impact plot was characterized by a high heterogeneity of radionuclide contamination (Table 1). The total amount of the primary contaminant ^{90}Sr was significantly higher than that of ^{137}Cs . The lowest stocks and ratios of radionuclides were recorded in the nearest, regularly flooded zone of the lake shore. Contents of radionuclides and the $^{90}\text{Sr}/^{137}\text{Cs}$ ratio in the soil increased with distance from the lake, with the ^{90}Sr stock in the soil approaching or exceeding that in the catchment area at a distance of only 20 m from the water edge. The greatest amount of ^{90}Sr in such areas was recorded in the old-arable native soil of the upland meadow.

Both the expansion of forests to the contaminated area and its anthropogenic disturbances proved to entail a decrease in the ^{90}Sr stock. In forests, this may be due to the fact that a certain part of contaminants is firmly fixed by tree bark (Tikhomirov, 1972). The smallest amount of ^{137}Cs was detected in anthropogenically disturbed soils. An analysis of radionuclide distribution over the soil profile showed that the bulk of radionuclides, up to 99%, was still retained in the 20-cm root layer 47 years after the accident, with 13–20% ^{90}Sr occurring beyond this layer only in the subaquatic soils of the lake shore and the soils exposed to anthropogenic impact.

Table 1. Radionuclide stocks in the soils of impact zone ecosystems, kBq/m²

Zone	Plot	⁹⁰ Sr	¹³⁷ Cs	⁹⁰ Sr/ ¹³⁷ Cs
Impact	Lake Berdyanish shore, distance from water edge, m:			
	4	270	58	4.6
	10	5036	300	16.7
	20	16690	700	23.9
	Upland herb-grass meadow:			
	native	14900	373	40.0
	anthropogenically disturbed	6740	201	33.5
Forest margin	Birch forest	10606	412	25.7
	Birch forest	8157	309	26.3
Buffer	Birch grove margin	20	10	2.0
	Birch grove	34	14	2.4
Background	Forest margin	2.5	4.5	0.6
	Old arable field	3.0	8.0	0.4

The results of radioecological studies in the buffer zone were described in detail previously (Aarkrog *et al.*, 1997; Molchanova and Karavaeva, 2001). Against the background of a general decrease in radionuclide stocks with an increase in the distance from the accident epicenter, a complicated pattern of the spatial distribution of radionuclides was observed. Nevertheless, the contents of radionuclides in soils along the central EURT axis decreased exponentially from 1500 to 20 kBq/m² for ⁹⁰Sr and from 100 to 8 kBq/m² for ¹³⁷Cs. In the plot chosen in the buffer zone, the total stock of radionuclides in soil was two to three orders of magnitude smaller (Table 1), and the ⁹⁰Sr/¹³⁷Cs ratio was lower due to additional ¹³⁷Cs input with contaminated silt and sand brought by winds from the Lake Karachai shore and to a relatively high mobility of ⁹⁰Sr in the ecosystem (Aarkrog *et al.*, 1997). In the background plot, soil contamination with ⁹⁰Sr resulted mainly from global fallout, and additional amounts of ¹³⁷Cs probably appeared after the Karachai or Chernobyl accidents.

A comparative analysis of the accumulative capacity of herbaceous plants along the radionuclide contamination gradient showed that ⁹⁰Sr concentrations in the above-ground phytomass in the impact zone exceeded those in the buffer and control plots by a factor of more than 1000 (Table 2), with the difference for ¹³⁷Cs being smaller. Concentrations of ⁹⁰Sr in plants were higher in the buffer than in the control plot: according to Student's *t*-test, differences in pairwise comparisons were statistically significant ($p = 0.05$). Of all the species studied, great nettle accumulated the greatest amounts of ⁹⁰Sr and ¹³⁷Cs. Common plantain is also capable of increased ⁹⁰Sr accumulation, which makes it a convenient test object for radiobiological research.

The accumulative capacity of different plant species was compared using the accumulation coefficient (AC), a formal criterion (see Table 2). Accumulation coefficients for ¹³⁷Cs were 5–10 times lower than those for ⁹⁰Sr in all cases, being more or less similar in all plots studied. It can be seen that the accumulation coefficients for ⁹⁰Sr in all species were significantly lower in the impact zone than in the buffer and background zones, being similar close in the same species growing in the last two zones. In our opinion, the fact that the accumulation coefficients for ⁹⁰Sr, the main pollutant, were lower in the impact zone is very important. It may well be that the plants have acquired this feature in the course of selection under the prolonged impact of the radiation factor. A decreased accumulative capacity allows the plants exposed to ionizing radiation to more effectively protect their above-ground parts and, in particular, the most sensitive reproductive organs. The mechanisms reducing the accumulative capability are different (Il'in and Syso, 2001), and more data are necessary for their adequate analysis.

