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RESEARCH ARTICLE

Genetic variation in natural *Melandrium album* populations exposed to chronic ionizing radiation

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Abstract The effect of radiation pollution on genetic variation in natural populations of *Melandrium album* was investigated at the head part of the East-Ural Radioactive Trace (EURT) and background areas. The highest genetic differentiation estimated using F_{ST} was revealed between compared pairs of the background and impact samples in populations of *M. album*. The highest rate of polymorphism was observed at the closest to nuclear accident, Impact-1 site. The unique alleles (*Mdh*-3^{104}, *Pgi*-2^{106}, *Lap*^{105}, *Mdh*-2^{96}, and *Dia*^{94}) were discovered at the EURT. Individuals from chronically low-level irradiated sites were genetically closer than to plants from background sites using *Nadhdh* locus. The increase of the frequency of unique homozygous and heterozygous genotypes was identified in populations of *M. album* growing under chronic radiation exposure conditions. The largest contribution to the group of unique heterozygous genotypes at the EURT was made by three loci – *Lap*, *Pgi*-2, and *Nadhdh*; the main role in interpopulation differentiation of samples was made by the alleles *Sod*-2^{115}, *Skdh*^{100}, and *Nadhdh*^{100}. Our results provide evidence for the correlation between the increase of genetic variation other than the «genetic erosion» and chronic radiation exposure factor in natural plant populations.

Keywords Kyshtym accident · Low-level radiation · *Melandrium album* (=*Silene latifolia*) · Genetic variation · Allozymes · Differentiation · Population structure

Introduction

Dramatic environmental changes are often associated with human activities (DiBattista 2008); and damaging effect of technogenic pollution is a stress-selective factor leading to the death of less-resistant specimens which induces changes in genetic diversity (e.g., proportion of polymorphic loci, number of alleles per locus, effective number of alleles, and expected heterozygosity) that may be determined based on allozyme studies (Chudzinska et al. 2014). Analysis of the variability of allozymes provides a convenient method to investigate genetic processes in plant populations; it is used to detect biological effects in natural populations chronically exposed to radiation after nuclear accidents (Geras'kin and Volkova 2014; Kalchenko et al. 1995a; Lysenko et al. 2000; Pozolotina et al. 2010; Shevchenko et al. 1998; Ul'yanova and Pozolotina 2004). The main role of genetic polymorphism of proteins may be associated with the processes of adaptation to temperate and local environmental factors, while monomorphic proteins provide species-specific constant of internal environment (Altukhov and Rychkov 1972).

Enhanced ionizing radiation in the environment can affect genetic structure of natural populations in many ways with potentially large long-term consequences. Theoretically, there are different ways in which contaminants can affect genetic variation: e.g. by increasing mutation rates, by directional selection of tolerant genotypes, by causing bottleneck events; thus resulting in increase of the rate of dispersion of genetic parameters keeping mean values unchanged, in loss («genetic erosion») or, in contrast, increase of genetic diversity (Prus-

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Glowacki et al. 1999; van Straalen and Timmermans 2002). Plants are expected to be especially affected by anthropogenic pollution (including radioactive material after accidents) because of their strong interaction with their immediate environment (Navarro et al. 2008) and mostly stationary lifespan scenario (Karimullina et al. 2015).

The Kyshtym accident took place at the Mayak production association in the Southern Urals in 1957. As a result, 7.4 PBq, which was 10 % of radioactive waste total volume, of man-made radionuclides have been released to the environment in the North-East direction and dispersed within a narrow territory of 23,000 km^2 (Romanov et al. 1990). According to the International Nuclear Event Scale (INES 2013), Kyshtym accident was recognized as serious accident (level 6). The Chernobyl accident occurred years later and the accident at the Fukushima-1 nuclear power plant in 1986 and 2011, respectively. Both events have been assigned as level 7 (major accident).

At present, the East-Ural Radioactive Trace (EURT) is a test site where living organisms have been exposed to emergency ionizing radiation for over half a century (Antonova et al. 2015; Pozolotina et al. 2010), representing an experimental ground suitable for investigation of effects of chronic low-level ionizing radiation in natural populations.

We have previously investigated viability, mutability, and radiosensitivity in seed progeny of white campion (*Melandrium album*) growing protractedly under chronic irradiation conditions (Antonova et al. 2013). It was shown that the interannual dynamics of survival of white campion seedlings was largely dependent on weather conditions during the formation of seeds. These effects were particularly pronounced in chronically irradiated samples, which may indicate a synergistic effect of interaction of radiation with other environmental factors. The number of abnormalities in the development of *M. album* seedlings was higher on EURT samples compared to background levels.

In the present study, we examined whether radionuclide pollution influence genetic variation in radiation-stressed natural plant populations of *M. album* using allozyme markers. To analyze the correlation between genetic parameters and total radiation absorbed whole-body doses, we assessed the radiation dose on the *M. album* populations using ERICA Tool (Beresford et al. 2007); within- and among-population genetic variation parameters were estimated conducting analysis of contingency tables, pair wise comparison of samples using F_{ST}, frequencies of alleles, genetic distance, and distribution of homo- and heterozygous genotypes.

The following hypotheses were tested: (1) some alleles (genotypes) in populations of *M. album* have a selective advantage under conditions of chronic exposure; (2) the proportion of rare alleles is higher in impact populations than in the samples from background; (3) among-population subdivision is greater than within-population variability.

Materials and methods

Test organism

White campion (*Melandrium album* (Mill.) Garcke = *Silene latifolia* Poirt = *Lychnis alba* Miller, *Silene alba*) is a dioecious annual or biennial herbaceous species of the Caryophyllaceae Family, a diploid ($2n = 24$, $x = 12$). These plant species are classified as a hemicryptophyte or therophyte and a mesophyte, tolerant to low air humidity. In the wild, the reproduction process is mainly due to seeds (Baker 1947; Gulenkova and Pyatunina 1997). This species is widely used as a model for studies in evolution and phylogenetics (Bernasconi et al. 2009; Rautenberg et al. 2010), reproductive biology and ecology (Jolivet and Bernasconi 2007), and, in particular, on sexual dimorphism (Delph et al. 2010; Steven et al. 2007; Taylor et al. 1999). Such interest received from specialists in different fields confirms the uniqueness of this species.

