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Intrapopulation Variation in the Quality of Dandelion Seed Progeny in Zones of Chemical and Radioactive Contamination

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Abstract—Interfamilial variation in metal tolerance and radioresistance of seed progeny from F_1 plants was studied in the dandelion from cenopopulations growing over several decades in gradients of radioactive or chemical contamination. The progeny from the impact zone of the Eastern Ural Radioactive Trace (EURT) proved to be highly viable but had latent injuries that accounted for a high sensitivity to additional technogenic impact. In the F_1 generation from the zone of chemical contamination, high seed viability was combined with increased tolerance to additional impact of radiation and heavy metals. No significant differences between responses to challenge with habitual and new factors were revealed in samples from either zone.

Key words: heavy metals, radioactivity, cenopopulations, dandelion, consequences for progeny, specificity of adaptation.

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Pathways of adaptation of living organisms to various influences are diverse and include not only universal but also specific components dependent on the nature of acting factor. Our previous studies on averaged samples of dandelion seed progenies from cenopopulations growing over several decades in gradients of radioactive contamination (the Eastern Ural Radioactive Trace, EURT) or chemical contamination (the impact zone of the Nizhnii Tagil Metallurgical Plant, NTMP) showed that seed viability in both samples equally decreased under increasing technogenic stress (Pozolotina et al., 2006).

The assessment of adaptation capacities of the seed progeny from each cenopopulation by means of acute challenging exposure (Pozolotina et al., 2006) showed that the background sample (formed beyond the zone of any impact) was relatively resistant to irradiation and very sensitive to heavy metals. The cenopopulations from the EURT and NTMP zones similarly responded to the effects of habitual and new factors. This similarity could be explained, in particular, by the genetic kinship of cenopopulations and consequent equivalence of their adaptive responses. However, allozyme analysis of both impact and control samples showed that all these cenopopulations were phenogenetically unique, differing in their genesis (Antonova and Pozolotina, 2007). Apparently, they had different sets of founder plants, whose descendants changed genetically in the course of more or less successful expansion over corresponding areas.

Similarity in tolerance to challenging chemical or radiation impact between dandelion samples from the EURT and NTMP zones can also be explained by intrapopulation polymorphism. It may well be that different group of plants, one tolerant to radiation and the other to heavy metals, have established themselves in each cenopopulation in the course of its adaptation to specific environmental conditions. If so, similarity in tolerance to different factors between cenopopulations exposed to long-term radiation or chemical stress indicates that the mechanisms of this tolerance are specific. On the other hand, it is also possible that the same group of plants in each cenopopulation is highly tolerant to different factors and, therefore, the mechanisms of tolerance and corresponding adaptation pathways are unspecific. The purpose of this study was to resolve this issue.

In addition, we compared remote consequences of chronic exposure to chemical pollutants and radiation as related to the viability of the next generation and its response to challenging exposure. Interfamilial variation in metal tolerance and radioresistance of dandelion seed progeny from F_1 plants was studied in samples from the EURT and NTMP cenopopulations in comparison with background samples in order to reveal specific features of progeny formation under exposure to technogenic factors of different nature.

MATERIAL AND METHODS

The dandelion (*Taraxacum officinale* s.l.) is a polycarpic perennial herb of the family Asteraceae. This plant is a facultative apomict, with embryos usually developing from unfertilized, unreduced ova and carrying no paternal chromosomes (Kashin, 2005). Therefore, seeds from one plant are actually a family, or a clone. Experiments were performed with seed progenies (F_1) of nine to ten families from each of four test cenopopulations (buffer-r and impact-r from the EURT zone and buffer-m and impact-m from the NTMP zone) and of 25 families from the background zone. Seeds of parental (P) plants were grown in a field experiment, on a "clean" agronomic background, until the next seed harvest. This approach makes it possible to obtain a large amount of genetically uniform material and reduce to a minimum variation related to the maternal effect, i.e., the aftereffect of any toxic exposure that usually manifests itself in the first year (*Maternal Effects...*, 1998).

