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Ljiljana B. Vapa[1]*, *Mihajla R. Djan*[1], *Dragana R. Obreht*[1], *Biljana M. Tošović-Marić*[1], *Milan M. Vapa*[2], *Miloš T. Beuković*[2]

[1] Faculty of Sciences, Trg Dositeja Obradovića 2
[2] Faculty of Agriculture, Trg Dositeja Obradovića 8;
21000 Novi Sad, Serbia and Montenegro

GENETIC VARIABILITY OF PHEASANT (*Phasianus* spp.) IN BREEDING STATION RISTOVACA

ABSTRACT: One of the possible reasons for pheasant population number decline in past several years might be loss of adaptability in populations originated from breeding stations caused by inbreeding depression. Due to fact that adaptability is a consequence of genetic structure of the populations, the aim of this paper was the analysis of genetic variability in pheasant population from breeding station Ristovaca using molecular markers. Allozyme variability of 20 putative gene loci was detected by polyacrylamide gel electrophoresis. Polymorphism was revealed in 5 loci: *Est-1*, *Pgd*, *Sod*, *Gpi-2* and *Odh*. The values of genetic variability measures — heterozigosity, polymorphism, fixation indices and H/P ratio indicate low level of genetic variability and possible presence of inbreeding depression within pheasant population.

KEY WORDS: allozyme, electrophoresis, genetic variability, pheasant

INTRODUCTION

Introduction of pheasant (*Phasianus* ssp.) into our area has started in mid fifties, and today it has become common and well adapted in our country, but also in large number of European hunting areas. Unfortunately, in the last decade, a remarkable decline of pheasant population number has been detected. In Central Serbia, population number in spring season has decrease 44%, and this trend continues. In Vojvodina, percentage of decline is lower, but population number in Vojvodina is at constant low level, mostly because of environmental conditions (less forest areas, more agricultural areas). Cause for named phenomenon can be high hunting pressure on the pheasant species in central part of Serbia (Ceranic, 2001).

Investigation of wild animal genomes is significant because of implementation of results in planing, hunting economy, conservation biology and lack of results published. Low genetic variability is usually related to inbreeding de-

pression and loss of heterozygosity. This leads to most of characteristics of population phenotype, such as metabolic efficiency, reproductive efficiency, disease resistance etc. (G i l p i n and S o u l e, 1986). In wild animal species, lower genetic variability can have larger consequences, because of small populations and high inbreeding. Loss of certain allele of genotype decrease chances for new better adapted genotypes, in case of environmental condition changes.

One of the most commonly used genetic markers in wild animal species are isozymes, present in different molecular forms, allozymes (V a p a et al., 1999, 2002).

The aim of this paper was estimation of genetic variability of pheasant (*Phasianus* ssp.) bred in breeding station Ristovaca, based on variability of isozymes systems.

MATERIAL AND METHODS

Material: Liver samples of forty three individuals were used in this research. Livers were frozen immediately after death of animal and kept in freezer at –20°C until electrophoresis.

Method: Tissue preparation and vertical polyacrylamide gel electrophoresis (PAGE) were performed according to G r i l l i t s c h et al. (1992) and M u n s t e r m a n n (1979). After electrophoresis gels were stained according to S e l a n d e r et al. (1971).

The following isozymes systems were examined (isozyme/-system, abbreviation, E.C. number and corresponding structural gene loci in parenthesis):

Lactate dehydrogenase (LDH, 1.1.1.27, Ldh-1, -2); Malate dehydrogenase (MOR, 1.1.1.37, Mor-1, -2); Malic enzyme (MOD, 1.1.1.40, Mod-1, -2); 6-phosphogluconate dehydrogenase (PGD, 1.1.1.44, Pgd); Octanol dehydrogenase (ODH, 1.1.1.73, Odh); Superoxid dismutase (SOD, 1.15.1.1, Sod); Aspartate aminotransferase (AAT, 2.6.1.1, Aat); Hexokinase (HK, 2.7.1.1, Hk-1, -2); Pyruvate kinase, (PK, 2.7.1.40, Pk); Creatine kinase (CK, 2.7.3.2, Ck-1, -2); Adenylate kinase (AK, 2.7.4.3, Ak-1, -2); Esterases (EST, 3.1.1.1, Es-1); Aldolase (ALDO, 4.2.1.3, Aldo); Glucose-6-phosphate isomerase (GPI, 5.3.1.9, Gpi-2).

Statistical analysis: Statistical evaluation of electrophoretic data was supported by the BIOSYS-1 program of Swofford and Selander (release 1.7, 1989). We used the BIOSYS-1 p.c. package to calculate allele frequencies, average heterozygosity (H_0-observed, H_e-expected), proportion of polymorphic loci (99% criterion) P, mean number of allele per locus (A), exact test of deviation of observed genotypes at polymorphic loci from Hardy-Weinberg expectation, as well as basic parameters of F statistics.

RESULTS AND DISCUSSION

Among 14 isozyme systems, represented by 20 putative loci, five loci were polymorphic. Polymorphism was revealed within loci: *Es-1, Gpi-2, Odh, Pgd* and *Sod*, with two to four alleles per locus (Tab. 1).

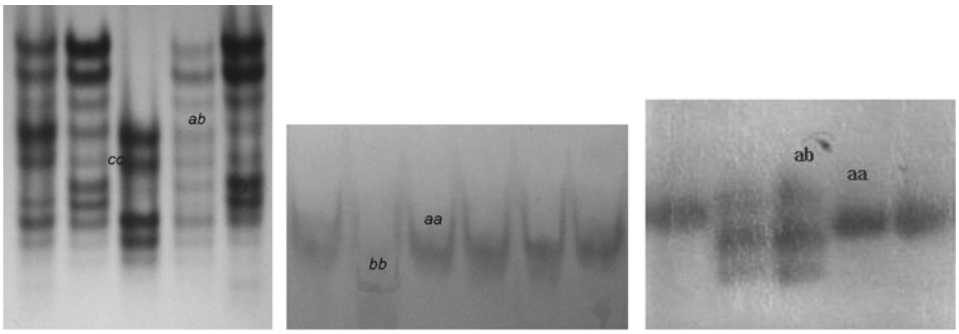
Table 1. Allele frequencies at polymorphic loci and indices of genetic variability in pheasant population from breeding station Ristovača (for acronyms see Material and Methods). H_o — observed population-specific heterozygosity; H_e — expected population specific heterozygosity; $P_{99\%}$ — rate of polymorphism (99% criterion); A — mean number of alleles per locus; n — number of individuals analyzed; F_{IS}, F_{IT} and F_{ST} — fixation indices

Locus	Allele	Ristovača n = 43
Es-1	a	0.174
	b	0.616
	c	0.209
Pgd	a	0.833*
	b	0.188
Gpi-2	a	0.333
	b	0.450
	c	0.200
	d	0.017
Odh	a	0.804*
	b	0.196
Sod	a	0.291
	b	0.570
	c	0.140
H_o		0.045
H_e		0.097
A		1.40
$P_{99\%}$		25%
H/P		0.18
F_{IS}		–0.135
F_{IT}		–0.097
F_{ST}		0.034

* significant deviation of genotype frequencies from Hardy-Weinberg expectation based on five polymorphic loci and exact Fisher test, criterion p < Pn

The mean H_e in various non-endangered bird species and subspecies, as calculated from Table 2. in Evans (1987), amounted to 0.06, with a range from 0.0 to 0.158, with more then 25 loci analyzed. The mean $P_{99\%}$ was 22.02%, and ranged between 0 and 54.2%. According to H_e = 0.097 and $P_{99\%}$ = 25% in our studied population, it clearly fits the values of genetic variability of non-endangered bird species. Hart and Pucek (1994) proposed the use of H/P ratio index, in order to overcome the differences in sample size and loci number analyzed in different mammal populations. In non-endangered bird species (calculated from Evans, 1987; regarding 53 studies with more than 24 loci analyzed) the mean H/P value was 0.303 and ranged between 0.104 and

0.5. Although with H/P value of 0.18, the pheasant population from breeding station in Ristovaca is slightly above bottom level for non-endangered bird species, it still have conserved some level of genetic variability. The lack of variability at 15 screened protein loci and genotype frequencies deviation in two, *Pgd* and *Odh* loci, indicate the restricted breeding range. The presence of inbreeding was also proved by negative F_{IS} = 0.135 and F_{IT} = –0.097 values. Considering the F_{ST} = 0.034 value, it can be concluded that pheasant population analyzed belongs to low genetic differentiated populations.



a) *Es-1* b) *Odh* c) *Pgd*

Figure 1. Zymogrames of polymorphic loci

The results of allozyme analysis in Golden Eagle population (S u c h e n - t r u n k et al., 1999) data showed H_e = 0.034, and $P_{99\%}$ = 10.8%, that reveal lower genetic variability in natural bird population. It can be due to the fact that in their research, variation of 31 isozyme systems represented by 37 putative loci was examined, but only 15 individuals were analyzed and rare alleles are likely to be missed. Number of individuals examined in our research was 43, and this high number of individuals gave us reliable results, because chances to miss rare allele are reduced, comparing with small sample size.

The analysis of breeding population of Common Snipe (Gallinago gallinago) revealed the average values of H_e = 0.461 and $P_{99\%}$ = 80% (P a u l a u - s k a s et al., 2002). Considering the smaller number of loci analyzed in this research (n = 6), we have calculated H/P ratio of 0.576. Comparing with our results for pheasants in breeding station (H/P = 0.18) it is clear that our pheasant population has lower level of genetic variability, comparing to other species breeding population.

Due to fact that common pheasant is a game species widely and increasingly used for restocking of natural populations depleted by hunting, more effort is done in using highly polymorphic molecular markers, e. g. microsatellites. B a r r a t i et al. (2001) reported on genetic variability detected in pheasant breeds by means of microsatellites obtained by heterologous amplification using primers specific to chicken and turkey. This analysis showed that actual heterozygosity of the pheasant populations was lower then expected under Hardy-Weinberg equilibrium (H_o = 0.191 and H_e = 0.271; H_o = 0.165 and H_e = 0.210). Same phenomenon occurred in our analyzed population (H_o = 0.045

and H_e = 0.097), with generally lower values, even with a less resolution power molecular markers. This was probably the effect of poor genetic management, e.g. the small number of founders, small population size. Inbreeding could lead to a rapid loss of individual fitness and genetic variability and reduced viability of populations utilized for restocking programs.

In order to be able to estimate influence of genetic variability in breeding program, it is necessary to continue further study of allozymic variability on wide set of isozyme systems, and in several generations of birds. Because of the limited part of genome that can be studied, additional RFLP analysis of nuclear and mitochondrial genome and DNA sequencing, respectively. The analysis of $tRNA^{Glu}$ gene at D-loop region of mtDNA in duck and chicken species (Liu et al., 1996) shows greatest sequence divergence in birds, and it could be relevant marker for estimating pheasant genetic variability.

CONCLUSION

The analysis of allozyme variability of 20 putative gene loci in pheasant population bred in breeding station Ristovaca was detected by polyacrylamide gel electrophoresis (PAGE). Polymorphism was revealed in 5 loci: *Est-1*, *Pgd*, *Sod*, *Gpi-2* and *Odh*. The values of genetic variability measures — heterozigosity (H_o = 0.045 and H_e = 0.097), polymorphism ($P_{99\%}$ = 25%), fixation indices (F_{ST} = 0.034) and H/P = 0.18 ratio indicate low level of genetic variability and possible presence of inbreeding depression within pheasant population.

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REFERENCES

Baratti, M., Alberti, A., Groenen, M., Veenendaal, T., Fulgheri, F. D. (2001): *Polymorphic microsatellites developed by cross-species amplifications in common pheasant breeds*, Animal Genetics 32: 222—225.

Ćeranić, A. (2001): *Fazan (Phasanius spp.)*. In: V. Šelmić, Ed., *Podaci o divljači, Program razvoja lovstva Srbije*, Lovački savez Srbije, Beograd, pp 32—37.

Evans, P. G. H. (1987): *Electrophoretic variability of gene products*. In: F. Cooke, P. A. Buckley, Eds., *Avian Genetics. A Population and Ecological Approach*, Academic Press, London, UK,pp 105—162.

Gilpin, M. E., Soule, M. E. (1986): *Minimum viable populations: Processes of species extinction*. In: M. E. Soule, Ed., *Conservation Biology, The Science of Scarcity and Diversity*, Sinayer Assoc. Inc. Sunderland, MA, pp 19—34.

Grillitsch, M., Hartl, G., Suchentrunk, F., Willing, R. (1992): *Allozyme evolution and the molecular clock in the Lagomorpha*, Acta theriologica 37: 1—13.

Hartl, G. B., Pucek, Z. (1994): *Genetic depletion in the European bison (Bison bonasus) and the significance of electrophoretic heterozygosity for conservation*, Conservation Biology 8: 167—174.

Liu, H. T., Hu, Y. H., Wang, C. T., Lin, L. Y. (1996): *Sequences and Comparisons of Duck Mitochondrial DNA Control Regions*, Comp. Biochem. Physiol., Vol. 115B, No. 2: 209—241.

Munstermann, L. (1979): *Isozymes of Aedes aegypti: Phenotypes, linkage, and use of genetic analysis of sympatric populations in East Africa*, Ph. D. Disertation, Univ. Notre Dame.

Paulauskas, A., Svazas, S., Danileviciute, A. (2002): *Multilocus allozyme analysis of Common Snipe (Gallinago gallinago) in Lithuania*, Acta Zoologica Lituanica, Vol. 12, No. 3: 318—332.

Selander, R. K., Smith, M. H., Yang, S. V., Johnson, W. E., Gentry, J. B. (1971): *Biochemical polymorphism and systematics in the genus Peromyscus I. variation in the old field mouse (Peromyscus polionotus)*, Studies in Genetics, VI. University of Texas Publication 7103: 49—90.

Suchentrunk, F., Haller, H., Ratti, P. (1999): *Gene pool variability of a golden eagle (Aquila chrysaetos) population from the Swiss Alps*, Biological Conservation 90: 151—155.

Swofford, D. L., Selander, R. B. (1989): *BIOSYS-1. A computer program for the analysis of allelic variation in population genetics and biochemical systematics. Release 1.7. Users Manual*, Champaign: Illinois Natural History Survey.

Vapa, M., Šelmić, V., Obreht, D., Vapa, Lj. (1999): *Allozyme variability in natural populations of hares (Lepus europaeus, Pallas)*, Zbornik radova Matice srpske za prirodne nauke 97: 85—91.

Vapa, Lj., Obreht, D., Vapa, M., Šelmić, V. (2002): *Genetic variability in brown hare (Lepus europaeus) populations in Yugoslavia*, Z. Jagdwissenschaft 48: 261—266.

ГЕНЕТИЧКА ВАРИЈАБИЛНОСТ ФАЗАНА (*Phasianus* spp.) ИЗ ФАЗАНЕРИЈЕ РИСТОВАЧА

Љиљана Б. Вапа[1]*, Михајла Р. Ђан[1], Драгана Р. Обрехт[1], Биљана М. Тошовић-Марић[1], Милан М. Вапа[2], Милош Беуковић[2]
[1] Природно математички факултет, Трг Доситеја Обрадовића 2
[2] Пољопривредни факултет, Трг Доситеја Обрадовића 8;
21000 Нови Сад, Србија и Црна Гора

Резиме

Један од могућих узрока опадања бројности фазана последњих година може бити губитак адаптибилности у популацијама пореклом из фазанерија услед парења у сродству, које води губитку генетичке варијабилности и смањењу хетерозиготности. Због чињенице да је адаптабилност последица генетичке структуре, циљ овог рада био је анализа генетичке варијабилности популације фазана из фазанерије Ристовача применом молекуларних маркера. Алозимска варијабилност 20 генских локуса детектована је полиакриламид гел електрофорезом (PAGE). Полиморфност је регистрована у оквиру 5 локуса: *Est-1*, *Pgd*, *Sod*, *Gpi-2* и *Odh*. Вредности мера генетичке варијабилности — хетерозиготност, полиморфност, индекси фиксације и H/P однос указују на низак ниво генетичке варијабилности и могућег парења у сродству у оквиру популације фазана.

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Mihajla R. Djan[1]*, *Ljiljana B. Vapa*[1], *Dragana R. Obreht*[1], *Milan M. Vapa*[2], *Vukoman R. Šelmić*[3]

[1] Faculty of Sciences, Trg Dositeja Obradovića 2, 21000 Novi Sad
[2] Faculty of Agriculture, Trg Dositeja Obradovića 8, 21000 Novi Sad
[3] Faculty of Forestry, Kneza Višeslava 1, 11000 Beograd; Serbia and Montenegro

ON GENE POOL DIVERGENCE OF THE BROWN HARE (*Lepus europaeus*, Pallas) IN VOJVODINA

ABSTRACT: Today it is widely accepted that a conservation of genetic diversity increases chances of animal survival. The aim of this study was investigation of gene pool divergence of the brown hare (*Lepus europaeus*) in Vojvodina. Allozymic diversity of 60 brown hares from different localities in Vojvodina was studied by polyacrylamide and starch gel electrophoresis at 31 putative structural gene loci. Only five loci: *Idh-2*, *Pgd*, *Pgm*, *Es-1* and *Es-D* were polymorphic, possessing 2 to 6 different alleles. The value of average heterozygosity (H_o) was 0.0427, while polymorphism ($P_{99\%}$) was 8.4%. Nei's values of genetic distance (ranged from 0.000 to 0.029) and modified Roger's distance (ranged from 0.030 to 0.181) were calculated among hare populations. Apart from the relatively high values of heterozygosity and polymorphism, the nuclear gene pool diversity of brown hare population in Vojvodina based on allozyme variation is low and corresponds to the data obtained for the populations in Austria and Central Europe.

KEY WORDS: allozymes, electrophoresis, *Lepus europaeus*, polymorphism

INTRODUCTION

It is widely accepted that the conservation of genetic diversity increases chances of animal survival and it has also been recognized that low genetic variability is associated with inbreeding depression and loss of heterozygosity, weakening the components of the population phenotype.

Brown hare, which primarily was forest-steppe game, and later resident of open steppe areas, in time adapted to „cultural" steppe, i.e. agroecosystems, which is main ecosystem in Vojvodina (Serbia and Montenegro) (Šelmić, 1997). Sudden regression of brown hare populations in sixties at its whole area in Europe, especially in the best biotopes, induced numerous investigations with different approach, in order to determine the cause of decline of brown hare. For all game species, especially for hare as species of permanent biologi-

cal interest and with important role in hunting economy in Vojvodina (V a p a and Š e l m i ć, 1997), it is necessary to conduct genetic and population studies.

Genetic variation in mammals can be characterized at three levels: within individuals, among individuals of a population and among populations, species and higher taxa. At all those levels, allozymes, genetically determined and structurally (not necessary functionally) different variants of enzymes, have played a major role in evolutionary and conservation genetics during the past 25 years (H a r t l et al., 1994). Allozymes have been implicitly or explicitly assumed to be a representative indicator of genomic variation in wild (G r i l - l i t s c h et al., 1992; H a r t l et al., 1993; S u c h e n t r u n k et al., 2000; V a p a et al., 2000) and domestic animals (P e t e r k a and H a r t l, 1992).

The aim of this paper was to estimate gene pool divergence of the brown hare population in Vojvodina based on allozymic variability.

MATERIAL AND METHODS

Allozymic diversity of 60 brown hares (*Lepus europaeus*, Pallas 1778) was studied by polyacrylamide and starch gel electrophoresis. Twenty two isozymes/-systems encoded by 31 hypothetical structural gene loci were assayed (Table 1).

Table 1. List of enzyme loci assayed for allozymic variation

Isozyme/-system	Abbreviation	E. C.	Locus
Lactate dehydrogenase	LDH	1.1.1.27	Ldh-1, -2
Malic enzyme	MOD	1.1.1.40	Mod-1, -2
Isocitrat dehydrogenase	IDH	1.1.1.42	Idh-1, -2
6-phosphogluconate dehydrogenase	PGD	1.1.1.44	Pgd
Glucose dehydrogenase	GDH	1.1.1.47	Gdh-2
Glucose-6-phosphate dehydrogenase	GPD	1.1.1.49	Gpd
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	1.2.1.12	Gapdh
Xanthine dehydrogenase	XDH	1.2.3.2	Xdh
Glutamate dehydrogenase	GLUD	1.4.1.3	Glud
Catalase	CAT	1.11.1.6	Cat
Superoxid dismutase	SOD	1.15.1.1	Sod-1, -2
Purine nucleoside phosphorilase	NP	2.4.2.1	Np
Aspartate aminotransferase	AAT	2.6.1.1	Aat-1, -2
Pyruvate kinase	PK	2.7.1.40	Pk
Creatine kinase	CK	2.7.3.2	Ck-1, -2
Adenylate kinase	AK	2.7.4.3	Ak-1, -2
Phosphoglucomutase	PGM	2.7.5.1	Pgm-1, -2
Esterases	ES	3.1.1.1	Es-1, -D
Fructose-1,6-diphosphatase	FDP	3.1.3.11	Fdp-1
Guanine deaminase	GDA	3.5.4.3	Gda
Aldolase	ALDO	4.2.1.3	Aldo
Fumarate hydratase	FH	4.2.1.2	Fh

Liver tissue samples of 60 brown hares were obtained from 15 sampling localities in Vojvodina (Serbia and Montenegro): Despotovo (DE), Begec (BE), Banatsko Arandjelovo (BA), Pacir (PA), Novo Milosevo (NM), Turija (TU), Donji Petrovac (DP), Futog (FU), Curug (CU), Padej (PD), Kulpin (KU), Hrtkovci (HR), Backi Jarak (BJ), Kovilj (KO) and Stara Moravica (SM). Tissue preparation, polyacrylamide and starch gel electrophoresis and staining procedure were performed according to Grillitsch et al. (1992) and modified method of Munstermann (1979) and Selander et al. (1986). For designation of alleles the nomenclature of Hartl et al. (1993) was used.

The BIOSYS-1 pc package, release 1.7 (Swofford and Selander, 1989) was used for calculation of allele frequencies, average heterozygosity (H_o — observed, H_e — expected), proportion of polymorphic loci (P, 99% criterion) and mean number of alleles per locus based on all 31 loci (A). Also, pairwise genetic D value (Nei, 1978) and modified Roger's distances were obtained. According to the results, unrooted Wagner dendogram based on modified Roger's distances, was created.

