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Isoenzymic Genetic Variability in Populations of *Messor structor* (Hymenoptera, Formicidae) from Bulgaria

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Abstract: Genetic variability in 36 populations of *Messor structor* (Latreille, 1798) from Bulgaria was studied using analysis of two enzymic systems corresponding to four loci (Sod-1, Sod-2, Sod-3 and Me-1). Three of the studied loci were found to be polymorphic. One of them was found to be monomorphic (Sod-3). One to three alleles were detected for the different isozymic systems: one allele at Sod-3 locus (Sod-3^{100}), two alleles at Sod-1 (Sod-1^{100} and Sod-1^{95}) and Sod-2 (Sod-2^{100} and Sod-2^{97}) loci and three alleles at Me-1 locus (Me-1^{100}, Me-1^{98} and Me-1^{96}). The observed and expected heterozygosities (H_o and H_e) ranged from 0.0 to 0.111 and from 0.168 to 0.372, respectively. Allele frequencies of all loci were used to estimate Nei's (1972) genetic distance, which was found to range from 0.001 (between Boliarino and Lubenova mahala) to 0.462 (between Chirpan and Merichleri). The estimated mean F_{IS} and F_{ST} values from isozyme data were 0.8738 and 0.1432, respectively. The Neighbour-joining method and Unweighted Pair Group Method with Arithmetic Mean phylogenetic trees were obtained using genetic distance matrix methods. Two of the studied populations were grouped separately in dendrograms as compared to all other populations which formed a large cluster consisting of three sub-clusters.

Keywords: *Messor structor*, isoenzyme genetic variability, population genetics

Introduction

Messor structor (Latreille, 1798) are harvester ants storing seeds in underground granaries. Being major seed consumers in xeric habitats they influence the vegetation by depleting and dispersing seeds (Azcarate, Peco 2003, MacMahon *et al.* 2000, Schlick-Steiner *et al.* 2005). According to Seifert (1996), Central European populations of the harvester ant genus *Messor* are currently considered to constitute a single species *Messor structor*. It has been reported that *M. structor* ants live mainly in polygynous colonies and could be members of unicolonial populations (Schlick-Steiner *et al*. 2005). *Messor structor* is one of the most widespread steppe species. It is well adapted to different conditions and occurs in all of Europe. Concerning the territory of Bulgaria, *M. structor* may be found all over the country in medium-sized populations. In Bulgaria harvester ants inhabit plain desolate terrains, mountainous slopes, outskirts in mixed woodland zone. They may be found also in open well-drained areas: abandoned fields, pastures, overgrown lawns.

Various genetic tools, such as DNA (microsatellite and mtDNA) analysis and isozyme electrophoresis, have been applied for the characterisation of the life history and genetics of ants (Cantagalli *et al*. 2010, Diehl *et al.* 2002, Hagen *et al.* 1988, Krieger *et al.* 1999, Pamilo *et al.* 1975, Pamilo *et al.* 2005, Ross *et al*. 2003, Ross *et al.* 2007, Schlick-Steiner *et al.* 2005, Schlick-Steiner *et al.* 2006, Tomaszewski *et al.* 1973). Although there are investigations on isozyme genotype-environment associations in populations of harvester ants (Tomaszewski

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et al. 1973) and on the isoenzymatic polymorphism in populations of other ant species (Cantagalli *et al.* 2010, Diehl *et al*. 2002), there is no information found concerning population-genetic characterisation of *M. structor* populations from Bulgaria and Europe based on isoenzymic analysis.

The present study aims at the detection and characterisation of the genetic variability among populations of *Messor structor* from various localities from Southern Bulgaria, based on isoenzymic analysis.

Materials and Methods

*Messor structo*r samples

In total about 1200 *Messor srtuctor* individuals were tested. Workers from 36 nests, each from different population from Southern Bulgaria (Fig. 1), were included in this study. Some (3-5) of the collected ants were used for morphological identification and others were stored at -20ºC for isoenzyme analysis. Thirty to 48 individuals per population (mean sample size per locus between 31.5 and 41.5) were analysed for two enzymatic systems.

Electrophoresis and isoenzyme analysis

The whole-body homogenisation, electrophoresis in 7.5% polyacrylamide gel, buffers and electrophoretic conditions for both studied enzymic systems were done following Davis (1964) with some modifications by Ivanova (1996). Two enzymic systems, corresponding to four loci, were studied: ME (malic enzyme, EC 1.1.1.40) and SOD (superoxide dismutase, EC 1.15.1.1.). Buffers and electrophoretic conditions for each enzymic system used were as in Shaw, Prasad (1970) and Korochkin *et al.* (1977). Enzyme activities were visualised by histochemical staining following Harris, Hopkinson (1976).