Characterization of sites

The EURT is located within the Trans-Ural forest-steppe, characterized by the alternation of steppe meadows, small groves of birch, and birch aspen, and pine forests (Gorchakovsky 1968). The soil is dominated by various subtypes of gray forest soils and chernozem soils of varying thicknesses and degrees of leaching. The greenery of the EURT represents a summation of synanthropic and semi-natural plant communities at different stages of successions. The steppe nature of the wild plants communities can be attributed to the increase of brightness and heating of the soil due to the dilution of the forest canopy that has resulted from the influence of radiation exposure (Pozolotina et al. 2012).

Two sites were chosen in the impact area. Impact site no. 2 [55° 46′ N 60° 53′ E] is located 9–12 km from the epicenter of the accident at the south-west bank of Lake Berdenish. Leached chernozem and subtypes of gray forest soils predominate at this site. The involvement of ruderal species in meadows and forest communities of the impact zone is reduced, while plants typical to the communities play significant roles. The total projective cover is 80–90 %. Impact site no. 1 [55° 45′ N 60° 50′ E] is the closest to the epicenter of the accident, located at a distance of 6–8 km. Atypical soils occur at this site, being similar in morphology to the brown forest soils. The most transformed plant community includes ruderals, lungwort, and thistle. The total projective cover is 100 %.

Three background sites are located outside the EURT area. The map (Fig. 1) and the characterization of sites is summarized here but is discussed elsewhere (Karimullina et al. 2013; Pozolotina et al. 2008; Pozolotina et al. 2012). Background site no. 1 [56° 41′ N 61° 02′ E] is located in the birch-pine

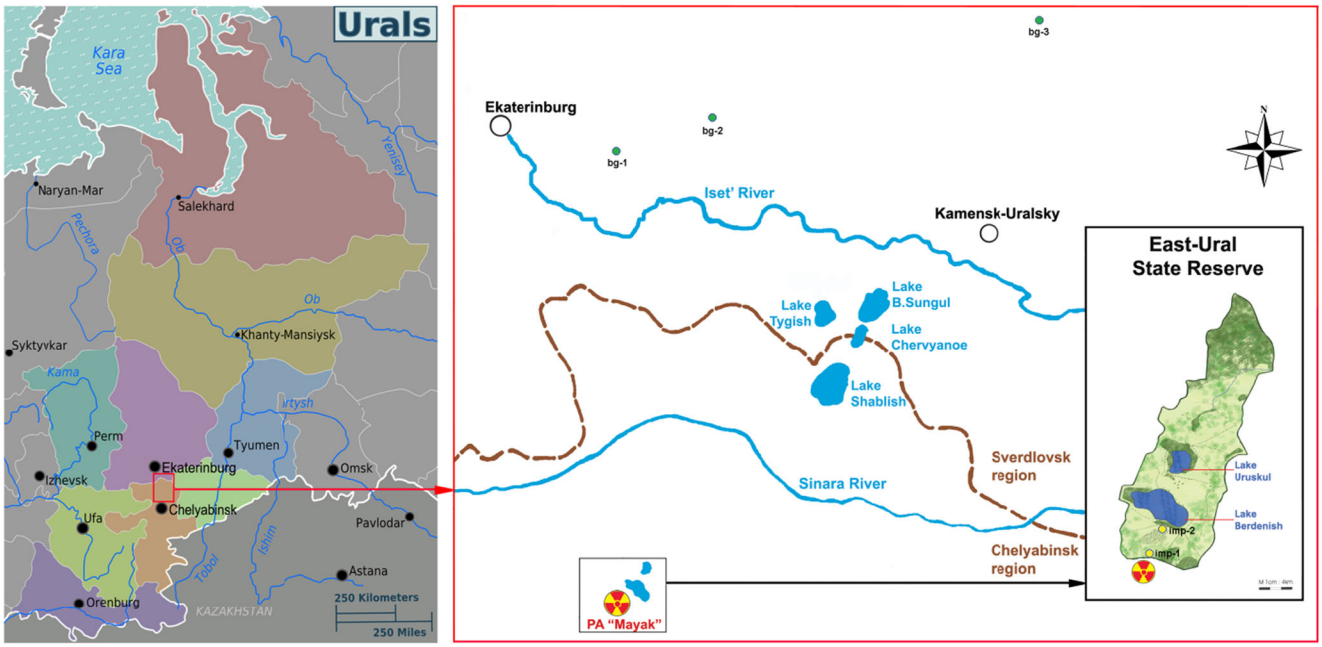

Fig. 1 Location of the sample site within the head part of East-Ural Radioactive Trace and background areas: *imp-2* – Impact 2 site, *imp-1* – Impact 1 site, *bg-3*, *bg-2*, *bg-1* – background plots

forest about 112 km from the epicenter of the accident. Grass cover is multi layer. The projective cover of the grass is 95 %. There is forest brown soil. The close proximity to a village area has led to a high abundance of the ruderals. Background site no. 2 [56° 47′ N 61° 18′ E] is located in the birch-pine forest mixed with some aspen 125 km away from the epicenter of the accident. The site soil is primitive-accumulative on the granite-gneiss weathering crust. The projective cover of the grass is 95 %. Background site no. 3 [57° 33′ N 62° 42′ E] is located 170 km from the western border of the EURT zone. The site has an upland forbs-grass meadow. The projective cover of the grass is 90–95 %. Soils are sod-podzolic.