RESULTS

Screening of 22 enzyme systems representing a total of 31 putative structural loci revealed polymorphism in the following 5 isoenzymes: *Idh-2*, *Pgd*, *Pgm-2*, *Es-1* and *Es-D*, for which allele frequencies were calculated in each brown hare population (Table 2). Allelic frequencies did not vary significantly from Hardy-Weinberg equilibrium.

Using BIOSYS-1 pc package, release 1.7 (Swofford and Selander, 1989) average heterozygosity (H_o — observed, H_e — expected), proportion of polymorphic loci (P, 99% criterion) and mean number of alleles per locus based on all 31 loci (A) were calculated for each sampling locality and the mean value for all localities analyzed (Table 3). The value of observed heterozygosity H_o ranged from 0.000 to 0.065, with a mean value $H_o = 0.042$. Expected heterozygosity value also ranged from 0.000 to 0.065, but with the mean value $H_e = 0.062$. The proportion of polymorphic loci based on 99% criterion ranged from 0 to 12.9%, with the mean value $P_{99\%} = 8.39\%$. Only one local population was polymorphic (Hrtkovci) probably due to low number of individuals analyzed.

Table 2. Allelic frequencies of polymorphic loci for each sampling locality (for abbreviation see Material and Methods)

Locus	Allele	Sampling localities														
		DE	CU	PA	KU	HR	BJ	KO	SM	BE	BA	PA	NM	TU	DP	FU
Idh-2	a	0.875	0.833	1.000	0.833	1.000	1.000	1.000	1.000	0.500	0.667	1.000	0.769	0.167	0.375	0.833
	b	0.125	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.500	0.333	0.000	0.192	0.833	0.625	0.000
	c	0.000	0.167	0.000	0.167	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.038	0.000	0.000	0.167
Pgd	a	0.875	1.000	1.000	1.000	1.000	1.000	0.833	0.714	1.000	1.000	1.000	0.885	0.833	1.000	1.000
	b	0.125	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.115	0.167	0.000	0.000
	c	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	d	0.000	0.000	0.000	0.000	0.000	0.000	0.167	0.143	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	e	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.143	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Pgm-2	a	0.875	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.900	1.000	1.000	1.000	1.000	1.000	1.000
	b	0.125	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.100	0.000	0.000	0.000	0.000	0.000	0.000
Es-1	a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.167	0.000	0.231	0.000	0.250	0.000
	b	0.000	0.500	0.500	0.333	0.000	0.500	0.500	0.429	0.000	0.167	0.000	0.423	0.333	0.500	0.667
	c	0.375	0.500	0.500	0.667	1.000	0.500	0.500	0.571	0.400	0.000	0.200	0.346	0.500	0.250	0.333
	d	0.250	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.167	0.100	0.000	0.167	0.000	0.000
	e	0.125	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.200	0.333	0.100	0.000	0.000	0.000	0.000
	f	0.250	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.400	0.167	0.600	0.000	0.000	0.000	0.000
Es-D	a	1.000	1.000	1.000	0.833	1.000	0.500	0.667	0.500	0.900	1.000	1.000	0.808	1.000	0.625	0.833
	b	0.000	0.000	0.000	0.167	0.000	0.500	0.333	0.500	0.100	0.000	0.000	0.192	0.000	0.375	0.167

Table 3. The values of H_o — direct count heterozygosity, H_e — expected heterozygosity, P — rate of polymorphism (99% criterion), A — mean number of alleles per locus per each sampling locality and the mean values (for acronyms see Material and Methods)

Locality	H_0	H_e	P	A
DE	0.040	0.051	12.90	1.19
BE	0.058	0.054	12.90	1.16
BA	0.054	0.047	6.45	1.16
PA	0.019	0.021	3.23	1.10
NM	0.050	0.051	12.90	1.19
TU	0.054	0.045	9.68	1.13
DP	0.065	0.058	9.68	1.13
FU	0.043	0.039	9.68	1.10
CU	0.032	0.041	9.68	1.10
PD	0.032	0.032	3.23	1.03
KU	0.043	0.039	9.68	1.10
HR	0.000	0.000	0.000	1.00
BJ	0.065	0.065	6.45	1.06
KO	0.043	0.047	9.68	1.10
SM	0.023	0.050	9.68	1.13
Mean	0.042	0.062	8.39	1.11

The genetic relationships of brown hares from Vojvodina sampling localities are summarized by an unrooted Wagner dendogram based on modified Rogers distances (Figure 1).

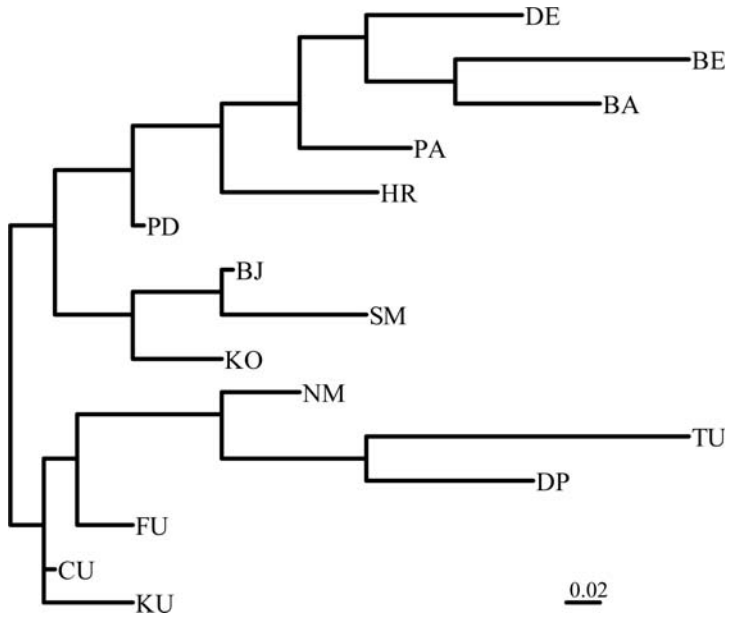

Figure 1. Unrooted Wagner dendogram based on modified Roger's distances

DISCUSSION

Values of observed heterozygosity H_0 = 0.042 and expected heterozygosity H_e = 0.062 (Table 3) were lower than in previous investigation of brown hare population in Vojvodina (V a p a et al., 2001; V a p a et al., 2002), due to higher number of analyzed enzyme systems, applied in this research. Level of allozymic diversity in brown hares from Bulgaria H_0 = 0.030 and H_e = 0.035 and from Austria, H_0 = 0.030 and H_e = 0.027 (S u c h e n t r u n k et al., 2000) revealed lower values of heterozygosity, but the overall rate of polymorphism (99% criterion) were higher in Bulgarian (P = 12%) and Austrian (10.7%) than in Vojvodinian brown hare population (P = 8.4%). Possible cause of these differences could lay in fact that this investigation (S u c h e n t r u n k et al., 2000) was performed on a larger number of individuals. Comparing to data obtained for Central Europe brown hare populations (H a r t l et al., 1993) the means of H_0 = 0.187 and P = 15.3% were higher than in Vojvodinian population (Table 3). In their research a total number of 469 brown hares from 20 sampling localities were screened for polymorphism at 54 loci, which brought to higher level of allozymic diversity than in our research.

Investigation revealed polymorphism at only 5 loci, possessing two to six different alleles, with average number of alleles per locus A = 1.11 (Table 3). Other analysis of European brown hare populations obtained the range of mean number of alleles per locus from A = 1.10 (S u c h e n t r u n k et al., 1999) to A = 1.17 (S u c h e n t r u n k et al., 2000). No new alleles were found in Vojvodinian brown hare population, comparing to previously published data on European brown hare populations (P e t e r k a and H a r t l, 1992; H a r t l et al., 1993; S u c h e n t r u n k et al., 2000).

N e i ’s (1978) unbiased genetic distance and modified Rogers distance (Wright, 1978). were calculated on the basis of allelic frequencies. Values of Nei’s distances ranged from 0.000 to 0.029, and values of modified Rogers distances 0.030 to 0.181 between different sampling localities in Vojvodina (Serbia and Montenegro). Relative low level of genetic distances can be explained by the fact that sampling localities analyzed are cited in the area with no significant geographical barriers and not far away from each other, which enable free migration of individuals between some close sampling areas.

Apart from the relatively high values of heterozygosity and polymorphism, it may be concluded that the nuclear gene pool diversity of brown hare population in Vojvodina based on allozyme variation is low (especially visible in the dendogram, Figure 1). It corresponds to the data obtained for the local population in eastern part of Austria (H a r t l et al., 1993), but it is lower than genetic variability based on nuclear gene pool divergence in Southerneastern Europe (S u c h e n t r u n k et al., 2000).

REFERENCES

G r i l l i t s c h, M., H a r t l, G., S u c h e n t r u n k, F., W i l l i n g, R. (1992): *Allozyme evolution and the molecular clock in the Lagomorpha*, Acta theriologica 37: 1—13.

Hartl, G., Suchentrunk, F., Nadlinger, K., Willing, R. (1993): *An integrative analysis of genetic differentiation in the brown hare Lepus europaeus based on morphology, allozymes, and mitochondrial DNA*, Acta theriologica 38: Suppl. 2: 33—57.

Hartl, G., Willing, R., Nadlinger, K. (1994): *Allozymes in mammalian population genetics and systematics: Indicative function of a marker system reconsidered*. In: Schirerwater et al., Eds., *Molecular Ecology and Evolution: Approaches and Applications*, Birkhauser Verlag Basel, Switzerland, pp: 299—310.

Munstermann, L. (1979): *Isozymes of Aedes aegypti: Phenotypes, linkage, and use of genetic analysis of sympatric populations in East Africa*, Ph. D. Disertation, Univ. Notre Dame.

Nei, M. (1978): *Estimation of average heterozygosity and genetic distance from a small number of individuals*, Genetics 89: 583—590.

Peterka, M., Hartl, G. (1992): *Biochemical-genetic variation and differentiation in wild and domestic rabbits*, Z. zool. Syst. Evolut. Forsch. 30: 129—141.

Selander, R. K., Caugant, D. A., Ochman, H., Musser, J. M., Gilmour, N., Whittam, T. S. (1986): *Methods for multilocus enzyme electrophoresis for bacterial population genetics and systematics*, Appl. Environ. Microbiol. 51: 873—884.

Suchentrunk, F., Polster, K., Giacometti, P., Ratti, P., Thulin, C., Ruhle, C., Vasilev, A., Slotta-Bachmayr, L. (1999): *Spatial partioning of allozyme variability in European mountain hares (Lepus timidus): gene pool divergence across a disjunct distributional range?* Z. Saugtierkunde 64: 308—318.

Suchentrunk, F., Michailov, C., Markov, G., Haiden, A. (2000): *Population genetics of Bulgarian brown hares Lepus europaeus: allozymic diversity at zoological crossroads*, Acta theriologica 45: 1—12.

Swofford, D., Selander, R. (1989): *A computer program for the analysis of allelic variation in population genetics and biochemical systematics release 1.7. User's manual*, Illinois natural History Survey, Champaign.

Šelmić, V. (1997): *Zec u savremenim ekosistemima*. In: V. Šelmić, Ed., *Zbornik radova: Zec i jarebica u savremenim agroekosistemima. Simpozijum iz lovstva*, Lovački savez Vojvodine, Novi Sad, pp: 1—19.

Vapa, Lj., Šelmić, V., Popović, T., Vapa, M. (2000): *Genetic identity in brown hare populations in Vojvodina*, Zbornik Matice srpske za prirodne nauke 99: 81—87.

Vapa, Lj., Obreht, D., Vapa, M., Šelmić, V. (2001): *Genetic identity of hare (Lepus Europaeus Pallas) populations*, Abstract book of XXVth International Congress of the I.U.G.B. Wildlife management in the 21th Century, Lesvos, Greece, pp: 55.

Vapa, Lj., Obreht, D., Vapa, M., Šelmić, V. (2002): *Genetic variability in brown hare (Lepus europaeus) populations in Yugoslavia*, Z. Jagdwissenschaft 48: 261—266.

Vapa, M., Šelmić, V. (1997): *Sadasnje stanje i buducnost evropskog zeca*. In: V. Šelmić, Ed., *Zbornik radova: Zec i jarebica u savremenim agroekosistemima. Simpozijum iz lovstva*, Lovački savez Vojvodine, Novi Sad, pp: 33—45.

Wright, S. (1978): *Evolution and genetics of populations. Vol. IV. Variability within and among populations*, Chicago. Univ. Chicago Press, 1—580.

ДИВЕРГЕНТНОСТ ГЕНСКОГ ФОНДА ПОПУЛАЦИЈЕ ЕВРОПСКОГ ЗЕЦА (*Lepus europaeus*, Pallas) У ВОЈВОДИНИ

Михајла Р. Ђан[1]*, Љиљана Б. Вапа[1], Драгана Р. Обрехт[1],
Милан М. Вапа[2], Вукоман Р. Шелмић
[1] Природно математички факултет, Трг Доситеја Обрадовића 2,
21000 Нови Сад
[2] Пољопривредни факултет, Трг Доситеја Обрадовића 8,
21000 Нови Сад
[3] Шумарски факултет, Кнеза Вишеслава 1,
11000 Београд; Србија и Црна Гора

Резиме

Очување генетичког диверзитета повећава шансу за преживљавање врста. Циљ овог рада било је истраживање дивергентности генског фонда европског зеца (*Lepus europaeus*, Pallas) у Војводини. Алозимска варијабилност 31 генског локуса 60 индивидуа зеца са различитих локалитета у Војводини анализирана је полиакриламидном и скробном гел електрофорезом. Само пет локуса: *Idh-2*, *Pgd*, *Pgm*, *Es-1* и *Es-D* било је полиморфно. Вредност просечне хетерозиготности износила је $H_o = 0.0427$, а полиморфности $P_{99\%} = 8.4\%$. Поред релативно високих вредности, диверзитет генског фонда европског зеца у Војводини на основу алозимске варијабилности је низак и одговара подацима из популација зеца Централне Европе.

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Snežana R. Radenković, Ante V. Vujić, Smiljka D. Šimić

University of Novi Sad, Faculty of Natural Sciences,
Department of Biology and Ecology, Trg Dositeja Obradovića 2,
21000 Novi Sad, Serbia and Montenegro

NEW DATA ON HOVERFLY DIVERSITY (INSECTA: DIPTERA: SYRPHIDAE) OF THE SPECIAL NATURE RESERVE THE OBEDSKA BARA MARSH (RAMSAR SITE IN SERBIA)

ABSTRACT: Hoverflies are well-investigated insects group on Obedska bara Marsh due to long-term investigations carried out by a scientific team from Faculty of Sciences, University of Novi Sad, Serbia (Vujić et al., 1998). After 15 years, monitoring of biodiversity in this Ramsar site is continued by scientific project 1770 of Ministry of Science and Environmental Protection, Republic of Serbia. Based on new investigations and current nomenclature changes, the following results were obtained: record of *Eupeodes goeldlini* Mazánek, Láska et Bičik, 1999 is the first for Serbia, nine species were recorded for the first time for Obedska bara Marsh, seven species were replaced by recently established junior synonyms and three were excluded from the faunal list of Obedska bara Marsh. These data complete the list of 93 hoverfly species registered on Obedska bara Marsh.

KEY WORDS: Diptera, Syrphidae, hoverflies fauna, the Obedska bara Marsh

INTRODUCTION

In 1977, the Special Nature Reserve Obedska bara, Marsh Stari Begej — Carska bara Marsh and Ludoško jezero, were designated as Wetlands of International Importance in Serbia according to the Ramsar convention. This seasonally inundated area of the Sava River floodplain, with marshes, ponds, wet meadows and an oxbow lake is located between the villages Obrež and Kupinovo in the Vojvodina Province (Serbia). It presents an important ornithological reserve on the Balkan Peninsula. Many bird species, as natural rarities, are included in the Red Lists of Serbia, and even in the Red Book of Europe and World. Vegetation includes reedbeds and *Salix-Populus* and *Quercus* woodland. Of 179 plant species, 25 are aquatic and 28 are swampy (Gajić and Karadžić, 1991). Hoverflies are well-investigated insects group in this area due to a seven-year investigation done by a scientific team from Faculty of

Natural Sciences, University of Novi Sad (Serbia). Of 87 registered hoverflies species, 29 are of special faunal, taxonomic and zoogeographical interest (Vujić et al., 1998). After 15 years, monitoring of biodiversity in this protected area is continued by scientific project 1770 of Ministry of Science and Environmental Protection, Republic of Serbia. The results gathered for hoverflies are presented here. Wetlands are among the world's most threatened ecosystems and this one has been seriously degraded, the most important problem being the overgrowing and disappearance of wet meadows. The aims of this paper are to complete the faunal data and to emphasize the necessity of permanent application of the biodiversity conservation policy.

MATERIAL AND METHODS

Material analyzed in this study has been collected during two periods, 1981—1990 and 2002—2004. It is deposited in the collection of Department of Biology and Ecology, Faculty of Natural Sciences, University of Novi Sad (Serbia).

Standard methods for collecting and preparation of hoverflies were used during this investigation (Vujić et al., 1998).

RESULTS AND DISCUSSION

The analysis of the recently collected material (2002—2004) and the review of the previously published records (Vujić et al., 1998) produced new data for the fauna of the Obedska bara Marsh.

Anasimyia (Eurimyia) lineata Fabricius, 1787

Vujić et al., 1998: as *Helophilus (Anasymia) lineatus* (Fabricius, 1787)

Remark: Claussen and Torp (1980) considered *Anasimyia* Schiner, 1864 as a good genus, not a subgenus of *Helophilus* Meigen, 1822.

Brachyopa bicolor (Fallén, 1817)

New data: Obedska bara, Debela gora, 21. 04. 2003, 1♂, leg. Vujić, A.

Remark: This is the first record of *Brachyopa bicolor* for the Obedska bara Marsh that is very rare in Serbia. Besides the Obedska bara Marsh, it has been registered in only two localities the Stara planina Mountain (Vujić, 1991; Šimić and Vujić, 1996) and the Petrovaradinsko-Karlovački rit Marsh (Vujić and Glumac, 1994). Data from Homolje (Glumac, 1955) have not been verified because corresponding samples lack from the collection of the Museum of Natural History in Belgrade (Serbia). It is distributed from Sweden and Finland in the north to northern Spain and from in the south Britain through central Europe (and the former Yugoslavia) into the European parts of Russia and on through Siberia to the Pacific. This species is largely arboreal and it occurs at sap runs on trunks of overmature and senile *Acer pse-*

udoplatanus, Fagus, Quercus and *Castanea*. There is no evidence that adults visits flowers (Speight, 2003). This may also be the reason why it is uncommon and rarely captured.

Brachyopa maculipennis Thompson, 1980

New data: Obedska bara, Debela gora, 21. 04. 2003, 1♂, leg. Vujić, A.

Remark: This very rare species has been found in Serbia in only two localities: the Stara planina Mountain (Vujić, 1991; Šimić and Vujić, 1996) and currently at the Obedska bara Marsh. It appears along streams in humid *Fagus* forests. The larva is still undescribed. Its range includes northern Germany, the Czech Republic, Austria, Slovakia, northern Italy, Romania, parts of the former Yugoslavia. It is probably extinct in Germany (Speight, 2003). This first record for the Obedska bara Marsh is important and it deserves special attention in biodiversity monitoring of Ramsar sites.

Cheilosia urbana (Meigen, 1822)

syn. *Cheilosia praecox* (Zetterstedt, 1843)

Remark: Claussen and Speight (1999) reinstated *Cheilosia urbana* (Meigen, 1822) as the senior valid synonym of *Cheilosia praecox* (Zetterstedt, 1843). This is the most abundant and widely distributed *Cheilosia* species on the Balkan Peninsula.

Chrysotoxum festivum (Linnaeus, 1758)

syn. *Chrysotoxum arcuatum* sensu Thompson, Vockeroth and Speight (1982) nec (Linnaeus, 1758)

Remark: Iliff and Chandler (2000) reinstated the names changed by Thompson et al. (1982) in the genera *Chrysotoxum* and *Xanthogramma*. Thompson et al. (1982) considered *Chrysotoxum festivum* of the former authors as junior synonym of *Chrysotoxum arcuatum* (Linnaeus, 1758). Iliff and Chandler (2000) proposed conservation of usage of the specific names by designation of neotypes for *Musca arcuata* Linnaeus, 1758 (currently *Chrysotoxum arcuatum*) and *Musca citrofasciata* Linnaeus, 1758 (currently *Chrysotoxum arcuatum*).

Chrysotoxum intermedium Meigen, 1822

Chrysotoxum aff. *intermedium* Meigen, 1822 by Vujić et al., 1998

Remark: Sommaggio (2001) examined the types of *Chrysotoxum* species from Giglio Tos' collection, and proposed several synonyms. He gave distinguishing characters for two closely related species, *Chrysotoxum intermedium* Meigen, 1822 and *Chrysotoxum lessonae* Giglio Tos, 1890. On the basis of these features, the male identified as *Chrysotoxum* aff. *intermedium* Meigen, 1822 (Vujić et al., 1998) belongs to *Chrysotoxum intermedium* Meigen, 1822.

Eristalis lineata (Harris, 1776)

syn. *Eristalis horticola* auct. nee (De Geer, 1776)

Remark: Hippa et al. (2001) reviewed the West Palaearctic species of the genus *Eristalis*. They cited that *Eristalis lineata* is the species usually named *horticola*, which is an unjustified replacement name for *Musca nemorum* Linnaeus, 1758. The latter is a synonym of *Eristalis arbustorum* Linnaeus, 1758.

Eristalis pertinax (Scopoli, 1763)

Published data: Šimić and Vujić, 1990 (without records)

New data: Obedska bara, Debela gora, 22. 09. 1981, 1♀, 10. 06. 1984, 2♀♀, 23. 04. 1986, 2♀♀, 23. 04. 1988, 2♂♂, 16. 04. 1989, 1♂.

Remark: Although the material from the Obedska bara Marsh determined as *Eristalis pertinax* was included in the list of *Eristalis* species recorded in Yugoslavia (Šimić and Vujić, 1990), it was omitted by Vujić et al. (1998). As *Eristalis pertinax* is common species, widely distributed in Serbia, Šimić and Vujić (1990) considered that it is not necessary to cite all records.

Eristalis similis Fallén, 1817

syn. *Eristalis pratorum* Meigen, 1822

Published data: Šimić and Vujić, 1990 (without records)

New data: Obedska bara, Debela gora, 23. 04. 1986, 1♀, 11. 06. 1986, 1♀, 23. 04. 1988, 1♂, 1♀, 16. 04. 1989, 2♂♂, Obrež — Ogar, 2. 07. 1987, 1♂.

