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COMMUNITY GENETICS: RAPD-PCR FRAGMENTS IN OAK MARKING HEAVY METAL ACCUMULATION AND HERBIVORE MICRO-COMMUNITY STRUCTURE

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ГЕНЕТИКА УГРУПОВАНЬ: RAPD-PCR ФРАГМЕНТИ ДУБА, ЩО МАРКУЮТЬ АКУМУЛЯЦІЮ ВАЖКИХ МЕТАЛІВ ТА СТРУКТУРУ МІКРОУГРУПОВАНЬ ФІТОФАГІВ

Уміст цинку, міді, свинцю й кадмію в листі дуба і щільність найбільш розповсюджених у його кроні комах-філофагів досліджували залежно від генотипу рослини-господаря, визначеної з використанням процедури випадково ампліфікованої поліморфної ДНК (RAPD) з праймером ОРА-14. Чотири з одинадцяти досліджених ДНК фрагментів (110, 210, 350 і 400 п.н.) маркірували мінливість щодо вмісту цинку, міді й кадмію в листі дуба. Дерева, що містять фракції ДНК завдовжки 250, 210 і 110 п.н., були в меншій мірі заселені зеленою дубовою листовійкою, а щільність непарного шовкопряда була майже в 2 рази нижчою на деревах, що містять фракцію завдовжки 110 п.н. Чим вищими були парні індекси генетичної схожості дерев, тим у більшій мірі збігалися на них щільність зеленої дубової листовійки і зимового п'ядуна. Отримані дані підтверджують гіпотезу про регулюючу роль генетичної інформації в екосистемних процесах, таких як кругообіг речовин і формування мікроугруповань.
Ключові слова: генетика угруповань, акумуляція важких металів, структура мікроугруповання.

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Contents of foliar zinc, copper, lead and cadmium in oaks and densities of the most common herbivore species in their crowns were studied in respect to the oak genotypes tested with random amplified polymorphic DNA procedure (OPA-14 primer). Four from eleven studied DNA fragments (110, 210, 350 and 400 bp) mark variation in zinc, copper and cadmium contents in oak leaves. Oaks with 250 bp, 210 bp and 110 bp fractions in their spectrums of amplified DNA were less infested by *T. viridana* than others and *L. dispar* density was nearly twice as lower in the oaks with 110 bp fragments in their spectrums than in others. Genetic similarity, calculated from pairwise comparisons of oak RAPD spectrums, was linked to similarity in densities of *T. viridana* and *O. brumata* on corresponding trees. These data confirm the hypothesis about regulating role of genetic information in the ecosystem process such as substance cycles and micro-community structure.
Key words: community genetics, heavy metal accumulation, micro-community structure.

Any biologist, independently from the area of his scientific interests, probably, would agree with an assumption that genetic information regulates biological processes at different levels from molecules up to populations. At the same time there are a number of theoretic works incorporating genetic patterns in description of ecological processes in ecosystems (Holubets, 1982, 2000; Ivashov, 1991). Their authors assume that genetic information execute managing role in biological community as a cybernetic system. After M. A. Holubets (1982), genetic system of biological community, which he named «genoplast», consists of functional assemblage of stochastically interacting genofonds of the community participants. Although this assumption is debatable (Chernov, 1996), experimental findings should argue for one or another point of view.

At the same time the theoretic constructions lean upon a number of empiric data, obtained from investigation of micro-communities in individual oak trees (Ivashov, 2001).

The whole micro-community with central tree (central determinant) was named «consortia» (Beklemishev, 1951). Ecological and genetic processes may vary significantly in their expressions and directions among the micro-populations or micro-communities and this could frequently result in their masking at higher scales. Micro-community in individual tree is, hence, the most adequate unit for investigation of detailed structure of forest ecosystems (Whitham, 1981).