Field sampling, preparation, and radionuclide analyses

Leaves without signs of pathologic damage were sampled in late August of 2008–2010. Each site comprised 46–56 plants. Only those plants growing at a distance of at least 5 m were analyzed. To avoid enzymatic activity loss, samples were immediately frozen in liquid nitrogen. Under laboratory conditions, leaves (50–100 mg) were triturated in the cold with sterilized mortars and pestles in 600 μl extraction buffer and centrifuged at +4 °C (13,000 *g* for 5 min). The storage temperature of enzyme extract was −80 °C.

The specific activity of ^{90}Sr in selected samples (soils and dried vegetative organs of plants) was determined by the radiochemical method (Molchanova 2006). The precipitating of ^{90}Sr in the oxalate form, and the separating of ^{90}Sr kept in balance with the daughter product of decay – ^{90}Y. The measurement of ^{90}Y was carried out with an alpha-beta radiometer (Russia); detection limit of 0.2 Bq. Procedural errors of the methods did not exceed 20 %. The specific activity of ^{137}Cs in samples was determined by a gamma-ray analyzer with a germanium semiconductor detector «Canberra Packard» (USA) with a detection limit of 0.1 Bq. For the correct assessment of radionuclides in samples collected in different years, correction for the radioactive decay was applied.

A modeling approach was used to estimate dose rates on *M. album.* Soil-to-organism transfer parameter values were delivered from empirical data of ^{90}Sr and ^{137}Cs soil and vegetative plant mass activity concentrations. External and internal whole-body dose rates were calculated using deterministic (The ERICA Tool-Tier 2) and probabilistic (The ERICA Tool-Tier 3) methods, as described earlier (Karimullina et al. 2013).

Genetic analysis

Twelve allozymes of *M. album* were investigated: ADH (E.C. 1.1.1.1), LAP (E.C. 3.4.11.1), NADHDH (E.C. 1.6.5.3), FDH (E.C. 1.2.1.2), GOT (E.C. 2.6.1.1), PGI (E.C. 5.3.1.9), SKDH (E.C. 1.1.1.25), PGM (E.C. 5.4.2.2.), DIA (E.C. 1.6.4.3); MDH (E.C. 1.1.1.37); SOD (E.C. 1.15.1.1); EST-UV (E.C.3.1.1.1). The analysis was conducted using the method for vertical 6.4 % polyacrylamide gel electrophoresis (PAAG) with TRIS-EDTA-borate system (Peacock et al. 1965). To label loci, the second and subsequent letters were replaced from uppercase to lowercase in abbreviated Latin symbols of the corresponding allozymes (Prakash et al. 1969). The

designation of alleles was given by the standard allele nomenclature by F. Ayala. The most common allele received numeric character 100. The rest alleles of the same locus were designated depending on their electrophoretic mobility relative to the most common allele encoded as 100 (Ayala 1982). Loci were numbered assigning the number 1 to that with the highest velocity of migration toward the anode. Histochemical staining of the samples was performed by standard methods (Harris and Hopkinson 1976). The total number of samples studied – 456.

Data analysis

For allozyme loci analysis, the values of the main indicators of variability were calculated. The expected heterozygosity (H_e) for each locus was calculated using the following equation: $H_e = 1\text{-}\Sigma x_i^2$, where x_i is the frequency of the i-th allele. The observed heterozygosity (H_o) was calculated by dividing the number of heterozygous plants by the total number of analyzed plants. The index of average heterozygosity (both expected and observed) was calculated as $N = 1/L\Sigma H$, where L is the number of loci studied. Polymorphic rate (P) was calculated by dividing the number of polymorphic loci by the total number of investigated loci. The average number of alleles per locus (N_A) was calculated by dividing the number of identified alleles by the total number of loci studied. Polymorphism was analyzed using both 99 % criterion (when the most common allele had a frequency not greater than 99 %) and 95 % criterions. The degree of genetic affinity between populations was calculated by the method proposed by M. Nei (Nei 1978).

To analyze the structure and the degree of subdivision of natural populations of enzymatic variability factor, the Wright's F-statistic was used. For the analysis of allelic frequencies, the hypotheses were tested using the Newcombe (*CI*) method for independent samples (Newcombe 1998). Bootstrap resampling (n = 1000) was performed to test the robustness of the dendrogram topology. Data were analyzed using BIOSYS-1+ (Swofford and Selander 1981), TFPGA (Miller 1997) and STATISTICA 6.0 (StatSoft 2001) software.

Results

Assessment of the radiation dose on the *Melandrium album* populations

The current state of soil contamination by ^{90}Sr and ^{137}Cs at the EURT zone can be found here (Molchanova et al. 2014). The radiation absorbed dose rate from external and internal radiation exposure in *M. album* populations from the EURT zone and background area were estimated (Table 1) using modeling approach (Beresford et al. 2007; Larsson 2008). Taking into account the summarized dose rates due to natural radiation and anthropogenic ^{137}Cs and ^{90}Sr radionuclide contamination, the dose rate per plant organism for *M. album* exceeded background sites values 330–400 times at the Impact EURT sites. These values exceed the 10 μGy h^{-1} dose screening value of chronic exposure, adopted as a universal value basing on data of radiation-induced biological effects in non-human biota summarized in the FASSET Radiation Effects Database (Garnier-Laplace et al. 2006). Exposure below this level ensures a negligible risk of adverse effects on wildlife. However, the total dose rates per plant organism for *M. album* were below the threshold of 400 μGy h^{-1} for terrestrial plants and aquatic biota (DOE-STD-1153 2002; IAEA 1992). These values are considered to be within the chronic low-level irradiation conditions for herbaceous species. The detailed description of the calculations of absorbed dose rates in *M. album* was previously described (Karimullina et al. 2013).