Remark: Šimić and Vujić (1990) cited *Eristalis pratorum* Meigen, 1822 for Yugoslavia, taking into consideration also the material from the Obedska bara Marsh. Vujić et al. (1998) omitted these records in their monograph on Syrphidae of Obedska bara. Nielsen (1995, 1999) has studied the types of *Eristalis similis* and found this name to be synonymous with *pratorum*.

Eupeodes (Eupeodes) corolae (Fabricius, 1794)

Vujić et al., 1998: as *Metasyrphus (Metasyrphus) corollae* (Fabricius, 1794)

Remark: Until recently, the European species of *Eupeodes* Osten-Sacken, 1877 has appeared under the generic name *Metasyrphus* Matsumura, 1917, but Vockeroth (1986) showed that there is no basis for segregating *Eupeodes* and *Metasyrphus* species into separate genera and pointed out that the generic name *Eupeodes* has precedence over *Metasyrphus* (Speight, 2003).

Eupeodes (Eupeodes) latifasciatus (Macquart, 1829)

Vujić et al., 1998: as *Metasyrphus (Metasyrphus) latifasciatus* (Macquart, 1829)

Remark: The same as for *E. corollae*.

Eupeodes (Lapposyrphus) lapponicus (Zetterstedt, 1838)

Vujić et al., 1998: as *Metasyrphus (Lapposyrphus) lapponicus* (Zetterstedt, 1838)

Remark: The same as for *E. corollae*.

Eupeodes goeldlini Mazánek, Laska et Bičik, 1999

Metasyphus nuba (Wiedemann, 1830) by Vujić et al., 1998

Remark: Mázanek et al. (1999) described two closely related *Eupeodes* species, similar to *Eupeodes bucculatus* (Rondani, 1957): *Eupeodes duseki* Mazánek, Laska et Bičik, 1999 from Scandinavia and *Eupeodes goeldlini* Mazanek, Láska et Bičik, 1999 from Central Europe and Far East of Russia. The female of *Eupeodes goeldlini* is still undescribed, but Mazanek (pers. comm.) prepared a description including material from the Obedska bara Marsh. This is the first published record of *Eupeodes goeldlini* for Serbia.

Fagisyrphus cinctus (Fallén, 1817)

Vujić et al., 1998: as *Melangyna (Meligramma) cincta* (Fallén, 1817)

Remark: Dušek and Laska (1967) described the monotypic genus *Fagisyrphus* on the basis of the type species *Scaeva cincta* Fallén, 1817. According to Catalogue of Palaearctic Diptera (Peck, 1988) the genus *Fagisyrphus* is congeneric with the genus *Melangyna* Verall, 1901 that is divided into two subgenera: *Melangyna* and *Meligramma* Frey, 1946. Most of the European syrphidologists (Ssymank et al., 1999; Nielsen, 1999) consider *Fagisyrphus* as good genus name and follow Dušek and Laska (1967).

Heringia (Neocnemodon) brevidens (Egger, 1865)

Vujić et al., 1998: as *Neocnemodon brevidens* (Egger, 1865)

Remark: Distinguishing *Heringia* females from females of *Neocnemodon* is uncertain. Vockeroth and Thompson (1987) took this fact into consideration while combining the two genera, under the earlier name *Heringia* (Speight, 2003). This practice is often followed. Claussen et al. (1994) also recognized two subgenera of the genus *Heringia* Rondani, 1856, based on the structure of the aedeagus: *Heringia* s.s. and *Neocnemodon*.

Merodon avidus Rossi, 1790 **B species** (Milankov et al., 2001)

Vujić et al. (1998): as *Merodon avidus* Rossi, 1790

Remark: Milankov et al. (2001) examined genetic divergence in the adult populations of *Merodon avidus* (Rossi, 1790) and detected two cryptic species, *Merodon avidus* A (Meditteranean) and *Merodon avidus* B (mountainous). The results of morphological analysis also confirmed the existence of two taxa. *Merodon avidus* complex is widely distributed and very frequent on the Balkan Peninsula. Because of significant variability, there are many taxa described under the name of *Merodon spinipes* (Fabricius, 1794), that is currently a junior synonym of *Merodon avidus*. Nomination of these two cryptic species demands study of the type material of all synonyms.

Merodon ruficornis Meigen, 1822

syn. *Merodon recurvus* Strobl, 1898

Remark: Dirickx (1994) erected *Merodon recurvus* Strobl, 1898 from a variety of *Merodon mucronatus* Rondani, 1857, and gave it species

status. Although this is a valid species, it has to be renamed. Study of the lectotype of *Merodon ruficornis* Meigen, 1822 and *Merodon mucronatus* var. *recurvus* Strobl, 1898 has shown that *Merodon recurvus* has to be considered as junior synonyms of *Merodon ruficornis* (Radenković et al., 2002).

Microdon analis (Macquart, 1842)

syn. *Microdon latifrons* Loew, 1856

Remark: Doczkal and Schmid (1999) revised the Central European taxa of the genus *Microdon* Meigen, 1803 and reviewed their synonymy. Speight (1978, 1984) considered *Microdon latifrons* Loew, 1856 conspecific with *Microdon eggeri* Mik, 1897, and later also with *Microdon analis* (Macquart, 1842) (Speight, 1994).

Paragus pecchiolii Rondani, 1857

syn. *Paragus majoranae* Rondani, 1857

Remark: Sommagio (2002) re-examined the type material and made a conclusion that *Paragus gorgus* Vujić et Radenković, 1999 is a junior synonym of *Paragus majoranae* Rondani, 1857, while *Paragus pecchiolii* Rondani, 1857 is the valid name for *Paragus majoranae* Rondani, 1857 sensu Goeldlin de Tiefenau (1976).

Parhelophilus versicolor (Fabricius, 1794)

Vujić et al., 1998: as *Helophilus (Parhelophilus) versicolor* (Fabricius, 1794)

Remark: Thompson (1997) revised the genus *Parhelophilus* Girschner, 1897 and discussed its nomenclature: Girschner (1897) divided the genus *Helophilus* Meigen, 1822 into subgenera. Verall (1901) noted that *Parhelophilus*, as construed by Girschner, was a heterogeneous group. Curran and Fluke (1926) designated a type species, recognized *Parhelophilus* as a distinct group, and treated the group as a genus. North American dipterists with Curan and Fluke, and so did European ones later on.

Pipiza luteibarba Vujić, manuscript name

Pipiza festiva Meigen, 1822 by Vujić et al. (1998), in part

Published data: Vujić et al., 1998 (the Obedska bara Marsh, Kupinske grede, 15. 04. 1990, 1♀, as *Pipiza festiva*).

New data: the Obedska bara Marsh, Kupinske grede, 23. 04. 1986, 1♀.

Remark: Among the material determined as *Pipiza festiva*, Vujić (manuscript) has recognized a sample of a still undescribed species. Description of *Pipiza luteibarba* is in a preparation (Vujić, manuscript). This species is one of the most enigmatic European *Pipiza*. It was collected at two lowland localities near Sava and Morava rivers in Serbia. *Pipiza luteibarba* probably presents a relict Moesian species. The preservation of its habitats is extremely important for protection of this species.

Rhingia rostrata (L i n n a e u s, 1758)

Rhingia campestris M e i g e n, 1822 by V u j i ć et al. (1998), in part

Published data: V u j i ć et al., 1998 (the Obedska bara Marsh, Kupinske grede, 15. 04. 1990, 1♂, as *Rhingia campestris*).

Remark: Re-examination of material determined as *Rhingia campestris* has shown that one male belongs to *Rhingia rostrata* (L i n n a e u s, 1758). It occurs in deciduous forest (*Quercus, Fraxinus/Fagus*) and scrubs, with a rich, tall-herb ground flora. This species is registered from southern Finland and Denmark to northern Spain; from Britain through Central Europe into the European parts of Russia, the Caucasus and western Siberia. Although frequent during the 19th century, this species disappeared from most parts of Europe during the 20th century and it should probably be regarded as threatened at the European level (S p e i g h t, 2003).

Scaeva selenitica (M e i g e n, 1822)

Scaeva pyrastri (L i n n a e u s, 1758) by V u j i ć et al. (1998)

Published data: V u j i ć et al, 1998 (Obedska bara, Kupinske grede, 3. 04. 1988, 1♀, as *Scaeva pyrastri*).

Remark: Redetermination of the only sample of the genus *Scaeva* collected at the Obedska bara Marsh has excluded *Scaeva pyrastry* from the list of species and added *Scaeva selenitica*. It appears in most types of deciduous forest, including scrub woodland and orchards, plus evergreen *Quercus ilex* forest in southern Europe. This species is distributed from Fennoscandia south to Iberia and the Mediterranean, including North Africa; from Ireland eastwards through much of Europe into Turkey and the European parts of Russia; from the Urals through Siberia to Cis-Baikal and on to Sachalin and the Kuril Isles (S p e i g h t, 2003).

Xanthogramma citrofasciatum (D e G e e r, 1776)

syn. *Xanthogramma festivum* (L i n n a e u s, 1758)

Remark: I l i f f and C h a n d l e r (2000) reinstated the names changed by T h o m p s o n et al. (1982) in the genera *Xanthogramma* and *Chrysotoxum*. In the genus *Xanthogramma*, the name *citofasciatum* is proposed as valid one.

Excluded and replaced names

Cheilosia praecox (Z e t t e r s t e d t, 1843) = *Cheilosia urbana* (M e i g e n, 1822). Synonymy.

Chrysotoxum arcuatum sensu T h o m p s o n, V o c k e r o t h and S p e i g h t (1982) nec (L i n n a e u s, 1758) = *Chrysotoxum festivum* (L i n n a e u s, 1758). Synonymy.

Chrysotoxum aff. *intermedium* M e i g e n, 1822 = *Chrysotoxum intermedium* M e i g e n, 1822. Misidentification.

Eristalis horticola auct. nee (D e G e e r, 1776) = *Eristalis lineata* (H a r r i s, 1776). Synonymy.

Merodon recurvus S t r o b l, 1898 = *Merodon ruficornis* M e i g e n, 1822. Synonymy.

Metasyphus nuba (W i e d e m a n n, 1830) = *Eupeodes goeldlini* M a z á n e k, L a s k a et B i č i k, 1999. Misidentification.

Microdon latifrons L o e w, 1856 = *Microdon analis* (M a c q u a r t, 1842). Synonymy.

Paragus majoranae R o n d a n i, 1857 = *Paragus pecchiolii* R o n d a n i, 1857. Synonymy.

CONCLUSION

Based on new investigations and current nomenclature changes, the following data of special importance for hoverfly diversity at the Obedska bara Marsh should be stated:

— this is the first published record for *Eupeodes goeldlini* in Serbia;

— nine species have been recorded for the first time at the Obedska bara Marsh: *Brachyopa bicolor, Brachyopa maculipennis, Chrysotoxum intermedium, Eristalis pertinax, Eristalis similis, Eupeodes goeldlini, Pipiza luteibarba, Rhingia rostrata, Scaeva selenitica*;

— seven species have been replaced by recently established junior synonyms: *Cheilosia urbana* (instead *Cheilosia praecox*), *Chrysotoxum festivum* (instead *Chrysotoxum arcuatum*), *Eristalis lineata* (instead *Eristalis horticola*), *Merodon ruficornis* (instead *Merodon recurvus*), *Microdon analis* (instead *Microdon latifrons*), *Paragus pecchiolii* (instead *Paragus majoranae*), *Xanthogramma citrofasciatum* (instead *Xanthogramma festivum*);

— three have been excluded from the faunal list of the Obedska bara Marsh: *Chrysotoxum* aff. *intermedium, Metasyphus nuba, Scaeva pyrastri*;

— currently, the above mentioned results complete the list of 93 hoverfly species registered at the Obedska bara Marsh.

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REFERENCES

C l a u s s e n, C., S p e i g h t, M. (1999): *On the identity of Cheilosia ruralis (Meigen, 1822) (Diptera, Syrphidae) — with a review of its synonymies*. Volucella 4 (1/2): 93—102.

C l a u s s e n, C., T o r p, E. (1980): *Untersuchungen über vier europaische Arten der Gattung Anasimyia Schiner, 1864 (Insecta, Diptera, Syrphidae)*. Mitt. Zool. Mus. Univ. Kiel 1(4): 1—16.

C l a u s s e n, C., G o e l d l i n d e T i e f e n a u, P., L u c a s, J. A. W. (1994): *Zur Identität von Pipizella heringii (Zetterstedt) var. hispanica Strobl, 1909 — mit Typenrevision der paläaktischen Arten der Gattung Heringia Rondani, 1856, sensu stricto (Diptera: Syrphidae)*, Bull. Soc. ent. Suisse, 67: 309—326.

C u r r a n, C. H., F l u k e, C. L. (1926): *Revision of the Nearctic species of Helophilus and allied genera*, Transaction of the Wisconsin Academy of Sciences, Arts and Letters 22: 207—281.

D i r i c k x, H. G. (1994): *Atlas des Diptères syrphides de la région méditerranéenne*, Doc. de Travail, Inst. roy. Sei. nat. Belg., Brüssel, 75: 314 pp.

D o c z k a l, D., S c h m i d, U. (1999): *Revision der mitteleuropäischen Arten der Gattung Microdon Meigen (Diptera, Syrphidae)*, Volucella 4 (1/2): 45—68.

D u š e k, J., L a s k a, P. (1967): *Versuch zum aufbau eines Naturlichen Systems mitteleuropäischer Arten der Unterfamilie Syrphinae (Diptera)*, Acta sc. nat. Brno 1: 349—390.

G a j i ć, M., K a r a d ž i ć, D. (1991): *Flora ravnog Srema sa posebnim osvrtom na Obedsku baru*, Šumarski fakultet Beograd, Šumsko gazdinstvo Sremska Mitrovica, Beograd, Sremska Mitrovica, pp 437.

G i r s c h n e r, E. (1897): *Über die Postalar-Membran (Schüppchen, Squamulae) der Dipteren*, Illustrierte Wochenschrift für Entomologie 2: 534—539, 553—559, 567—571, 586—589, 603—607, 641—645, 666—670.

G l u m a c, S. (1955): *Osolike muve Srbije (Syrphidae, Diptera) iz zbirke Prirodnjačkog muzeja srpske zemlje u Beogradu*, Zaštita bilja 27: 3—43.

H i p p a, H., N i e l s e n, T. R., S t e e n i s, J. (2001): *The West Palaearctic species of the genus Eristalis Latreille (Diptera, Syiphidae)*, Norw. J. Entomol. 48: 289—327.

I l i f f, D. A., C h a n d l e r, P. J. (2000): *Musca arcuata and M. festiva Linnaeus, 1758 (currently Chrysotoxum arcuatum and C. festivum) and M. citrofasciata De Geer, 1776 (currently Xanthogramma citrofasciatum) (Insecta, Diptera): proposed conservation of usage of the specific names by the designation of neotypes for M. arcuata and M. festiva*, Bulletin of Zoological Nomenclature 57 (2): 87—93.

M a z á n e k, L., L á s k a, P., B i č i k, V. (1999): *Two new Palaearctic species of Eupeodes similar to E bucculatus (Diptera, Syrphidae)*, Volucella 4 (1/2): 1—9.

M i l a n k o v, V., V u j i ć, A., L u d o š k i, J. (2001): *Genetic divergence among cryptic taxa of Merodon avidus (Rossi, 1790) (Diptera: Syrphidae)*, Int. J. Dipteral. Res. 12 (1): 15—24.

N i e l s e n, T. R. (1995): *Studies on some northern Eristalis species (Diptera, Syrphidae)*, Dipterological Research 6: 129—133.

N i e l s e n, T. R. (1999): *Check-list and distribution maps of Norwegian Hoverflies, with description of Platycheirus laskai nov. sp. (Diptera, Syrphidae)*, NNA Fagrapport 35: 1—99.

P e c k, L. V. (1988): *Syrphidae*. In: A Soos, L Papp, Eds., Catalogue of Palaearctic Diptera, 8, Akad. Kiado, Budapest, pp 11—230.

R a d e n k o v i ć, S., V u j i ć, A., Š i m i ć, S. (2002): *On the identity and synonymy of two species from Merodon ruficornis group (Diptera: Syrphidae)*, Acta entomologica serbica 7 (1/2): 51—57.

S o m m a g g i o, D. (2001): *The species of the genus Chrysotoxum Meigen, 1822 (Diptera, Syrphidea) described by Giglio Tos*, Boll. Mus. reg. Sci. not. Torino 18 (1): 115—126.

S o m m a g g i o, D. (2002): *Paragus gorgus Vujić & Radenković, 1999: a junior synonym of P. majoranae Rondani, 1857, and reinstatement of P. pecchioli Rondani, 1857 (Diptera, Syrphidae)*, Volucella 6: 53—56.

Speight, M. C. D. (1978): *A check list of Irish Syrphidae (Diptera)*, Bulletin of the Irish Biogeografical Society 2: 26—31.
Speight, M. C. D. (1984): *Liste provisoire des (Syrphides Diptères) de la plaine d'Alsace et des Voges*, Bulletin de la Société entomologique de Mulhouse 57—64.
Speight, M. C. D. (1994): *Rèvision des syrphes de la faune de France: II — Les Microdontidae et les Syrphidae Milesiinae (in part.) (Diptera, Syrphidoidea)*, Bulletin de la Société entomologique de France 99 (2): 181—190.
Speight, M. C. D. (2003): *Species accounts of European Syrphidae (Diptera): special commemorative issue*, Second International Workshop on the Syrphidae, Alicante, June 2003. In: MCD Speight, E Castella, P Obrdlik, S Ball, Eds., *Syrph the Net, the Database of European Syrphidae*, Vol. 38, Syrph the Net publications, Dublin, pp 231.
Ssymank, A., Doczkal, D., Barkemyer, W., Claussen, C., Löhr, P. W., Scholz, A. (1999): *Syrphidae*. In: H Schumann, R Bährmann, A Stark, *Checkliste der Dipteren Deutschlands*, Supplement 2, Studia dipterologica, Halle, pp 195—203.
Šimić, S., Vujić, A. (1990): *Vrste roda Eristalis Latreille, 1804 (Diptera Syrphidae) iz zbirke Instituta za biologiju u Novom Sadu*, Glasnik Prirodnjačkog muzeja u Beogradu 45: 115—126.
Šimić, S., Vujić, A. (1996): *Hoverflies fauna (Diptera: Syrphidae) of the southern part of the mountain Stara planina, Serbia*, Acta entomologica serbica 1(1/2): 21—30.
Thompson, F. C. (1997): *The genus Pleskeola Stackelberg (Diptera: Syrphidae) a junior synonym of Parhelophilus Girschner*, Proc. entomol. Soc. Wash. 99: 628—640.
Thompson, F. C., Vockeroth, J. R., Speight, M. C. D. (1982): *The Linnaen species of flower flies (Diptera: Syrphidae)*, Mem. Entomol. Soc. Wash., 10: 150—165.
Verrall, G. H. (1901): *Platypezidae, Pipunculidae and Syrphidae of Great Britain*. In: GH Verrall, Eds., *British Flies*, 8, London, pp 691.
Vockeroth, J. R. (1986): *Nomenclatural notes on Nearctic Eupeodes (including Metasyrphus) and Dasysyrphus (Diptera: Syrphidae)*, Can. Ent. 118: 199—204.
Vockeroth, J. R., Thompson, F. C. (1987): *Syrphidae*. In: JF McAlpine, Eds., *Manual of Nearctic Diptera*, 2, Agriculture Canada, Ottawa, pp 713—743.
Vujić, A. (1991): *Vrste roda Brachyopa Meigen 1822 (Diptera: Syrphidae) u Jugoslaviji*, Glasnik Prirodnjačkog muzeja u Beogradu 46: 141—150.
Vujić, A., Glumac, S. (1994): *Fauna osolikih muva (Diptera: Syrphidae) Fruške gore*, Monographs of Fruška gora, Matica srpska, Novi Sad, pp 163.
Vujić, A., Šimić, S., Milankov, V., Radović, D., Radišić, P., Radnović, D. (1998): *Fauna Syrphidae (Insecta: Diptera) Obedske bare, značaj i potreba zaštite*, Zavod za zaštitu prirode Srbije, Beograd, pp 71.

НОВИ ПОДАЦИ О ДИВЕРЗИТЕТУ ОСОЛИКИХ МУВА (INSECTA: DIPTERA: SYRPHIDAE) СПЕЦИЈАЛНОГ РЕЗЕРВАТА ПРИРОДЕ ОБЕДСКЕ БАРЕ (РАМСАРСКОГ ПОДРУЧЈА У СРБИЈИ)

Снежана Р. Раденковић, Анте В. Вујић, Смиљка Д. Шимић
Универзитет у Новом Саду, Природно-математички факултет,
Департман за Биологију и екологију, Трг Доситеја Обрадовића 2,
21000 Нови Сад, Србија и Црна Гора

Резиме

На Обедској бари осолике муве представљају добро истражену инсекатску групу захваљујући дугогодишњим истраживањима научног тима са ПМФ-а, Универзитета у Новом Саду, Србија (В у ј и ћ и сар., 1998). Након 15 година, мониторинг биодиверзитета овог Рамсарског подручја се наставио у оквиру пројекта 1770, Министарства за науку и заштиту животне средине, Републике Србије. На бази нових истраживања и номенклатурних промена добијени су следећи резултати: налаз врсте *Eupeodes goeldlini* представља први податак за Србију, девет врста је регистровано по први пут за Обедску бару, за седам врста су наведени недавно успостављени синоними, а три врсте су искључене са листе фауне осоликих мува Обедске баре. На основу ових података, новоустановљена листа броји 93 врсте сирфида за Обедску бару.

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Vesna R. Milankov, Jasmina Lj. Ludoški, Ante A. Vujić

Department of Biology and Ecology, University of Novi Sad,
Trg Dositeja Obradovica 2, 21000 Novi Sad, Serbia and Montenegro

GENETIC DIFFERENTIATION BETWEEN CONSPECIFIC POPULATIONS OF *MERODON AVIDUS* A (*DIPTERA*, *SYRPHIDAE*)

ABSTRACT: Allozyme variability of populations of *Merodon avidus* A (Milankov et al., 2001) from Dubašnica Mountain (Serbia), Morinj Bay (Montenegro) and Pindos Mountain (Greece) was analysed. The influence of gene flow on genetic differentiation among populations from the three biogeographical regions was also investigated. Genetic differentiaiton quantified by the F_{st} value, which is an inverse function of gene flow between populations, seemed to be correlated to both geographic and genetic distance (*D*, Nei, 1978), Namely, in the population pairs Morinj — Dubašnica (253 km air distance), Morinj — Pindos (390km air distance) genetic differentiation and genetic distance increased with the geographic distance (F_{st} = 0.133, D = 0.022 and F_{st} = 0.309, D = 0.052, respectively). The exception was the population pair Dubašnica — Pindos (500 km air distance), where a lower degree of genetic differentiation was observed (F_{st} = 0.266; D = 0.047) than was expected based solely on the geographic distance. Results of this study suggest that that genetic differentiation among conspecific populations depends not only on the number of migrants (i. e. gene flow), but also on different selection pressure in different habits.