Preliminary findings on the micro-community patterns within individual tree have shown that genetic information plays here critical role and the genoplast of consortia may serve as a cybernetic compartment, regulating stochastic interactions among organisms of different species. Any organism interacts with an environment in accordance to its genetic constitution, raises unique requirements to the environment and in its own manner interacts with representatives of other species. Thus, intra-populative genetic structure and variation should strongly influence integrative ecological pattern of the population in the community or ecosystem.

In this respect, our present work is focused on the investigation of the relationships between intra-population genetic variation in oaks and such the «ecosystem» parameters as heavy metal accumulation and micro-community structure in individual oak consortias.

MATERIALS AND METHODS

The study was performed in the testing ground «Lavrovoe» situated in 15 km to Northeast from Yalta (South Coast of the Crimean peninsular). Representatives of two oak species, *Quercus pubescens* Willd. and *Q. petreae* Mattush. /Liebl., grow in this general area. Totally 16 trees of *Q. pubescens* (trees 1–13) and *Q. petreae* (trees 14–16) served as the models. Leaves were sampled from model trees in May 2004. Simultaneously, densities of the most common insect herbivores were documented in the model trees. Following species were taken into the account: *Tortrix viridana* L., *Lymantria dispar* L., *Operophthera brumata* L., *Toeniocampa gothica* L., *Agrotera nemoralis* Sc. The insect densities were expressed in numbers of found individuals per 100 apical points.

RAPD-PCR analysis was performed using DNA from each of the model trees. DNA was extracted from 0.5 cm^2 of fresh leaf tissue in accordance with standard method (Sambrook, Fritisch & Maniatis, 1989). OPA-14 primer, TCTGTGCTGG (Operon Technologies, USA), was used for RAPD-PCR. Reaction was initiated in 25 μl of the mixture with PCR reagents from GenePak^{TM} PCR Universal (IsoGen, Moscow) at the «Tercyc» amplyficator (DNA-Technology, Russia). Amplification was carried out under fallowing conditions: 95 ^{0}C for 5 min, followed by 45 cycles of 95 ^{0}C for 1 min, 36 ^{0}C for 1 min, 72 ^{0}C for 2 min and final extension 72 ^{0}C for 10 min.

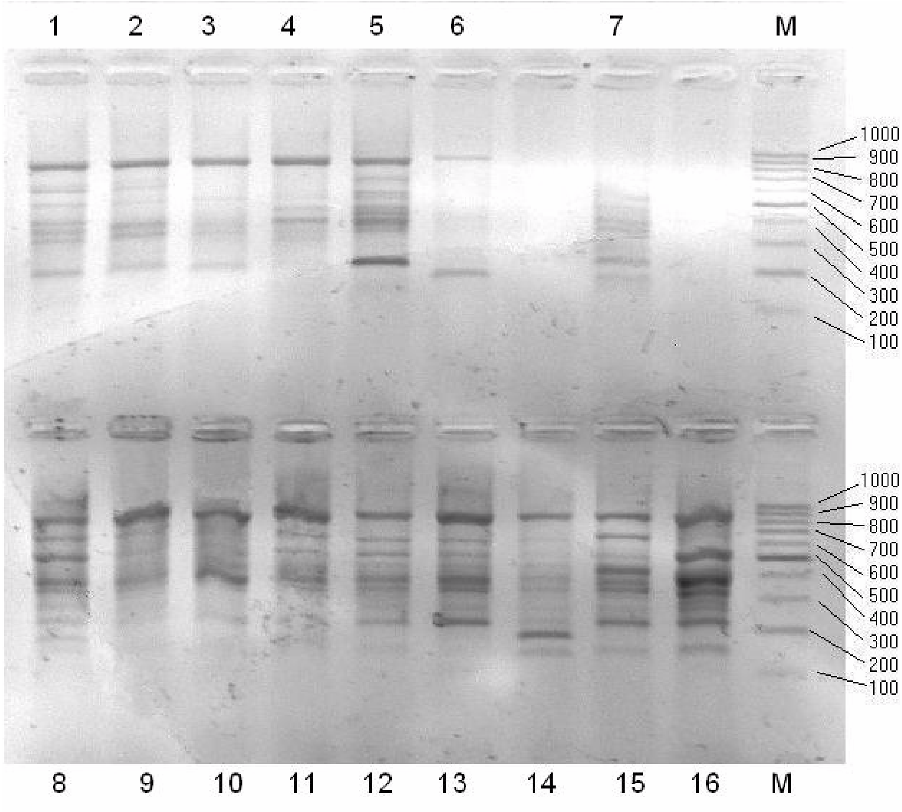
The amplification products were separated by electrophoresis in 1.8 % TBE agarose gel (Sambrook, Fritisch & Maniatis, 1989), stained with ethidium bromide and photographed under the UV light. DNA-markers M 100 (IsoGen, Moscow) including fragments with length of 100, 200,…, 900, 1000 bp, were used as the molecular weight markers.

