

EVOLUTIONARY DIVERGENCE OF SEXUAL *OTIORHYNCHUS ALPICOLA* POPULATIONS ON THE SOUTHEAST DINARIDS

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Abstract -The present distribution of weevil *Otiorhynchus alpicola* at the Southeast Europe is disjunct (vicariant), restricted to open habitats above the timberline around the tops of the highest mountains. In order to estimate the level of genetic differentiation and patterns of evolutionary divergence over these postglacial vegetational disjunctions, allozyme variation of ten populations were analyzed. The analysis of genetic differentiation among populations revealed a moderate degree of intrapopulation variation and a rather high level of genetic differentiation. The results of hierarchical Wright's statistics ($F_{DT}=0.338$; $F_{DS}=0.278$ and $F_{ST}=0.083$) and a low level of estimated number of migrants *per* population ($N_e m$) suggest the prevalence of the genetic differentiation of the local populations over regional genetic differentiation. Results of estimation of genetic distances between populations are concordant with the history of the high altitude environments in Southeast Europe during Pleistocene.

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INTRODUCTION

Otiorhynchus alpicola is one of the most widely distributed high altitude weevil species in Europe and the most abundant alpine-subalpine species of the genus *Otiorhynchus* in the region of the Southeast Dinarids (Apfelbeck 1929; Osella 1977; Freude *et al.* 1981)(Fig. 1). On the mountains of Balkan Peninsula this polytypic species inhabits alpine and subalpine habitats from the eualpine zone (Apfelbeck 1896). In Europe, the eualpine zone is characterized by waste space covered by mountain meadows above the timberline situated at the altitudes of 1800-2500 *m a.s.l.* (Mani 1968). In the region of the Alps this species inhabits the continual high-altitude communities in the zone at 2000-2500 *m a.s.l.*, whereas its distribution in the Southeast Europe is restricted to disjunct habitats at elevations over 1800 *m.* (Apfelbeck 1896; Kovačević 1971) (Fig. 1). The sizes of these islands-like habitats range from about 10 *km*² (Bjelašnica Mt. and Visočica Mt.) to several hundred *km*² (Prokletije Mt.).

The populations of *O. alpicola* which inhabit the Dinarian mountains are considered relicts of an ancestral northern stock that was dispersed along the corridors when Pleistocene glacial advances have caused displacements of high altitude vegetation to lower elevations and altitudes (Janković 1984). With

subsequent warm-dry interglacials, the northward and upward elevational displacement of vegetation (combined with local tectonic) has dispersed this inferred ancestral stock through the Dinarian mountain ranges.

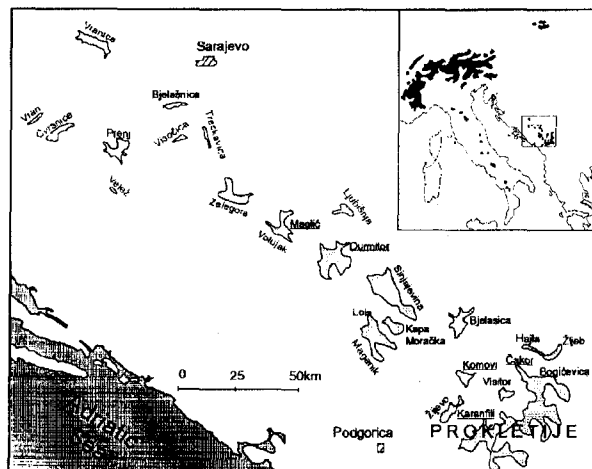


Fig. 1. Distribution of the *Otiorhynchus alpicola* Boh. in Europe summarized from different sources (Kovačević 1971; Osella 1977; Freude *et al.* 1981; Mesaroš, unpublished data). Areas of distribution in the Southeast Europe are emphasized according to isohypses over 1800 *m a.s.l.* Analysed localities and/or regions are underlined.

Individuals of *Otiorhynchus alpicola* are characterized by the absence of wings, while elytrae are fused, forming a chitinized capsule that protects the soft parts of the body. This characteristic makes their dispersion to broader regions impossible (Wagner and Liebherr 1992). Dramatic differences of day and night temperatures these beetles are abiding spending a daytime under the stones where changes of the microclimate are mainly alleviated. These adaptations to the environmental heterogeneity in the high mountains are well known (Mani 1968; Edwards 1987) and result in a characteristic population structure of this species (Mesaroš 1993). *Otiorhynchus alpicola* reproduces sexually and asexually. Somatic cells of bisexual individuals have 22 chromosomes. It was noticed that within the populations of this species parthenogenetic races of different ploidy level occur sympatrically or allopatrically (Jahn 1941; Mesaroš 1993).

