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LINKAGE DISEQUILIBRIUM IN POPULATIONS OF *MERODON AVIDUS A (DIPTERA, SYRPHIDAE)*

ABSTRACT: Analysis of the genetic structure in the populations of *Merodon avidus A*, originated from the Dubašnica Mountain, Serbia (AADUB), Pindos Mountain, Greece (AAPIN) and Morinj, Montenegro (AAMOR) was done. Based on the polyacrylamide gel electrophoresis out of 16 analysed loci 10 izozymic loci were monomorphic: *Aat*, *Fum*, *Hk-2*, *Hk-3*, *Idh-2*, *Mdh-1*, *Pgm*, *Sod-1*, *Sod-2*, *Sod-3*. Nonrandom association between alleles of the *Ao* and *Me* in the AAMOR population has been registered. Significant association among alleles of the *Ao* and *Mdh-2* loci in AADUB and *Ao* and *Gpd-2* loci in AAPIN was found as well. Percent of the locus with observed significant gametic disequilibrium was from 16.7% in AAMOR to significant association of the only variable loci pair in AADUB and AAPIN.

KEY WORD: Linkage equilibrium, *Merodon avidus A*, Population genetics, *Syrphidae*

INTRODUCTION

For the understanding of the adaptive relevance of the genetic polymorphism and concept of coadapted gene complex is very important to study a complex interaction among individuals of the particular local group (deme; sub/population), including intrapopulation relation, interaction to the other organisms of the sympatric and synchronic population as well as their numerous interactions to the environment. The most important interaction among individuals is a process of reproduction which provide a setting up a gene pool of offspring generation. Occasional reproduction transport of allele at particular locus might be independent from the allele of another locus, so, in that case, their fitnesses are also independent. However, since relation between genotype and phenotype is often complex authors consider that there is no relation „one gene — one characteristic” (Lewontin, 1974). In the multilocus system during random reproduction, alleles at different loci are being randomly combined, forming genotypes with no frequency deviation from the expected ones, based on the Hardy-Weinberg proportion. Similarly, alleles at different loci

constitute a random association. But, alleles at particular loci could form non-random association, named as gametic disequilibrium or linkage disequilibrium (Lewontin and Kojima, 1960), which is a short sentence of the „gametic phase disequilibrium” (Crow and Kimura, 1970). Since that, measure of the deviation from random association between alleles is D (deviation) or gametic disequilibrium (Lewontin and Kojima, 1960). Origins and maintenance of the allelic nonrandom association are result of population structuring and an influence of many of factors like chromosomal place of genes, natural selection to the multilocus system, epistatic interaction and genetic drift. Contrary to this, allelic nonrandom association due to the random sexual reproduction is being reduced. Also, gametic disequilibrium is very important factor for the evolution of population pointed out the natural selection to one locus has impact to the genotype frequency of another locus (Lewontin, 1974).

In the study of the molecular mechanism of the adaptive biochemical evolution, quantification of the genetic variability and analysis of the influence of evolutionary mechanisms play an important role. So far, study of the genetic structure of population of the *Merodon avidus* group, family *Syrphidae*, is based on the identification and separation of the cryptic taxa *M. avidus* A and *M. avidus* B, measure of the genetic variability of populations from the Balkan Peninsula (Milankov et al., 2001) and analysis an impact of the evolutionary mechanisms on the distribution of genetic variation and genetic divergence between conspecific populations of *M. avidus* A (Milankov et al., in press). Population-genetic analyse was showed that gametic disequilibrium exist in almost all analysed loci (Milankov et al., in press), which indicate necessity of additional studies.

Aim of this paper was analysis a (non)random association of alleles among allozyme polymorphic loci in three populations of the *Merodon avidus* A species from three different biogeographical region (based on Matvejev and Puncer, 1989: Biome of evergreen Mediterranean maritime woodlands and maquis — locality Morinj in Adriatic bay Boka Kotorska, Biome of South European mostly deciduous woodlands — Dubašnica Mountain and Biomes of stony grounds, pastures and woods on stony grounds of (oro)mediterranean mountains — Pindos Mountain).