KEY WORD: Allozyme, Evolutionary mechanisms, Genetic differentiation, *Merodon avidus* A, *Syrphidae*

INTRODUCTION

The genus *Merodon* Meigen, 1803 of the family *Syrphidae*, subfamily *Milesiine*, tribe *Eumerini*, is the third-largest European genus. It comprises about 55 species that occur throughout the continent (except its most northerly parts), but predominate in the southern and Mediterranean areas (Hurkmans, 1993; Dirickx, 1994). On the Balkan Peninsula more than 50 *Merodon* species have been found which represents approximately 10% of hoverflies diversity in this area.

The group *avidus* of the genus *Merodon* has been defined based on the apomorphic characters of the abdomen and anterior lobe of surstylus (Hurkmans, 1993), but there is no identification key that includes all known Euro-

pean taxa from this group, with females being particularly difficult to separate (S p e i g h t, 2003). Ever since its first description, *M. avidus* has been the subject of taxonomic debates due to its great variaiton of the coloration anennae, thorax, legs and abdomen (H u r k m a n s, 1993). This resulted in 24 known synonyms for the *M. avidus* species today (H u r k m a n s, 1993).

A recent study of allozyme variability in populations of the *M. avidus* taxon identified two cryptic taxa, *Merodon avidus* A and *Merodon avidus* B (M i l a n k o v et al., 2001). Based on the diagnostic species-specific alleles at *Idh-2* and *Aat* loci, a Mediterranean species *M. avidus* A was delineated from the mountaine taxon *M. avidus* B. In addition, results of morphological analysis of tergite II and III, tibiae, and mesoscutum confirmed the existence of at least of two taxa. *M. avidus* A was also registered at several localities in the central part of the Balkan Peninsula with Mediterranean faunal elements.

Specimens of *M. avidus* have been recorded throughout Europe: from southern Sweeden in the North, to the Mediterannean and North Africa in the South, and from Spain in the West, to Turkey and European parts of Russia and in Asia Minor in the East (S p e i g h t, 2003). As the wide distribution of *M. avidus* and varying environmental conditions in different habitats likely contributed to this species' variability, the goal of this paper was twofold: to analyze genetic differentiation of populations of *M. avidus* A using allozymes, and to study evolutionary mechanisms which influenced the genetic divergence of geographically distant populations. Populations of *M. avidus* from three different biogeographical regions (based on M a t v e j e v and P u n c e r, 1989) were chosen: Biome of evergreen Mediterranean maritime woodlands and maquis (Morinj Bay on the Adriatic coast), Biome of South European mostly deciduous woodlands (Dubašnica Mountain), and Biome of stony grounds, pastures and woods on stony grounds of (oro)mediterranean mountains (Pindos Mountain).

MATERIAL AND METHODS

Sample collection

Specimens *Merodon avidus* A were collected from Dubašnica Mountain, Serbia (AADUB; 14 specimens), Morinj, Montenegro (AAMOR; 30) and Pindos Mountain, Greece (AAPIN; 9) (Fig. 1).

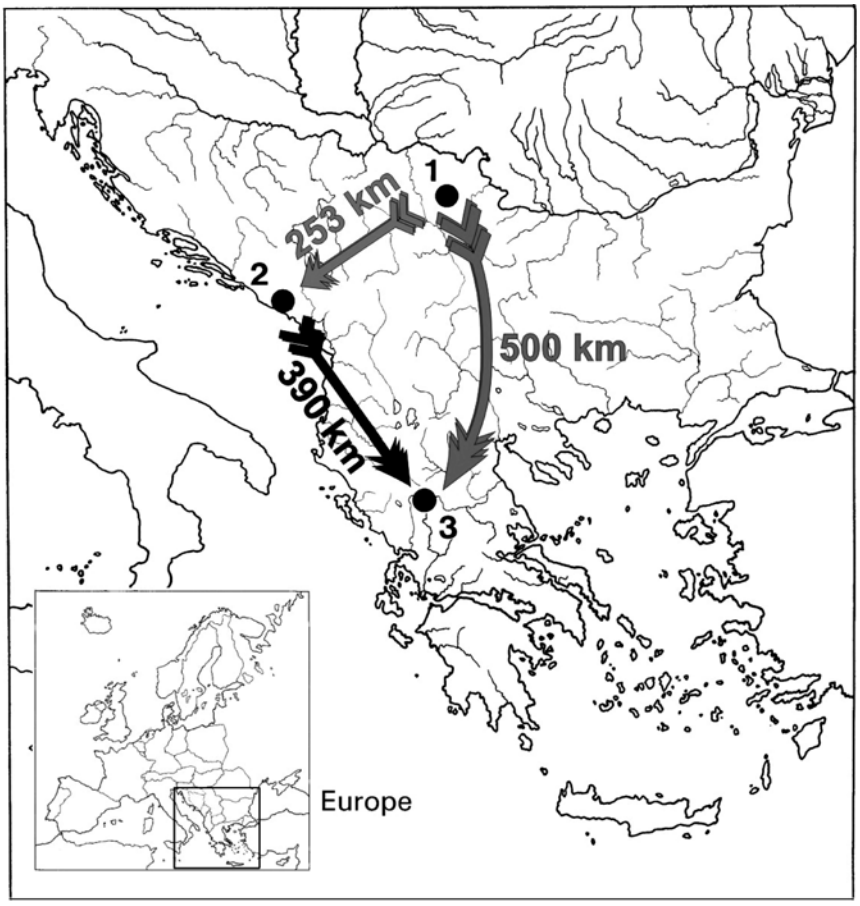

Figure 1. Map of the Balkan Peninsula. Origin of the analysed populations: 1. Dubašnica Mountain, Serbia (E 21°59', N 44°01'); 2. Morinj bay, Montenegro (E 18°40', N 43°29'30''); Pindos Mountain, Greece (E 20°37', N 39°14')

Allozyme analysis

Allozyme variability of aldehyde oxidase, aspartate amino transferases, β-hydroxy acid dehydrogenase, fumarate hydratase, α-glycerophosphate dehydrogenase, glucosephosphate isomerase, hexokinase, isocitrate dehydrogenase, malate dehydrogenase, malic enzyme, phosphoglucomutase and superoxide dismutase (Tab. 1) was studied using the method of polyacrylamide gel electrophoresis (PAGE) according to Munstermann (1979) and Pasteur et al. (1988) with slight modifications (Milankov, 2001).

Table 1. Buffer systems, enzymes (EC— number and name) and loci

Buffer system	EC* Number	Enzyme	Locus
TC**	2.6.1.1	aspartat amino transpherase (AAT)	*Aat*
TBE***	1.2.3.1	aldehide oxidase (AO)	*Ao*
TBE	4.2.1.2	fumarate hydratase (FUM)	*Fum*
TC	1.1.1.8	α-glycerophosphate dehydrogenase (GPD)	*Gpd-2*
TBE	5.3.1.9	glucose phosphate isomerase (GPI)	*Gpi*
TBE	2.7.1.1	hexokinase (HK)	*Hk-2* *Hk-3*
TC	3.1.1.31	β-hidroksiacide dehydrogenase (HAD)	*Had*
TC	1.1.1.42	isocitrate dehydrogenase (IDH)	*Idh-2*
TBE	1.1.1.40	malic enzyme (ME)	*Me*
TC	1.1.1.37	malate dehydrogenase (MDH)	*Mdh-1* *Mdh-2*
TBE	2.7.5.1	phosphoglucomutase (PGM)	*Pgm*
TBE	1.15.1.1	superoxide dismutase (SOD)	*Sod-1* *Sod-2* *Sod-3*

* EC number — Enzyme Commision
** TC buffer — 1M Tris-citric buffer pH = 7.1
*** TBE buffer — 1M Tris-boric-EDTA pH = 8.9

Analysis

Statistical analysis of electrophoretic variability data was performed using the BIOSYS-2 computer program (Swofford and Selander, 1989). The influence of gene flow on genetic divergence of populations was inferred from values of pairwise F_{st} from the formula of Wright (1951): $F_{st} = 1/(1 + 4 N_e m)$, where $N_e m$ being the measure of gene flow between populations (Slatkin and Barton 1989). The relation between genetic differentiation, measured by allelic variance of the particular population or Wright's F_{st} value, and geographic distance (air distance between localities in kilometers; Fig. 1), as well as F_{st} value and Nei's genetic distance (*D*; Nei, 1978) have been analysed as well. The resence of rare alleles (frequency < 0.1) was used tom evaluate interpopulation divergence.

RESULTS

Out of 16 analysed izozymic loci, six loci were monomorphic (with a common allele) in all populations: *Ao, Gpd-2, Gpi, Had, Mdh-2, Me*. Based on the geographic distribution of the allelic frequency, unique, rare and major alleles, spatial variation of the *M. avidus* A has been studied (Milankov et al., 2001). With the exception of the *Gpi* locus, same major alleles (frequency

> 0.5) were found at all other analysed loci. Rare alleles *Gpd-2*^{i} and *Had*^{j} were detected only in the heterozygous combinations with common alleles. Unique alleles, specific alleles, present only in one population, were recorded at four loci: *Gpd-2, Gpi, Mdh-2* and *Me* (Fig. 2).

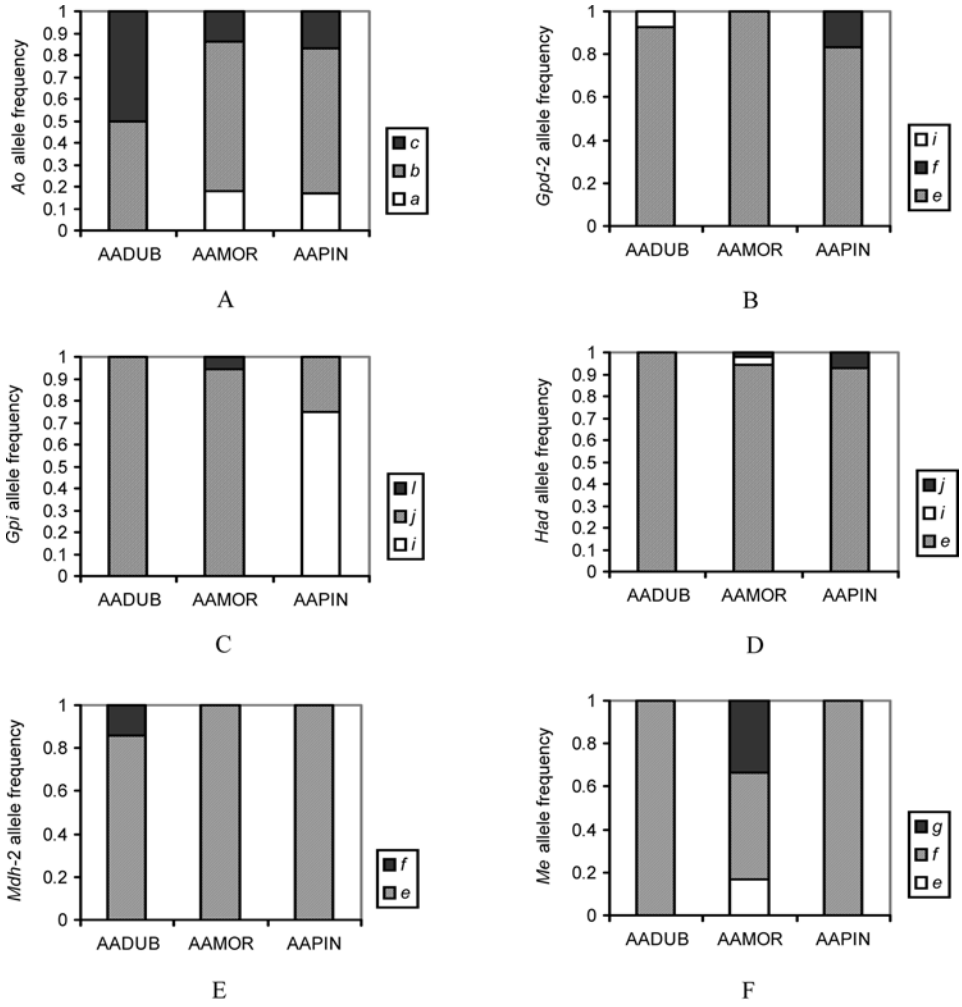

Figure 2. Distribution of the allelic frequencies at *Ao* (A), *Gpd-2* (B), *Gpi* (C), *Had* (D), *Mdh-2* (E) and *Me* (F) loci in AADUB, AAMOR and AAPIN populations of the *Merodon avidus* A species

Difference between all genotypic classes based on Hardy-Weinberg values was statistically significant for all variable loci in all populations. The genotypic fixation index, F_{is}, indicated excess homozygosity ($F_{is} > 0$) in all populations at each variable loci except *Gpd*-2 in the AADUB population and *Had* in the AAPIN population. These results were in accordance with Selan-

der's *D* — coefficient, since *D* was 0 for *Gpd-2* in AADUB and *Had* in AAPIN (M i l a n k o v et al., 2001; Tab. 2).

Table 2. Deviation of genotipic frequencies at 6 variable loci from Hardy-Weinberg equilibrium of *Merodon avidus* A

Locus	Population	H_o	H_e	F_{is}	*D*	χ^2	*P*
Ao	AADUB	0	3.273	1.000	–1.000	7.14	—
	AAMOR	0	10.884	1.000	–1.000	40.69	—
	AAPIN	0	3.273	1.000	–1.000	12.85	—
Gpd-2	AADUB	1	1.000	–0.077	0.000	0.00	ns.
	AAPIN	0	1.818	1.000	–1.000	7.14	—
Gpi	AAMOR	0	1.943	1.000	–1.000	19.04	—
	AAPIN	0	1.714	1.000	–1.000	5.22	—
Had	AAMOR	1	2.922	0.651	–0.658	17.83	***
	AAPIN	1	1.000	–0.077	0.000	0.00	ns.
Mdh-2	AADUB	0	1.846	1.000	–1.000	8.12	—
Me	AAMOR	0	14.979	1.000	–1.000	45.21	—

H_o = Observed heterozygosity; H_e = Expected heterozygosity over all loci; F_{is} = Fixation index (W r i g h t, 1951); *D* = Selander's coefficient; *P* = Level of significance (*** = significant at $P \leq 0.001$)

The degree of genetic differentiation among conspecific populations was quantified using parameters of the *F*-statistics (W r i g h t, 1951), that partition genetic variaiton of all hierarchical levels — from individual level, through population to the whole species. Although representing only a part of the total variability, F_{st} as a measure of genetic differentiation between populations of *M. avidus* A was the most important parameters in explaning the distribution of genetic variation (Tab. 3).

Table 3. χ^2 differences of the allelic frequency at variable loci between populations of *Merodon avidus* A

Locus	No. of alleles	χ^2	*d.f.*	*P*	F_{st}
Ao	3	8.812	4	ns.	0.000
Gpd-2	3	13.955	4	**	0.104
Gpi	3	39.522	4	***	0.639
Had	3	2.750	4	ns.	0.000
Mdh-2	2	4.779	2	ns.	0.022
Me	3	24.034	4	***	0.255
Total		93.853	22	***	0.223

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

Genetic differentiation among conspecific populations of *M. avidus* A was mainly caused by statistically significant difference in the allele frequency

at *Gpi* (F_{ST} = 0.639), followed *Me* (F_{ST} = 0.255) and *Gpd-2* (F_{ST} = 0.104) loci (Tab 3).

Significant difference in allele frequencies at *Gpi* had the strongest influence on genetic differentiation of populations originated from localities Dubašnica — Pindos and Morinj — Pindos, followed by the difference in allele frequency at *Gpd-2* (MOR-PIN) and *Me* (DUB-MOR and MOR-PIN) (Tabs. 4—6).

Table 4. χ^2 differences of the allelic frequency at variable loci between AADUB and AAMOR populations of *Merodon avidus* A

Locus	No. of alleles	χ^2	*d.f.*	*P*	F_{st}
Ao	3	8.485	2	*	0.077
Gpd-2	2	3.198	1	ns.	0.098
Gpi	2	0.581	1	ns.	0.000
Had	3	0.846	2	ns.	0.000
Mdh-2	2	3.619	1	ns.	0.082
Me	3	14.143	2	***	0.251
Total		30.872	9	***	0.133

d.f. — Degree of freedom; *P* — Level of significance (ns. = not significant; * = significant at $P \leq 0.05$; *** = $P \leq 0.001$)

Table 5. χ^2 differences of the allelic frequency at variable loci between AADUB and AAPIN of *Merodon avidus* A

Locus	No. of alleles	χ^2	*d.f.*	*P*	F_{st}
Ao	3	4.286	2	ns.	0.000
Gpd-2	3	3.257	2	ns.	0.000
Gpi	2	11.250	1	***	0.706
Had	2	1.037	1	ns.	0.000
Mdh-2	2	1.257	1	ns.	0.000
Total		21.087	7	**	0.266

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

Table 6. χ^2 differences of the allelic frequency at variable loci between AAMOR and AAPIN of *Merodon avidus* A

Locus	No. of alleles	χ^2	*d.f.*	*P*	F_{st}
Ao	3	0.076	2	ns.	0.000
Gpd-2	2	7.605	1	**	0.247
Gpi	3	31.302	2	***	0.746
Had	3	1.532	2	ns.	0.052
Me	3	12.800	2	**	0.239
Total		53.316	9	***	0.309

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

Genetic differentiation, quantified by the F_{st} values, was positive correlated to geographic distance and genetic distance for populations pairs Dubašnica-Morinj (253 km; F_{st} = 0.133; D = 0.022) and Morinj-Pindos (390 km; F_{st} = 0.309; D = 0.052). Not following this trend was the population pair Dubašnica — Pindos (500 km). The degree of genetic divergence between AADUB and AAPIN populations was lower (F_{st} = 0.266; D = 0.047) than expected based on the geographic distance (Figs. 3, 4). Additionally, the number of migrants

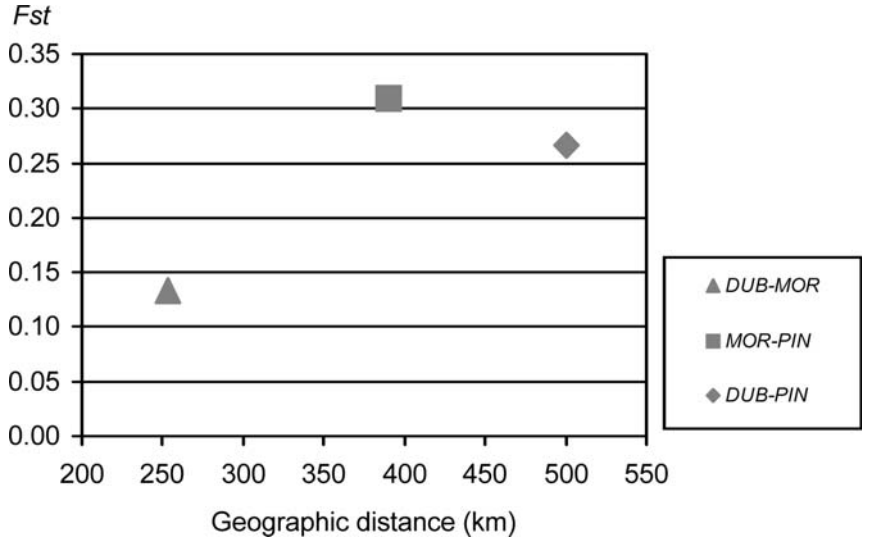

Figure 3. Correlations between F_{st} values and geographic distance of conspecific populations of *Merodon avidus* A

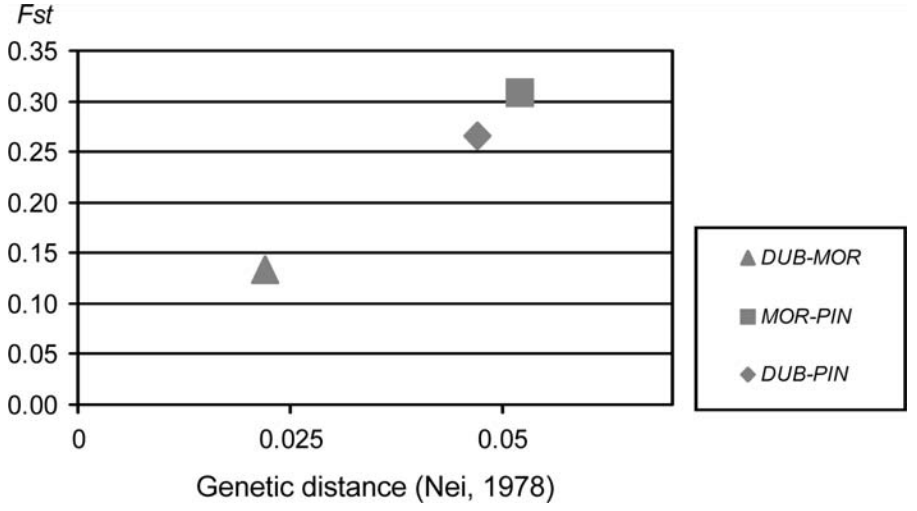

Figure 4. Correlations between F_{st} values and genetic distances (N e i, 1978) of *Merodon avidus* A

between more geographically distant populations (AAMOR-AAPIN and AADUB--AAPIN) was lower than the number registered for AAMOR-AADUB. F_{st} values indicated that the rate of migration (Nm) was approximately 1 individue per 2 generations in AAMOR-AAPIN population pairs and AADUB-AAPIN population pairs (0.559, 0.690, respectively), and almost 2 individuals per generation (Nm = 1.630) in AAMOR-AADUB pair.

DISCUSSION

Geographic structure of the species is one of the basic components of the ecological and evolutionary studies, which contains demographic and genetic structure. A complex population structure, including set of populations, clinal and peripheral populations and zone integration, is a common trait of the hierarchical level of the species. Geographic variability of the species is a general occurance and inevitable result of the habitat spatial discontinuity, geographic variation of the available habitat and effect of the evolutionary mechanisms. Different evolutionary processes such as gene flow, natural selection, mutation, genetic drift and historical effects may cause differentiation of the genotypes, differences between allelic frequencies and unique alleles in the spatially divergent subpopulations (R o d e r i c k, 1996).