The sites of seed sampling and characteristics of contamination in each plot are described in detail in our previous study (Pozolotina et al., 2006). Here, it should be noted that total toxic load on plants due to soil pollution with heavy metals (Pb, Cd, Zn, Cu) around the NTMP exceeded the background level by a factor of 8 in the buffer zone and by a factor of 33 in the impact zone. In the EURT, the annual absorbed doses of radiation (^{90}Sr and ^{137}Cs) to plagiotropic plant parts reached 28 cGy in the buffer zone and 2412 cGy in the impact zone, compared to a background dose of only 1.4 cGy. Three background (control) populations were from areas not exposed to any technogenic impact.

The experiment consisted of three blocks: (1) seeds from different populations were grown in vessels with "clean" soil suspension in distilled water (1 : 50) (control); (2) conditions were the same, but the seeds before sowing were γ-irradiated at a dose of 250 Gy in an Issledovatel' unit; and (3) seeds were grown in the same way, but the suspension was prepared from the soil in which the contents of Cu, Cd, Pb, and Zn were ten times higher than in the control.

The F_1 seedlings were grown in roll cultures for three weeks at constant temperature and artificial illumination. Culture vessels were rerandomized every day and replenished with water to maintain constant growing conditions. Seed progenies were tested for germination rate, seedling survival, the rate of true leaf formation, and root length. Variants involving challenge with additional irradiation (R) or heavy metal exposure (HM) were compared with the control by these parameters to estimate metal tolerance and radioresistance of each family in all cenopopulations studied. Experiments in each variant were performed in three replications.

Experimental data were processed statistically using the methods described by Newcombe (1998) for analyzing proportions and by Wilson (1972) for independent samples. The null hypothesis (H_0) was as follows: $P_1 - P_2 = 0$, where P_1 and P_2 are the proportions of two samples under comparison. When the 95% confidence interval (*CI*) for the difference between these proportions did not pass through zero, the difference was considered statistically significant (i.e., H_0 was rejected at $p = 0.05$). In addition, the hypothesis was tested using one- and two-way ANOVA and Scheffe's multiple comparisons test.

RESULTS

Viability of seed progeny. Our previous study (Pozolotina et al., 2006) showed that the viability of seeds from P plants developing in the EURT and NTMP zones, in gradients of different deleterious factors, decreased in a similar way, with the same deleterious effect being caused by an increase in radiation dose by two orders of magnitude and an increase in heavy metal concentrations by one order of magnitude.

Data on the viability of seed progenies from F_1 plants grown under "clean" conditions are shown in Table 1. Viability parameters of three background cenopopulations were pooled into a single sample (25 families). The survival and numbers of seedlings with true leaves proved to be significantly lower in the background sample than in the impact sample from the EURT zone (the lower limit of *CI* for the difference of proportions varied from between –0.1932 and –0.1697 to 0.0052–0.0307, and the upper limit, from between –0.0988 and –0.1360 to 0.0895–0.1122) and the NTMP zone (from between –0.2164 and –0.1596 to between –0.1005 and –0.1446). Differences in root length between the background sample and samples from the EURT and NTMP zones lacked statistical significance (Scheffe's test, $p = 0.225$–0.962), except for the impact-r cenopopulation from the EURT ($p = 0.0019$).

Since average values do not provide a complete idea of interfamilial variation in viability parameters within each cenopopulation, the limits of variation in each parameter are also shown in Table 1. It can be seen that the variation range in background samples was markedly wider; i.e., the species reaction norm was expressed to a lesser extent in experiments with EURT and NTMP samples than with the background sample. Therefore, although the P generation from the EURT and NTMP zones was formed under chronic technogenic stress and was relatively weak (Pozolotina et al., 2006), plants of this generation grown under clean conditions produced a high-quality seed progeny.