Statistical Analyses

Allele frequencies, mean number of alleles per locus, proportion of polymorphic loci, observed (H_o) and expected (H_e) heterozygosity, deviation from the Hardy-Weinberg equilibrium, Nei's genetic distance (D; Nei 1972) and fixation indexes – F_{IT} and F_{ST}, (Wright 1965) were calculated using BIOSYS-1 (Swofford, Selander 1981) software package. Phylogenetic Unweighted Pair Group Method with Arithmetic Mean – UPGMA (Sneath, Sokal 1973) and neighbour-joining (NJ; Saitou, Nei 1987) trees were constructed using Nei's (1972) genetic distance and the PHYLIP (Felsenstein 1993) software package.

Results

In total, for all the studied populations of *M. structor* from Bulgaria, products of three Sod and one Me genes were detected: SOD-1, SOD-2, SOD-3 and ME-1. Data about the isoenzyme polymorphism de-

Fig. 1 Sampling sites (scale: 1cm ≈ 25km)

Table 1. Allele frequencies in the studied *Messor structor* populations

	Locus							
Population Localities	**Sod-1**		**Sod-2**		**Sod-3**	**Me-1**		
	Sod-1^{100}	Sod-1^{95}	Sod-2^{100}	Sod-2^{97}	Sod-3^{100}	Me-1^{100}	Me-1^{98}	Me-1^{96}
Nova Zagora I	0.200	0.800	0.364	0.636	1.000	0.400	0.233	0.367
Bryastovo	0.078	0.922	0.219	0.781	1.000	0.156	0.313	0.531
Lub. Mahala	0.183	0.817	0.283	0.717	1.000	0.049	0.451	0.500
Plovdiv I	0.083	0.917	0.403	0.597	1.000	0.216	0.405	0.378
Graf Ignatievo	0.176	0.824	0.378	0.622	1.000	0.167	0.833	0.000
Chernozemen	0.000	1.000	0.294	0.706	1.000	0.000	0.735	0.265
Kaloyanovo	0.167	0.833	0.300	0.700	1.000	0.323	0.452	0.226
Belozem	0.117	0.883	0.790	0.210	1.000	0.100	0.200	0.700
Orizovo	0.108	0.892	0.389	0.611	1.000	0.300	0.567	0.133
Boliarino	0.139	0.861	0.278	0.722	1.000	0.028	0.444	0.528
Granit	0.100	0.900	0.167	0.833	1.000	0.167	0.833	0.000
Choba	0.067	0.933	0.667	0.333	1.000	0.400	0.600	0.000
Velingrad	0.063	0.938	0.359	0.641	1.000	0.063	0.531	0.406
Elenovo	0.222	0.778	0.208	0.792	1.000	0.111	0.792	0.097
Stara Zagora	0.234	0.766	0.328	0.672	1.000	0.063	0.250	0.688
Chirpan	0.237	0.763	0.184	0.816	1.000	0.000	1.000	0.000
Trankovo	0.071	0.929	0.537	0.463	1.000	0.073	0.634	0.293
Pazardjik	0.088	0.912	0.176	0.824	1.000	0.500	0.500	0.000
Parvomai	0.076	0.924	0.470	0.530	1.000	0.156	0.594	0.250
Calapica	0.150	0.850	0.567	0.433	1.000	0.183	0.467	0.350
Sadovo	0.274	0.726	0.419	0.581	1.000	0.300	0.633	0.067
Milevo	0.100	0.900	0.567	0.433	1.000	0.500	0.500	0.000
Topolovo	0.281	0.719	0.677	0.323	1.000	0.281	0.719	0.000
Zvanichevo	0.183	0.817	0.600	0.400	1.000	0.167	0.367	0.467
Dimitrovgrad	0.233	0.767	0.433	0.567	1.000	0.167	0.267	0.567
Iambol	0.250	0.750	0.758	0.242	1.000	0.152	0.515	0.333
Haskovo	0.083	0.917	0.208	0.792	1.000	0.292	0.486	0.222
Tvarditsa	0.329	0.671	0.276	0.724	1.000	0.263	0.553	0.184
Karlovo	0.200	0.800	0.314	0.686	1.000	0.067	0.683	0.250
Kalugerovo	0.113	0.887	0.226	0.774	1.000	0.367	0.467	0.167
Hisaria	0.446	0.554	0.300	0.700	1.000	0.133	0.600	0.267
Oryahovitsa	0.213	0.788	0.429	0.571	1.000	0.229	0.329	0.443
Merichleri	0.183	0.817	0.733	0.267	1.000	0.133	0.000	0.867
Pesnopoi	0.000	1.000	0.774	0.226	1.000	0.103	0.474	0.423
Plovdiv II	0.152	0.848	0.516	0.484	1.000	0.031	0.281	0.688
Nova Zagora II	0.303	0.697	0.203	0.797	1.000	0.600	0.400	0.000