Due to differences in growing conditions, radiation loads on plants differ as well. Below, radiation loads and the response of cenopopulations to them are considered using the example of our test object, *P. major*.

Evaluation of radiation loads. Calculating the dose of radiation received by the plant, we proceeded from the premise that the most sensitive meristematic tissues in *P. major* virtually do not extend above the soil surface. Hence, it was acceptable to use a simple model assuming that the growing point is within a homogeneously contaminated soil volume. The contributions of ⁹⁰Sr + ⁹⁰Y and ¹³⁷Cs were estimated separately. The absorbed dose rate was calculated as $M = q_1 L_{(^{90}\text{Sr} + ^{90}\text{Y})} + q_2 L_{(^{137}\text{Cs})}$, where q_1 and q_2 are the specific activities of each radionuclide measured in the surface soil layer,

Table 2. Concentrations and accumulation coefficients of radionuclides in the air-dry phytomass of some plant species

Plot	Species	⁹⁰ Sr		¹³⁷ Cs	
		Bq/kg	AC	Bq/kg	AC
Impact	<i>Urtica dioica</i> L.	134148	1.5	960	0.4
	<i>Plantago major</i> L.	71600	0.8	149	0.06
	<i>Rumex confertus</i> Willd.	51230	0.6	458	0.2
	<i>Tanacetum vulgare</i> L.	34850	0.4	283	0.1
	<i>Achillea millefolium</i> L.	20190	0.2	190	0.1
	Meadow herbs	18260	0.2	144	0.06
	Forest herbs	53460	0.7	218	0.08
Buffer	<i>Urtica dioica</i> L.	150	8.8	5	0.1
	<i>Plantago major</i> L.	40	13.0	N.a.	N.a.
	<i>Rumex confertus</i> Willd.	37	3.4	3	0.1
	<i>Solochloa festucacea</i> (Willd.) Link.	22	1.1	3	0.1
	Meadow herbs	29	1.2	6	0.1
Background	<i>Urtica dioica</i> L.	57.5	5.7	1	0.1
	<i>Plantago major</i> L.	13.0	1.6	2	0.1
	<i>Rumex confertus</i> Willd.	7	1.0	4	0.4
	<i>Galium verum</i> L.	20	3.4	6	0.4
	<i>Filipendula umaria</i> (L.) Maxim.	7	1.0	2	0.1
	Forest herbs	20	3.4	7	0.4

Table 3. Concentrations of ⁹⁰Sr and ¹³⁷Cs in the 5-cm soil layer and resulting radiation loads on *P. major* meristematic tissues

Zone	Plot	Concentration, Bq/kg		Dose rate, mR/h	Annual dose, mGy
		⁹⁰ Sr	¹³⁷ Cs		
Impact	Upland herb-grass meadow: native	113135	5141	3813	334.0
	anthropogenically disturbed	122750	5516	4135	362.2
	Birch forest margin	141550	7040	4792	419.7
Buffer	Birch grove	646	314	31.7	2.8
Background	Old arable field	15	31	1.6	0.14

and L is the absorbed dose rate (cGy/s) produced by this radionuclide within the homogeneously contaminated volume at $q_0 = 3.7 \times 10^4$ Bq/g (Gorshkov, 1967). It follows from Table 3 that the contributions of artificial radionuclides to the radiation load in the buffer and impact zones of the EURT were, respectively, 20 and 2600 times higher than in the background plot. The longest individual life span in *P. major* is close to 20 years, and the total radiation doses absorbed by plants over this period amount to approximately 7 Gy for the impact cenopopulation, 0.06 Gy for the buffer cenopopulation, and 0.003 Gy for the background cenopopulation.

Viability of seed progeny. The highest indices of viability were recorded for the background cenopopulation.