The patterns of recent geographical distribution and the extent of differentiation in populations of *O. alpicola* are important issues in the explanation of historical zoogeography of the Southeast Dinarids.

MATERIAL AND METHODS

Adults from ten different populations of *Otiorhynchus alpicola* were collected in late June and early July of 1991 at the region of Southeast Dinarids (Fig. 1). The localities of collecting, their regional appurtenance and the number of analyzed individuals (in parenthesis) were as follows:

- Prenj Mt. (32);
- Bjelašnica Mt. (40);
- Sedlo - Durmitor Mt. (95);
- Ališnica - Durmitor Mt. (32);
- Štuoc - Durmitor Mt. (32);
- Žijevo Mt. - West Prokletije (32);
- Komovi Mt. - West Prokletije (68);
- Karanfili - East Prokletije (20);
- Bogičevica Mt. - East Prokletije (32);
- Čakor - East Prokletije (31)

The samples were transferred live to the laboratory where all inside organs were dissected out and deep frozen (-70°C) for electrophoresis of isoenzymes. Eight enzyme systems were assayed by horizontal starch gel electrophoresis: *Adenylate kinase* (Ak), *Aspartate aminotransferase* (Aat), *Isocitrate dehydrogenase* (Idh), *Malate dehydrogenase* (Mdh), *Phosphoglucosomerase* (Pgi), *Phospho-glucomutase* (Pgm), *Amylase* (Amy), *Esterase* (Est) and *Superoxide*

dismutase (Sod). Details of the isoenzyme separation and the staining procedure were published elsewhere (Mesaroš *et al.* 1995).

Twelve zones of activity have been reliably scored on the gels in the samples. In order to compare genetic similarities between analyzed populations of *O. alpicola* (according to the allozyme frequencies), a widely used Nei's coefficient of genetic identity, modified for small samples, was applied (Nei 1978).

To estimate the regional genetic differentiation of populations within and between the mountain complexes from the Southeast Dinarids the hierarchical F-statistics was used (Wright 1978). The variance component that partitions the variability in the total sample (T), regional appurtenance (S) and populations (D) were calculated. The three F-statistics are interrelated so that the $F_{DT} = F_{DS} (1 - F_{ST})$, where F_{DS} and F_{DT} are measures of deviation from Hardy-Weinberg proportion within regions and the total sample respectively, whereas F_{ST} is a measure of the genetic differentiation among regions, mountain complexes in this situation.

At the same hierarchical levels, the estimated migratory rate (m) and the number of migrants *per* generation ($N_e m$) were calculated. The $N_e m$ was calculated using the formula:

$$N_e m = (1/F_{ST})/4$$

where $N_e m$ is the effective population size, F_{ST} is the estimated level of genetic differentiation among populations and m is the gene flow rate (Wright, 1943). The migratory rates were estimated according to the mean genetical identity (\hat{I}) at the same level (Nei 1987), using the equation:

$$\hat{I} = m/(m+v)$$

where \hat{I} was the average genetic identity and $v = 2 \cdot 10^{-6}$. These quantities were calculated only for those regions that were represented by more than one population (Durmitor, West Prokletije and East Prokletije).

Genetic identities and genetic distances were clustered using an unweighted pair-group method of arithmetic averages (UPGMA) (Swofford 1989) and the obtained results were presented as the dendrogram.

RESULTS

Observed heterozygosity within investigated populations ranged from 4.2% (Bogičevica) to 15.3% (Bjelašnica Mt.) while the percentage of polymorphic loci varied from 28.6 (Karanfili) to 71% (Sedlo) (Table 1). These results of the within population genetic vari-

Table 1. Parameters of within population genetic variability of *Otiirhynchus alpicola* estimated according to 14 codominant loci.