tected and the allele frequencies calculated are presented in Table 1. Mean sample size per locus, mean number of alleles per locus, proportions of polymorphism, observed and expected heterozygosity in the populations tested are presented in Table 2.

Sod-1 locus was polymorphic with two alleles (Sod-1^{100} and Sod-1^{95}). We detected that Sod-1^{95} allele had quite high frequency in almost all of the studied populations. This allele was fixed in two of the populations (Chernozemen and Pesnopoi). At the same time, the frequency of Sod-1^{100} allele varied largely: between 0 and 0.446 (in Hisaria population; Table 1). Sod-2 locus was polymorphic with two alleles: Sod-2^{100} and Sod-2^{97}. Their frequencies

Table 2. Mean sample size per locus, Mean number of alleles per locus, Percentage of polymorphic loci, Observed and expected heterozygosity in the populations tested (Standard errors are included)

Population	Mean sample size per Locus	Mean no. of alleles per locus	Percentage of loci polymorphic	Mean heterozygosity – Direct-Count (Ho)	Mean heterozygosity – HdyWbg Expected (He)
Nova Zagora I	31.5 ±0.9	2.0 ±0.4	75.0	0.050 ±0.050	0.364 ±0.140
Bryastovo	32.0 ±0.0	2.0 ±0.4	75.0	0.023 ±0.023	0.275 ±0.131
Lub. Mahala	32.8 ±2.8	2.0 ±0.4	75.0	0.039 ±0.021	0.317 ±0.117
Plovdiv I	36.3 ±0.3	2.0 ±0.4	75.0	0.035 ±0.026	0.324 ±0.150
Graf Ignatievo	35.3 ±1.8	1.8 ±0.3	75.0	0.020 ±0.020	0.263 ±0.098
Chernozemen	34.0 ±0.0	1.5 ±0.3	50.0	0.000 ±0.000	0.204 ±0.118
Kaloyanovo	30.3 ±0.3	2.0 ±0.4	75.0	0.017 ±0.017	0.340 ±0.136
Belozem	30.5 ±0.3	2.0 ±0.4	75.0	0.033 ±0.024	0.254 ±0.100
Orizovo	35.0 ±1.7	2.0 ±0.4	75.0	0.027 ±0.027	0.315 ±0.133
Boliarino	36.0 ±0.0	2.0 ±0.4	75.0	0.042 ±0.042	0.295 ±0.115
Granit	30.0 ±0.0	1.8 ±0.3	75.0	0.033 ±0.033	0.187 ±0.067
Choba	30.0 ±0.0	1.8 ±0.3	75.0	0.017 ±0.017	0.267 ±0.120
Velingrad	32.0 ±0.0	2.0 ±0.4	75.0	0.039 ±0.030	0.286 ±0.134
Elenovo	36.0 ±0.0	2.0 ±0.4	75.0	0.111 ±0.076	0.260 ±0.087
Stara Zagora	32.0 ±0.0	2.0 ±0.4	75.0	0.016 ±0.009	0.320 ±0.109
Chirpan	36.0 ±2.0	1.5 ±0.3	50.0	0.039 ±0.025	0.168 ±0.098
Trankovo	41.5 ±0.3	2.0 ±0.4	75.0	0.048 ±0.028	0.288 ±0.130
Pazardjik	34.0 ±0.0	1.8 ±0.3	75.0	0.015 ±0.015	0.241 ±0.107
Parvomai	32.8 ±0.3	2.0 ±0.4	75.0	0.045 ±0.036	0.304 ±0.138
Calapica	30.0 ±0.0	2.0 ±0.4	75.0	0.050 ±0.029	0.349 ±0.140
Sadovo	30.8 ±0.3	2.0 ±0.4	75.0	0.056 ±0.056	0.353 ±0.120
Milevo	30.0 ±0.0	1.8 ±0.3	75.0	0.017 ±0.017	0.298 ±0.125
Topolovo	31.8 ±0.3	1.8 ±0.3	75.0	0.078 ±0.059	0.316 ±0.106
Zvanichevo	30.0 ±0.0	2.0 ±0.4	75.0	0.008 ±0.008	0.356 ±0.136
Dimitrovgrad	30.0 ±0.0	2.0 ±0.4	75.0	0.017 ±0.017	0.363 ±0.130
Iambol	33.5 ±0.3	2.0 ±0.4	75.0	0.007 ±0.007	0.341 ±0.126
Haskovo	36.0 ±0.0	2.0 ±0.4	75.0	0.028 ±0.011	0.282 ±0.137
Tvarditsa	38.0 ±0.0	2.0 ±0.4	75.0	0.053 ±0.044	0.363 ±0.128
Karlovo	33.5 ±1.2	2.0 ±0.4	75.0	0.068 ±0.026	0.309 ±0.108
Kalugerovo	30.8 ±0.3	2.0 ±0.4	75.0	0.056 ±0.056	0.297 ±0.133
Hisaria	34.8 ±1.7	2.0 ±0.4	75.0	0.014 ±0.008	0.372 ±0.127
Oryahovitsa	39.8 ±1.7	2.0 ±0.4	75.0	0.057 ±0.026	0.372 ±0.140
Merichleri	31.0 ±1.0	1.8 ±0.3	75.0	0.025 ±0.025	0.234 ±0.085
Pesnopoi	33.0 ±2.0	1.8 ±0.5	50.0	0.045 ±0.045	0.237 ±0.145
Plovdiv II	32.3 ±0.5	2.0 ±0.4	75.0	0.031 ±0.018	0.306 ±0.115
Nova Zagora II	32.0 ±0.7	1.8 ±0.3	75.0	0.053 ±0.043	0.311 ±0.109