Assessment of adaptive potential. The next step was aimed at revealing cryptic variation in responses to challenging factors (HM and R). Analyzing the interfamilial diversity of these responses in each cenopopulation, it was possible to estimate the consequences of

Table 1. Assessment of viability of F_1 seed progeny from different cenopopulations

Parameter	Cenopopulation				
	Background	EURT		NTMP	
		buffer	impact	buffer	impact
Survival of seed progeny, %	68.2	66.4	84.7	81.2	82.5
	(2.0–94.0)	(47.3–80.7)	(74.0–90.0)	(26.3–96.1)	(57.5–98.0)
Proportion of seedlings with leaves, %	38.9	33.1	52.3	56.3	57.0
	(0–85.7)	(16.0–45.3)	(36.0–65.3)	(15.8–75.0)	(0–80.0)
Root length, mm	19.7	18.0	14.4	19.9	18.8
modal	20	15	10	20	20
median	20	18	15	20	20
	(3–120)	(3–47)	(3–60)	(3–48)	(3–96)
Sample size, ind.	2203	633	1131	1178	1074

exposure to chronic technogenic stress and differences between samples in a contamination gradient.

Families from all cenopopulations showed approximately similar resistance to challenge with HM and R, with parameters of their survival remaining in the vicinity of the bisecting line (Fig. 1). The initial range of survival in the background sample was slightly wider, but there was no principal difference between manifestations of metal tolerance and radioresistance in buffer and impact samples from the EURT and NTMP zones and background samples. It is noteworthy that, by the criterion of seedling survival, their resistance to R and HM challenge was similar ($R^2 = 0.76$, $p = 0.00001$, $y = 11.47 + 0.85x$).

A different situation was observed with true leaf formation, an important prognostic character. By this criterion, background samples proved to be resistant to R challenge but sensitive to HM challenge (Fig. 2a). Most values characterizing the resistance of families from the EURT zone are below the bisecting line, which is evidence for significant inhibition of growth processes upon challenge with either factor. A similar trend was previously observed in the P generation (Pozolotina et al., 2006).

Responses to challenge in samples from the NTMP zone were highly diverse by this criterion: families from the buffer zone responded in approximately the same way as families from the background samples, while most families from the impact zone proved to be quite resistant to additional irradiation (Fig. 2b). The same conclusion follow from analysis with respect to root length, although differences from background samples in families from the impact zone of NTMP are less distinct in this case.

A comparison of the effects of the two challenging factors (HM and R) showed that suppression of growth processes was stronger after HM challenge than after irradiation in samples from both EURT and NTMP zones (Fig. 3).

Analyzing each sample for the proportion of families tolerant to habitual and new factors, it is possible to estimate specificity or universality of adaptation mechanisms in populations from the zones of chemical and radioactive contamination. The progeny of a family may be (1) resistant to both R and HM, (2) sensitive to both R and HM, (3) sensitive to R but resistant to HM, and (4) sensitive to HM but resistant to R. All families were conventionally divided into these four groups (Table 2). The inhibitory effect of any challenge was considered significant at 5% confidence level. Families showing no inhibition of growth or its stimulation were pooled into an individual group. The response was estimated from the survival of seedlings, the rate of leaf formation, and root length, recalculating absolute values into percentages relative to the internal control (without challenge).

The data on seedling survival (Table 2) show that responses to challenge in background samples were highly diverse, without prevalence of any definite effect. The same follows from the data on the rate of leaf formation and root length.

No definite conclusion could be drawn for the buffer samples from the EURT and NTMP zones (buffer-r and buffer-m). Judging by the survival of seedlings, the group contained a large proportion of families where none of the challenging factors caused any inhibitory effect; on the other hand, both factors proved to retard leaf formation in most of these families. By the criterion of root length, 60% of families in the buffer-m sample from the NTMP zone showed no inhibition under the effect of both factors; in the buffer-r sample from the EURT zone, more than half