Data on the heavy metal contents were obtained after dry mineralization procedure with following detection of copper, zinc, cadmium and lead using atomic absorption method (Methodic instructions…, 1989). Each analysis was carried out in three replicates. The index of genetic similarity (F) was calculated for all pairs of genotypes using Nei & Li's matching coefficient method: $F=2N_{jk}/(N_j+N_k)$, where N_{jk} is the number of bands shared by both genotypes and N_j and N_k are the numbers of bands shared by genotypes j and k, respectively (Nei, 1987).

RESULTS

RAPD-PCR polymorphism in model oak trees

Amplification of the oak leaf DNA with the OPA-14 primer has given up to 12 electrophoretically detectable RAPD-markers (*Figure*). The set of the markers could specifically characterize each of the model trees. Obtained markers are not specific for studied oak species and give no possibility to separate them each from other. Thus we may analyze total oak sample.



Individual electrophoretic spectrums of random amplified polymorphic DNA from *Q. pubescens* (1–13) and *Q. petraea* (14–16) with OPA-14 primer; M – markers of molecular weights (100 – 1000 bp)

Model trees exhibit wide polymorphism on the obtained RAPD-markers. Individual spectrum could contain from 4 to 12 amplified DNA fragments.

RAPD fragments, marking heavy metal contents in oak leaves

Table 1 presents the data on copper, zinc, cadmium and lead contents in leaves of the model trees in respect to occurrence or absence of one or another band in their RAPD-spectrums. Significant differences were found for the copper, zinc and cadmium contents and no differences were found for the lead contents. Oaks with 400 bp fractions in their spectrums accumulate more zinc and copper in their leaves than the oaks without these fractions. Presence of the 350 bp fraction in the spectrum coincides with the low cadmium contents in the oak leaves (Table 1).

Amplified fragments also marked variances of the metal contents. Variation in the copper contents was marked by 400 bp fraction, variation in the zinc contents was marked by 400 bp and 210 bp fractions and variation in the cadmium contents was marked by 400 bp, 350 bp, 210 bp and 110 bp fractions (Table 1).

Copper and zinc showed close trends in respect to variation of their contents in dependence from genotype, and both them vary in contrast with cadmium (Table 1). As a rule, presence of a RAPD fraction coincides with high contents of copper and zinc or with their high variation, and with low cadmium contents or with low its variation and *vice versa*.

Two oak groups may be formed in agreement with likeness of their RAPD spectrums. One group (A) includes oaks 8, 12, 13 and 16, and another (B) consists of oaks 1-5, 9 and 11. Spectrums of the A-group participants include at mean 9.75 fractions, while oaks from the B-group have at mean 7.13 fractions in their spectrums. These oak groups significantly differ each from other by the variation of zinc and cadmium contents (Table 2).