varied between 0.167 and 0.790 for Sod-2^{100} and between 0.210 and 0.833 for Sod-2^{97}. Three alleles were detected at Me locus in the populations of *M. structor* from Bulgaria. Me-1^{98} was more or the most frequent allele in 21 of the populations studied and it was fixed in one of them (Chirpan). In addition we observed that for the Pasardjik and Milevo populations Me-1^{100} and Me-1^{98} alleles were with equal frequencies. In other populations Me-1^{100} or Me-1^{96} were detected as more frequent alleles (Table 1).

The mean number of alleles per locus varied from 1.5 (Chernozemen and Chirpan) to 2.0 (Nova Zagora I, Bryastovo, Lubenova mahala, Plovdiv I, Kaloyanovo, Belozem, Orizovo, Boliarino, Velingrad,

Table 3. F statistics for polymorphic loci studied

F - statistics for individual alleles		F(IS)	F(ST)	F(IT)
	Sod-1^{100}	0.5896	0.0661	0.6167
Locus: Sod-1	Sod-1^{95}	0.5896	0.0661	0.6167
	Sod-2^{100}	0.9378	0.1368	0.9463
Locus: Sod-2	Sod-2^{97}	0.9378	0.1368	0.9463
	Me-1^{100}	0.9751	0.1347	0.9784
Locus: Me-1	Me-1^{98}	0.9637	0.1543	0.9693
	Me-1^{96}	0.9619	0.2525	0.9715
Summary of F - statistics at all loci		F(IS)	F(ST)	F(IT)
Locus: Sod-1		0.5896	0.0661	0.6167
Locus: Sod-2		0.9378	0.1368	0.9463
Locus: Me-1		0.9662	0.1827	0.9724
Mean		0.8738	0.1432	0.8919

Elenovo, Stara Zagora, Trankovo, Parvomai, Calapica, Sadovo, Zvanichevo, Dimitrovgrad, Iambol, Haskovo, Tvarditsa, Karlovo, Kalugerovo, Hisaria, Oryahovitsa and Plovdiv II). The estimated percentage of polymorphic loci ranged from 50% (Chernozemen, Chirpan and Pesnopoi) to 75% (all the other populations) using the 0.95 criterion. (Table 2). The observed and expected heterozygosity (H_o and H_e) ranged from 0.0 (Chernozemen) to 0.111 (Elenovo) and from 0.168 (Chirpan) to 0.372 (Hisaria and Oryahovitsa), respectively (Table 2). The expected heterozygosity (H_e) by polymorphic loci was higher than the observed one (H_o) in all of the tested populations. There were significant deviations of genotype frequencies from Hardy-Weinberg expectations at all loci in most populations ($0.05 \geq P$). Chi-Square (df: 1-3) tests showed that the deviations were generally in favour of homozygotes. This fact, in addition to the deficit of heterozygotes, suggests the presence of inbreeding effect.