Analysis of *Melandrium album* allozyme variation

A total of 14 loci for *M. album* were analyzed. Four loci were monomorphic: *Adh*, *Fdh*, *Sod*-1, and *Mdh*-1. The following 10 allozymes had polymorphic loci:

- *Lap* (leucine aminopeptidase) – one locus (four alleles were revealed).
- *Got* (glutamate oksaloasetat transaminase) – no heterodimer locus was determined on the gel, therefore two loci were identified. Locus *Got*-1 has at least two alleles. However, we were unable to take them to analysis due to low quality of separation. Locus *Est-UV*-1, *Pgi*-1 and *Sod*-1 were also excluded for the same reason. Locus *Got*-2 (two alleles were designated).
- *Dia* (diaphorase) – one locus (two alleles).
- *Nadhdh* (nicotinamide adenine dinucleotide dehydrogenase) – one locus (three alleles).
- *Est-UV*-2 (fluorescent esterase) – one locus (three alleles).
- *Sod*-2 (superoxide dismutase) – one locus (three alleles).
- *Pgi*-2 (phosphoglucoisomerase) – one locus (three alleles).
- *Mdh* (malate dehydrogenase) had three loci, two of which (*Mdh*-2 and *Mdh*-3) were designated by two strips on gel reflecting their monomeric structure.

The highest number of alleles was observed in *Skdh* (shikimate dehydrogenase) allozyme. Five alleles were revealed.

Within population genetic variation in *Melandrium album*

The results of the analysis of contingency tables (Table 2) show that the frequencies of alleles in background *M. album* populations are genetically close to each other. The significant difference was observed in two loci (*Sod*-2 and *Skdh*) only.

Table 1 Absorbed dose rates (μGy h^{-1}) from external and internal radiation exposure in *M. album* populations from the EURT zone and background area

Site	Absorbed dose rate without regard to NR[a]			With regard to NR
	External	Internal	Total	
Background area[b]	2.63×10^{-3}	1.39×10^{-3}	4.02×10^{-3}	0.092
Impact 2	0.39	37.9	38.3	38.39
Impact 1	1.39	28.7	30.1	30.19

[a] NR is the level of natural background radiation, which in the Ural region averages 0.088 μGy h^{-1} (Antonova et al. 2013)

[b] Three background sites were merged due to equality of dose rates

Samples from impact sites were different from those from control sites in most loci. The only exceptions were three loci: *Est*-2, *Pgi*-2, and *Got*-2. The comparison of chronically irradiated impact samples together revealed a significant difference in 50 % of loci (*Lap*, *Est*-2, *Mdh*-2, *Skdh*, *Dia*). Each population undergoes unique multidirectional changes of allele frequencies.

There are two patterns of genetic variation that can be distinguished in *M. album* populations:

1) Some alleles are found only in the chronically irradiated samples and absent in the background populations. These atypical alleles are *Mdh*-3^{104}, *Pgi*-2^{106}, *Lap*^{105}, *Mdh*-2^{96} and *Dia*^{94}.
2) Other alleles are present in all populations of *M. album*. However, the frequency of their occurrence in the background and impact samples differed significantly.

On the one hand, we have seen an upward trend in the frequency of the following alleles along a pollution gradient – *Sod*-2^{115}, *Skdh*^{100}, *Nadhdh*^{100}, *Lap*^{90}, *Pgi*-2^{94}, *Est*-2^{104}, and *Nadhdh*^{95}. These alleles had higher frequency rate at the EURT sites compared with background ones. Differences were significant in *Sod*-2^{115}, *Skdh*^{100}, and *Nadhdh*^{100} (lower limits of the difference of proportions *CI* ranged from −0.674 to −0.257, upper ones were from −0.466 to −0.013). On the other hand, an inverse relationship was observed alleles *Lap*^{100} (lower limits of *CI* ranged from 0.005 to 0.076; upper limits ranged from 0.195 to 0.307). These alleles were more frequent in the background areas. We suggest that for the first 2 years after the Kyshtym accident the selection pressure on these alleles could be large, resulting in a decrease of allele frequency in impact populations.

The rare alleles (with frequency less than 1 %) were absent in populations of *M. album.* The only exceptions were *Skdh*^{84} at Background-3 and *Pgi*-2^{106} at the Impact-2 sites. There were seven alleles that had frequency rate of not more 5 %. They were distributed randomly in populations (Table 2).

On the basis of the allele frequency of allozyme loci, the values of the main parameters of genetic variability in *M. album* populations were calculated (Table 3). Fifty percent (P_{95},%) and 54.3 % (P_{99},%) of the loci were polymorphic. The highest rate of polymorphism (64 and 71 %, respectively) was observed at the closest to nuclear accident spot, Impact-1 site. The average number of alleles per locus (N_A) in populations was 1.94, which is close to the value of the parameter in the background samples and is lower than N_A in the chronically irradiated populations (2.0–2.07). The observed heterozygosity had no significant difference from the expected, indicating the absence of breeding barriers. On the whole, the EURT impact populations of *M. album* are characterized by increased gene polymorphism of allozyme loci associated with the presence of a higher number of alleles per locus compared to background samples and with the presence of atypical alleles.

Intraspecific (among-population) genetic variation in *Melandrium album*

We examined intraspecific differentiation in *M. album,* using F_{ST} test in the following aspects: (1) for the whole species, analyzing all the data set; (2) for the whole species, estimating interloci differences only; (3) the pair wise comparison of samples, analyzing all the data set; (4) the pair wise comparison of samples based on their interloci differences. In sum, 10.6 % of the total genetic variation is attributed to interpopulation component (F_{ST} = 0.106, p = 0.001). The analysis of individual loci revealed differences in contribution of examined loci to interpopulation differentiation of *M. album*. We suggest that some of these loci are selectable (or are linked to selectable markers) and depend on selection processes. It was shown that loci with the average F_{ST} (0.096) are likely neutral markers; loci with low F_{ST} are maintained by balancing selection leveling allele frequencies; loci with high F_{ST} are maintained by disruptive selection (Altukhov 2003). According to this classification, balancing selection is common for most loci studied in populations of *M. album* (F_{ST} = 0.015–0.069, p = 0.001–0.03). Disruptive selection is induced for *Mdh*-2, *Shkdh* and *Sod*-2 loci (F_{ST} varies from 0.132 to 0.305, p = 0.001). We conducted a paired comparison of samples (10 pairs) using the total set of loci. The greatest differences (F_{ST} = 0.115–0.187) were observed between pairs composed