Table 1

Contents of some heavy metals in the leaves of oaks with occurrence or absence of a DNA fraction in their RAPD-spectrums (OPA-14 primer)

RAPD-маркер	400 bp			350 bp	210 bp		110 bp
Heavy metal	zinc	copper	cadmium	cadmium	zinc	cadmium	cadmium
Absence of the fraction							
Mean (mg/kg of dry weight)	21.98	9.93	0.0310	0.0312	25.72	0.0264	0.0289
Standard error	1.58	0.87	0.0077	0.0047	1.26	0.0040	0.0046
Variance	9,97	3.04	0.00024	0.00013	14.37	0.00015	0.00015
N	4	4	4	6	9	9	7
Occurrence of the fraction							
Mean (mg/kg of dry weight)	28.31	14.51	0.0204	0.0188	27.98	0.0197	0.0186
Standard error	1.52	1.62	0.0019	0.0017	3.26	0.0019	0.0018
Variance	27.74	31.60	0.00005	0.00003	63.93	0.00002	0.00003
N	12	12	12	9	6	6	9
t-criterion for the means	2.89	2.49	–	2.46	–	–	–
P	0.02	0.03	–	0.05	–	–	–
F-criterion for the variances	–	10.39	5.22	5.03	4.45	6.92	5.25
P	–	0.04	0.02	0.02	0.03	0.02	0.02

Only significant values of the t and F criterions are given

In this variant of comparison, zinc and cadmium showed opposite trends in variation of their contents. It may be due to well-known fact of antagonistic interaction between these elements (Alekseeva-Popova, 1983). All the more zinc and cadmium both belong to the same element group in the Mendeleev's periodic table. Besides it is well known that cadmium, zinc and lead ions jointly are less toxic for organisms than each of the ions separately (Coughtrey & Martin, 1978).

Table 2

Contents of some heavy metals in the leaves of oaks separated into two genetically distinct groups (A and B) in accordance with likeness of their summary RAPD spectrums (OPA-14 primer)

Heavy metal	zinc	copper	lead	cadmium
A group (mean numbers of bands 9,75)				
Mean (mg/kg of dry weight)	26.2	13.8	0.253	0.0188
Standard error	5.0	3.6	0.081	0.0017
Variance	100.0	53.0	0.026	0.000012
N	4	4	4	4
B group (mean numbers of bands 7,13)				
Mean (mg/kg of dry weight)	26.7	14.5	0.411	0.027
Standard error	1.4	1.9	0.054	0.004
Variance	16.2	30.3	0.024	0.00016
N	8	8	8	8
F-criterion	6.15	–	–	13.81
P	0.02	–	–	0.03

Only significant values of the F criterions are given

Relation between contents of the metals and herbivore densities

Densities of some studied oak herbivores exhibit significant relation with contents of the microelements. Density of *O. brumata* larvae in the tree was negatively related with overall contents of copper and zinc ($R = -0.51$; d.f. = 14; $P < 0.05$). Other analyzed variants in absolute evaluations showed no significant correlations.

Different herbivore species not equally contribute to the total herbivore density. The same is true for contribution of different metals to their total contents. In this relation we searched the ties between ranged data sets of metal concentration and herbivore density when natural digital number was conferred to each measure in relation to its value. This procedure allows revealing a number of significant correlations. Gypsy moth density in a tree was positively related with sum of ranges describing cadmium and lead contents in oak leaves ($R = 0.5$; d.f. = 14; $P = 0.05$). Sum of ranged densities of *L. dispar*, *O. brumata* and *T. gothica* correlates with ranged data of cadmium contents in the oak leaves ($R = 0.51$; d.f. = 14; $P < 0.05$).

RAPD fragments, marking the herbivore densities

Microelement accumulation in oak leaves was related with genetic determinants of the oak. In its turn, densities of some oak herbivores were correlated with the microelement contents in the oak leaves. Thus, the next logic step is to detect possible relation between oak genetic determinants and the composition or densities of the herbivores inhabiting it.