	Percent of polymorphic loci	Heterozygosity	
		Obtained	Expected
1) Prenj Mt.	35.7	0.1120.05	0.1350.06
2) Bjelašnica Mt.;	64.3	0.1530.06	0.2160.06
3) Sedlo (Durmitor Mt.);	71.4	0.1270.04	0.2120.06
4) Ališnica (Durmitor Mt.);	64.3	0.1620.04	0.1680.05
5) Štuoc (Durmitor Mt.);	57.1	0.1020.4	0.1530.06
6) Žijevu Mt. (West Prokletije);	35.7	0.1070.7	0.0790.05
7) Kom Mt. (West Prokletije);	57.1	0.1100.04	0.1320.05
8) Karanfili (West Prokletije);	28.6	0.0720.04	0.0970.05
9) Bogićevica Mt. (East Prokletije);	42.9	0.0600.04	0.0870.04
10) Čakor (East Prokletije)	42.9	0.0470.02	0.0690.3

ability had significantly higher values than those accepted as an average for invertebrates (Nevo *et al.* 1984). The details on the allele frequencies of polymorphic loci in diploid *O. alpicola* populations can be found elsewhere (Mesaroš *et al.* 1995).

differentiation between populations within a particular mountain were somewhat lower ($F_{DS} = 0.278$). The genetic differentiation between different mountains, however, was very low ($F_{ST} = 0.083$), which suggests the prevalence of the genetic differentiation of the local

Table 2. Coefficients of genetic identity (below the diagonal) and genetic distances (above the diagonal (Nei 1978) for 10 sexual population of *O. alpicola* from south-east Dinarids. The numbers of populations are as in Table 1.

Pop.	1	2	3	4	5	6	7	8	9	10
1	-	.185	.073	.140	.201	.300	.124	.235	.103	.132
2	.831	-	.061	.054	.019	.041	.046	.111	.082	.070
3	.930	.940	-	.022	.064	.130	.031	.134	.085	.082
4	.869	.947	.978	-	.044	.111	.016	.104	.087	.067
5	.818	.982	.938	.957	-	.054	.045	.103	.080	.069
6	.741	.960	.878	.895	.947	-	.109	.170	.167	.145
7	.884	.955	.969	.984	.956	.896	-	.067	.034	.019
8	.791	.895	.875	.901	.902	.844	.935	-	.086	.072
9	.902	.921	.919	.916	.932	.846	.966	.918	-	.006
10	.876	.933	.921	.935	.933	.865	.981	.930	.994	-

Relations between diploid populations according to their genetic similarities/distances are shown in Table 2. The mean coefficient of genetic identity (\bar{i}) and the mean genetic distance (\bar{D}) between diploid populations were 0.913 and 0.093, respectively, suggesting an average level of genetic differentiation.

The estimation of genetic divergence between the populations of *O. alpicola* by means of Wright's hierarchical F-statistics exposed that the highest level of genetic differentiation occurred between local populations ($F_{DT} = 0.338$), while the genetic

populations of *O. alpicola* over regional genetic differentiation.

The results of population differentiation and estimations of a gene flow within various mountains showed that genetic differentiation between populations on Durmitor Mt. was somewhat lower ($F_{ST} = 0.125$) and the number of migrants *per* generation somewhat higher ($N_e m = 1.750$) than the one observed in other regions (Table 3). However, the genetic differentiation was the highest ($F_{ST} = 0.402$) and the gene flow the lowest ($N_e m = 0.0372$) between populations from Prokletije Mts. among all inter-regional comparisons.

Table 3. Coefficients of genetic differentiation (F_{ST}) and estimated gene flow ($N_e m$) between the geographic regions in southeast Dinarids. - average genetic identity; m - migration rate calculated from $m = m/(m+v)$ where v was $2 \cdot 10^{-6}$.

Region	F_{ST}	$N_e m$	\hat{i}	m
Durmitor Mt.	0.125	1.750	0.958	$4.6 \cdot 10^{-5}$
West Prokletije Mts.	0.287	0.621	0.896	$1.7 \cdot 10^{-5}$
East Prokletije Mts.	0.310	0.556	0.947	$3.6 \cdot 10^{-5}$
Prokletije Mts.	0.402	0.372	0.875	$1.4 \cdot 10^{-5}$

DISCUSSION

Similarly to the majority of high altitude insect species from Himalayas (Mani and Giddings 1980), a gathering of individuals of *O. alpicola* in the limited regions was observed. This leads to a highly structured organization of their populations with mosaically distributed local groups (demes). Observations in the field showed that the number of individuals within one deme varies from several dozens to several thousands. Due to a short period of reproductive activity and low mobility of these organisms, contacts of individuals between the neighbouring demes are certainly rare. The dispersion of individuals of this species is rather limited and interactions of individuals from different populations within the same mountain are almost negligible. The upper forest line represents an insurmountable obstacle for this species, hence the migration of individuals and exchange of individuals between the populations from different mountains (high altitude island) is completely lacking.