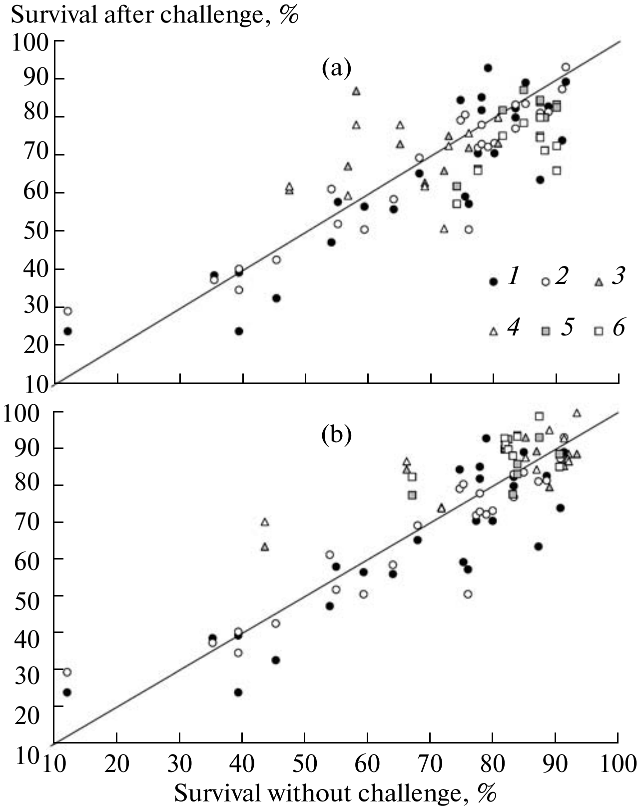

Fig. 1. Familial analysis of survival in F_1 seed progenies from (a) EURT and (b) NTMP zones after challenging exposure to heavy metals (HM) and radiation (R). Samples: (*1*, *2*) background zone, HM and R; (*3*, *4*) buffer zone, HM and R; (*5*, *6*) impact zone, HM and R (here and in Fig. 2).

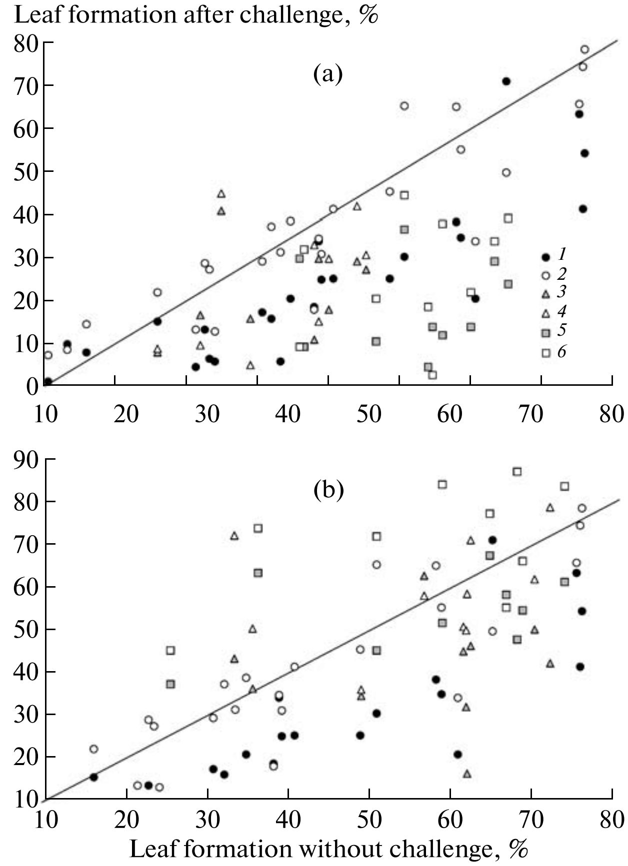

Fig. 2. Familial analysis of leaf formation in F_1 seed progenies from (a) EURT and (b) NTMP zones after challenging exposure to heavy metals (HM) and radiation (R). Samples: (*1*, *2*) background zone, HM and R; (*3*, *4*) buffer zone, HM and R; (*5*, *6*) impact zone, HM and R.

of families were inhibited by the habitual factor (R) but showed no response to the new factor (HM).

In the impact-r cenopopulation from the EURT zone, the greater proportion of families showed growth inhibition by all three criteria in response to challenge with either R or HM, with all remaining families lacking resistance to the habitual factor (R) (Table 2). This is evidence that the adaptive potential of F_1 generation from the impact EURT zone was significantly lower than in the progenies from other cenopopulations. Noteworthy is the striking discordance between the high viability of seed progeny (see Table 1) and its low resistance to adverse factors (Table 2). Apparently, long-term growing in the zone of radioactive contamination result in latent injuries that are transmitted to the progeny and manifest themselves upon additional stress exposure.