MATERIAL AND METHODS

Sample collection

Natural populations of the *Merodon avidus* A species originated from Dubašnica Mountain (E 21°59', N 44°01'), Serbia (AADUB; 14 specimens), Morinj, (E 18°40', N 43°29'30''), Montenegro (AAMOR; 30 specimens), Adriatic sea in the Mediterranean area; and Pindos Mountain (E 20°37', N 39°14'), Greece (AAPIN; 9 specimens) were analysed.

Allozyme analysis

Genetic variation of 16 allozymic loci was studied by standard 5% polyacrylamide gel electrophoresis (Munstermann, 1979; Pasteur et al., 1988) with slight modifications (Milankov, 2001). Tris-Boric-EDTA (pH 8.9) buffer was used to assay aldehyde oxidase (AO; E.C. 1.2.3.1), fumarate hydratase (FUM; 4.2.1.2), glucocephosphate isomerase (GPI; 5.3.1.9), hexokinase (2.7.1.1. HK; two loci: *Hk-2*, *Hk-3*), malic enzyme (ME; 1.1.1.40), phosphoglucomutase (PGM; 2.7.5.1), superoxide dismutase (1.15.1.1. SOD; three loci: *Sod-1*, *Sod-2*, *Sod-3*). Tris-Citric (pH 7.1) buffer was used to assay aspartate amino transferases (AAT; 2.6.1.1), α -glycerophosphate dehydrogenase (1.1.1.8; GPD; *Gpd-2*), β -hydroxy acid dehydrogenase (HAD, 1.1.1.30), isocitrate dehydrogenase (1.1.1.42; IDH; *Idh-2*) and malate dehydrogenase (1.1.1.37. MDH; two loci: *Mdh-1*, *Mdh-2*).

The electrophoresis of individual insects from different populations was performed in the same gel for direct interpopulation and intertaxon comparison. Loci were numbered and alleles marked alphabetically with respect to increasing anodal migration.

Depending on metabolic function and regional distribution of enzymes, different body regions were used for analysis of isozyme variability (head + 0.15 ml homogenate: AAT, FUM, GPI, HAD, MDH, ME; thorax + 0.2 ml homogenate: AO, GPD, HK IDH, PGM, SOD). The duration of electrophoretic run at 90 mA (141—210 V) was 2.5—4 hrs.

Analysis

In this paper linkage disequilibrium coefficients (D) for multiple alleles at polymorphic loci using BIOSYS-2 (Swofford and Selander, 1989) were quantified. According to Ohta (1982) the observed gametic associations on the whole data set (D_{it}^2) was decomposed in estimations within (D_{is}^2 and $D'_{is}{}^2$) and between subpopulations (D_{st}^2 and $D'_{st}{}^2$). The ratio of D_{is}^2/D_{st}^2 and $D'_{is}{}^2/D'_{st}{}^2$, was measured for testing which of factors — epistatic nature selection or population subdivision — is responsible for registered deviation from random association between alleles at polymorphic enzymatic loci. Analysis of the linkage disequilibrium in subdivided populations was tested by the critical value of P from the chi-square analysis with the 0.05 default value.

RESULTS

Analysis of the genetic structure of populations of *M. avidus* A out of 16 izozymic loci 6 polymorphic loci was revealed. Locus *Ao* was polymorphic in all analysed populations, but *Gpd-2* locus was only in AADUB and AAPIN. In contrary to the other populations *Gpi* and *Had* loci were monomorphic in AADUB, the *Me* locus in AADUB and AAPIN, *Mdh-2* in AAMOR and AAPIN (Milankov et al., 2001).

Table 1. Analysis of linkage disequilibrium in AADUB, AAMOR and AAPIN populations of *Merodon avidus* A

AADUB					
Loci compared	No. of comparison	Common correlation	χ^2	<i>d.f.</i>	<i>P</i>
<i>Ao</i> : <i>Mdh-2</i>	4	0.999	28.44	1	***
AAMOR					
<i>Ao</i> : <i>Had</i>	18	0.137	2.37	2	ns.
<i>Ao</i> : <i>Me</i>	16	0.258	19.39	4	***
<i>Gpi</i> : <i>Had</i>	18	0.070	0.43	2	ns.
<i>Gpi</i> : <i>Me</i>	18	0.203	5.94	2	ns.
<i>Had</i> : <i>Me</i>	24	0.148	8.81	4	ns.
AAPIN					
<i>Ao</i> : <i>Gpd-2</i>	5	0.999	31.25	1	***

d.f. — Degree of freedom; *P* — Level of significance (ns. = not significant; *** = significant at $P \leq 0.001$)