Table 4 shows the averaged data on ten plants whose seeds germinated individually. According to the results of statistical analysis (Fisher's exact test), the viability of seeds from the buffer sample was minimal ($N_{\text{buf}} = 1500$, $p = 0.0026$). Significant differences between plants from the impact and background samples were revealed with respect to seed vigor, germination capacity, and the survival of seedlings ($N_{\text{imp}} = 1500$ and $N_{\text{bg}} = 1500$; $p = 0.0075$). The impact sample was close to the background sample in the number of seedlings with true leaves ($p = 0.33$) and in root length ($p = 0.66$).

Individual variation in the viability in seed progeny was greater in chronically irradiated samples: the variation coefficient for seedling survival in the background cenopopulation was only 16.5%, compared to 50.5 and

Table 4. Viability of *P. major* seed progeny

Cenopopulation	Seed vigor	Germination capacity	Survival	Number of seedlings with leaves	Root length, mm
	%				
Background	8.3 ± 1.1	64.3 ± 6.1	56.8 ± 7.4	9.1 ± 4.5	13.1 ± 1.4
Buffer	1.9 ± 1.3	35.1 ± 12.9	33.1 ± 10.8	4.1 ± 2.4	9.8 ± 1.7
Impact	5.8 ± 0.8	52.3 ± 7.8	48.6 ± 7.6	7.5 ± 3.2	12.7 ± 1.6

19.5% in the buffer and impact cenopopulations, respectively.

Radiosensitivity of seeds. Additional acute irradiation of seeds in a wide dose range was used to estimate the adaptive potentials of plants from different populations. Interesting data were obtained on the survival of seedlings depending on radiation dose, with absolute values being recalculated into proportions (%) relative to the internal nonirradiated control (Fig. 1a). In the background sample, this dependence was described by a classic S-shaped curve. For the buffer sample, a zigzag dose-effect curve was obtained, and an equally paradoxical dose-effect relationship was characteristic of the impact sample: the survival of seedlings was the same as in the

control upon irradiation at the largest dose but proved to be very low at smaller doses. These results may be indicative of a high instability of the plant genome under conditions of chronic radiation exposure.

It was interesting to study the effect of radiation on the rate of growth processes in plants from different cenopopulations. The background and buffer samples were characterized by similar dose-effect relationships by the "root length" parameter: the growth of roots was suppressed upon irradiation in both cases (Fig. 1b), and this suppression was stronger in the buffer cenopopulation ($p = 0.004-0.008$). In seedlings of the impact sample, root length upon irradiation at any dose was similar to that in the nonirradiated control. This fact may be regarded as evidence for a preadaptation of seeds from the impact cenopopulation to radiation impact.

Taking into account all the variety of anomalies in the development of plantain seeds (changes in the color of seed lobes and leaves, deformations of all organs, and necroses), we found that the proportion of seedlings with anomalies was significantly higher in chronically irradiated cenopopulations than in the background cenopopulation. For example, the background frequency of seedlings with root necroses was 0.5%, but necroses in the buffer and impact samples were found 50 and 100 times more often ($p = 0.00001-0.00015$). The limits of variation in this parameter in plants of the background cenopopulation were 0.1-0.8%, compared to 13.8-40.8 and 6.4-93.3% in the buffer and impact cenopopulations, respectively.

The cenopopulations from the EURT zone markedly differed in the character of root necrosis development. For example, most disturbances in the buffer samples were concealed and manifested themselves only upon additional acute irradiation: the number of necroses increased by a factor of three relative to the internal nonirradiated control (Fig. 2). In the impact cenopopulation, conversely, the proportion of seedlings with root necroses was high (about 55%) both in the control and in variants with irradiation.

Thus, *P. major* cenopopulations in the contamination gradient significantly differed both in the pattern of radionuclide accumulation in the biomass and in viability, mutation rate, and radiosensitivity of the seed progeny, which could be due to differences in their genetic structure. To reveal these differences, we performed an analysis of the allozyme patterns in these cenopopulations.