In the study of the importance of a particular evolutionary mechanism in genetic divergence among conspecific populations, from the family *Syrphidae*, temporal allozyme variability was also taken into account (L u d o š k i et al., 2002; M i l a n k o v et al., 2002; S t a m e n k o v i ć et al., in press). Recent studies of the geographic distribution of variability showed that genetic changes during independent evolution of the conspecific populations of the *Cheilosia vernalis* species was mainly a result of differential selection in various parts of the population habitat, and partially a conseqence of the population substructuring, while gene flow, historical effects and genetic drift probably had a minor influence (M i l a n k o v et al., unpublished). Contrary to the discontinued spatial variation of the *C. vernalis* species, clinal continual variability of the *Merodon avidus* B species has been observed (M i l a n k o v et al., 2001).

A high value of the local inbreeding (F_{is}; W r i g h t, 1951) and deficit of heterozygotes in populations of *M. avidus* A, suggest the effect of the genetic drift and limited rate of migration. A direct consequence of the population substructuring are Wahlund's effect (W a h l u n d, 1928) and local inbreeding, resulting in excess of homozygosity, high value of the Index fixation (F_{is}) and a presence of rare alleles. The highest number of rare alleles were registered in the AAMOR population (*Gpi*^{l}, Had^{i}, Had^{j}), while in AADUB and AAPIN only one rare allele (*Gpd*-2^{i} and *Had*^{j}, respectively) was identified.

Considering that the effect of genetic drift, historical effect and gene flow are almost equal at all loci, which depend on biology of a particular species, geographic distance between populations, barriers, similar habitats of subpopulations (J o h a n n e s s o n and T a t a r e n k o v, 1997), it could be expected that F_{st} would be a similar in all polymorphic loci. However, results in this

study indicate that genetic drift and gene flow have not been a major factor in the observed distinct genetic divergence between conspecific populations. Additionally, if these mechanisms had been decisive factor of the allelic frequencies, the variance of the polymorphic loci would have been equal. In other words, in that case, genetic changes during independent evolution of the analysed population would be uniform and the same rate of changes in all loci. As opposed to this, genetic divergence between conspecific populations, measured by F_{st} parameter, ranged from 0.133 (between AADUB and AAMOR), 0.266 (AADUB and AAPIN) to 0.309 (AAMOR and AAPIN) and was caused by differences of the allelic frequencies at *Ao* (AADUB-AAMOR), *Me* (AADUB--AAMOR; AAMOR-AAPIN), *Gpi* (AADUB-AAPIN; AAMOR-AAPIN) and *Gpd*-2 (AAMOR-AAPIN). Differences of the allelic frequencies at *Had* and *Mdh*-2 were not significant for the divergence of all pairs of conspecific populations. Loci that had the strongest influence on differentiation were *Gpd*-2, *Gpi* and Ao. Based on the results herein it could be hypothesized that genetic changes during independent evolution of conspecific populations of *M. avidus* A have not been equal and uniform. Except the *Had* and *Mdh*-2 (no registers differences in allelic frequencies), and *Me* (similar standard variance among all analysed conspecific populations) loci, at all other analysed loci similar value of the genetic changes were not observed. So, alleles of those loci possessed different fitnesses in different habitats. Also, occurrence of the locality to different biogeographical areas (L o p a t i n and M a t v e j e v, 1995) indicate the influence of the natural selection to the divergence between conspecific populations.

Furthermore, absence of strong relationships between geographic distance and values of the standard variance (F_{st}) as well as between gene flow, measuring by Nm coefficient (W r i g h t, 1951), and geographic distance show that historical effect and reduce of gene flow have not been important mechanism responsible for the observed genetic divergence. Thus, genetic differentiation among the populations from localities with similar history, such as 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) (L o p a t i n and M a t v e j e v, 1995) was smaller than expected ones, based on their geographic distance. Also, distribution of the genetic variability of the *M. avidus* A taxon is likely to be a results of the biotic factors. In that habitats and during a simultaneous period of adult activities the mountainous population of the *M. avidus* B cryptic taxon has been registered (M i l a n k o v et al., 2001). Moreover, characteristic of AAMOR population are: it is a typical Mediterranean population from a typical Mediterannean habitat, without a contact with the mountaine population. Also, a high variability is a specific for the AAMOR population. Namely, the analysis of the morphological traits of a large population from Morinj showed the great variability which is correlated with the allozyme variation, and some specimens were very close to the montane „phenotype" (M i l a n k o v et al., 2001). In all probability, the importance of the population structuring to the genetic differentiation pointed to registered differences among spring — early summer generation (V—VI) and late summer generation (VII—IX), so spring

Mediterranean samples and some specimens in Mediterranean population were almost inseparable from slightly variable montane specimens (Milankov et al., 2001). Likewise, higher degree of the differences between sympatric populations of the Mediterranean *M. avidus* A and mountane *M. avidus* B taxa from Dubašnica Mountain, as well as great degree of variability of the alopatric population of *M. avidus* A from Morinj might be a result both a character displacement and a presence of the cryptic taxon, especially belonging to the great variable mediterranean taxon, *M. avidus* A.

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REFERENCES

Dirickx, H. (1994): *Atlas des Diptees syrphides de la region mediterraneenne*, Studiedocumenten van het Koninklijk Belgish Instituut voor Natuurweten, 75, Brussel, 317.

Hurkmans, W. (1993): *A monograph of Merodon (Diptera: Syrphidae)*, Part 1. Tijdschrift voor Entomologie 136: 147—234.

Johannesson, K., Tatarenkov, A. (1997): *Allozyme variation in a snail (Littorina saxatilis) — deconfounding the effects of microhabitat and gene flow*, Evolution 51(2): 402—409.

Lopatin, I. K., Matvejev, S. D. (1995): *Zoogeography, principles of biogeography and ecology of the Balkan Peninsula*, Univerzitetski udžbenik, Ljubljana, 166. (in Serbian).

Ludoški, J., Milankov, V., Vujić, A. (2002): *Genetic diversity and differentiation between montane populations of Cheilosia urbana (Diptera: Syrphidae)*, An International Journal of Dipterological Research 13 (2): 135—140.

Matvejev, S. D., Puncer, I. J. (1989): *Karta bioma. Predeli Jugoslavije i njihova zaštita*, Prirodnjački muzej, posebna izdanja 36, Belgrade, 76 (in Serbian with English abstract).

Milankov, V. (2001): *Evolutionary relationships of the ruficornis and aeneus groups of species of the genus Merodon Meigein, 1803 (Diptera: Syrphidae)*, Ph. D. Thesis. University of Novi Sad, Novi Sad. (in Serbian with English abstract).

Milankov, V., Stamenković, J., Vujić, A., Šimić, S. (2002): *Geographic variability of Cheilosia vernalis (Fallen, 1817) (Diptera: Syrphidae)*, Acta Zoologica Academiae Scientarum Hungaricae 48 (4): 255—267.

Milankov, V., Vujić, A., Ludoški, J. (2001): *Genetic divergence among cryptic taxa of Merodon avidus (Rossi, 1790) (Diptera: Syrphidae)*, An International Journal of Dipterological Research 12 (1): 15—24.

Munstermann, L. E. (1979): *Isozymes of Aedes aegypti: Phenotypes, linkage, and use of genetic analysis of sympatric population in East Africa*, Ph. D. Thesis. University of Notre Dame, Notre Dame.

Nei, M. (1978): *Estimation of average heterozygosity and genetic distance from a small number of individuals*, Genetics 89: 583—590.

Pasteur, N., Pasteur, G., Bonhomme, F., Catalan, J., Britton-Davidian, J. (1988): *Practical isozyme genetics*, Chichester (Ellis Horwood Limited), 205.

Roderick, G. K. (1996): *Geographic structure of insect populations: gene flow, phylogeography, ad their uses*, Annual Review of Entomology 41: 325—352.

Slatkin, M., Barton, H. (1989): *A comparison of three indirect methods for estimating average levels of gene flow*, Evolution 43: 1349—1368.

Speight, M. C. D. (2003): *Species accounts of European Syrphidae (Diptera)*, 2002. In: M. C. D. Speight, E. Castella, P. Obrdlik, S. Ball, Eds., *Syrph the Net, the database of European Syrphidae*, Vol 37, Syrph the Net publications, Dublin, 1—213.

Stamenković, J., Ludoški, J., Milankov, V., Vujić, A. (In press): *Genetic differentiation between two populations of Cheilosia himantopus (Panzer, 1798) (Diptera: Syrphidae)*, An International Journal of Dipterological Research.

Swofford, D. L., Selander, R. B. (1989): *BIOSYS-2: a computer program for the analysis of allelic variation in population genetics and biochemical systematic*, Champain, Illinois Natural History Service, Illinois.

Wahlund, S. (1928): *The combination of populations and the appearance of correlation examined from the standpoint of the study of heredity*, Hereditas 11: 65—106. (in German)

Wright, S. (1951): *The genetical structure of populations*, Annals of Eugenics 15: 323—354.

ГЕНЕТИЧКА ДИФЕРЕНЦИЈАЦИЈА ИЗМЕЂУ КОНСПЕЦИФИЧНИХ ПОПУЛАЦИЈА *Merodon avidus* A *(Diptera, Syrphidae)*

Весна Р. Миланков, Јасмина Љ. Лудошки, Анте А. Вујић
Департман за биологију и екологију, Универзитет у Новом Саду,
Трг Доситеја Обрадовића 2, 21000 Нови Сад, Србија и Црна Гора

Резиме

У раду је анализиран утицај протока гена на дистрибуцију фреквенције алела у популацијама врсте *Merodon avidus* A (Миланков и сар., 2001) пореклом са локалитета Дубашница планине (Србија), Мориньског залива (Црна гора) и планине Пиндос (Грчка). У раду је утврђено да је генетичка диференцијација, квантификована F_{st} параметром, а која кореспондира са вредностима Nm, у корелацији са географском дистанцом: Морињ — Дубашница (253 km; F_{st} = 0,133), Морињ — Пиндос (390 km; F_{st} = 0,309) и вредностима генетичке удаљености (D; Nei, 1978) која је за популације првог наведеног пара локалитета износила 0,022, а другог 0,052. Једино одступање је забележено за популације са локалитета Дубашница — Пиндос које су, иако међусобно удаљене 500 km ваздушне линије, у мањем степену генетички диференциране (F_{st} = 0,266; D = 0,047) у односу на очекиване вредности. Резултати указују да на генетичку дивергенцију конспецифичких популација утичу не само број миграната (= проток гена), него и различити селекциони притисци присутни у различитим окружењима.

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Vesna R. Milankov, Jasmina Lj. Ludoški, Ante A. Vujić

Department of Biology and Ecology, University of Novi Sad,
Trg Dositeja Obradovica 2, 21000 Novi Sad, Serbia and Montenegro

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 a 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" (L e w o n t i n, 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 nonrandom association, named as gametic disequilibrium or linkage disequilibrium (L e w o n t i n and K o j i m a, 1960), which is a short sentence of the „gametic phase disequilibrium" (C r o w and K i m u r a, 1970). Since that, measure of the deviation from random association between alleles is *D* (deviation) or gametic disequilibrium (L e w o n t i n and K o j i m a, 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 (L e w o n t i n, 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 (M i l a n k o v 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 (M i l a n k o v et al., in press). Population-genetic analyse was showed that gametic disequilibrium exist in almost all analysed loci (M i l a n k o v 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 M a t v e j e v and P u n c e r, 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), glucosephosphate 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

Loci compared	No. of comparison	Common correlation	χ^2	*d.f.*	*P*
		AADUB			
Ao : Mdh-2	4	0.999	28.44	1	***
		AAMOR			
Ao : Had	18	0.137	2.37	2	ns.
Ao : Me	16	0.258	19.39	4	***
Gpi : Had	18	0.070	0.43	2	ns.
Gpi : Me	18	0.203	5.94	2	ns.
Had : Me	24	0.148	8.81	4	ns.
		AAPIN			
Ao : Gpd-2	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	*d.f.*	*P*
Ao : Gpd-2	25	0.272	18.08	2	***
Ao : Gpi	19	0.207	5.81	2	ns.
Ao : Had	25	0.120	2.54	2	ns.
Ao : Mdh-2	14	0.393	18.32	2	***
Ao : Me	23	0.195	17.15	4	**
Gpd-2 : Had	34	0.039	0.31	4	ns.
Gpd-2 : Me	33	0.115	3.82	4	ns.
Gpi : Had	26	0.661	0.90	4	ns.
Gpi : Me	28	0.187	21.02	4	***
Had : Me	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 (O h t a, 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)*
Ao : Gpd-2	0.082	0.472	0.186	0.014	0.486
Ao : Gpi	0.000	0.756	0.466	0.018	0.774
Ao : Had	0.003	0.417	0.234	0.003	0.420
Ao : Mdh-2	0.161	0.194	0.044	0.046	0.240
Ao : Me	0.075	0.572	0.255	0.051	0.623
Gpd-2 : Had	0.000	0.034	0.047	0.000	0.034
Gpd-2 : Me	0.000	0.439	0.087	0.003	0.441
Gpi : Had	0.000	0.326	0.281	0.000	0.327
Gpi : Me	0.007	0.779	0.289	0.022	0.801
Had : Me	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 (W a d e and G o o d n i g h t, 1998). So, as the opposite to the Fisher's theory (F i s h e r, 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, W r i g h t (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 fundament as well as an epistasis and pleiotropy are essential in the genetic basic of the evolutionary change (L e w o n t i n, 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 method in the study of coadapted gene complex, a sets 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, analyse of the allelic association of polymorphic loci in spatial 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 explain due to the existing of many factors which influenced on its origins and maintenance. An insufficient available information about a genetic system in hoverflies are existed as well. So, until now, just only for a few species a number of its chromosomes have been known (B o y e s et al., 1972, 1973; R o ž e k et al., 1996), while the other essential information, such as linkage map and recombination rate are totally unknown. Linkage disequilibrium dates in populations of *Cheilosia vernalis* are only available. In the spatial 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 (M i l a n k o v 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 enzyme MDH, ME and GPD (G i l l e s p i e and K o j i m a, 1968) are not in the tightly relation with AO, which is have 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 been explained by the function of encoded allozymes.

An integral part in the debate of nonrandom association of alleles at allozymic loci is analysis a linkage between map distance and linkage disequilibrium. It is known that *D* (coefficient of deviation) is a 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 contrary to the study of the population of *C. vernalis*, in which the linkage between a certain genotypes and males as well as between genotypes and genotypic nonrandom association were registered (M i l a n k o v 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 (H e d r i c k 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 (W a d e and G o o d n i g h t, 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 (M i l a n k o v 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 (M i l a n k o v et al., 2002; M i l a n k o v 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 (M i l a n k o v et al., 2001; M i l a n k o v 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 (O h t a, 1982), with one exception, was $D_{is}^2 < D_{st}^2$; $D'^2_{is} > D'^2_{st}$ 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 (M i l a n k o v 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 (W r i g h t, 1931; W a d e and G o o d n i g h t, 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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REFERENCES

Boyes, J. W., Boyes, B. C., Van Brink, J. M., Vockeroth, J. R. (1973): *Cytotaxonomy of south American Syrphinae (Diptera: Syrphidae)*, Genetica 44: 368—415.

Boyes, J. W., Brink, J. (1972): *Chromosomes of Syrphidae. VI. The tribe Pipizini*, Genetica 43: 321—333.

Crow, J. F., Kimura, M. (1970): *An introduction to population genetics theory*, Harper & Row, New York.

Fisher, R. A. (1958): *The genetical theory of natural selection*, Dover Publications, Toronto.

Gillespie, J. H., Kojima, K. (1968): *The degree of polymorphisms in enzyme involved in energy production compared to that in non-specific enzymes in two Drosophila ananassae populations*, Proceedings of the National Academy of Sciences USA 61: 582.

Hedrick, P. W., Jain, S., Holden, L. (1978): *Multilocus systems in evolution*, Evolutionary Biology 11: 101—182.

Lewontin, R. C. (1974): *The genetic basis of evolutionary change*, Columbia University Press, New York.

Lewontin, R. C., Kojima, K. I. (1960): *The evolutionary dynamics of complex polymorphisms*, Evolution 14: 458—472.

Matvejev, S. D., Puncer, I. J. (1989): *Karta bioma. Predeli Jugoslavije i njihova zaštita*, Prirodnjački muzej, posebna izdanja 36, Belgrade, 76 (in Serbian with English abstract).

Milankov, V. (2001): *Evolutionary relationships of the ruficornis and aeneus groups of species of the genus Merodon Meigein, 1803 (Diptera: Syrphidae)*, Ph.D. Thesis. University of Novi Sad, Novi Sad. (in Serbian with English abstract).

Milankov, V., Ludoški, J., Vujić, A. (In press): *Genetic differentiation between conspecific populations of Merodon avidus A (Diptera, Syrphidae)*, Proceedings for Natural Sciences, Matica Srpska, Novi Sad.

Milankov, V., Stamenković, J., Vujić, A., Šimić, S. (2002): *Geographic variation of Cheilosia vernalis (Fallen, 1817) (Diptera: Syrphidae)*, Acta Zoologica Academiae Scientarum Hungaricae 48(4): 255—267.

Milankov, V., Vujić, A., Ludoški, J. (2001): *Genetic divergence among cryptic taxa of Merodon avidus (Rossi, 1790) (Diptera: Syrphidae)*, An International Journal of Dipterological Research 12 (1): 15—24.

Munstermann, L. E. (1979): *Isozymes of Aedes aegypti: Phenotypes, linkage, and use of genetic analysis of sympatric population in East Africa*, Ph. D. Thesis. University of Notre Dame, Notre Dame.
Ohta, T. (1982): *Linkage disequilibrium due to random genetic drift in finite subdivided populations*, Proceedings of the National Academy of Sciences USA 79: 1940—1944.
Pasteur, N., Pasteur, G., Bonhomme, F., Catalan, J., Britton-Davidian, J. (1988): *Practical isozyme genetics*, Chichester, Ellis Horwood Limited.
Rožek, M., Lachovwska, D., Marcos Garcia, M. A. (1996): *The C-banded karyotypes of Metasyrphus corollae Fabr. and Spherophoria scripta (L.) (Diptera: Syrphidae)*, Folia biologica (Krakow) 44: 11—13.
Swofford, D. L., Selander, R. B. (1989): *BIOSYS-2: a computer program for the analysis of allelic variation in population genetics and biochemical systematic*, Champain, Illinois Natural History Service, Illinois.
Wade, M. J., Goodnight, C. J. (1998): *Perspective: The theories of Fisher and Wright in the context of metapopulations: when nature does many small experiments*, Evolution 52(6): 1537—1553.
Wright, S. (1931): *Evolution in Mendelian populations*, Genetics 16: 97—159.
Wright, S. (1978): *Evolution and the genetics of populations*, University Chicago Press, Chicago.

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

Весна Р. Миланков, Јасмина Љ. Лудошки, Анте А. Вујић
Департман за биологију и екологију, Универзитет у Новом Саду,
Трг Доситеја Обрадовића 2, 21000 Нови Сад, Србија и Црна Гора

Резиме

Проучавање генетичке структуре популација врсте *Merodon avidus* A пореклом са планина Дубашница, Србија (AADUB) и Пиндоса, Грчка (AAPIN) и из Морињa, 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).

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Daliborka Đ. Barjaktarov

Natural History Museum, Njegoševa 51,
11000 Beograd, Serbia and Montenegro

ORNITHOLOGYCAL IMPOTRANCE OF GRUŽA ACCUMULATION

ABSTRACT: Damming the middle part of Gruža River, in order to supply drinking and technical water for Kragujevac District caused appearance of Gružanska accumulation. This lake fills the depression of Knićko Polje between the Gledićke Mts and Kotlenik. As the ornithofauna of accumulation lakes, both in this country and abroad, is poorly studied, this paper presents the data on the state of ornithofauna of Gružansko Lake. The lake has an important role in migration of both native and foreign bird populations, but also as a wintering area for certain species of ducks and geese, as it is situated on the assumed Morava—Vardar migration route. During the research, 78 bird species were recorded and for 25 species it was proven that they breed in the area. Most species were recorded in the zone of strong anthropogenic influence, which is understandable as before the building of the dam and the artificial lake the Knićko Polje was dominated by agricultural land, mowed meadows and orchards.

KEY WORDS: birds migration, Gružanska accumulation, ornithofauna.

INTRODUCTION

Gružansko Lake is an artificial lake, which originated after the flooding of a river valley when the hydro-accumulative dam was built on the river Gruža, in order to provide water supply for industry in Kragujevac District (Marković 1980). Besides the developmental-economic significance for this area, Gružanska accumulation represents a new and suitable habitat, both for wetland birds and the other birds that use Eastern European migratory pathways. During the migration, this modified area, situated in the Morava—Vardar migratory pathways, enables the birds to get some rest, recharge their strength and renew the energetic supplies before they continue the migration.

The ornithofauna of accumulative lakes in Serbia (Vasić and Šoti 1979), but also former Yugoslavia (Vasić 1979; Lukač 1983; Mužinić 1976), was quite rarely studied, primarily due to lack of interest in ornithologists for such ecosystems, which are as a rule poor in bird diversity. Therefore, the ornithofauna of divide of river Gruža and the surroundings of Kra-

gujevac was also rarely and sparsely studied (Matvejev 1938; 1950, Marinković 1995; 1997). Therefore, this paper presents the two years of research efforts on collecting the data on state of ornithofauna at Gružansko Lake and its surroundings.

CHARACTERISTICS OF GRUŽANSKO LAKE

Gružansko (also known as Knićko) Lake originated by damming the middle part of the river Gruža, in order to supply the nearby District of Kragujevac with drinking and technical water and for other versatile needs (Pantović and Madžarević, 1999). The lake fills the depression of Knićko Polje and is situated between Gledićke Mts at the east and Kotlenik at the west (Veljović 1967). The building of the dam started in 1979 and it was completely finished in 1985. The lake covers the surface area of 934 ha, representing one of the largest water surfaces in the area of Šumadija. The total length of the lake is about 10 km, while the width varies between 300 and 2800 m. In certain places, depth of the lake is only 1.3 m, while the greatest depth is immediately below the dam — 31 m. Oscillation of water levels within the accumulation is 3—5 m, depending on the season (Milojević, 1994). Besides the atmospheric water and the water from Gruža River, the lake also takes water from Boračka River, which previously used to be the right tributary of Gruža. Most of the accumulation has the character of lowland accumulations, with small depth and shores surrounded with agricultural fields and meadows. Only the small part of the lake near the dam has some characteristics of steep--shore cliffs, overgrown with forest vegetation. The lake is rich in fish.