Table 2 The frequencies of alleles of polymorphic loci in *M.album* populations from the EURT zone and background area and evaluation of the significance of differences between samples

Locus	Allele	Site					The results of the analysis of contingency tables (number of site) χ^2 (*df*); *p*-level		
		Background -1 (1[a])	Background -2 (2[a])	Background -3 (3[a])	Impact -2 (4[a])	Impact -1 (5[a])			
Lap	105	0	0	0	0.282	0.077	(1–2) 0.34 (2); 0.845	(2–3) 0.11 (2); 0.946	**(3–4) 52.6 (3); 0.000**
	100	0.783	0.808	0.813	0.618	0.692	(1–3) 0.27 (2); 0.872	**(2–4) 51.9 (3); 0.000**	**(3–5) 10.6 (3); 0.014**
	95	0.163	0.154	0.143	0	0.154	**(1–4) 47.2 (3); 0.000**	**(2–5) 10.3 (3); 0.016**	**(4–5) 30.0 (3); 0.000**
	90	0.054	*0.038*	0.045	0.100	0.077	**(1–5) 8.04 (3); 0.045**		
Est-2	104	0.065	0.087	0.071	0.196	0.087	(1–2) 1.95 (2); 0.378	(2–3) 1.53 (2); 0.464	**(3–4) 13.8 (2); 0.001**
	100	0.870	0.798	0.857	0.804	0.760	(1–3) 0.06 (2); 0.968	**(2–4) 16.1 (2); 0.000**	(3–5) 4.15 (2); 0.126
	96	0.065	0.115	0.071	0	0.154	**(1–4) 13.1 (2); 0.001**	(2–5) 0.68 (2); 0.710	**(4–5) 20.2 (2); 0.000**
							(1–5) 4.48 (2); 0.106		
Nadhdh	105	0.239	0.250	0.250	*0.036*	0	(1–2) 3.68 (2); 0.159	(2–3) 4.36 (2); 0.113	**(3–4) 24.6 (2); 0.000**
	100	0.761	0.712	0.750	0.918	0.913	(1–3) 0.03 (1); 0.856	**(2–4) 20.3 (2); 0.000**	**(3–5) 37.5 (2); 0.000**
	95	0	*0.038*	0	*0.045*	0.087	**(1–4) 21.7 (2); 0.000**	**(2–5) 30.6 (2); 0.000**	(4–5) 5.19 (2); 0.075
							(1–5) 34.2 (2); 0.000		
Got-2	100	0.946	0.942	0.884	0.964	1.00	(1–2) 0.02 (1); 0.903	(2–3) 2.26 (1) 0.133	**(3–4) 5.04 (1); 0.025**
	85	0.054	0.058	0.116	*0.036*	0	(1–3) 2.42 (1); 0.119	(2–4) 0.58 (1); 0.446	**(3–5) 12.8 (1); 0.000**
							(1–4) 0.38 (1); 0.535	**(2–5) 6.21 (1); 0.013**	(4–5) 3.82 (1); 0.051
							(1–5) 5.76 (1); 0.016		
Skdh	106	0.098	0.096	0	0	0.077	(1–2) 0.13 (4); 0.998	**(2–3) 98.7 (4); 0.000**	**(3–4) 57.3 (2); 0.000**
	100	0.348	0.337	0.598	0.918	0.673	**(1–3) 91.3 (4); 0.000**	**(2–4) 80.7 (4); 0.000**	**(3–5) 48.1 (4); 0.000**
	96	0.065	0.058	0.393	0	0.077	**(1–4) 75.4 (4); 0.000**	**(2–5) 29.5 (4); 0.000**	**(4–5) 27.9 (4); 0.000**
	90	0.130	0.144	0	0	0.058	**(1–5) 25.7 (4); 0.000**		
	84	0.359	0.365	*0.009*	0.082	0.115			
Pgi-2	106	0	0	0	*0.009*	*0.048*	(1–2) 0.03 (1); 0.872	(2–3) 1.40 (1); 0.237	(3–4) 1.02 (2); 0.602
	100	0.967	0.971	0.938	0.927	0.856	(1–3) 0.96 (1); 0.326	(2–4) 2.44 (2); 0.295	**(3–5) 6.52 (2); 0.038**
	94	*0.033*	*0.029*	0.063	0.064	0.096	(1–4) 1.88 (2); 0.391	**(2–5) 9.48 (2); 0.009**	(4–5) 3.90 (2); 0.142
							(1–5) 7.99 (2); 0.018		
Sod-2	115	0.256	0.087	0.223	0.400	0.433	**(1–2) 69.4 (2); 0.000**	**(2–3) 37.6 (2); 0.000**	**(3–4) 14.6 (2); 0.001**
	100	0.367	0.913	0.563	0.536	0.481	**(1–3) 8.93 (2); 0.012**	**(2–4) 38.4 (2); 0.000**	**(3–5) 0.19 (2); 0.001**
	85	0.378	0	0.214	0.064	0.087	**(1–4) 29.9 (2); 0.000**	**(2–5) 46.9 (2); 0.000**	(4–5) 0.83 (2); 0.659
							(1–5) 24.2 (2); 0.000		
Dia	100	1	1	1	0.936	1	**(1–4) 6.10 (1); 0.014**	**(2–4) 6.88 (1); 0.009**	**(3–4) 7.40 (1); 0.007**
	94	0	0	0	0.064	0			**(4–5) 6.88 (1); 0.009**
Mdh-2	100	1	1	1	0.682	1	**(1–4) 35.4 (1); 0.000**	**(2–4) 39.5 (1); 0.000**	**(3–4) 42.3 (1); 0.000**
	96	0	0	0	0.318	0			**(4–5) 39.5 (1); 0.000**
Mdh-3	104	0	0	0	0.127	0.058	**(1–4) 12.6 (1); 0.001**	**(2–4) 14.1 (1); 0.001**	**(3–4) 15.2 (1); 0.000**
	100	1	1	1	0.873	0.942	**(1–5) 5.50 (1); 0.019**	**(2–5) 6.21 (1); 0.013**	**(3–5) 6.68 (1); 0.010**
									(4–5) 3.00 (1); 0.083

Significant differences that are shown are marked in bold; rare alleles are marked in italic

[a] Figures in brackets represent numbering introduced for pair wise comparisons

of only the background and impact samples (Table 4). This indicates a difference between the background and the chronically exposed to radioactive substances populations at the EURT area. The average F_{ST} for the contaminated and reference sites was 0.063 and 0.082, respectively.