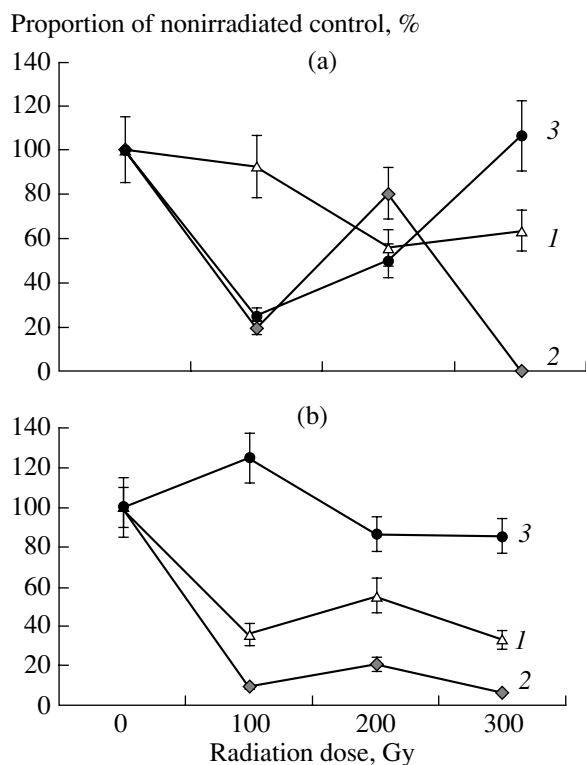


Fig. 1. Changes in (a) survival and (b) root length of *P. major* seedlings from (1) background, (2) buffer, and (3) impact cenopopulations depending on the dose of additional acute irradiation.

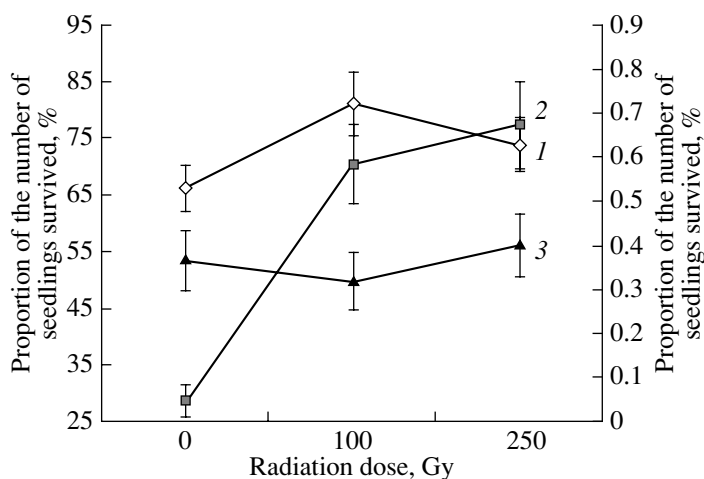


Fig. 2. Proportions of *P. major* seedlings with root necrosis in (1) background, (2) buffer, and (3) impact cenopopulations depending on the dose of additional acute irradiation. The right ordinate shows values for the background cenopopulation.

Polymorphism of enzyme systems in *P. major* cenopopulations. Three allozyme loci appeared to be polymorphic: *Adh* (two alleles), *Got-1* (two alleles), and *Got-2* (three alleles); the remaining loci were monomorphic. The ratio of *Adh* alleles was balanced only in the background cenopopulation (Table 5). The frequencies of allele *Adh*₃ in chronically exposed samples, being similar to one another ($p = 0.1909$), significantly exceeded the background level ($p = 0.0010$ – 0.0162). The background sample was close to the impact sample in the frequency of the *Got-1*₂ allele ($p = 0.1059$), differing from the buffer sample ($p = 0.0000$). The background and buffer samples were similar in the frequency of the *Got-2*₂ allele but differed from the impact sample in this parameter ($p = 0.0002$). In the aggregate, these data indicate that the impact and background cenopopulations are close to one another, with the genetic distance between them being $D = 0.005$ (Nei, 1973). Consequently, it may well be that the polymorphism of two *Got* loci is independent of the radiation factor.

An analysis of contingency tables revealed significant differences between the cenopopulations in the frequencies of loci *Adh* and *Got-2* (Table 5). The difference between the background and impact cenopopulations in *Got-1* frequency was insignificant. Having calculated the expected genotype frequencies in the background and buffer cenopopulations, we found that the observed frequencies, irrespective of the locus, never corresponded to the Hardy–Weinberg equilibrium. However, no deviations from this equilibrium were observed in the impact sample, which could be due to the absence of homozygotes with the high-mobility allele at the two *Got* loci and a small number of heterozygotes carrying this allele. Significant differences between the expected and observed genotype frequencies were revealed only at locus *Adh* (Table 5).

