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Ecogenetic Variation of White Clover (*Trifolium repens* L.) in Natural Populations in the Middle Ob Region[1]

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Abstract – The maximum lengths of seedlings of white clover were studied in two nonpolluted populations and two populations polluted with gas and oil in the middle Ob region. Seeds were collected by families (i.e., separately from each maternal plant). The seeds were germinated in water, gibberellin solution, or ammonium nitrate solution. In the course of analysis of variation, an ecological aftereffect was taken into account; this consisted of linear regression of the length of seedlings versus the seed mass in the family. The variation pattern of the trait appeared to be the same in all populations: the effect of the germination medium accounted for 72.5 - 83.1% of variation; that of the family, for 2.3 - 4.9%; and that of the medium–family interaction, for 2.6 - 4.1% of variation. The average lengths of the seedlings were higher for the polluted populations, irrespective of the germination medium. The results obtained are explained in terms of the conditions of plant growth in anthropogenically altered areas, which are not related to pollution.

INTRODUCTION

As a result of intense oil production, the western Siberian territory undergoes complex anthropogenic alterations, including coarse mechanical deterioration of lands and pollution with oil products and salt waters. Delayed genetic consequences (i.e., population genetic ones) of these effects generally receive little attention. We found such effects in white clover. When studying the character "leaf pattern" in the middle reaches of the Ob, we found considerable interpopulational variations, in contrast to the clover populations in northwestern Russia (Maksimenko *et al.*, 1993). Populations of clover in old Russian villages in the middle Ob region are characterized by greater genotypic variability with regard to leucylamine peptidase isozymes as compared to newly emerging populations on fill lands in this region (Maksimenko, 1991; Denisova, 1991). These differences may be interpreted in terms of the founder effect (Mayr, 1965).

While germinating *Phalaroides arundinacea* seeds by families on ecologically contrasting backgrounds, L.F. Semerikov and N.S. Zav'yalova (Semerikov and Zav'yalova, 1990) found the change in population variation of the trait "length of the seedling" that was determined by oil pollution itself. For a nonpolluted population, components of variation were found that were connected with the effects of genotype and genotype–environment interaction. These components were earlier described for a number of species under natural conditions (Glotov, 1983; 1992). In the population that was moderately polluted with oil, the authors did not reveal the component determined by genotype–environment interaction, and in a heavily polluted population neither genotypic nor genotype–environment interaction components were found. In this study, we attempted to establish this effect in white clover.

MATERIAL AND METHODS

We collected the seeds of clover by families (i.e., separately from each maternal plant) in nonpolluted populations and in populations polluted with oil and gas. In nonpolluted populations of the old Russian villages Tundrino and Cheuskino, clover (*Trifolium repens* L.) grows on the outskirts as a continuous carpet together with *Plantago major* L., *Poa pratensis* L., *Polygonum aviculare* L., *Matricaria matricarioides* Parter, and other species. The other two populations, those of the Karkateevy and Yuzhnyi Balyk villages, occupy fill lands of oil-refining facilities, where soil is heavily polluted with oil products, and air – with gases. Clover is one of the pioneer species settling on these lands; it grows in clusters.

After determining the mass of 100 seeds in a family, we scarified them by treating them with boiling water for 45 s. Then we placed the seeds in a Petri dish, and the seeds that were ready for germination were placed into a roll of filter paper impregnated with the corresponding solution. Next we placed the roll in a damp chamber at a temperature of approximately 20°C. We conducted the germination in water, 0.001% gibberellin, or 0.5% ammonium nitrate (Glotov and

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Table 1. Structure of variation of the seedling length in white clover (%)

Population (number of families)	Source of variation			
	medium	family	interaction	error
Tundrino (15)	83.1	2.9	3.6	10.6
Cheuskino (16)	73.5	2.8	4.1	19.6
Karkateevy (20)	77.6	2.3	2.6	17.5
Yu. Balyk (16)	72.5	4.9	3.4	19.2

Gritsenko, 1983). After 12 days, we measured the lengths of the seedlings (from the cotyledonous bend to the top of the radicle). As we found in preliminary experiments, this point of time corresponded to the maximum seedling length. We conducted the experiments with the seeds from all populations and families simultaneously and in duplicate. For statistical treatment, we used a logarithm of the seedling length in order to stabilize the dispersion of the feature.