Adaptations of *O. alpicola* to a high altitude environment have conspicuously affected the pattern of genetic variation within its populations. Out of approximately 40 eggs laid into the ground, adults appear after two years. At the larval stage there is no dispersion and accordingly adults copulate immediately after the eclosion from the pupae, making inbreeding rather possible and frequent. The analysis of genetic differentiation among sexual populations of *O. alpicola* actually revealed a moderate degree of intrapopulation variation (mean heterozygosity was approximately 0.11) and a rather high level of genetic differentiation. The average value for F_{ST} of 0.347 was among the highest ones found for insect populations. The estimated number of migrants *per* generation was rather small (Table 4). Liebherr (1988) also recorded a high level

of genetic differentiation in local populations of some high altitude ground beetles (Carabidae). This author compared the gene flow between lowland and high altitude ground beetle species and found that in high mountains genetic differentiation of local populations increases, while the gene flow decreases. He found that at the high mountain species of apterous *Platinus angustatus* the genetic differentiation F_{ST} is 0.27 while the estimated gene flow amounted $N_e m = 0.50$. A high concordance of obtained data for different beetle species (weevils and ground beetles) from similar habitats demonstrates that adaptations to the high altitude environment have the similar effect on the genetic structure of their populations. These processes certainly play a crucial role in the increase of endemism, well known for insects from high altitude habitats (Mani 1968; Somme 1989; Wagner and Liebherr 1992).

Results of estimation of genetic distances between *O. alpicola* populations indicate an interesting pattern. Our cluster analysis showed that populations of *O. alpicola* from Prenj and Karanfili Mts. are genetically distinct from all other studied populations (Fig. 2). A separate position of the population from Prenj Mt. on the dendrogram could be causally related to the distribution of the mountain tundra type communities during Pleistocene. We must take into account that during the Pleistocene glaciations the populations of *O. alpicola*, as well as the tundra like high altitude communities underwent extensive horizontal and vertical shifts. It is believed that, during the glaciations, there were 3-5 large "islands" of mountain tundra communities at the Dinarids: the littoral and subcontinental mountains of the middle Adriatic (Dinara, Cincar, Bitovnja, Čvrsnica and Čabulja Mts.); Prenj complex of mountains (Velež and Prenj Mts.) and Vranica Mt. isolated in central Bosnia. It seems that the remaining part of the Southeast Dinarids was differentiated in two sub-regions: the Durmitorian

"island", including the mountains from Bjelašnica Mt. to Sinjajevina Mt. and the Prokletijan "island" with the mountains from Lola Mt. to Bogičevica Mt. including Bjelasica Mt. The processes that took place during Pleistocene were characterized by numerous warm and cold periods, combined with local tectonic and erosive effects of the glaciers, which modified the plasticity of the relief (Stevanović *et al.* 1992). That certainly enabled shorter or longer contacts and a gene flow between populations from different "islands". With the retreat of ice-sheets and return of more xeric climates, the high altitude communities become disjunct, finding refuge at higher elevations. This hypothesis is not new and was discussed in details on montane mammal fauna of North America (Patterson 1982). The period of genetic divergence of Prenj population is estimated to over 1 million years which could suggest the separation of high altitude communities on Prenj Mt. during early or even the prediluvian period.

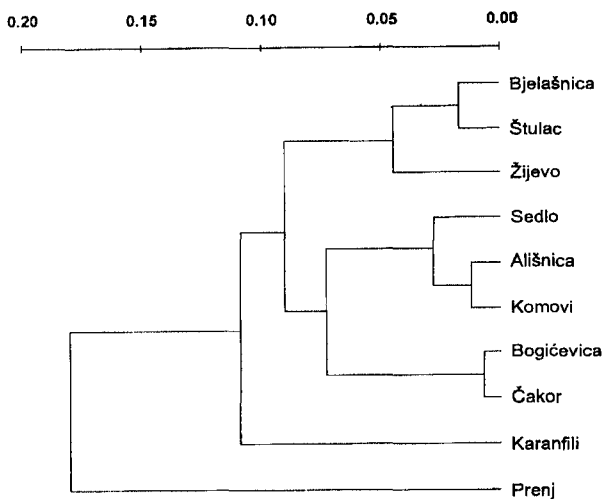


Fig. 2. Dendrogram showing genetic relationships among sexual populations of *Otiorynchus alpicola*. Clusters were extracted using UPGMA algorithm on minimum genetic distances between the populations (Nei 1978).