Using the data on allozyme allele frequencies, we calculated the main parameters of genetic variation in *P. major* cenopopulations (Table 6). The average number of alleles per locus (N_A) was 1.30. A similar value (1.24) was determined earlier for the American and European populations of common plantain (Van Dijk *et al.*, 1988). This number in the background sample ($N_A = 1.36$) was greater than in other samples, because the *Got-1*₃ allele occurred only in the background population. The observed heterozygosity was also higher in the background cenopopulation. According to Van Dijk *et al.* (1988), average heterozygosity in *P. major* is 0.047, which agrees well with the value determined for the background sample.

The smallest and largest proportions of polymorphic loci (at 95% significance level) were revealed in the impact and buffer cenopopulations, respectively. A similar situation was observed when we calculated the effective number of alleles. The proportion of polymorphic loci in the impact sample at P_{99} was three times greater than that at P_{95} , providing evidence for the presence of rare alleles in this sample (Table 6).

In all cenopopulations studied, the observed heterozygosity (H_O) was 1.4–3.7 times lower than the expected heterozygosity (H_E), which was indicative of a high coefficient of inbreeding. On the one hand, this phenomenon may be explained by the fact that individuals from different subpopulations were combined in the sample; on the other hand, this may be a consequence of selection in favor of homozygotes in *P. major* cenopopulations. The latter assumption is confirmed by Wright's fixation index (Wright, 1951): the genotypes homozygous at all loci prevail in the background and buffer cenopopulations ($F_{Wt} = 0.343$), and homozygotes at locus *Adh* prevail in the impact cenopopulation ($F_{Wt} = 0.359$). Inbreeding coefficient relative to cenopopulation as a whole (F_{ST}) is 53.5%, with the index of

Table 5. Allele frequencies at three polymorphic loci in the upland *P. major* cenopopulations from the EURT zone

Locus	Allele	Cenopopulation			Results of analysis of contingency tables
		background (1)	buffer (2)	impact (3)	
Adh	2	0.500	0.225	0.323	$\chi^2_{1,2} = 15.87, df = 2, p = 0.0014$
	3	0.500	0.775	0.677	$\chi^2_{1,3} = 10.54, df = 2, p = 0.0051$
	<i>N</i>	96	60	82	$\chi^2_{2,3} = 8.99, df = 2, p = 0.0111$
	χ^2_{HW}	$\chi^2 = 38.13; df = 1; p = 0.0001$	$\chi^2 = 36.06; df = 1; p = 0.0001$	$\chi^2 = 10.96; df = 1; p = 0.001$	
Got-1	2	0.047	0.333	0.010	$\chi^2_{1,2} = 26.49, df = 2, p = 0.0000$
	3	0.953	0.667	0.990	$\chi^2_{1,3} = 3.42, df = 2, p = 0.1806$
	<i>N</i>	96	60	96	$\chi^2_{2,3} = 36.04, df = 2, p = 0.0000$
	χ^2_{HW}	$\chi^2 = 18.97; df = 1; p = 0.0001$	$\chi^2 = 44.40; df = 1; p = 0.0001$	$\chi^2 = 0.005; df = 1; p = 0.942$	
Got-2	2	0.141	0.250	0.031	$\chi^2_{1,2} = 10.19, df = 4, p = 0.0374$
	3	0.792	0.750	0.969	$\chi^2_{1,3} = 23.89, df = 4, p = 0.0001$
	<i>N</i>	0.068	0.000	0.000	$\chi^2_{2,3} = 23.16, df = 2, p = 0.0000$
	χ^2_{HW}	96	60	96	
	2	$\chi^2 = 31.16; df = 3; p = 0.0001$	$\chi^2 = 19.30; df = 1; p = 0.0001$	$\chi^2 = 0.083; df = 1; p = 0.774$	
Sum total by loci		$\chi^2 = 88.26; df = 5; p = 0.0001$	$\chi^2 = 94.76; df = 3; p = 0.0001$	$\chi^2 = 11.05; df = 3; p = 0.002$	

Note: *N* is sample size, χ^2_{HW} is deviation of genotype distribution from the Hardy–Weinberg equilibrium.