At the lake itself, four main zones are differentiated: the zone of permanently open water, which includes most of the surface area, zone of flooded water, zone of forested and steep cliffs and the zone of strong anthropogenous influence. The zone of emergent vegetation includes only a smaller area and stretches as a belt, 5—10 m wide along the shore. It is dominated by Reed (*Phragmites communis*) and Sedge (*Scirpus lacuster*). At the north side of the lake, at the places where Gruža and Boračka rivers flow into the lake, there are formations of flooded wetland meadows as a belt up to 100 m in width (Fig. 1). The dominant species in association of wetland meadows, which are underwater for major part of the year, is *Trifolium resupinaum* (Veljović 1967). Somewhat further away, in the shallower coastal belt, there is also a development of submerged macrophytic vegetation composed of: Floating-leaved Pondweed (*Potamogeton natans*), Meadow Bistort (*Polygonum bistorta*), Common Hornwort (*Ceratophyllum demersum*) and Eurasian Water Milfoil (*Myriophyllum spicatum*).

The eastern and the southern shore of the lake are stony and steep, and only in certain spots they are slightly inclined. The shores are overgrown with xero-mesophytic Oak forest from association *Quercion frainetto* and the sparse shrubby vegetation (Stevanović and Stevanović 1995). The western shore of the lake is slightly undulating, covered with orchards, cultivated fields under vegetables (cabbage and potato) and mowed meadows. The main pro-

blem of Gružanska accumulation is the relatively increased process of euthrophization and aging, caused by negative anthropogenous factors, due to intensive use of agrotechnic measurements on the surrounding cultivated land, also the influx of sewage waters from households etc. (Pantović and Madžarević, 1999).

Fig. 1. Flooded meadows near Gružansko Lake nearby the mouth of Gruža

MATERIAL AND METHODS

The main method of data collecting was the „minimal transect" as defined by Matvejev (1976). Besides the faunistic data, this method also provided data on structure of bird community within the studied locality. Presence of certain bird species, whose whole life cycle is connected with water, was determined by census (Campbell and Lack 1985). Besides, the birds were caught in ornithological nets in order to be ringed. The results of this were diagnosing the species in area of Gružansko Lake as well as possibility of follow-up of bird migration.

Data on ornithofauna include only the Gružansko Lake, its shores and the immediate vicinity (wetland meadows, cultivated land, open grassland and forest communities). This paper includes only the spring-summer aspect, while the autumn-winter aspect was not studied.

RESULTS AND DISCUSSION

Area of greatest part of Serbia, including Šumadija, belongs to the valley type of ornithofauna. This type of ornithofauna includes lowlands and river

valleys, modified and artificial areas of water habitats, orchards and vineyards, gradually climbing into hills (M a t v e j e v 1950). In the area of Gružansko Lake and its closest surroundings, elements of all mentioned biomes may be found. In order to study the ornithofauna more easily, we determined several zones and biotopes:

I Zone — Permanent lake water

a) Biotope of open water (limnal) represents the lake surface free of aquatic vegetation. During the year, considering presence or absence of vegetation, this biotope is least susceptible to changes, but is in the same time under the worst influence of strong winds.

b) Biotope of macrophytic vegetation includes a combination of floating and submerged plants: Floating-leaved Pondweed and Meadow Bistort (*Potamogeton natans* and *Polygonum bistorta*), Common Hornwort (*Ceratophium demersum*) and Eurasian Water Milfoil (*Myriophyllum spicatum*).

II Zone — Flooded waters

a) Biotope of emergent vegetation occupies the narrow belt around the lakeshores where the dominant species are Reed (*Phragmites communis*) and Sedge (*Scirpus lacuster*).

b) Biotope of wetland meadows is present around the mouth of Gruža and Boračka rivers. It is composed of hygrophilic vegetation dominated by association *Trifolium resupinati*, strewn with sparsely spaced willow (*Salix* sp.) trees. This is a very suitable biotope for feeding and breeding of birds, so it leads to the greatest diversity of species.

III Zone — Forested and steep shores

This zone includes associations of Oak forest that overgrow the eastern and southern shores of the lake. In certain places, the shores become steep and inaccessible, overgrown with sparse woody vegetation.

IV Zone — zone of strong anthropogenous influence

a) Biotope of orchards includes cultivated areas around the lake, represented by forest remnants, planted orchard trees and sparse shrubby vegetation.

b) Biotope of cultivated farmland is under the strongest anthropogenous influence. It is characterized by the cultivated areas, mostly under vegetables.

c) Biotope of open grassland communities is characterized by grass vegetation, which is mowed in appropriate intervals (mowed meadows). Also present are occasional hedgerows, lone fruit trees and lone trees of forest species remaining after the forest was cleared.

Based on the field studies, Table 1 presents the list of species by zones, their abundance and status on the lake, as well as the protection status in Europe.

Explanation of abbreviations in the table:

I a — biotope of open water;

I b — biotope of macrophytic vegetation;

II a — biotope of emergent vegetation,
II b — biotope of wetland meadows;
III — zone of forested and steep shores;
IV a — biotope of orchards,
IV b — biotope of cultivated farmland,
IV c — biotope of open grassland communities.

Status:
B — *breeding* — species that breed in the area;
P — *passage* — species that pass through the area during migration or wandering;
W — *wintering* — species that spend winter in the area
V — *visitor* — species that are occasionally recorded in the area during the reproductive period, but do NOT breed there (breeding species of other habitats nearby);
Rg — *regular* — regularly recorded (during breeding, migration or during winter);
S — *secure* — species with stable population numbers (neither increasing nor decreasing);
D — *decline* — species with decreasing population numbers;
R — *rare* — species threatened with extinction;
V — *vulnerable* — species with decreasing population numbers AND threatened with extinction;
(P) — Temporary status

Table 1. List of species in area of Gružansko Lake and its surroundings

No.	Species	Presence in the zone	Max. No. of recorded or estimated individuals	Status of threat/protection	
				On the lake	In Europe
1.	*Tachybaptus ruficollis*	I a	15	P	S
2.	*Podiceps cristatus*	I a; I b	100	RgB	S
3.	*Phalacrocorax carbo*	I a; III	70	RgB	S
4.	*Nycticorax nycticorax*	II b	3	V	D
5.	*Ardeola ralloides*	II a, II b	5	V	V
6.	*Egretta garzetta*	II a	3	Rg	S
7.	*Egretta alba*	II a	20	RgB	S
8.	*Ardea cinerea*	II a, II b, III	60	RgB	S
9.	*Ciconia ciconia*	II b	6	RgV	V
10.	*Anas platyrhynchos*	I a, I b	1000	RgB W	S
11.	*Aythya ferina*	I a, I b	30	RgB	S
12.	*Haliaeetus albicilla*	I a	1	RgV	R
13.	*Circus aeruginosus*	II a, II b	3	Rg	S
14.	*Accipiter gentilis*	III	2	Rg	S
15.	*Accipiter nisus*	III	2	Rg	S
16.	*Buteo buteo*	IV b, IV c	6	Rg	S

17.	*Falco tinnunculus*	IV a, IV b	2	RgB	D
18.	*Phasianus colhicus*	IV c	10	RgB	S
19.	*Fulica atra*	I b, II a	50	RgB	S
20.	*Vanellus vanellus*	II b	12	RgB	S (P)
21.	*Philomachus pugnax*	II b (II a)	25	P	S (P)
22.	*Tringa totanus*	II b (II a)	15	P	D
23.	*Tringa ochropus*	II b (II a)	50	P	S
24.	*Tringa glareola*	II b (II a)	15	P	D
25.	*Larus ridibundus*	I a	20	Rg	S
26.	*Chlidonias niger*	I a, I b	20	Rg	D
27.	*Columba palumbus*	III	8	Rg	S
28.	*Sreptopelia decaocto*	IV a, IV b	20	Rg	S (P)
29.	*Streptopelia turtur*	IV a, IV b	8	Rg	D
30.	*Cuculus canorus*	II a, II b	10	Rg	S
31.	*Alcedo atthis*	II a	4	Rg	D
32.	*Upupa epos*	IV a, IV b	6	Rg	S
33.	*Picus viridis*	III	3	Rg	D
34.	*Dendrocopos major*	III	4	Rg	S
35.	*Dendrocopos syriacus*	III	3	Rg	S (P)
36.	*Galerida cristata*	IV c	7	Rg	D (P)
37.	*Lullula arborea*	IV c	4	Rg	V
38.	*Hirundo rustica*	I, II, IV	30	RgV	D
39.	*Delichon urbica*	I, II, IV	25	RgV	S
40.	*Anthus trivialis*	IV b, IV c	4	Rg	S
41.	*Motacilla flava*	II a, II b	10	RgB	S
42.	*Motacilla alba*	II a, II b	8	RgB	S
43.	*Erithacus rubecula*	III	8	RgB	S
44.	*Luscinia megarhynchos*	II b	20	RgB	S
45.	*Turdus merula*	II b, IV a	30	RgB	S
46.	*Acrocephalus palustris*	II a	4	Rg	S
47.	*Acrocephalus arundinaceus*	II a	10	Rg	S (P)
48.	*Hippolais palida*	IV a, IV b	4	P	V (P)
49.	*Sylvia communis*	IV a, IV b	20	RgB	S
50.	*Sylvia borin*	IV a, IV b	16	RgB	S
51.	*Sylvia atricapilla*	IV a, IV b	18	RgB	S
52.	*Philooscopus sibilatrix*	III	4	P	S (P)
53.	*Philloscopus collybita*	III	8	Rg	S (P)
54.	*Muscicapa striata*	III	5	P	D
55.	*Aegithalos caudatus*	IV a, IV b	15	Rg	S
56.	*Parus palustris*	III	5	Rg	S
57.	*Parus caeruleus*	III	15	Rg	S
58.	*Parus major*	III	25	Rg	S
59.	*Sitta europea*	III	5	Rg	S
60.	*Oriolus oriolus*	II b, III	7	Rg	S

61.	*Lanius collurio*	IV a, IV b	15	RgB	D (P)
62.	*Garrulus glandarius*	II b, III	10	Rg	S (P)
63.	*Pica pica*	IV a, IV b	20	RgB	S
64.	*Corvus monedula*	IV b, IV c	20	Rg	S (P)
65.	*Corvus frugilegus*	IV b, IV c	35	Rg	S
66.	*Corvus corone cornix*	IV b, IV c	20	Rg	S
67.	*Corvus corax*	III	5	V	S (P)
68.	*Sturnus vulgaris*	IV a, IV b	40	RgB	S
69.	*Passer montanus*	IV b, IV c	20	Rg	S
70.	*Fringilla coelebs*	III	20	RgB	S
71.	*Carduelis chloris*	IV a, IV b	5	Rg	S
72.	*Carduelis carduelis*	IV a, IV b	13	Rg	S(P)
73.	*Coccothraustes coccothraustes*	IV a, IV b	8	Rg	S
74.	*Emberiza citrinella*	IV a, IV b	20	RgB	S (P)
75.	*Emberiza hortulana*	IV b, IV c	11	RgB	V (P)
76.	*Emberiza cirlus*	IV a, IV b	7	RgB	S (P)
77.	*Miliaria calandra*	IV b, IV c	15	RgB	S (P)

Presence of several different habitats in area of Gružansko Lake enables greater bird diversity, both in breeders and migratory species. According to Matvejev (1976), fauna of Gružansko Lake and its surroundings can be considered a Valley type of ornithofauna, the subtype of cultivated fields at foothills. Remembering the presence of species connected with water, this type of ornithofauna is modified in certain sense, and the method of census provided data on their abundance and status.

During the research of spring-summer aspect of Gružansko Lake, altogether 78 bird species were recorded, and 25 of these were determined to be regular breeding species at the lake and its immediate surroundings. Considering the species distribution throughout zones, most species (30) were recorded in the zone of strong anthropogenous influence, that is, in the zone of modified habitats (cultivated fields, orchards, mowed meadows etc.) A somewhat smaller number (25 species) was recorded in the zone of flooded water, while in the zone of forested and steep shore cliffs 20 species were recorded. Only 11 bird species were recorded in the zone of permanent lake water. The reason for such a distribution of species is the fact that flooding of the Gruža River valley and building of the accumulation led to the significant changes in ecosystems of this area. That way, the species that used to breed in meadows and cultivated land just next to Gruža, moved to surrounding areas, and the newly formed lake was occupied by various species of wetland birds. In this way, the ornithofauna of this area was enriched with new species that are not characteristic for Valley type of ornithofauna.

As birds are mobile organisms that in order to satisfy their basic needs do not dwell exclusively in one biotope but move from one to another (Table 1), especially during breeding season, when they find food in one biotope and often breed in another, the distribution of ornithofauna in Gružansko Lake by zones should not be considered a strictly closed but a dynamic and variable

system, dictated by ecological demands by species and environmental conditions.

List and abundance of species presented in Table 1 are not final, as the research did not include the autumn-winter aspect. However, this list might also include Woodcock (*Scolopax rusticola*) and Snipe (*Gallinago gallinago*), who were observed on spring migration in vicinity of Kragujevac by Matvejev (1938). As the small water surfaces in vicinity of Kragujevac are places where during winter (Marinković, 1997) species from order *Anseriformes*, especially ducks (*Anas penelope*, *Anas crecca*, *Anas querquedula*, *Anas platyrhynchos*, *Anas acuta*, *Aythya ferina* and *Aythya nyroca*) may be observed, it is very possible that during migration they also appear on Gružansko Lake. This is supported by the fact that on October 27th, 2001, 1000 individuals of *Anas platyrhynchos* were recorded on the lake, and this species also breeds in this locality.

The ornithological value of artificial water ecosystems is reflected in the fact that they are often situated on the migration pathway of many bird species, and that as such they represent the only water surfaces where the birds may rest, renew their strength and the fatty deposits. During the spring migration and winter, in such localities it is possible to record some very rare bird species, so the importance of artificial lakes considering the species diversity is extraordinary (Vasić and Šoti, 1979; Mužinić, 1986). Besides, the artificial water surfaces are new and certainly interesting habitats, not only during migration but also during the breeding season, as most of the lakes are stocked with fish and represent suitable conditions for feeding of many birds, primarily the ichthyofagous species (Vasić, 1979; Grubač and Grubač, 2002).

Diversity of bird species on an artificial lake may be very great, representing a real richness of ornithofauna for a region. The value of studied wetland locality for many bird species might decrease, on one hand due to huge anthropogenic activity (presence of a large number of angler fishermen and picnickers) and on the other hand due to presence of cultivated surfaces that descend to the very lake, and the surplus of pesticide-laden water directly goes into the lake. This problem is present on almost all artificial accumulations that are not under a strict protection regimen or are insufficiently studied (Lukač, 1983; Mužinić, 1986; Grubač and Grubač, 2002).

Properly used artificial water ecosystems can easily turn into „small centers" of biological diversity, which can be important for preservation of many rare species, not only birds but also amphibians (Paunović et al., 2003). Therefore, it is necessary to better protect the existing artificial accumulations, especially as they are valuable and often the only resting places for migratory birds.

CONCLUSION

As the ornithofauna of Gružansko Lake and its surroundings was not studied so far, during the two years of this research special efforts were made to collect data on state of ornithofauna at this accumulation.

During the research in spring-summer aspect, 78 bird species were recorded, and for 25 species it was determined that they breed in the area. The list of species is not complete, as it should also include the birds appearing on the lake during the autumn migration and winter months.

Most species were recorded in the zone of strong anthropogenous influence, as before the building of the dam and artificial lake, Knićko Polje was dominated by cultivated land, mowed meadows and orchards, so the species from these biotopes probably only moved to nearby localities. The novelty for this area is the species of water habitats, occupying the very surface of the lake.

Direct drainage of pesticides from the surrounding agricultural land, as well as the increased anthropogenic influence at the lake itself and around it, are negatively influencing the species richness, in terms of destroying and degradation of breeding habitats of birds.

As Gružansko Lake is on the assumed Morava—Vardar migratory pathway, it plays an important role in migration of both native and foreign bird populations, as well as the wintering area for certain species of ducks and geese.

Studies of Gružansko Lake are in no means the final research, and must be continued not only during breeding and migration season, but also during the winter aspect.

REFERENCES

Campbell, B., Lack, E. (1985): *A Dictionary of Birds*, T &A D Poyser. London.

Grubač, B., Grubač, S. (2002): *Bogatstvo diverziteta faune ptica centralnog Pomoravlja na primeru jezera i ribnjaka kod Paraćina*, Ciconia 10: 77—92. Novi Sad.

Hagemeijer, E. J. M., Blair, M. J. (1997): *The EBCC Atlas of European Breeding Birds: Their Distribution and Abundance*, T &A D Poyser. London.

Lukač, G. (1983): *Ornitofauna Ormoškog akumulacionog jezera*, Larus 33—35, pp.: 173—194, Zagreb.

Marinković, Đ. (1997): *Pregled ptica iz reda Anseriformes na širem području Kragujevca u Šumadiji*, Ciconia 6: 46—50, Novi Sad.

Marković, J. (1980): *Regionalna geografija SFR Jugoslavije*, pp.: 277—316. Građevinska knjiga. Beograd.

Matvejev, S. D. (1976): *Pregled faune ptica balkanskog poluostrva I deo detlići i ptice pevačice*, Srpska akademija nauka i umetnosti, odeljenje prirodno-matematičkih nauka, knjiga 46, Beograd.

Milojević, M. (19994): *Neka iskustva stečena tokom sedam godina aeracije akumulacije „Gruža"*, Konferencija „Kvalitet voda", Zbornik radova: 111—120. Čačak.

Mužinić, J. (1986): *Ptice močvarice umjetnih jezera u Bedekovčini (Hrvatsko zagorje)*, Larus 36—37: 101—109, Zagreb.

Pantović, N., Madžarević, S. (1999): *Kragujevac u borbi za vodu*, Fond za finansiranje izgradnje vodovodnog sistema „Gruža" — Kragujevac i RO „Vodovod i Kanalizacija".

Paunović, A., Barjaktarov, D., Vasić, V. (2003): *Modifikovani veštački predeli vodenih staništa značajna za očuvanje biodiverziteta vodozemaca i ptica u*

centralnim delovima Srbije (Šumadija), Ekološka istina 2003, Zbornik radova: 94—97. Donji Milanovac.

S t e v a n o v i ć, V., S t e v a n o v i ć, B. (1995): *Osnovni klimatski, geološki i pedološki činioci biodiverziteta kopnenih ekosistema Jugoslavije*. U: Vasić, V., Stevanović, V. (urednici): *Biodiverzitet Jugoslavije*, pp: 75—95. Ecolibri i Biološki fakultet, Beograd.

V a s i ć, V. (1979): *Kolonije srebnastog galeba — Larus argentatus michahellis Naumann, 1840 (Laridae) i gaka — Nycticorax nycticorax (Linnaeus, 1758, Ardeidae) na Bilećkom jezeru u Hercegovini*, Biosistematika 5: 187—200, Beograd.

V a s i ć, V. (1995): *Diverzitet ptica Jugoslavije*. U: Vasić, V., Stevanović, V. (urednici): *Biodiverzitet Jugoslavije*, pp: 471—516. Ecolibri i Biološki fakultet, Beograd.

V a s i ć, V., Š o t i, J. (1979): *Višegodišnje promene u sastavu ornitofaune Vlasine*, II Kongres ekologa Jugoslavije, Saopštenja, pp.: 1691—1704. Zadar.

ОРНИТОЛОШКИ ЗНАЧАЈ ГРУЖАНСКЕ АКУМУЛАЦИЈЕ

Далиборка Ђ. Барјактаров
Природњачки музеј, Његошева 51, 11000 Београд, Србија и Црна Гора

Резиме

Преграђивањем средњег тока реке Груже, ради снабдевања општине Крагујевац пијаћом и техничком водом, настала је Гружанска акумулација. Језеро испуњава депресију Книћког поља и између Гледићких планина и Котленика. Како је орнитофауна акумулационих језера код нас али и у свету слабо проучавана, у раду су приказани подаци о стању орнитофауне Гружанског језера. Језеро има важну улогу током сеобе страних и завичајних популација птица, али и као зимовалиште за неке врсте патака и гусака, јер се налази на претпостављеном моравско-вардарском миграторном путу. Током истраживања забележено је 78 врста птица, од чега је за 25 врста утврђено да се гнезде на овом подручју. Највећи број врста забележен је у зони јаког антропогеног утицаја, што је и разумљиво јер је пре изградње бране и акумулационог језера у Книћком пољу преовладавало обрадиво земљиште, ливаде кошенице и воћњаци.

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Miloš C. Kapetanov, Dušan B. Orlić, Maja J. Velhner, Dubravka V. Potkonjak, Sava M. Lazić

* Miloš Kapetanov, DVM PhD, Dušan Orlić, DVM PhD, Maja Velhner, DVM PhD, Dubravka Potkonjak, DVM, Sava Lazić, DVM PhD
Scientific Veterinary Institute „Novi Sad", Rumenački put 20, Novi Sad, Serbia and Montenegro

CLINICAL AND LABORATORY INVESTIGATION OF EXPERIMENTALY INFECTED BROILERS WITH CIAV

ABSTRACT: Chicken infectious anemia (CIA) is widespread viral disease in countries with the intensive poultry industry. In susceptable birds CIAV causes anemia, subcutaneous and intramuscular hemorrhages, lymphoid tissue atrophy, immunosuppression, cachexia and increased mortality. Protection of progeny relies not only on age resistance but also on maternally delivered antibodies (Mabs) so possesing the information on level and persistence of Mabs is of great significance. In our study experimental infection with CIAV was performed on one and seven days old broiler chickens from naturally infected parent flock during the rearing period. In infected birds, clinical signs, hematological findings and humoral immune response were examined. After euthanasia, we looked for specific pathomorphological and histopathological changes that indicate the presence of CIAV infection. In all one and seven days old chickens maternally derived antibodies were established. No clinical signs of CIA were observed, hematological findings showed no deviation from referent values, and there were no specific pathomorphological and histopathological changes at postmortem examination. According to previous knowledge, only serological negative flock if infected in time of laying represent risk for vertical transmission to progeny where typical disease with mortality will apear. The absence of Mabs in one day old chikens is critical point in break of disease. Typical clinical picture in day old chickens rises only when vertical transmission occurs.