During analysis of the data, a problem emerged concerning an allowance for the ecologic aftereffect. The seeds were formed on a certain maternal plant. Therefore, their quality and, as a result, characteristics of the plants developing from them (at least those occurring in early ontogeny) were partly determined by the growth microconditions and the viability of their maternal plant (the environment-related parent–offspring correlation). The mass of seeds is often used to characterize their quality. An analysis of the literature revealed that, within normal limits, seedling length either depended linearly on the seed mass or followed a pattern of the saturation curve (Kuperman, 1969; Kizilova, 1974; Khaideker, 1978; Schaal, 1980; Weiss, 1982; Gross, 1984; Temme, 1986). In our study, we found that the squared seed mass in the regression equation had a negative coefficient. Therefore, we restricted our corrections by excluding the aftereffect via an allowance for the linear regression. This was made by rotating the regression line into a position parallel to the abscissa axis around the point (0, a), where a is a constant term of the equation of linear regression. As a rule, the association between the seedling length and the seed mass was statistically significant. The extent of this association considerably varied, determining 1 - 30% of the total variation. Therefore, we further analyzed the values

$$y'_{ij} = y_{ij} - bx_i,$$

where y_{ij} is the value of trait y in the jth seedling from the ith family, b is the coefficient of linear regression in the given series of the experiment on the given germination medium, and x_i is the mass of 100 seeds in the ith family.

We analyzed the structure of variation using three-factor dispersion analysis (with the germination medium and the series as fixed factors and the family as a random factor and assuming a nonorthogonal complex, the number of observations per cell being 4 - 10). We used SAS statistical software package and GLM procedure for the general linear model (Afifi and Azen, 1979). The components of dispersion were estimated from the mathematical expectations of mean squares for the given model of dispersion analysis (Scheffe, 1959). Then we combined the components of dispersion and estimated the contributions of the medium, family, and medium–family interaction.

RESULTS AND DISCUSSION

We analyzed the structure of variation using either original data or the data corrected for the ecological aftereffect. Both approaches yielded virtually the same results. This seems to be accounted for by a similar regression pattern for all variants of the experiment. Table 1 shows the results of the second type of analysis. As one can see from the table, the structures of variation of the trait in the nonpolluted and polluted populations were almost the same. The effects of the family

Table 2. Average lengths of seedlings in different media

Medium	Parameter	Population			
		Tundrino	Cheuskino	Karkateevy	Yu. Balyk
Water	Sample size	235	267	329	278
	Mean ± error (log)	1.364 ± 0.009	1.385 ± 0.007	1.426 ± 0.006	1.450 ± 0.006
	Mean (mm)	23.1	24.3	26.7	28.2
Gibberellin	Sample size	242	289	346	291
	Mean ± error (log)	1.433 ± 0.007	1.439 ± 0.006	1.478 ± 0.006	1.510 ± 0.005
	Mean (mm)	27.1	27.5	30.1	32.4
Ammonium nitrate	Sample size	216	262	316	280
	Mean ± error (log)	1.098 ± 0.007	1.099 ± 0.006	1.142 ± 0.006	1.194 ± 0.006
	Mean (mm)	12.5	12.6	13.9	15.6

(genotype) and the family–medium (genotype–medium) interaction were always highly significant ($P < 0.001 - 0.01$), and contributions by these two sources to the total variation were approximately the same, as was the case for all species studied earlier (Glotov, 1993; 1992). These results may be explained using the data represented in Table 2 (the average seedling lengths for different media). It is evident that the average values for the polluted populations (Karkateevy and Yuzhnyi Balyk) were significantly higher than in the nonpolluted ones (Tundrino and Cheuskino) for all three media (even for ammonium nitrate solution, which considerably suppresses seedling growth).

White clover is characterized by a very good ability to adapt to a wide range of abiotic conditions, including extreme ones. In particular, this accounts for the wide distribution of white clover on deteriorated lands, where it is often one of the pioneer species. However, as soon as other species begin to settle there, it appears that white clover is not competitive with them. Clover remains, if at all, in the succession communities that undergo continuous anthropogenic pressure. It is more resistant to mechanical damage, primarily to trampling, as compared to other species. In this respect, clover has less favorable growth conditions in nonpolluted populations. A sort of equilibrium occurs: in nonpolluted areas of the types studied, abiotic conditions are better, but a cenosis-related stress is more acute; in polluted areas (at the studied level of pollution), although toxic effects of oil products occur, the competition is almost entirely excluded. As a result, there is no cause for changing vectors of selection or for dramatic plastic reactions. On the contrary, *P. arundinacea* is able to tolerate a phytocenosis-related stress, often suppressing other species. Toxic abiotic effects occurring in polluted populations appear to alter the structure of variation. Therefore, we believe that the different responses of *P. arundinacea* and *T. repens* L. to the conditions of oil-polluted lands are connected with different biological characteristics and different strategies of these species.

On the one hand, the results obtained are useful for more sophisticated research and complete confirmation of the hypothesis suggested. On the other hand, they indicate the necessity of involving in analysis the delayed population genetic consequences of anthropogenic deterioration of the western Siberian territory for many plant and animal species, which differ in their biological characteristics and systematic position.

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