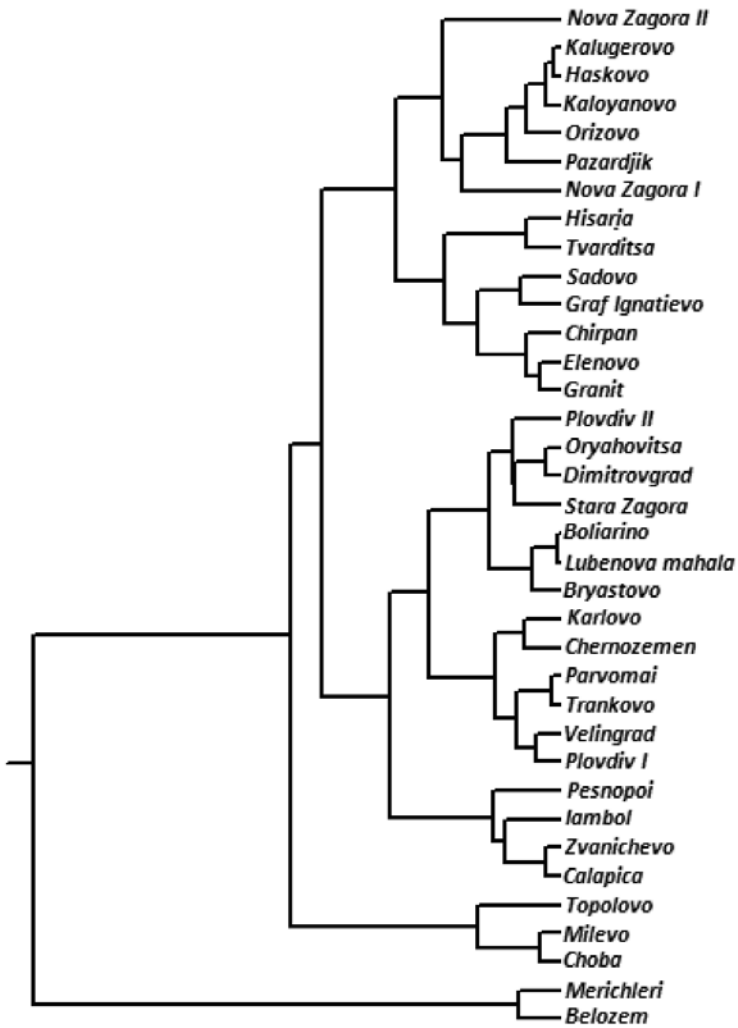

Fig. 2 Relationships of *Messor structor* populations studied as shown in UPGMA (Sneath and Sokal, 1973) dendrogram

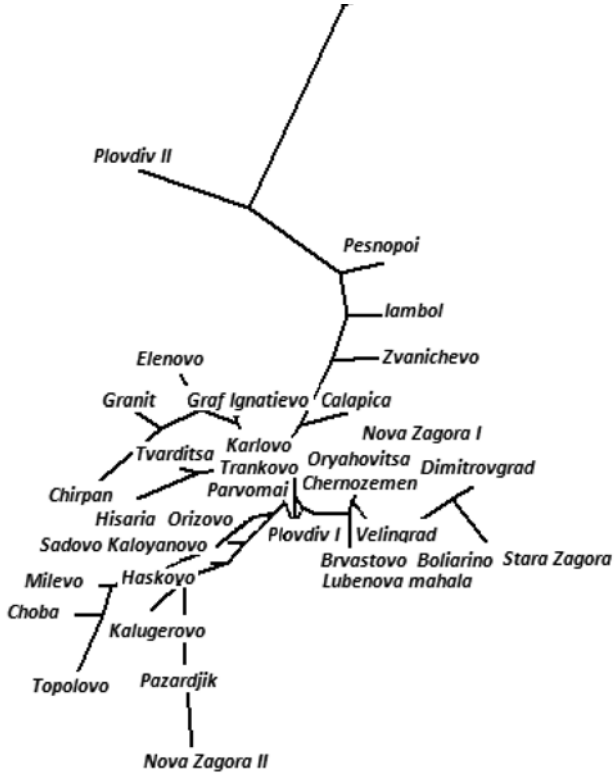

Fig. 3 Relationships of *Messor structor* populations studied as shown in Neighbour-joining (Saitou and Nei, 1987) dendrogram

F-statistics for the polymorphic loci in the populations, as well as in all populations considered together, are presented in Table 3. The estimated mean F_{IS} and F_{ST} values were 0.8738 and 0.1432, respectively.