A different situation was observed in the impact-m sample from the NTMP zone (Table 2): in the greater proportion of families, the survival or seedlings and the rate of root growth remained unchanged after either HM or R challenge; according to the criterion of leaf formation, however, resistance to the habitual factor (HM) was observed in only 33% of families. In general, we can conclude that growing on a clean agronomic background abolished the consequences of chemical stress, which were previously manifested in the low viability of P plants from the NTMP zone. In seedlings of the F_1 generation, high viability was combined with resistance to challenge with both factors.

DISCUSSION

The above data provide a basis for conclusion about specificity of pathways leading to adaptation of dandelion cenopopulations to different deleterious factors. In the impact-r cenopopulation from the EURT zone, the adaptive potential of F_1 generation was so low that almost all families showed increased sensitivity to both radiation and heavy metal exposure. In the impact-m cenopopulation from the NTMP zone, the majority of families proved to be resistant to challenge with either factor. The proportions of families with resistance to the habitual factor alone were small in all cenopopulations exposed to technogenic impact. Thus, in dandelion progeny from the EURT and NTMP zones, resistance to one deleterious factor proved to correlate with that to the other factor.

Nevertheless, remote consequences of exposure to factors of different physical nature have certain specific features, which manifest themselves quite distinctly. Dandelion families descending from P plants exposed to long-term radiation stress (the impact-r population from the EURT) produce progenies with latent injuries. Thus, the proportion of families showing inhibition of seedling growth upon challenge with either HM or R reaches 80–100%. These injuries are apparently genetic by nature, since preliminary cultivation of plants under clean conditions failed to eliminate them.

On the other hand, such cultivation proved to have an effect on families derived from P plants exposed to chemical pollution (the NTMP impact zone): after this procedure, they produced seed progeny that was both highly viable and resistant to challenge with different factors. Thus, F_1 generations from the zones of chemical and radioactive contamination showed opposite responses to HM and R challenge. It should also be noted that plant homeostasis in both EURT and NTMP samples was disturbed, and metal tolerance and radioresistance of progenies from these zones differed from those in the background sample.

The causes of this difference apparently lies in specific action mechanisms of heavy metals and radiation. The fact that ionizing radiation is mutagenic was discovered long ago. In particular, its effect is manifested in an increased frequency of mutations, chromosomal aberrations, and changes in gene expression (Grodzinskii, 1989; Dubinin, 2000). In addition, radiation induces genomic instability in cells, which is transmitted to their distant progeny and accounts for increased frequencies of mutations and chromosomal aberrations over 30–100 generations (Dubinin, 2000; Mazurik and Mikhailov, 2001; Little, 2007). Molecular mechanisms of DNA-nontargeted effects differ from those operating in the irradiated nucleus. Such effects are related to changes in gene expression and, in particular, are mediated through regulation of oxidative metabolism (Spitkovskii, 1992; Little, 1998; Mothersill and Seymour, 1998; Morgan, 2003; Shao et al., 2004).

The toxic effect of heavy metals on cells is explained by their ability to bind proteins and amino acid as well as to substitute for ions with similar physicochemical properties contained in various enzymes (Evseeva et al., 2005; Geras'kin et al., 2005). Moreover, heavy metals al low concentrations have an indirect mutagenic effect, impairing the efficiency of DNA repair systems (Li and Rossman, 1989; Steinkellner et al., 1998; Rogstad et al., 2003). Special attention should be paid to the ability of heavy metals to generate reactive oxygen species in cells, which may also aggravate the genetic load (Murnane, 1995; Waisberg et al., 2003).