Table 6. Parameters of genetic variation in allozyme loci in *P. major* cenopopulations

Cenopopulation	P ₉₅ , %	P ₉₉ , %	N _A	H _O	H _E	<i>n_e</i>
Background	18.18	27.27	1.36 ± 0.20	0.043 ± 0.025	0.086 ± 0.052	1.09
Buffer	27.27	27.27	1.27 ± 0.14	0.029 ± 0.017	0.107 ± 0.056	1.12
Impact	9.09	27.27	1.27 ± 0.14	0.033 ± 0.025	0.047 ± 0.040	1.05
Average	18.18	27.27	1.30 ± 0.16	0.035 ± 0.022	0.080 ± 0.049	1.09

Note: P₉₅ and P₉₉ are the proportions of polymorphic loci at 95% and 99% significance levels; N_A is the average number of alleles per locus; H_O and H_E are the observed and expected values of average heterozygosity; *n_e* is the effective number of alleles. Standard errors are shown for N_A, H_O, and H_E.

inbreeding per individual relative to the species (*F_{IT}*)—57.4%. The index of inbreeding per population relative to species as a whole (*F_{ST}*) shows that genetic variation is observed mainly at the intrapopulation level (91.5%), with only 8.5% being distributed among cenopopulations.

The level of gene flow calculated on the basis of the *F_{ST}* index was low, approximately three migrants per generation. It should be noted that the *F_{ST}* value for European and American populations of *P. major* L. averages 0.232 (Morgan-Richards and Wolff, 1999) or, according to other data, 0.216 (Van Dijk *et al.*, 1988).

Table 7. Indices of Wright's E-statistics for *P. major* cenopopulations from the EURT zone

Locus	F _{IS}	F _{IT}	F _{ST}
Adh	0.5611	0.5853	0.0552
Got-1	0.7045	0.7600	0.1879
Got-2	0.3852	0.4257	0.0659
Average for three loci	0.5347	0.5742	0.0848

It is probable that higher values of F_{ST}, compared to those from the Ural populations, may be due to geographic variation of plantain.

We determined the genotypes of plants with respect to the combination of three polymorphic loci. There were a total of 20 genotypes: 17 in the background cenopopulation, 13 in the buffer cenopopulation, and 6 in the impact cenopopulation. The plants with different homozygous genotypes dominated in all cenopopulations. This is evidence that the genotypic diversity in *P. major* cenopopulations decreases along the ascending gradient of radionuclide contamination, and a certain genotype gains increasing dominance. Similar data were previously obtained for dandelion cenopopulations, in which clonal diversity also decreased, whereas the proportion of rare enzyme morphs increased along a contamination gradient (Ul'yanova and Pozolotina, 2004). As a rule, selection in favor of radioresistant individuals is more active in populations of radiosensitive species (including *P. major*) than in populations of radioresistant species (Shevchenko and Pomerantseva, 1985). This fact may account for the decreased genotypic variation in the impact sample and the effect of preadaptation observed upon acute irradiation of the seed progeny.

Thus, the results obtained in the impact part of the EURT show that the stocks of ⁹⁰Sr and ¹³⁷Cs in the root layer of gray forest soils prevailing in this area amount to 6700–15000 and 200–400 kBq/m², respectively, concentrating in the 20-cm soil layer. Accordingly, the concentrations of ⁹⁰Sr in different species of herbaceous plants in the impact plot are three orders of magnitude higher, whereas the accumulation coefficients are lower than in the buffer and background zones. The accumulative capability of plants has probably decreased in the course of selection as an adaptation to chronic radiation exposure.

The study of *P. major* cenopopulations in the gradient of radionuclide concentrations showed that the seed progeny of plants from the impact cenopopulation was highly similar to that from the background cenopopulation in viability and growth parameters, but these parameters in the buffer cenopopulation were low. The range of individual variation and the mutation rate in the seed progeny proved to be increased in both chronically irradiated cenopopulations. Their response to additional acute irradiation was unstable with respect to

most characters studied. Additional radiation exposure significantly reduced the rate of growth processes in seedlings from the buffer cenopopulation, whereas the corresponding parameters in the impact sample were the same as in the nonirradiated control.

The data on the allozyme structure show that all *P. major* cenopopulations are deficient in heterozygous genotypes, but this deficiency is less pronounced in the impact sample. Variation in allozyme loci is maximal in the buffer zone and minimal in the impact zone. In general, genotypic variation decreases in the ascending gradient of radiation load. This may be a consequence of stringent selection in the impact cenopopulation in favor of individuals better adapted to the complex of conditions existing in its habitat, including a high level of background radiation.

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