KEY WORDS: Broiler chickens, chicken infectious anemia, experimental infection, maternally derived antibodies

INTRODUCTION

Chicken infectious anemia (CIA), anemia-dermatitis syndrome or blue--wing disease refers to hemorrhagical syndrome manifested with: anemia, subcutaneous and intramuscular hemorrhages, immunosuppression, lymphoid tissue atrophy, cachexia and increased mortality (Rozypal et al., 1997; Goryo

et al., 1987; F a r k a š et al., 1992). The etiological agent of disease is circovirus, first isolated in Japan by Y u a s a et al. (1979).

After contact with microorganisms, cellular and humoral immune response efectors are produced by immune system. In serology diagnostic procedures for various infectious particulary viral diseases, the most often determined characteristics are presence of specific antibody titar. Those values demonstrate transportation of maternal immunity. Determination of persistence and protective effect of Mabs in progeny is very important for establishing immunoprophilaxis measures. Regarding to CIA, Mabs persist and have protective effect until 3 weeks of age (P a g e s et al., 1997; Y u a s a et al., 1980; B ü l o w, 1988; L u c i o et al, 1989; O t a k i et al., 1992). CIA is widespread in countries with intensive poultry industry, both in heavy and light lines, that was established during serological investigations in many countries and by isolation of virus. In our country, CIAV has not been isolated yet. Preliminary investigations taken on several poultry farms gave serologically positive results that indicates the presence of CIAV infection in our flocks (K a p e t a n o v et al., 1999).

The aim of this study was to determine the efficancy of Mabs against CIAV through experimental infection of broiler chickens one and seven days of age, originated from naturally infected parent flock. Knowledge of maternall transfer of antibodies could represent substantial base for adequate control and immunoprophilaxis programme in flocks infected with CIAV.

MATERIAL AND METHODS

Broiler chickens

Broilers were provided from farm with 54 weeks of age parent flock at the end of exploatation period. Experimental groups consisted of 10 broilers 1 and 7 days old. Broilers in farm facilities served as controls.

Virus

For establishment of efficancy of maternal immunity against CIAV in experimentally infected broilers referent Cux-1,27 CIAV strain with 10^7 TCD_{50}/ml titer, provided by Veterinary Academy, Budapest was used for challenge test. Working virus was obtained after two passages on SPF chickens, and was stored at –20°C until the time of infection. During infection procedure 0,2 ml of virus was inoculated by intramuscular route.

Experimental model

The experiment was performed in experimental facilities of Scientific Veterinary Institute „Novi Sad", Novi Sad. One and seven days old broilers were

infected with CIAV by intramuscular injection in the area of tight. Weight, food conversion ratio and mortality rate were calculated weekly. Broilers were vaccinated against NCD and IBD. Blood samples were taken before infection (at 1 and 7 days of age) and 14 days after the infection (at 14 and 21 days of age) and sent in laboratory for serology and hematology analysis. Fourteen days PI broilers were euthanised, necropsised and tissue samples were examined histopathologically. The level of thymus damage was determined from Thymus Body Index calculation. Experiment lasted 14 days.

Serology

Ten samples of blood sera from each experimental group were sent to virology laboratory at Scientific Veterinary Institute „Novi Sad", Novi Sad where the presence of specific antibodies against CIAV was determined using commercial ELISA set kit (IDEXX).

Hematology

In blood samples obtained by punction of wing vein RBC, WBC, hemoglobin, hematocrit, MCH and MCV were determined using standard methods (Rusov, 1984).

Pathomorphology

After postmortem examination of experimental and control groups of broilers, macroscopic examination and description of changes, samples of bone marrow, thymus and bursa of Fabricius were collected for histopathology. Five broilers from both experimental and control groups were weighted, and after euthanasia five samples of thymus were weighted and Thymus Body Index (TBI) was calculated. The value of TBI less than 0,7 indicated thymus atrophy.

Statistical analysis

Descriptive statistics was used to analyse serology and hemathology data.

RESULTS

After every day clinical examination, determined weight and mortality rate we could not find any sign that indicated the presence of CIAV infection.

Results of serology of one day old broiler chickens are presented in Tab. 1. The presence of antibodies was determined in all chickens.

Tab. 1. Serology investigation of blood sera from one day old broilers on presence of Mabs against CIAV using ELISA

No	OD1	SN2	results
neg. cont.	0,118	—	– +
pos. cont.	0,06	—	
1	0,058	0,49	+
2	0,062	0,52	+
3	0,065	0,55	+
4	0,060	0,50	+
5	0,061	0,51	+
6	0,060	0,50	+
7	0,064	0,54	+
8	0,058	0,49	+
9	0,064	0,54	+
10	0,62	0,52	+
x	Sd	CV	IV
0,516	0.022	0,22	0,49—0,55

[1] OD — optical density
[2] SN — speciment mean value and negative control ration

Mean values of extinctions of Mabs in one day old broilers were 174% lower than mean values of extinctions of Abs in their parents in age of 54 weeks, as shown in Fig. 1.

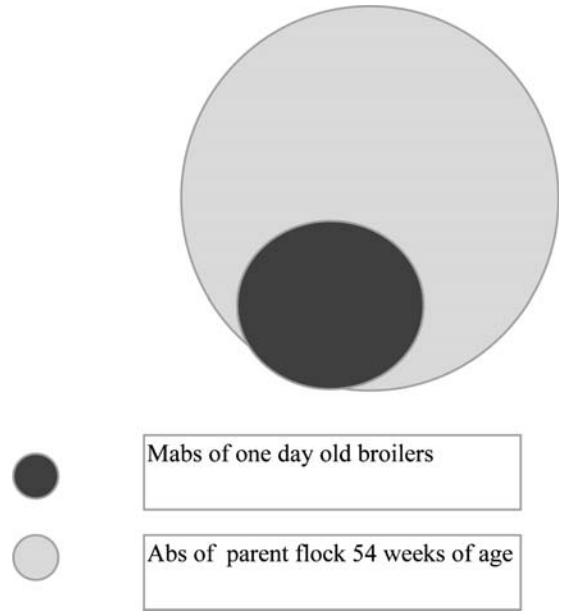

Fig. 1. Relation of mean antibodies extinction of 54 weeks old parent flock and their one day old broiler progeny presented as circle

In all seven days old chickens antibodies were found as presented in Tab. 2.

Tab. 2. Serology investigation of blood sera from 7 days old broilers on presence of Mabs against CIAV

No	OD	SN	results
neg. cont.	0,118	—	– +
pos. cont.	0,060	—	
1	0,058	0,49	+
2	0,061	0,51	+
3	0,060	0,50	+
4	0,060	0,50	+
5	0,062	0,52	+
6	0,065	0,55	+
7	0,065	0,55	+
8	0,066	0,55	+
9	0,069	0,58	+
10	0,068	0,57	+
$\bar{x}$	Sd	CV	IV
0,532	0,032	0,32	0,49—0,58

Results of hemathology of 1 and 7 days old broilers (before infection) i.e. at 14 and 21 days of age (after infection) are shown in Tab.3 and Tab.4. There were no deviations from referent values.

Tab. 3. Hemathological findings of one and seven day old broilers before infection

No	**RBC** T/1 2,5—3,5		**WBC** G/1 12,0—30,0		**Hemoglobin** g/l 70—130		**Hematocrit** 1/1 0,22—0,35		**MCH** Pg 33—47		**MCV** F1 90—140	
	1	7	1	7	1	7	1	7	1	7	1	7
1.	2.58	3.15	16.82	16.85	79.12	112.61	0.38	0.45	35.16	35.75	168.89	142.86
2.	2.53	3.39	12.54	16.14	85.74	112.61	0.28	0.42	38.97	33.22	127.27	123.89
3.	2.33	2.33	10.60	13.48	121.07	102.30	0.24	0.40	33.82	43.91	146.34	171.67
4.	2.88	2.70	15.11	12.67	100.46	109.30	0.35	0.38	46.08	40.48	160.55	140.74
5.	3.23	2.84	11.87	12.67	97.52	79.12	0.30	0.33	30.19	27.86	92.88	116.20
6.	2.5	2.94	13.68	11.81	83.90	126.22	0.33	0.33	33.56	42.93	132.00	112.24
7.	2.53	2.04	11.87	15.67	104.93	132.48	0.23	0.24	37.37	64.94	174.24	117.65
8.	2.31	3.19	12.11	16.12	108.61	126.22	0.26	0.48	38.66	39.57	112.55	150.47
9.	2.94	3.78	12.91	13.85	124.02	101.20	0.24	0.41	39.62	26.77	115.38	108.47
10.	2.90	3.09	16.96	16.18	104.88	105.25	0.42	0.31	36.17	34.06	144.83	100.32
X	**2.67**	**2.95**	**13.45**	**14.54**	**101.03**	**110.73**	**0.30**	**0.38**	**36.96**	**38.95**	**137.49**	**128.45**

Tab. 4. Hemathological findings of 2 and 3 weeks old broilers 14 days PI

No	**RBC** T/1 2,5—3,5		**WBC** G/1 12,0—30,0		**Hemoglobin** g/l 70—130		**Hematocrit** 1/1 0,22—0,35		**MCH** Pg 33—47		**MCV** F1 90—140	
	2	3	2	3	2	3	2	3	2	3	2	3
1.	2.48	2.81	14.39	15.85	80.96	79.86	0.30	0.29	44.51	28.42	202.70	103.20
2.	2.65	2.69	7.83	12.32	67.71	70.66	0.26	0.24	34.97	35.15	143.70	119.40
3.	3.16	2.66	16.81	12.99	76.54	68.08	0.26	0.28	28.19	30.39	115.56	125.00
4.	2.65	2.75	12.94	13.53	77.17	86.85	0.28	0.26	41.63	31.58	125.68	94.55
5.	2.00	2.49	14.90	12.36	77.28	77.65	0.27	0.29	33.77	31.18	143.84	116.47
6.	2.66	2.59	16.08	15.31	69.55	77.28	0.28	0.26	20.89	39.03	108.11	131.31
7.	2.07	2.69	13.38	16.43	72.13	76.18	0.27	0.25	35.55	24.90	124.29	92.94
8.	2.47	2.49	16.97	7.50	84.64	70.29	0.28	0.27	28.33	28.57	112.68	118.42
9.	2.39	2.62	16.77	10.86	83.90	79.12	0.29	0.27	45.90	38.22	129.03	130.43
10.	2.49	2.57	15.22	9.05	83.54	78.75	0.28	0.26	34.84	40.80	132.98	134.72
X	**2.50**	**2.64**	**14.53**	**12.62**	**77.28**	**76.47**	**0.28**	**0.27**	**34.86**	**32.82**	**133.85**	**116.64**

Samples of thymus were of normal size and appearance for age of birds with pronounced segmented appearance, and there was no atrophy of bursa of Fabricius. Also no histopathological changes were determined in thymus and bone marrow samples. TBI values summarised in Tab.7 indicate that there was no thymus atrophy.

Tab. 5. TBI of experimentally infected broilers and control groups

Experimentally infected broilers			Control groups		
Weeks of age	2	3	Week of age	2	3
TBI	1,000	0,978	TBI	0,998	1,000

DISCUSSION

In this experiment we investigated persistence and level of maternally derived antibodies in blood sera of broiler chickens, originated from 54 week old parent flock, in the beginning (1 and 7 days of age) and in the end (14 and 21 days of age) of trial. In 100% of 1 and 14 days old broilers we established the presence of Mabs against CIAV. In older broilers (3 weeks of age) we couldn't find Mabs against CIAV. These results coincide with investigations of Pages-Mante et al. (1997) that the highest mean values of Mabs against CIAV were in population of one day old broilers from vaccinated 30 weeks old parent flock and the lowest in 30 days old chickens from vaccinated 60 weeks old parent flock, respectively. Same researchers observed that mean values of Mabs decrease in linear regression line with approximately 10 days halflife.

Yuasa et al. (1980) and Otaki et al. (1992) emphasize the necessity of high level of antibodies in parents that are transsmited to their progeny and

protect them from infection in first two weeks of life i. e. until the age resistence is developed.

The virulence of CIAV strain influence the rapidness and efficancy of age resistence development (Yuasa et al., 1980; Goryo et al., 1985). Immunosupresion by simultaneous infection with IBDV or in bursectomised chickens impede the age resistence (Yuasa et al., 1980; Yuasa et al., 1988).

Hemathological findings in experimentally infected broilers (1 and 7 days old and 14 days PI) and their uninfected controls remained in referent values. The presence of protective Mabs transmited from naturally infected parent flock in rearing period could explain the absence of clinical CIA in broilers after experimental infection in this trial. Also pathomorphological and histopathological examination revealed not one evidence of pathogenic effect of CIAV as well as thymus damage based on TBI from 0,998 to 1, that coincede with findings of Pages-Mantesa et al. (1997).

According to previous knowledge on transmission of virus and time of occurrence of disease, only serological negative flock if infected in time of laying represent risk for vertical transmission to progeny where typical disease with mortality will apear (Yuasa et al., 1980).

CONCLUSION

1. Maternally derived antibodies against CIAV were established in one day old broilers from naturally infected parent flock and were present until they were 7 days old.

2. Mabs from naturally infected parent flock completely protected broiler chickens in their first week of life.

3. In experimentally infected broilers with CIAV no clinical signs of disease were observed. Hemathological findings showed no deviation from referent values. At postmortem examination there were no pathomorphological changes that indicate CIA.

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REFERENCE

Bülow, V. (1988): *Unsatisfactory sensitivity and specificity of indirect immunofluorescence testes for the presence or absence of antibodies to chicken anaemia agent (CAA) in sera of SPF and broiler breeder chickens*, J Vet Med B 35: 594—600.

Farkaš, T., Dren, C., Nemeth, I., Dobos-Kovacs, M. and Saghy, Erzsebet (1992): *Acta Veterinaria Hungarica*, 40 (3): 207—223.

Goryo, M. H., Sugimura, S., Matsumoto, T., Umemura and C. Itakura (1985): *Isolation of agent inducing chicken anemia*, Avian Pathol 14: 483—496.

Goryo, M., Y. Shibata, T. Suwa, T. Umemura and C. Itakura (1987): *Outbreak of anemia associated with chicken anemia agent chicks*, Jpn J Vet Sci 49: 867—873.

Kapetanov, M., Orlić, D., Gagić Maja, Palić, T. (1999): *Ispitivanja prisustva antitela za virus anemije pilića*, Nauka u živinarstvu 1—2: 61—66.

Lucio, B., K. A. Schat, and H. L. Shivaprasad (1989): *Identification of the chicken anemia agent, reproduction of the disease, and serological survey in the United States*, Proc 38th West Poult Dis Conf: 35—38.

Otaki, Y., Saito, K., Tajima, M. Nomura, Y. (1992): *Persistence of maternal antibody to chicken anemia agent and its effect on the susceptibility of young chickens*, Avian Pathology 21: 147—151.

Pages, A., N. Saubi, C. Artigas, S. E. (1997): *Experimental evaluation of an inactivated vaccine against chicken anemia virus*, Avian Pathology 26: 721—729.

Rozypal, T. L., Skeeles, J. K., Dash, J. K., Anderson, E. J., Beasley, J. N. (1997): *Identification and partial characterization of Arkansas izolates of chicken*, Avian Dis. Jul-Sep 41 (3): 610—6.

Rusov, Č. (1984): *Osnovi hematologije životinja*, Naučna knjiga, Beograd.

Yuasa, N., T. Taniguchi, and I. Yoshida (1979): *Isolation and some characteristics of an agent inducing anemia in chickens*, Avian Dis 23: 366—385.

Yuasa, N., T. Noguchi, K. Furata, and I. Yoshida (1980): *Maternal antibody and its effect on the susceptibility of chicks to chicken anemia agent*, Avian Dis 24: 197—201.

Yuasa, N., K. Imai, and K. Nakamura (1988): *Pathogenicity of chicken anaemia agent in bursectomised chickens*, Avian Pathol 17: 363—369.

КЛИНИЧКА И ЛАБОРАТОРИЈСКА ИСПИТИВАЊА НАКОН ВЕШТАЧКЕ ИНФЕКЦИЈЕ БРОЈЛЕРСКИХ ПИЛИЋА ВИРУСОМ ЗАРАЗНЕ АНЕМИЈЕ

Милош Ц. Капетанов, Душан Б. Орлић, Маја Ј. Велхнер,
Дубравка В. Поткоњак, Сава М. Лазић
Научни Ветеринарски институт „Нови Сад", Руменачки пут 20,
Нови Сад, Србија и Црна Гора

Резиме

Заразна анемија пилића (ЗАП) распрострањена је у свим земљама света са интензивном живинарском производњом. Болест је вирусне етиологије и код пријемчивих пилића изазива анемију, субкутана и интрамускуларна крварења, атрофију лимфоцитног ткива, имуносупресију, кахексију и повећан морталитет. Заштита потомства зависи не само од старосне резистенције, него и од нивоа матерналних антитела. Стога је познавање њиховог нивоа и перзистенције веома важно. У овом истраживању, вирусом заразне анемије пилића вештачки смо инфицирали бројлерске пилиће узраста 1 и 7 дана, пореклом од природно инфицираног родитељског јата у одгоју. Пратили смо клиничку слику, хематолошке параметре и хуморални имунолошки одговор код инфицираних пилића. Након жр-

твовања, утврђивали смо патоморфолошке и патохистолошке промене које би указивале на ЗАП. Код свих једнодневних и седмодневних пилића доказано је присуство матерналних антитела. Код вештачки инфицираних пилића нису уочени клинички симптоми ЗАП, резултати хематолошких испитивања нису одступали од референтних вредности, а након жртвовања нису утврђене специфичне патоморфолошке или патохистолошке промене органа. Према досадашњим сазнањима о начину инфекције и времену појављивања болести, само серолошки негативна јата инфицирана у периоду проношења представљају опасност за преношење болести на потомство и обољење у типичном облику са пратећим угинућем. Критичан моменат у настанку болести је недостатак наслеђених антитела код једнодневних пилића, односно пилићи обољевају са типичном клиничком сликом само ако до инфекције дође вертикалним путем.

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Igor M. Stojanov, Maja J. Velhner, Dušan B. Orlić

Scientific Veterinary Institute, Rumenački put 20,
21000 Novi Sad, Serbia and Montenegro

PRESENCE OF CAMPYLOBACTER SPP. IN NATURE

ABSTRACT: Presence of bacteria from Campylobacter spp. in nature and their importance encourages many researchers to study their biology.

The aim of this study was to understand the epizootiology of these bacteria by analyzing all available data and our own results. This would help to follow their movements and prognosticate the outbreak of the disease caused by *Campylobacter*.

In this experiment we used parts of the digestive tracts and reproductive organs of poultry and intestines of pigs and cattle. Isolation methodology was adjusted to the conditions favorable for *Campylobacter* spp. by providing selective mediums and microaerophilic conditions. Final determination of the isolates was done with Api Campy strips and appropriate software.

Of the 60 samples analyzed, 42 were positive for the presence of *Campylobacter* spp.: *Campylobacter jejuni* in 28 and *Campylobacter coli* in 14 cases. After examining samples from the reproductive tract, 4 birds were positive, with *Campylobacter jejuni* in 3 and *Campylobacter coli* in 1 sample. Of the 12 samples from pigs, 7 were positive (*Campylobacter jejuni*). Of the 6 samples from calves, *Campylobacter jejuni* was present in 4 cases.

For the sake of clarity, the overall epizootiological situation, established on the basis of literature data and the obtained results, may be viewed as consisting of three interactive parts. The first one is the territorial prevalence of *Campylobacter* spp. The second is their presence in different kinds of animal and their systems (organs), and the third are health problems that occur in consequence to their presence.

KEY WORDS: epizootiology, *Campylobacter* spp.

INTRODUCTION

Distribution of biological and non-biological agents, which are widely spread in nature, which are mutually interlaced and which may cause outbreaks of diseases, are permanently in the focus of attention of researchers, especially epizootiologists. Human medicine studies problems of epidemiology (Greek words *epi* = above, *demos* = people, *logia* = science) in humans as if they were separated from nature and need not be viewed as a part of the overall ecosystem.

Maybe the concept of epizootiology (*epi* = above, *zoo* = animal, *logia* = science) is more comprehensive, because it investigates the diseases that attack

animals and humans alike since the latter, due to their biological and physiological features, are part of the animal kingdom. Veterinary epidemiology includes many factors, ambience and events (V a l č i ć, 1998) that threaten animal populations and influence their normal development and homeostasis. Complexity of the problems that disturb a normal physiological and morphological state of species (a definition of a disease given in *Mala enciklopedija*), demands deductive observation and analytical investigation of the reality. This does not mean that individual perception will solve a problem in general, but it will facilitate its understanding and help view all parts of the whole.

Among the many aspects that are important for monitoring the presence of *Campylobacter* spp., their enormous distribution deserves to be singled out. Presence of *C. jejuni* has been detected (L e u c h t e f e l d and W a n g, 1981) in poultry and other bird species, in carnivores, herbivores and omnivores, in domestic and wild animals, in mammals and reptiles, fish, crabs and shells. However, the fact remains that the large distribution of this microorganism is not in correlation with the outbreak of the disease, at least not with the clinical manifestation of the disease. Epizootiology, as an important part of biomedicine research on *Campylobacter* spp. infection, must assess the importance of direct presence of these bacteria and the role of other biological and nonbiological factors.

Presence of bacteria from this genus *Campylobacter* in nature has encouraged many researchers to investigate their biology. It seems equally important to know where they can be found in nature and how they circulate within the ecosystem.

The aim of this study was to contribute to the understanding of the epizootiology of these bacteria by analyzing the places where they may be found. This would facilitate the monitoring of their movement and the forecast of outbreaks of the disease caused by *Campylobacter*.

MATERIAL AND METHODS

Parts of digestive tracts and reproductive organs from poultry and intestines from pigs and cattle were used for examination. A total of 60 samples from poultry digestive tract and the same number of samples of reproductive organs were examined. There were 12 samples from pigs and 6 samples from the digestive tract (abomasum, intestine) of calves. The methodology of isolation was adjusted to conditions necessary for *Campylobacter* spp. *Campylobacter* isolation was done with Columbia agar enriched with 5% of defibrinated sheep blood and an antibiotic-selective medium (bioMerieux) that contains Cefoperayon 3 mg, Colistan 2000 U, Vankomicine 2 mg and Amphotericina 0.4 mg per 200 ml of Columbia agar (Q u i n n et al., 1998). Microaerophilic conditions were provided by gas packs of the same producers. After making the preparation and staining according to Gram, oxidase and catalase tests (P e n e r, J. 1991), determination of *Campylobacter* was performed by Api-Campy strips (bioMerieux) and appropriate software.