The pair wise comparison of samples using F_{ST}, carried out for each locus separately, revealed that in 44.3 % of cases, the samples from background sites were significantly different from those from the impact the EURT zone as well (F_{ST} varies from 0.021 to 0.341, p = 0.001–0.044). Only in 15.7 % of these cases the differentiation between populations was weak (F_{ST} = 0.016–0.03, p = 0.083–0.381). The largest contribution (60 %) to the difference between the sample from background and impact sites was made by three loci: *Nadhdh*, *Sod*-2 and *Skdh*. Similar to the analysis of the frequencies of alleles, the presence or absence of differentiation between samples from the impact sites was low and nearly equal (4.3 and 5.7 %, respectively). In contrast, the samples from three background sites were close to each other in 22.9 % of cases (F_{ST} = 0.0001–0.015, p = 0.110–0.352) (Table 4). Significant differentiation was detected only in 7.1 % of the pair wise comparisons of loci (F_{ST} = 0.001–0.0043, p = 0.001–0.007).

The analysis of genetic distance (Nei 1978) also confirms the omni-directional processes in different loci in populations of *M. album.* Consequently, the calculation of the index, using total amount of polymorphic loci, revealed no correlation between the rate of polymorphism and radioactive contamination (Fig. 2a). Interestingly, the construction of dendrograms for individual allozymes showed that, using only the *Nadhdh*

Table 3 Parameters of genetic variability in *M. album* populations from the EURT zone and background area

Site	P_{95},%	P_{99},%	N	$N_A \pm S.E.$	$H_O \pm S.E.$	$H_E \pm S.E.$**
Background-1	42.9	50.0	46	1.93 ± 0.32	0.164 ± 0.082	0.181 ± 0.068
Background-2	42.9	50.0	52	1.93 ± 0.32	0.120 ± 0.059	0.154 ± 0.060
Background-3	50.0	50.0	56	1.79 ± 0.24	0.121 ± 0.051	0.169 ± 0.056
Impact-2	50.0	50.0	52	2.07 ± 0.35	0.146 ± 0.059	0.180 ± 0.060
Impact-1	64.3	71.4	55	2.00 ± 0.21	0.127 ± 0.044	0.193 ± 0.052
Mean	50.0	54.3	52.2	1.94 ± 0.29	0.137 ± 0.059	0.175 ± 0.059

P_{95} and P_{99} represent the rate of polymorphic loci using 95 %-th and 99 %-th criterions of polymorphism, N – sample size, N_A – the average number of alleles per locus, H_O – average observed, and H_E – average expected heterozygosity; ** – unbiased estimator

locus, chronically irradiated samples from Impact sites are genetically closer to each other than to background ones (Fig. 2b). Bootstrap values were considered high when the resampling occurrence was more than 50 %. Within this, the most robust feature of the grouping separated the background and impact samples (100 %) into individual clusters. As mentioned above, the frequency of *Nadhdh*^{100} allele was increased along the gradient of ionizing radiation (see Table 2). In addition, *Nadhdh*^{95} allele was observed in both impact sites at the EURT area, while being rare or absent in background samples.

The distribution analysis of homo- and heterozygous genotypes (Table 5) in populations of *M. album* growing along the radioactive contamination gradient showed that general for background and impact samples homozygous genotypes were observed with the same frequency as the heterozygous ones (27.3 and 29.5 %, respectively). We found seven genotypes of rare (incidence rate is less than 5 %) which was less abundant in impact samples. The unique genotypes were detected for EURT area. The proportion of unique homozygotes at the EURT area was 11.4 %, which is 2.5 times higher compared to samples from background sites. Similar results were obtained in the frequency of unique heterozygous genotypes in samples at impact and background sites (20.5 vs. 6.8 %, respectively). At EURT, the main contribution was made by *Lap*, *Pgi*-2 and *Nadhdh* loci. At the same time, the increase of the proportion of heterozygous genotypes was observed in all sites along the pollution gradient with *Lap*, *Pgi*-2 loci, while heterozygous genotypes of *Nadhdh* locus were present only in the EURT area. Thus, the increase of the frequency of unique homozygous and heterozygous genotypes was identified in populations of *M. album* growing under chronic radiation exposure conditions.

Discussion

The observed patterns of genetic variability in each population of *M. album* can equally be the result of neutral mutations, isolation, level of migration, genetic drift, or founder effect (Jolivet and Bernasconi 2007). In small populations of *M. album*, the changes of genetic structure may occur due to removal of inbreeding depression and the increase of gene flow (Richards et al. 2003). Low genetic variability may be associated with low levels of migration and genetic drift, especially in marginalized populations (Hoffmann and Blows 1994; Soule 1973) as well as the effect of the bottleneck (Nei et al. 1975), although this effect can contribute to opposite effects (Willis and Orr 1993). High levels of genetic variation in natural populations may be associated with a wide variability of ecological niche (Babbel and Selander 1974; Prentice et al. 1995) or local adaptation processes in response to changing environmental conditions (Jolivet and Bernasconi 2007).

The results of our study suggest an increase of genetic variability in the populations of *M. album* growing at chronic low-level irradiation conditions, which supports our hypothesis. It should be noted that the dose rates in the first years after the accident was due to large short-lived radionuclides (^{95}Zr + ^{95}Nb; ^{106}Ru + ^{106}Rh; ^{144}Ce + ^{144}Pr), which disintegrated rapidly. According to some authors's estimations, the dose rate for living organisms exceed current levels 8800 times in the study areas (Nikipelov et al. 1989). It is these doses that might cause mutagenic effects.