Table 2. Analysis of linkage disequilibrium in the total population of *Merodon avidus* A

Loci compared	No. of comparison	Common correlation	χ^2	<i>d.f.</i>	<i>P</i>
<i>Ao</i> : <i>Gpd-2</i>	25	0.272	18.08	2	***
<i>Ao</i> : <i>Gpi</i>	19	0.207	5.81	2	ns.
<i>Ao</i> : <i>Had</i>	25	0.120	2.54	2	ns.
<i>Ao</i> : <i>Mdh-2</i>	14	0.393	18.32	2	***
<i>Ao</i> : <i>Me</i>	23	0.195	17.15	4	**
<i>Gpd-2</i> : <i>Had</i>	34	0.039	0.31	4	ns.
<i>Gpd-2</i> : <i>Me</i>	33	0.115	3.82	4	ns.
<i>Gpi</i> : <i>Had</i>	26	0.661	0.90	4	ns.
<i>Gpi</i> : <i>Me</i>	28	0.187	21.02	4	***
<i>Had</i> : <i>Me</i>	38	0.086	4.34	4	ns.

d.f. — Degree of freedom; *P* — Level of significance (ns. = not significant; ** significant at $P \leq 0.01$; *** = significant at $P \leq 0.001$)

Nonrandom associations of the alleles at *Ao* variable locus with alleles at *Mdh-2* in the AADUB population, alleles at *Me* in AAMOR and alleles at the *Gpd-2* locus in AAPIN were detected (Tab. 1). Percent of the significant gametic disequilibrium in relation to the whole analyse at the population level was from 16.7% (AAMOR) to the significant nonrandom association alleles at the only variable loci pair in AADUB and AAPIN (Tab. 2). Out of 10 pairs of loci 4 loci were in the significant gametic disequilibrium on the species level (Tab. 2).

Ohta's analysis of linkage disequilibrium in subdivided total population (Ohta, 1982), forming from 3 subpopulations, has been found out that in all pairs of loci value of the expected variance of linkage disequilibrium within

subpopulation (D_{is}^2) was lower than the variance of the correlation of genes of two loci of different gametes relative to the total population (D_{st}^2) and D'_{is}^2 (variance of the correlation of two loci of one gamete in a subpopulation relative to that of the total population) greater than D'_{st}^2 (variance of the disequilibrium of the total population), only with the exception of the *Ao — Mdh-2* loci pair (Tab. 3). Results in this study indicate that nonrandom association of the alleles at particular variable loci (except *Ao — Mdh-2*) is mainly caused by limited migration and random process (genetic drift). However, for the *Ao — Mdh-2* pair the relation between coefficients of the linkage disequilibrium ($D_{is}^2 > D_{st}^2$; $D'_{is}^2 < D'_{st}^2$) showed that epistatic natural selection is the mechanism responsible for the revealed significant allelic association (Tab. 3).

Table 3. Variance components of linkage disequilibrium in the total population of *Merodon avidus* A

Loci compared	Within subpopulation components		Between subpopulation components		Total population components
	$D(IS)$	$D'(IS)$	$D(ST)$	$D'(ST)$	$D(IT)$
<i>Ao : Gpd-2</i>	0.082	0.472	0.186	0.014	0.486
<i>Ao : Gpi</i>	0.000	0.756	0.466	0.018	0.774
<i>Ao : Had</i>	0.003	0.417	0.234	0.003	0.420
<i>Ao : Mdh-2</i>	0.161	0.194	0.044	0.046	0.240
<i>Ao : Me</i>	0.075	0.572	0.255	0.051	0.623
<i>Gpd-2 : Had</i>	0.000	0.034	0.047	0.000	0.034
<i>Gpd-2 : Me</i>	0.000	0.439	0.087	0.003	0.441
<i>Gpi : Had</i>	0.000	0.326	0.281	0.000	0.327
<i>Gpi : Me</i>	0.007	0.779	0.289	0.022	0.801
<i>Had : Me</i>	0.003	0.362	0.108	0.001	0.363