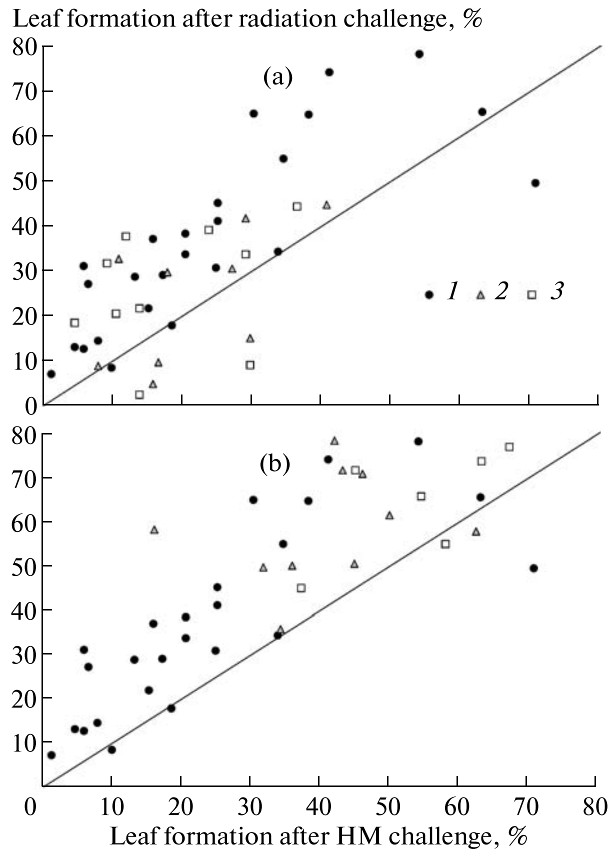

Fig. 3. Comparison of the effects of challenge with heavy metals (HM) and radiation on seed progenies from (a) EURT and (b) NTMP zones. Samples: (*1*) background, (*2*) buffer, and (*3*) impact zones.

Effects observed at the cell level cannot be directly extrapolated to organisms and populations. Many barriers for eliminating defective cells or organisms exist at every level of biological organization, since events regarded as a loss of reliability at a certain level are prerequisites for the maintenance and transmission of valid genetic information at a higher level. However, there is evidence that all these barriers do not ensure elimination of all alterations induced by technogenic factors (Dubinin, 2000). For example, the consequences of one-time irradiation of dandelion seeds proved to manifest themselves in five consecutive generations, affecting plant viability, mutability, and radioresistance (Pozolotina, 2003).

The results of this study that genetic disturbances induced in parental plants by chronic radiation exposure are differently expressed in their progeny. At a contamination level exceeding that of natural background radiation by two orders of magnitude, their progeny is highly viable and has a high growth rate but

Table 2. Proportions of families with different types of response to challenging exposure to heavy metals (HM) and radiation (R) in dandelion from different cenopopulations

Cenopopulation	Type of response*			
	HM "–" R "–"	HM "+" R "–"	HM "+" R "+"	HM "–" R "+"
	Survival of seedlings			
Background	0.32	0.20	0.36	0.12
Buffer-r	0.22	–	0.56	0.22
Impact-r	0.60	0.40	–	–
Buffer-m	–	0.10	0.70	0.20
Impact-m	–	0.11	0.78	0.11
	Number of seedlings with true leaf			
Background	0.40	0.04	0.16	0.40
Buffer-r	0.78	–	0.11	0.11
Impact-r	1.00	–	–	–
Buffer-m	0.50	–	0.30	0.20
Impact-m	0.11	–	0.33	0.56
	Root length			
Background	0.36	0.32	0.20	0.12
Buffer-r	0.33	0.56	–	0.11
Impact-r	0.80	–	0.10	0.10
Buffer-m	0.20	0.10	0.60	0.10
Impact-m	0.11	0.22	0.56	0.11

* (–) suppressive effect of challenging exposure exceeds 5%; (+) no suppressive effect or a stimulating effect.

carries latent injuries, which manifest themselves in reduced resistance to additional technogenic factors.

In the zone of chemical pollution, plants suffer mainly from the direct toxic effect of heavy metals, since this component has proved to level off after growing F_1 plants under clean conditions. The progenies of most families show increased resistance to challenge with different factors, which is evidence for disturbances in plant homeostasis and may result from selection for more resistant individuals.

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