Our findings are in agreement with a quantitative literature review of allozyme and microsatellite estimates confirming the evidence of the increased genetic variation due to anthropogenic pollution (DiBattista 2008). Such observations may be associated with increased mutation events (including the increase of the activity of mobile genetic elements), or with contribution of DNA methylation occurring in the population due to radiation impact (Kovalchuk et al. 2003), which may result in the formation of unique alleles, not typical for the background samples. Previously, such changes were observed in populations of *Taraxacum officinale* s.l. from the EURT zone (Ulyanova and Pozolotina 2006). Genetic effects in chronically exposed to radiation natural populations can be measured by the speed of mutation of allozyme loci (Kal'chenko and Fedotov 2001). Using allozyme assay, the mutagenesis of greater knapweed (*Centaurea scabiosa* L.) was studied under chronic exposure conditions (Kalchenko

Table 4 The results of pairwise comparison of *Melandrium album* populations from the EURT zone and background area using F_{ST}

Site	Background-1	Background-2	Background-3	Impact-2	Impact-1
Background-1					
Background-2	0.084				
Background-3	0.065	0.097			
Impact-2	0.072	*0.115*	0.061		
Impact-1	*0.157*	*0.187*	*0.128*	0.063	

The number of permutations = 999, significant differences ($p = 0.001$) are marked in italic

et al. 1983): the experimental data showed the presence of mutant variants in a diploid seedlings knapweed which were the progeny of plants homozygous for the normal *Lap* allele; the magnification of irradiation dose 3×10^{-4}, 40×10^{-4}, 70×10^{-4} Gy day^{-1} resulted in the 10, 35 and 83 times increase of mutation frequency in *Lap* locus, respectively. Also, in the first years after the Chernobyl accident, plants of scots pine growing in the exclusion zone led to an increase of the rate of mutational events per locus, and hence to the tightening of selection and an increase of radioresistant individuals in the population (Kalchenko et al. 1995b). The dependence of the rate of mutation on the absorbed dose was nonlinear which is explained by selection pressure. The rate of mutation in certain loci was also heterogeneous and its highest value present in the highly polymorphic loci *Adh*-1, *Adh*-2, *Got*-1, *Got*-2, *Lap*-1 and *Lap*-2 (Kal'chenko and Fedotov 2001).

More than 10 % of the total genetic variation was attributed to interpopulation component in our study. The same ($F_{ST} = 0.11$) substantial genetic differentiation was reported among European and American populations of *M. album* using microsatellite markers (Barluenga et al. 2011). Slightly higher values using allozyme markers ($F_{ST} = 0.134$) were reported in the study of *M. album* populations in Virginia, USA (McCauley 1994). This may be due to methodological differences: polyacrylamide gel, that we used, has a higher resolution, while the authors utilized agarose gel, which allowed selecting only one locus in MDH allozyme. Also the different set of quantitative and qualitative allozymes was analyzed for this species (McCauley 1997; McCauley et al. 1995).

As shown above, the frequency of *Nadhdh*^{100} allele was increased along the gradient of radioactive contamination. The allele of *Nadhdh*^{95} was observed in both impact sites at the EURT area, while being rare or absent in background samples. A similar observation of genetic similarity in populations chronically exposed to radiation using individual locus for analysis with the absence of the effect when considering the total amount of loci has been shown earlier in mosquitofish (*Gambusia affinis*) transplanted into a pond which was heavily contaminated with radionuclides metabolism (Theodorakis and Shugart 1997). Out of the 12 studied allozymes, the differentiation of «clean» and irradiated samples was revealed with nucleoside phosphorylase locus only which is an important enzyme of cellular metabolism.

In the remote period after accident, values of genetic variability (heterozygosity, frequency of polymorphic loci, the index of Zhivotovsky) and frequency, resulting in the loss of enzyme activity, significantly increase with the dose absorbed by the radiosensitive organs of plants (Geras' kin et al. 2009). Allozyme analysis revealed elimination of alleles *Per*-1 incapable of intensive peroxides utilization in *Arabidopsis* samples collected during 1986–1991 (Lysenko et al. 2000).

The increase of genetic diversity (some authors link it to genetic load) may be associated with a high frequency of rare alleles in populations of herbaceous plants at the EURT (Lysenko et al. 1999; Pozolotina et al. 2010). However, in our study, rare alleles were absent in the populations of *M. album* from impact sites at the EURT. We suggest that alleles appeared de novo after Kyshtym disaster, and are rarely encountered in the beginning, eventually could entrench in

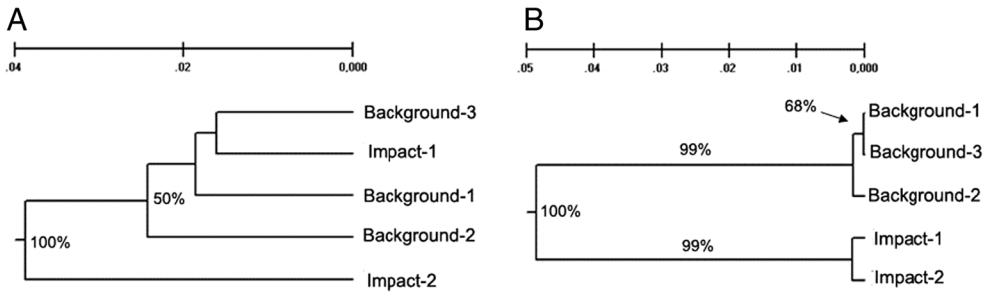

Fig. 2 Dendrograms of genetic distance estimates in *Melandrium album* populations between background and impact sites, built in the TFPGA program: **a** Using 10 polymorphic loci. **b** Using locus *Nadhdh.* Numbers are the percentage bootstrap values (50 % and higher) from 1000 replicates over loci