$$D_{is} < D_{st}; D'_{is} > D'_{st}$$

DISCUSSION

Apprehension of the role and importance of the certain mechanisms in the process of the evolutionary changes mainly depend on the definition of the central problem of evolutionary theory, major processes of evolutionary change, ecological context of evolution, genetic basis of evolutionary change and process of speciation (Wade and Goodnight, 1998). So, as the opposite to the Fisher's theory (Fisher, 1958) which regard that evolutionary changes is based on the additive genetic effect, that their context is a large, panmictic population and that mutation and mass selection are mechanisms by which it operates, Wright (1978) consider that the environment of any species is no simple. Namely, continual evolutionary changes in subdivided population (i.e. metapopulation) are results not only changes and interaction between biotic and abiotic components, but a genotypic background, interaction between ge-

nes and epistasis. Bearing in mind that the context and interaction are fundamental as well as an epistasis and pleiotropy are essential in the genetic basis of the evolutionary change (Lewontin, 1974), analysis of allelic association at different loci play a major role in the study of the adaptive genetic polymorphism and a concept of coadapted gene complex.

One of the methods in the study of coadapted gene complex, a set of alleles held together by epistatic selection, is an analysis of the genes encoded enzymes of the fundamental importance in the metabolism, the main points of a particular metabolic pathway. In this study, analysis of the allelic association of polymorphic loci in spatially fragmented population of *Merodon avidus* A, found out the significant high percent of allelic association at different loci, ranged from 16.7% (AAMOR) to 100% (AADUB and AAPIN), while on the species it was 40%. Additionally, based on the revealed linkage allozyme loci is a quite difficult to explain due to the existence of many factors which influenced on its origins and maintenance. An insufficiently available information about a genetic system in hoverflies exists as well. So, until now, just only for a few species a number of its chromosomes have been known (Boyes et al., 1972, 1973; Rožek et al., 1996), while the other essential information, such as linkage map and recombination rate are totally unknown. Linkage disequilibrium data in populations of *Cheilosia vernalis* are only available. In the spatially fragmented population of *C. vernalis*, the high percent of linkage alleles, ranging from 60% to 75%, were evaluated. These results pointed out that low migration level, population substructuring and natural selection were highly influenced to the genetic divergence among the conspecific populations (Milanek et al., unpublished).

In this study potential coordination between observed allelic linkage and a point in metabolic pathway of allozyme were analysed as well. However, it is known that glucose-metabolizing enzymes MDH, ME and GPD (Gillespie and Kojima, 1968) are not in the tight relation with AO, which has a different function. So, registered allelic nonrandom association at the *Ao* locus, on the one side, and the *Mdh-2*, *Me* and *Gpd-2* loci, on the other, it could not be explained by the function of encoded allozymes.

An integral part in the debate of nonrandom association of alleles at allozymic loci is analysis of linkage between map distance and linkage disequilibrium. It is known that *D* (coefficient of deviation) is higher when whatever of which evolutionary mechanisms — natural selection, genetic drift, migration, genetic hitchhiking — influenced on the nonlinked loci. But, without any information of genetic maps in hoverflies it is very difficult to discuss about a relation between gene location and their epistatic interaction. The eventual connection between sex and linked genotypes of the particular loci were observed as well. But, inconsistency in the analysed number of female and male (AAMOR: 22 m, 8 f; AADUB: 9 m, 5 f; AAPIN: 5 m, 4 f) have caused difficulty. In contrast to the study of the population of *C. vernalis*, in which the linkage between certain genotypes and males as well as between genotypes and genotypic nonrandom association were registered (Milanek et al., unpublished), in this study the strong relation between specific genotypes and sex has not been revealed. Since it has been considered that inhi-

bitory effect of crossing over due to inversion heterokaryotypes and that there is a tight connection between alleles and inversion, especially in the well-studied the *Drosophila* group (Hedrick et al., 1978), it could be supposed that some mechanisms which regulate the rate of recombination in the hoverflies species are also exist. It is noteworthy to mention that pericentric inversion and translocation in the insects formed a linkage blocks of parental genes, maintained in the population under the influence of natural selection.