Table 3 presents the data on the densities of some oak herbivores on the trees with presence or absence of one or another band in their RAPD spectrums. Oaks with 250 bp, 210 bp and 110 bp fractions in their spectrums of amplified fragments were less infested by *T. viridana* than others. Besides *L. dispar* density was nearly twice as lower in the oaks with 110 bp fragments in their spectrums than in others (table 3).

Table 3

Densities of some oak herbivores on the oaks with occurrence or absence of a DNA fraction in their RAPD-spectrums (OPA-14 primer)

RAPD-marker	350 bp	250 bp	210 bp			110 bp	
Species	L. dispar	T. viridana	T. viridana	O. brumata	A. nemoralis	T. viridana	L. dispar
Absence of the fraction							
Mean (individuals/100 a.p.)	41.0	35.7	33.8	2.1	3.2	37.7	48.6
Standard error	3.6	3.4	3.2	0.5	1.1	4.1	4.6
Variance	76.3	102.0	93.6	2.3	10.2	118.1	145.4
N	6	9	9	9	9	7	7
Occurance of the fraction							
Mean (individuals/100 a.p.)	32.5	20.4	17.4	4.5	3.6	22.2	26.5
Standard error	6.8	5.1	3.5	1.4	0.5	4.0	4.0
Variance	410.4	184.1	74.7	11.0	1.5	147.3	143.2
N	9	7	6	6	6	9	9
t-criterion for the means	–	2.58	3.45	–	–	2.69	3.65
P	–	0.022	0.005	–	–	0.017	0.003
F-criterion for the variances	5.38	–	–	4.72	7.03	–	–
P	0.040	–	–	0.026	0.023	–	–

Only significant values of the t and F criterions are given; a.p. – apical point

Genetic determinants of the tree are also related with variance of the insect densities. For instance, variation of gypsy moth density was high among the oaks with 350 bp fractions in their RAPD spectrums. Analogous relation was found between 210 bp fraction and variation in *O. brumata* density, while this oak DNA fragment marks low variation in *A. nemoralis* density (table 3).

Summary RAPD spectrums showed relation with only variation in *O. brumata* density. Variation in *O. brumata* density was higher among the trees from A-group (variance = 15.05) in comparison with trees from B-group (variance = 2.48; F=6.08; P=0.023).

Genetic similarity, calculated from pairwise comparisons of oak RAPD spectrums, was related to similarity in densities of *T. viridana* (R = 0,38, d.f. = 118, P<0,01) and *O. brumata* (R = 0,23, d.f. = 118, P<0,05). No significant relations were found for other species.

DISCUSSION

The data obtained show that there are some relations among, at first sight, so distant each from other parameters as tree genotype, accumulation of heavy metals in its leaves and structure of the insect micro-community inhabiting this tree. Genetic determinants detected with RAPD-PCR method are an innate tree characteristic. Heavy metal contents in oak trees on the one hand may be considered as their individual features but on another hand heavy metal accumulations contribute to flow of microelements in the ecosystem, extend beyond the limits of plant organism and may be included in the biogeochemical cycle as its elements. The set of species and the ratio of their densities are concerned to biological assemblage of organisms.

The question arises in respect to determination of causal relations among the established ties. Obviously genetic information might play a role of the major regulating factor. It is well known that genetic patterns regulate functions of cells, organs, organisms and populations. Recent results allow extending regulating role of genetic information up to highest levels of biological organization such as biological community or ecosystem (Holubets, 1982; Ivashov, 1991).

Genetic information determines all the processes associated with basic or secondary metabolisms (Schweitzer et al., 2004). Hence, it may be respected that accumulation of microelements, which execute visible role in an organism functioning, should be under the gene control. Thus, genetic patterns of oak tree, the central determinant of the consortia, might influence heavy metal accumulation in its organism and therefore determine flow of the microelements throughout the micro-community of the consortia. In this case genetic structure of whole oak population may play significant role in regulation of microelement flow throughout the local ecosystem. This is also an instance that itraspecific genetic variation could affect ecosystem processes (Whitham et al., 2003).