The analysis of genetic distances also demonstrated close similarity of the populations from the Eastern Prokletije (Bogičevica Mt. and Čakor). According to the magnitude of genetic distances, the populations from Durmitor Mt. are positioned on the dendrogram somewhere between populations from Bjelašnica and populations from the Western part of the Prokletije Mts. (Žijevo and Komovi Mt.). The position of the populations from Western and Eastern parts of Prokletije Mts. is rather unexpected because of their geographic closeness. But due to the distribution of Plav glacier during the Pleistocene glaciations (Asev *et al.* 1982; Petrović 1985), which interrupted the

communication between western and eastern parts of Prokletije Mts., it is reasonable to suppose that the populations from Komovi Mt. and Žijevo are genetically closer to the populations from Durmitor Mt. since they could communicate with each other through the massifs of Lola and Kapa Moraka (Fig. 1).

The analysis of the regional genetic differentiation showed a high level of genetic differentiation of local populations within the particular mountains. Genetic differentiation between mountains was much lower with regard to the total gene fund of the species.

A withdrawal of the glaciers in the Southeast Europe started about 15000 years ago and it is presumed that the present distribution of communities was established some 12000 years ago (Stevanović *et al.* 1992). Since the development of *O. alpicola* takes about two years, the age of high altitude populations distributed on the tops of the Dinaric mountains is estimated to approximately 6 thousand generations. However, the conditions of microenvironment have not been changed radically and thus, individuals were not exposed to a significant prolonged influence of directional selection. A rather high level of genetic polymorphism and their coarse grained utilization of the environment impose the assumption that populations of this species are permanently exposed to constant effect of stabilizing selection. Historical parameters suggest that the populations of *O. alpicola* were recently passed through a dramatic decrease in size of populations (bottleneck effect) that could explain a relatively low heterozygosity level (Chakraborty and Nei 1977). Genetic constitution of recent populations could imply that the result of genetic differentiation of the local populations were the result of the Wright's shifting balance process (Wright 1980). Populations of *O. alpicola* are highly structured in heterogeneously distributed groups of individuals of various sizes. Within these groups (demes) genotypes are harmoniously balanced through pleiotropic and epistatic interactions between the genes and therefore individuals are distributed near the peaks in an adaptive landscape. Negative effects of the drift are amortized by non-additive interactions, maintaining a relatively high level of genetic variability. As a result of interaction between natural selection and a drift (with a constant mutation rate) one can expect a further gradual genetic differentiation of local populations. According to low level of gene flow, a much slower genetic differentiation at the regional level could be expected.

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ЕВОЛУЦИОНА ДИВЕРГЕНЦИЈА СЕКСУАЛНИХ ПОПУЛАЦИЈА ВРСТЕ *OTIORHYNCHUS ALPICOLA* НА ПОДРУЧЈУ ЈУГОИСТОЧНИХ ДИНАРИДА

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Данашње распрострањење врсте *Otiorhynchus alpicola* на подручју југоисточне Европе је дисјунктно, ограничено на заједнице изнад горње шумске граница на највишим планинама југоисточних Динарида. Са циљем да се утврди ниво генетичке варијабилности ових популација и начин њихове еволутивне дивергенције, извршена је анализа аллозимске варијабилности десет популација ове врсте. Анализа генетичке варијабилности између популација показала је умерен ниво унутар популационе генетичке варијабилности и висок ниво генетичке диференцијације -

Резултати хијерархијске Рајтове статистике ($F_{DT}=0.338$; $F_{DS}=0.278$ и $F_{ST}=0.083$) и низак ниво процене броја јединки које мигрирају у току генерације (N_{em}) указују да је у популацијама *Otiorhynchus alpicola* генетичка диференцијација далеко више изражена на локалном, него на регионалном нивоу. Резултати процене генетичких дистанци између анализираних популација су у складу са претпостављеном глацијалном историјом заједница које насељава врста *Otiorhynchus alpicola*.