In the point of view of neutralists and selectionists the role and importance of natural selection to maintain of linkage disequilibrium are still under discussion. As opposed to the neutralist view random genetic drift has a large effect to the nonrandom association at tightly linked loci, selectionist considered that natural selection is more likely to cause nonrandom association with tight linkage (Wade and Goodnight, 1998). The importance of natural selection in populations of *Cheilosia vernalis*, based on the registered unique alleles and genotypes, spatial distribution of genotypes and gene complexes was observed (Milankov et al., 2002). Furthermore, genetic changes during independent evolution of the conspecific populations of *C. vernalis* have not been occurred equally and uniformly at all loci. Additionally, the value of Wright's F_{st} coefficient was showed that different selection pressures were probably caused a geographic pattern of genotypic and allelic frequencies (Milankov et al., 2002; Milankov et al., unpublished).

Observed linkage disequilibrium in this study is likely to be results of combination of many evolutionary forces. Impact of the population structuring and effect of the local inbreeding were expressed by excess homozygosity, high value of the Fixation index (F_{is}) and the presence of rare alleles (Milankov et al., 2001; Milankov et al., unpublished), as well as increase a measure of the deviation from random association between alleles. Bearing in mind that ratio of Ohta's variance components of linkage disequilibrium (Ohta, 1982), with one exception, was $D_{is}^2 < D_{st}^2$; $D'_{is}^2 > D'_{st}^2$ low rate of migration was probably been an effective mechanism in the rise of correlation of nonallelic genes within populations and differentiation of gametes among subpopulations, limited effective size. However, not only reduced gene flow, but also natural selection was an important factor which caused a quantified distribution of the genetic variability. The importance of the epistatic selection to the pattern of genetic variation in populations of *M. avidus* A has been showed by variance of the allelic frequencies at each locus (F_{st}), especially for the *Ao*, *Me*, *Gpi* and *Gpd-2* loci (Milankov et al., in press). As a part of the total genetic variability at the species level, F_{st} value showed that differences between population were not equal and uniform.

Origins and maintenance of the linkage disequilibrium, as a part of the adaptive evolution, are very important to study. Existing of the epistasis effect of one allele in the combination of the suitable set of alleles at other loci in one deme under a particular conditions of the environment and influence of drift and selection it could be completely different in other local populations due to the local drift, selection and genetic background which are being a sensitive to changes (Wright, 1931; Wade and Goodnight, 1998). Finally, based on the tight and complex connection between linkage disequilibrium

and adaptive gene complex under the changeable biotic and abiotic environments, with strong effect of genetic drift and natural selection in subdivided populations (under the low rate of migration) possible answer to the distribution of genetic variability of *M. avidus* A might be found.

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ГАМЕТСКА НЕРАВНОТЕЖА У ПОПУЛАЦИЈАМА *Merodon Avidus A (Diptera, Syrphidae)*

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Резиме

Проучавање генетичке структуре популација врсте *Merodon avidus A* пореклом са планина Дубашница, Србија (AADUB) и Пиндоса, Грчка (AAPIN) и из Мориња, Бока Которски залив, Црна гора (AAMOR) полиакриламид гел електрофорезом утврђено је да је 10 локуса било мономорфно (*Aat*, *Fum*, *Hk-2*, *Hk-3*, *Idh-2*, *Mdh-1*, *Pgm*, *Sod-1*, *Sod-2*, *Sod-3*) од укупно 16 анализираних изозимских локуса. Анализом везаности алела варијабилних локуса AAMOR популације врсте *M. avidus A* уочена је неслучајна асоцијација само алела пара локуса *Ao* и *Me*. Такође је утврђена статистички сигнификантна везаност алела локуса *Ao* са алелима *Mdh-2* локуса популације AADUB и алелима локуса *Gpd-2* популације AAPIN врсте *M. avidus A*. Процентуална заступљеност статистички значајног присуства гаметске неравнотеже у односу на укупну анализу на нивоу популације била је од 16,7% (AAMOR) до статистички значајне асоцијације алела јединог пара варијабилних локуса (AADUB и AAPIN).