Significance of the tree genetics for composition and ratios of phytophagous insects, inhabiting it, is less obvious, but may be also easily described. Any individual tree forms highly heterogeneous environment for organisms inhabiting it (Whitham, 1981). Tree serves as fodder resource for herbivores and whole the diapason of its variation represents herbivores' potential niche. Competition among them leads to the niche partitioning. Niche partitioning occurred even inside the single oak tree (Simchuk, Ivashov, 2005). In its turn tree heterogeneity is determined by its genetic constitution. Thus the tree genotype may indirectly influence composition and ratios of the species in the micro-community inhabiting the tree.

Existence of relations between heavy metal contents in oak leaves and ratios of some herbivores eating these leaves show that the microelement accumulation might serve as one of possible mediators for transition of ordering information from the tree's genetic determinants to the herbivore micro-community. There is some conformity among relations of DNA markers with heavy metal contents, DNA markers with species ratios and heavy metal contents with species ratios. For example, 350 and 110 bp DNA fragments mark low gypsy moth density or low its variation (see table 3). These markers also coincide with low cadmium contents or low its variation in oak leaves (see table 1). And finally, gypsy moth density in a tree shows positive correlation with cadmium contents in its leaves. Analogous reasoning could be performed in respect to the oak 210 bp DNA marker, zinc contents in oak leaves and *O. brumata* density in its crown.

At the same time no all the variants of established insects' density differences could be explained by means of changes in the heavy metal contents as mediators between tree genotype and the insect. No any such the explanation may be proposed for *T. viridana* and *A. nemoralis*. This is not a surprise because a great complex of tree features affects the herbivores inhabiting it. Nevertheless, obtained results clearly show that intrapopulative genetic variation in oaks has ecosystem consequences and influences higher level of biological organization, the ecosystem level.

Wide empiric work in this field, named community or ecosystem genetics (Antonovics, 2003), is beginning only now, while theoretic basis was built much earlier (Holubets, 1982; Ivashov, 1991; Antonovics, 1992). The role of intrapopulative genetic variation for ecosystem structure was empirically studied on the instance of such the processes as litter decomposition and nitrogen mineralization (Madritch & Hunter, 2002; Whitham et al., 2003). For the data explanation authors introduced the term «extended phenotype», meaning ecosystem consequences of intrapopulative variation in a keystone or a dominant species in the community.

By our opinion, the concept of «extended phenotype» approaches in its essence to the much more definite and elaborated concept of consortia. In particular, it has been shown that the oak tree, for example, as a consortia determinant influences genetic structure of the herbivores inhabiting it (Ivashov, 2001). The presented paper continues the above mentioned works and shows that peculiarities of a consortia determinant is able to influence features of «ecosystem» level such as flow and accumulation of microelements and densities of the herbivores inhabiting it.

Extended phenotypes or consortias describe, as a rule, direct interactions among closest links in the complex web of interspecies interactions in ecosystem and, thus, in the genetic sense they are only elements of the community genetic system. These elements are very suitable and useful for investigation, but we should keep it in mind that they are only simplified elements.

The data obtained show that oaks' genetic determinants influence herbivore densities. Earlier we have shown that herbivore genetics also determine its numerical dynamics (Simchuk et al., 1999). Hence, herbivore dynamics is a result of interaction between genetic determinants of both the tree and the herbivore. Besides, one herbivore competes with other herbivores in respect to its and their genetic compositions (Simchuk, Ivashov, 2005), and, thus, transfers to them the information about its interaction with the tree. The most susceptible trees, on which the herbivores are prevailed, are thought to lose in the straggle for existence and will not bring their genes to the next generations or will do this worse than others. Hence, genetic patterns of the herbivores could alter genetic structure of their host plant. We see here instances of complex web of ties among the genofonds of interacting species. As ties determine a system, we could describe genetic system of a biological community with genofonds of interacting species as its elements, and this system was named Genoplast (Holubets, 1982).

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