Table 5 The frequency of occurrence of homo- and heterozygous genotypes in *Melandrium album* populations from the EURT zone and background area

Loci	Genotypes	Background-1	Background-2	Background-3	Impact-2	Impact-1
Lap	11 (105–105)				12.7[b]	
	12 (105–100)				20.0[b]	
	14 (105–90)				10.9[b]	15.4[b]
	22 (100–100)	65.2	67.3	71.4	47.3	63.5
	23 (100–95)	15.2	19.2	10.7		11.5
	24 (100–90)	10.9	7.7	8.9	9.1	
	33 (95–95)	8.7	5.8	8.9		9.6
Est-2	11 (104–104)				19.6[b]	
	12 (104–100)	2.2[a]	1.9[a]			17.3
	13 (104–96)	10.9[c]	15.4[c]	14.3[c]		
	22 (100–100)	84.8	75.0	85.7	80.4	51.9
	23 (100–96)	2.2[a]	7.7			30.8
Dia	11 (100–100)	100.0	100.0	100.0	87.3	100.0
	12 (100–94)				12.7[b]	
Nadhdh	11 (105–105)	23.9[c]	25.0[c]	25.0[c]		
	12 (105–100)				7.3[b]	
	22 (100–100)	76.1	71.2	75.0	83.6	88.5
	23 (100–95)				9.1[b]	5.8[b]
	33 (95–95)		3.8[a]			5.8
Got-2	11 (100–100)	89.1	88.5	76.8	92.7	100.0
	12 (100–85)	10.9	11.5	23.2	7.3	
Sod-2	11 (115–115)				12.7[b]	9.6[b]
	12 (115–100)	17.8	17.3	23.2	41.8	50.0
	13 (115–85)	33.3		21.4	12.7	17.3
	22 (100–100)	6.7	82.7	33.9	32.7	23.1
	23 (100–85)	42.2[c]		21.4[c]		
Pgi-2	12 (106–100)				1.8[a,b]	
	13 (106–94)					9.6[b]
	22 (100–100)	93.5	94.2	87.5	85.5	80.8
	23 (100–94)	6.5	5.8	12.5	12.7	9.6
Shkdh	12 (106–100)	19.6	19.2			5.8
	15 (106–84)					9.6[b]
	22 (100–100)	4.3[a]	3.8[a]	42.9	83.6	55.8
	23 (100–96)			32.1[c]		
	24 (100–90)	8.7	7.7			7.7
	25 (100–84)	32.6	32.7	1.8[a]	16.4	9.6
	33 (96–96)	6.5	5.8	23.2		7.7
	45 (90–84)	17.4	21.2			3.8[a]
	55 (84–84)	10.9[c]	9.6[c]			
Mdh-2	11 (100–100)	100.0	100.0	100.0	60.0	100.0
	12 (100–96)				16.4[b]	
	22 (96–96)				23.6[b]	
Mdh-3	11 (104–104)				12.7[b]	5.8[b]
	22 (100–100)	100.0	100.0	100.0	87.3	94.2

[a] Rare genotypes

[b] Unique genotypes, occurring in impact populations only

[c] Unique genotypes, occurring in background populations only

herbaceous populations. The present study extends previous work (Geras'kin et al. 2010; Ul'yanova and Pozolotina 2004; Volkova and Geraskin 2014), which showed the elimination of certain alleles and directional shift of allele frequencies in the populations of several plant species at chronic low-level irradiation conditions.

A fixation of certain alleles *Sod*-2^{115}, *Skdh*^{100} and *Nadhdh*^{100} was shown in populations of *M. album* from impact sites at the EURT. We suggest that these alleles might encode subunits of allozyme that perform their function more successfully under chronic radiation stress. Open discussion regarding the *pro* and *contra* of a high level of allelic diversity of populations is reflected with respect of genotypic variability. Previously, we revealed the deficit of heterozygous genotypes at an inbreeding, rosette-forming perennial species *Plantago major* populations growing under radiation stress (Pozolotina et al. 2005). A number of other studies have shown that an excision of recessive lethals occurs in the progeny of highly heterozygous parents, consequently producing a large amount of non-viable seeds (Altukhov 2003; Korshikov and Kalafat 2004). The number of heterozygous loci negatively correlated with coefficients of variation of three fitness components (Ostermeijer et al. 1995). Our data indicate an increase of the incidence of unique heterozygous and homozygous genotypes in populations of *M. album* from impact sites at the EURT.

Conclusions

The absorbed dose rate for plants of impact of *M. album* populations was 30.19–38.39 μGy h^{-1} at the EURT. These values are within the low doses rates for non-human biota.

The patterns of genetic structure in populations of *M. album* from background and chronically exposed to radionuclide pollution were investigated using allozyme assay. The analysis revealed allelic diversity of unique alleles, present only at EURT (*Mdh*-3^{104}, *Pgi*-2^{106}, *Lap*^{105}, *Mdh*-2^{96} and *Dia*^{94}), as well as the increase in the frequency of *Sod*-2^{115}, *Skdh*^{100} and *Nadhdh*^{100} alleles along the pollution gradient. The consequence of the above was the increase of polymorphism of allozyme loci in natural *M. album* populations exposed to chronic ionizing radiation.

The highest genetic differentiation estimated using F_{ST} was revealed between compared pairs of the background and impact samples in populations of *M. album*. The analysis of genetic distances with *Nadhdh* also contributed to observation that individuals from chronically irradiated sites are genetically closer than to plants from background sites. The increment of the frequency of unique homozygous and heterozygous genotypes was observed in populations of *M. album* under chronic radiation exposure. The largest contribution to the group of unique heterozygous genotypes at the EURT was made by three loci – *Lap*, *Pgi*-2 and *Nadhdh*; the main role in interpopulation differentiation of samples was made by the following alleles – *Sod*-2^{115}, *Skdh*^{100} and *Nadhdh*^{100}.

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