The values for the genetic distance (Nei, 1972) were calculated using the allele frequencies and ranged from 0.001 (between Boliarino and Lubenova mahala) to 0.462 (between Chirpan and Merichleri).

In both, UPGMA and NJ dendrograms, two of the populations, Merichleri and Belozem, were grouped separately from all the others which formed a large cluster consisting of three sub-clusters (Figs. 2 and 3).

Discussion

We found no information in the available literature concerning isoenzyme polymorphism in *Messor structor* ants neither from Bulgaria, nor from Europe. Ward, Taylor (1981) reported that SOD and ME isoenzyme systems were monomorphic in the primitive ant *Nothomirmecia macrops*.

Three of the four enzyme loci analysed in our study were found to be polymorphic (except Sod-3 locus) in almost all of the studied populations of *M. structor* . A total of seven alleles were obtained from the four loci analysed. The allele frequencies showed considerable differences among the populations for both the Sod and Me loci. Concerning the different loci, our results showed that the most frequent alleles in the most of the populations were Sod-1^{95}, Sod-2^{97} (except in the Belozem, Iambol and Pesnopoi populations), Me-1^{98}, (except in the Nova Zagora I and II, Bryastovo, Lubenova mahala, Belozem, Boliarino, Stara Zagora, Zvanichevo, Dimitrovgrad, Oryahovitsa, Merichleri and Plovdiv populations, where Me-1^{100} or Me-1^{96} were detected as more frequent alleles than Me-1^{98}). In accordance with Ward, Taylor (1981), the third locus, Sod-3, was monomorphic in all of the studied populations.

The expected heterozygosity (H_e) by polymorphic loci was higher than the observed one (H_o) in all of the tested populations (Table 2). At the same time, H_e ranged from 0.168 to 0.372, which was much higher than the values calculated by other authors using electrophoretic markers for populations of other ant species (Ross *et al*. 1987, Shoemaker *et al*. 1992, Ward, Taylor 1981). The differences between observed and expected heterozygosity obtained may be explained by the small number of the analysed loci (only four in this investigation). The low values obtained for the heterozygosity in ants may be explained also by the kin selection theory (Hamilton 1964), social way of life and climate stability (Pamilo *et al.* 1975), nest stability (Pamilo *et al*. 1978), haplodiploidy and low dispersal which promotes inbreeding (Cantagalli *et al.* 2010).

It must be noted that among the tested populations lower percentage of polymorphic loci was found for the Chernozemen, Chirpan and Pesnopoi populations. This result, together with the fixation of Sod-1^{95} allele in Chernozemen and Pesnopoi and of Me-1^{98} allele in the Chirpan population, and the low values of H_o and H_e enhance the possibility that this harvester ant populations may maintain some specific characteristics (Tables 1 and 2).

Our results on the F-statistics showd that the calculated mean F_{IS} value (between 0.5896 and 0.9637 for the different loci) over all loci was quite high (0.8738). This demonstrated high level of inbreeding within populations (Conner, Hartl 2004). This fact was correlated to a low level of observed heterozygosity compared to the expected one (Tables 2 and 3).

The estimated mean F_{ST} value was 0.1432 (between 0.0661 and 0.2525 for the different loci studied) which showed that 14.32% of the overall genetic diversity observed was among populations, as opposed to 85.68% within populations. This value of mean F_{ST} indicated relatively moderate level of

genetic differentiation among the populations for polymorphic loci studied (Table 3).

The values of the genetic distance (Nei 1972) and the topology of both dendrograms (Figs.2 and 3) clearly confirmed the genetic differentiation among the studied populations of *M. structor* .

This study provides new information on the genetic variability of harvester ant *Messor structor* from Bulgaria on the basis of isoenzymic analysis. The isozyme genetic markers indicated in this study could be used appropriately for comparisons, discrimination and characterisation of Bulgarian and European *Messor structor* populations.

Further investigations, increasing the number of analysed loci, are needed in order to provide additional information on the genetic structure and the genetic relations between *Messor structor* populations.

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