RESULTS AND DISCUSSION

Of the 60 examined samples of the cecum and rectum, as parts of the digestive tract, and the magnum and uterus as parts of the reproductive tract, 42 birds were positive for the presence of *Campylobacter* spp., with *Campylobacter jejuni* present in 28 and *Campylobacter coli* present in 14 cases. The samples were taken from broiler breeders flock that exhibited no symptoms or changes in the production of eggs. These results represent a total finding of *Campylobacter* spp. in all of the examined birds. The examination of the reproductive tract in the same samples showed that 4 birds were positive: *Campylobacter jejuni* in 3 samples and *Campylobacter coli* in 1 sample (Table 1).

Table 1. Presence of *Campylobacter* spp. in poultry

Material	Number of examined samples	Positive findings	Isolate	
			Campylobacter jejuni	*Campylobacter coli*
Digestive tract — cecum + rectum	60	42 (70.0%)	28	14
Reproductive tract — uterus + magnum	60	4 (6.66%)	3	1

The obtained results correspond to the results reported by other authors for poultry material. Prevalence of *Campylobacter* spp. in poultry flocks ranges up to 100% (Blaser et al., 1981). Our results are within the range reported by other authors (Di Modugno et al., 1997). It may also be noticed that the places where *Campylobacter* spp. were found (i.e., the organs from which they were isolated) correspond to the results of other authors (Di Modugno, 1997).

There were 12 samples for examination of intestines (the ileum) in pigs. The samples were taken from animals with intestinal changes characterized by large amounts of mucus and blood in the intestinal content and dilated blood vessels of mucosis (Table 2).

Table 2. Presence of *Campylobacter* spp. in pigs

Material	Number of examined samples	Positive samples	Isolate	
			Campylobacter jejuni	*Campylobacter coli*
Digestive tract — ileum	12	7 (58.3%)	7	—

The findings of *Campylobacter* spp. in the digestive tract of pigs were in agreement with the results of the authors who analyzed parenchymatose organs and digestive tract of slaughtered animals (Weber, 1985). There is a large number of different *Campylobacters* that have been isolated in pigs. Such flora is considered to be able to cause a disease of animals (*C. hyointestinalis*; Bi-

berstein and Zee, 1990) or it has not been proved capable of causing disturbances (*C. mucosalis*; Biberstein and Zee, 1990).

The results of the laboratory investigation of the bovine material are presented in Table 3.

Table 3. Presence in *Campylobacter* spp. in calves

Material	Number of examined samples	Positive samples	Isolate	
			Campylobacter jejuni	*Campylobacter coli*
Digestive tract — jejunum	4	3 (75.0%)	3	—
Digestive tract — abomasum	2	1 (50.0%)	1	—

Table 3 shows that *Campylobacter* was isolated in 66.66% of the samples from calves. Different *Campylobacter* spp. were isolated, including *Campylobacter jejuni* (Luechtefeld and Wang, 1981).

The epizootiological situation was presented from three different aspects that should not be considered separately but as segments of a whole. The aspects were introduced in order to see more clearly mutual relationships among the places where these bacteria may be found, mechanisms of their transmission, their reservoirs and consequences they may cause. The first aspect of the epizootiological situation of Campylobacteriosis is its territorial distribution, the second concerns health problems that may be caused by *Campylobacter* spp. and the third describes the hosts and organs in which *Campylobacter* spp. can be found.

Regarding territorial distribution, the literature shows that *Campylobacter* spp. is present in almost all countries around the world. Presence of *Campylobacter* on our territory was confirmed in papers of Vakanjac (1994) which report that different Campylobacter species had been found in poultry material. Blaser et al. (1981) report on the occurrence of *Campylobacter* in USA, isolated in human faeces, running water, fresh shells and turkeys. There are reports about isolation of *Campylobacter* in Asia (Itoh et al., 1982) and there is evidence of its presence in Tokyo (Japan), in materials of animal and human origin, as well as in Bangladesh (Glass et al., 1982). The presence of *Campylobacter* in Europe is reported in papers from Sweden (Kaiser and Svedhem, 1980), Norway (Maeland, 1982), Great Britain (Young, 1982), and Greece (Danielides et al., 1981). A bacteriological examination of faecal samples from an Australian hospital confirmed the presence of *Campylobacter* in Australia too (Mc Gechie et al., 1982).

Previous results have shown that the presence of *Campylobacter* in a host may cause health problems. In many cases there were no problems although *Campylobacter* was isolated in certain materials, or at least there was no correlation between bacteriological findings of *Campylobacter* spp. and clinical symptoms. *Campylobacter jejuni* (Pener, 1991) causes abortion in sheep as well as fever and enteritis in humans. It may cause intestinal problems in calves, lambs and other animals. They are part of the normal intestinal flora in

young cattle, sheep, goats, dogs, rabbits, monkeys, cats, poultry, gulls, blackbirds, starlings and sparrows. In certain situations *Campylobacter coli* can be pathogenic (P e n e r, 1991; A d e s i y u n et al., 1992), when it was isolated from digestive tracts of pigs, poultry and humans. *Campylobacter laridis* was isolated in people suffering from diarrhoea, but it cannot be described with certainty as a pathogen of humans and animals.

Both subspecies of *Campylobacter fetus* (H o l t, 1984) are infective agents that cause sporadic abortion of animals and extra-intestinal infections, meningitis, salpingitis, embryo infection and abortion in humans. *Campylobacter hyointestinalis* (B i b e r s t e i n and Z e e, 1990) is considered to be a cause of proliferative ileitis of pigs, and it was also isolated in human faeces, from patients with watery diarrhoea. Some biotypes of *Campylobacter sputorum* may be found as commensal microorganisms in the oral cavity of humans, as part of the bacterial flora in the faeces of healthy humans, as well as in the prepuce of males and the genital tract of female cattle or in the sperm of bulls. Its role in the above mentioned humans and animals has not been explained. *Campylobacter mucosalis* is responsible for intestinal lesions in pigs, but this could not be proved in bacteriological experiments. *Campylobacter cryaerophilia* was found in genital tracts of cows, sheep, pigs and horses, in faeces of different animals and in milk from cows infected by mastitis. It has been discovered in the digestive tract of humans complaining of abdominal pain and diarrhoea.

C. jejuni was discovered in faeces of dogs and cats as reported by B r u c e (1982). It was isolated in animals with clinically manifested diarrhoea or haemorrhagic enteritis as well as in healthy animals, but in different percentages.

The presence of *Campylobacter* in certain bodily systems and products of cattle was described by many authors (L u e c h t e f e l d and W a n g, 1981). *Campylobacter* (*C. fetus subsp. fetus*) was isolated in the placenta and abdominal content of aborted calves, and it was also discovered in the digestive tract of calves with intestinal problems. Its presence was reported in the blood, bile ducts and milk of cows. A typical place where some *Campylobacter* species (*C. fetus subsp. venerealis*) may be found is bull prepuce, but without any symptoms. It is also found in the semen of bulls and the vaginal mucous membrane of cows. Infection is caused perorally and through mating, but also through the vagina, cervix, uterus and ductus deferens.

In their research on campylobacteriosis in sheep, L a t i n o v i ć et al. (1985) established the presence of several kinds of *Campylobacter* spp. (*C. jejuni, C. fetus subsp. fetus, C. sputorum spubsp. bubulus*) in vaginal swabs, faeces and parenchymatitis organs. *Campylobacter* was isolated in the placenta and intestinal content of aborted sheep embryo (P e n e r, 1991) and it was found cause intestinal problems in lambs. Also, it may be found in the normal intestinal flora of lambs.

S t i c h (1982) found *Campylobacter* spp.s in faeces of healthy pigs awaiting to be slaughtered. Similarly, W e b e r et al. (1985) found *Campylobacter* spp. in faeces of pigs before slaughtering, its concentration varying with season.

Campylobacter spp. were isolated in human faeces, from patients suffering from diarrhoea, blood, abscesses and cerebrospinal fluid. They were described as cause of meningitis, salpingitis embryo infection and abortion. They were also found in the oral cavity. The infection spreads perorally. Neal and Slack (1995) report that in humans, the outbreak of Campylobacter infection depends on season and it occurs most frequently in the fall.

Fenlon et al. (1982) investigated possible causes of campylobacteriosis. *Campylobacter* was present in gulls, city pigeons, crows, wild gees, and even herrings. Pener (1991) found *Campylobacter* spp. in shells and crabs.

All papers emphasize the importance of *Campylobacter* spp. from the point of possible transmission of these bacteria among animals, but also to humans. Peroral transmission and transmission by mating are the most frequent means of transmission of *Campylobacter* spp. among animals from one species. Transmission of infection between different species is exclusively peroral.

CONCLUSION

1. *Campylobacter* spp were found in the reproductive and digestive tracts in 76.66% of the poultry material, in 58.33% of the cases in the digestive tract of pigs and in 66.66% of the cases in the material taken from calves.

2. The obtained results on *Campylobacter* species, their distribution and organs where they may be found in animal samples were in agreement with the literature data.

3. Our results could neither prove nor disprove that the described health problems and clinical picture were due to the studied microorganisms, whose presence we could prove, nor could we prove that these microorganisms were the sole reason for the development of such a state.

4. On the basis of the available literature and obtained results, the epizootiological situation was interpreted from three different aspects that should not be considered separately but as segments of a whole. One aspect presents prevalence on territory. The other their presence in different animal species and their systems (organs) and the third aspect present health problems that are the consequence of their presence. The first aspect is the territorial distribution of Campylobacter species, the second concerns health problems that may be caused by *Campylobacter* spp. and the third describes the hosts and organs in which Campylobacter spp. can be found.

5. The following pattern of transmission from animal to humans should be taken into consideration: environment (water courses, vegetated areas, game) — pets and domestic animals — animal secretions and excretions — products from animals — humans.

REFERENCES

Adesiyun, A. A., Kaminjolo, J. S., Loregnard, R. Kitson-Piggott, W. (1992): *Campylobacter infections in calves, piglets, lambs and kids in Trinidad*, Br-Vet-J. Nov-Dec; 148(6): 547—56.

Biberstein, L., Ernst, Zee Chung Yuan (1990): *Review of Veterinary Microbiology*, Blackwell Scientific Publications Inc. Boston, Oxford, London, Edinburgh, Melbourne, Chapter 13, p. 125—129

Blaser, J. M., Feldman, A. R. and Wells, G. J. (1981): *Epidemiology of endemic and epidemic campylobacter infections in the United States*. In: Newell, D. C. (ed). Campylobacter: Epidemiology, Pathogenesis, Biochemistry. MTP Press Limited, Lancaster, 1982.

Bruce, D. (1982): *Campylobacters in cats and dogs*. In: Newell, D. C., (ed). Campylobacter: Epidemiology, Pathogenesis, Biochemistry. MTP Press Limited, Lancaster, 1982.

Danielides, V., Augoustidou-Savvopoulou, P., Manios, S., Sidira, G. and Kanzouzidou, A. (1982): *Campylobacter enteritis in Northern Greece*. In: Newell, D. C., (ed). Campylobacter: Epidemiology, Pathogenesis, Biochemistry. MTP Press Limited, Lancaster, 1982.

Di Modugno, G., Nasti, R. and Camarda, A. (1997): *Isolation of campylobacter jejuni from laying hens oviduct: Preliminary researches. Abstracts*, XI International Congress of the World Veterinary Poultry Association. 309, 18—22 August, Budapest.

Fenlon, R. D., Reid, S. M. T. and Porter, A. I. (1982): *Birds as a source of campylobacter infections*. In: Newell, D. C., (ed). *Campylobacter*: Epidemiology, Pathogenesis, Biochemistry. MTP Press Limited, Lancaster, 1982.

Glass, I. R., Huq, I., Stoll, J. P., Kibriya, G. and Blaser, J. M. (1982): *Epidemiologic features of campylobacter enteritis in Bangladesh.* In: Newell, D. C., (ed). *Campylobacter*: Epidemiology, Pathogenesis, Biochemistry. MTP Press Limited, Lancaster, 1982.

Holt, J. G. (1984): *Bergey's Manual of Systematic Bacteriology*, Williams and Wilkins Co. Baltimore, Vol 1.

Itoh, T., Saito, K., Yanagawa, Y., Sakai, S. and Ohashi, M. (1982): *Campylobacter enteritis in Tokyo*. In: Newell, D. C. (ed). *Campylobacter*: Epidemiology, Pathogenesis, Biochemistry. MTP Press Limited, Lancaster, 1982.

Kaiser, B. and Svedhem, A. (1980): *Campylobacter jejuni: a common cause of diarrhoea in Sweden*, Journal of Infectious Diseases, 142, 353.

Latinović, V., Popović, Miroslava, Nevjestić, A. (1985): *Prvi slučajevi izolacije bakterije roda Campylobacter kod goveda i ovaca u SR BiH*, Veterinaria 34, 3—4, 367—375, Sarajevo.

Leuchtefeld, N. W. and Wang, W. L. (1981): *Campylobacter fetus subsp. jejuni in a turkey processing plant*, J. Clin. Microbiol. 13, 266.

Maeland, A. (1982): *Some epidemiological features of campylobacter infections in Oslo*. In: Newell, D. C. (ed). *Campylobacter*: Epidemiology, Pathogenesis, Biochemistry. MTP Press Limited, Lancaster, 1982.

Mala enciklopedija Prosveta (1968): *Opšta enciklopedija*, Prosveta — Beograd, Ed. II, vol. 1, str. 177.

McGechie, B. D., Teoh, B. T. and Bamford, W. V. (1982): *Campylobacter enteritis in Hong Kong and Western Australia*. In: Newell, D. C. (ed). *Campylobacter*: Epidemiology, Pathogenesis, Biochemistry. MTP Press Limited, Lancaster, 1982.

Neal, K. R. and Slack, R. C. (1995): *The autumn peak in campylobacter gastro-enteritis. Are the risk factors the same for travel- and UK-acquired campylobacter infections?*, J. Public Health Med. Mar; 17(1): 98—102.

Pener, L. John (1991): *Campylobacter, Helicobacter and Related Spiral Bacteria*, Chapter 39. In: Lennette, Albert Balows et al. (eds), Manual of Clinical Microbiology, 5th Ed. pp: 402—408 American Society for Microbiology, Washington, D. C.

Quinn, J. P., Carter, E. M., Markey, B., Carter, R. G. (1998): *Clinical Veterinary Microbiology, Mosby*. London, Philadelphia, St. Louis, Sydney, Tokyo.

Stich-Groh, V. (1982): *Campylobacters in pig faeces*. In: Newell, D. C. (ed). *Campylobacter*: Epidemiology, Pathogenesis, Biochemistry. MTP Press Limited, Lancaster, 1982.

Vakanjac, Slobodanka (1994): *Preživljavanje kampilobaktera živine u prirodnoj sredini i eksperimentalnim uslovima*, magistarska teza. Beograd.

Valčić, A., Miroslav 1998: *Opšta epizootiologija*, Fakultet veterinarske medicine, Beograd.

Weber, A., Lembke, C. und Schafer, R. (1985): *Nachweis von Campylobacter jejuni und Campylobacter coli in Kotproben gesunder Schlachtschweine in Abhangigkeit von der Jahreszeit*, Zbl. Vet. Med. B, 32, 40—45.

Young, J. E. S. (1982): *Human campylobacter infections 1977—1980: national data based on routine laboratory reporting*. In: Newell, D. C. (ed). *Campylobacter*: Epidemiology, Pathogenesis, Biochemistry. MTP Press Limited, Lancaster, 1982.

ПРИСУСТВО КАМПИЛОБАКТЕР ВРСТА У ПРИРОДИ

Игор М. Стојанов, Маја Ј. Велхнер, Душан Б. Орлић
Научни институт за ветеринарство Нови Сад, Руменачки пут 20,
21000 Нови Сад, Србија и Црна Гора

Резиме

Присуство бактерија из рода кампилобактер у природи и њихов значај су разлог који подстиче многе истраживаче да изучавају њихову биологију.

Циљ рада је да се анализирањем података где се све ове бактерије налазе и делом сопствених резултата допринесе разумевању њихове епизоотиологије чиме би се омогућило лакше праћење кретања ових бактерија и боље прогнозирање када је у питању избијање болести изазване кампилобактерима.

У раду смо као материјал користили делове дигестивног тракта и делове репродуктивних органа живине, црева свиња и говеда. Методологија изолације је била прилагођена неопходним условима предвиђеним за кампилобактер врсте обезбеђивањем селективних подлога и микроаерофилних услова. Крајњу детерминацију изолата урадили смо уз помоћ апистрипова ApiCampy и одговарајућег софтвера.

Од укупно 60 прегледаних узорака 42 јединке су биле позитивне на присуство кампилобактер врста, од тога *Campylobacter jejuni* је заступљен у 28 и *Campylobacter coli* у 14 случајева. После прегледа репродуктивног тракта 4 јединке су биле позитивне, и то *Campylobacter jejuni* у 3 и *Campylobacter coli* у 1 узорку. Из испитаних 12 свињских материјала позитивно је било 7 (*Campylobacter jejuni*), а код 6 материјала телади 4 је имало *Campylobacter jejuni*.

На основу прегледане литературе и добијених резултата епизоотиолошка слика кампилобактер врста може да се интерпретира у три целине, које се никако не смеју посматрати одвојено него као подела која омогућава лакше сагледавање проблематике везане за ове бактерије. Једну целину чинила би територијална распрострањеност кампилобактер врста. Другу — њихово присуство у различитим животињским врстама и њиховим системима (органима) и трећу целину чинили би здравствени поремећаји који се јављају као последица присуства ових бактерија.

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Svetlana D. Dragojević-Dikić[1], *Saveta Draganić*[2], *Srđan S. Dikić*[3], *Vladimir Pilija*[4]

[1] Gynecology-Obstetrics Clinic, Narodnog Fronta 62, 11000 Belgrade, Serbia and Montenegro;
[2] Institute of Mental Health, University of Belgrade, Medical School, 11000 Belgrade, Serbia and Montenegro;
[3] University Clinic, University of Belgrade, 11000 Belgrade, Serbia and Montenegro;
[4] Medical Center of Novi Sad, Institute of Forensic Medicine, Hajduk Veljkova 7, 21000 Novi Sad, Serbia and Montenegro

ETHICAL AND LEGAL DILEMMAS IN INFERTILITY TREATMENT

ABSTRACT: One of the main characteristics of the new millennium is the affirmation of human rights in all aspects of human existence, with the intention of turning declarative statements into reality. Development of up-to-date assisted reproductive technologies (ART) and their application in infertility treatment have raised numerous ethical, legal, religious, social and other questions. In vitro fertilization, donation of gametes, embryos and pre-embryos, cryopreservation of gametes, embryos, ovarian and testicular tissues, embryo transfer, genetic reproductive techniques, cloning and other sophisticated methods used in infertility treatment require cooperation between the medical and legal professions. Ethical aspects of human reproduction and assisted fertilization are based on full respect of the life of an individual even before conception, from pre-embryo stage, via embryo stage and fetus stage to a newborn infant. Regarding investigative and clinical projects, this standpoint implies the legalization of all ART procedures, unencumbered exchange of information and consensus about their application, and adherence to the basic ethical principles of autonomy, benefit, justice and common welfare. Ethical postulates provide unequivocal directions in the creation of new life and resolve all possible ethical dilemmas, protecting the rights of doctors and participant in relevant procedures alike and reasserting the crucial principle — respect of human dignity.

KEY WORDS: infertility, ethics, jurisprudence

INTRODUCTION

Infertility is a topical problem in gynecology, which requires delicate approach, analysis and treatment. Causes of infertility, presently encountered in about 15—20% of couples, are numerous: tubal, ovarian, anatomical, immuno-

logical, endometriosis, cervical, psychological, idiopathic and male factors, whereat the infertility is most often multifactorial in nature. Developments in reproductive biology have enabled infertility treatment by means of assisted reproductive techniques (ART) and/or introduction of up-to-date methods for reproductive function restoration. The term „assisted reproduction" is associated with certain treatments which give hope to many — patients, clinical personnel, researchers — but which also open numerous ethical, legal, religious and social questions (P a p p, 2000).

In vitro fertilization (IVF), donation of gametes, embryos and pre-embryos, cryopreservation of gametes, embryos, ovarian and testicular tissues, embryo transfer, genetic reproductive techniques, cloning and other sophisticated methods used in infertility treatment require cooperation of medical, ethical and legal professions, with the aim of combing research work and individualized clinical approach. Ethical aspects of human reproduction and assisted fertilization are based on full respect of the life of an individual even before conception, from pre-embryo stage, via embryo stage and fetus stage to a newborn infant. Regarding investigative and clinical projects, this standpoint implies the legalization of all ART procedures, unencumbered exchange of information and consensus about their application, and adherence to the basic ethical principles of autonomy, benefit, justice and common welfare.

In 1990 in the U. K., the Parliament passed the Human Fertilization and Embryology Act (HUFE) which provides legislation for the control of the procedures of assisted reproduction. In our country, Ethical Committee of the Yugoslav Section for Fertility and Sterility made a draft of the ethical code on assisted reproduction, which is going to be legislated in the near future (1990, M i l a č i ć, 2000). The HUFE Act requires the experts engaged in assisted reproduction to consider „the welfare of the child born as a result of the treatment (including the child's need for a father), and any other child that may be affected by its birth". The Human Fertilization Act permits the use of embryos for research in five categories: for promoting the treatment of infertility; for improving the knowledge of the causes of congenital diseases; for improving the knowledge of the causes of miscarriages; for developing more effective techniques of contraception, and for developing methods of detection of the presence of gene or chromosome abnormalities in embryos before implantation. After 23 years from the birth of the first IVF child, the necessity for embryo research, and its implications for the status of the embryo as an entity, is less challenging than in the previous decades, in particular at a time when the therapeutic potentials of embryo stem cells provides convincing arguments regarding its necessity and further analysis (S t r o n g, 1998).

Law and ethics are indeed in an inevitable interaction with each other, as two systems of normative ordering, which sometimes overlap and are sometimes in conflict. On one hand, the law may seem a more powerful instrument than ethics, because its provisions are more authoritatively and comprehensively presented by political legislation and courts, more systematic and more transparent, while its use is more practical, instrumentally versatile and institutionally challengeable. On the other hand, the law is seen to lack an ethical dimension, to be crudely pragmatic at best, and impoverished in its capacity to

educate and inspire those it governs to distinguish the right conduct from wrong. The law sets a framework for practical utilization of ethical choices, but the ethics sets limitations that are voluntarily obeyed, as expressed through respect for the law, which in its turn asserts the merits of the society it governs (D i c k e n s, 1999).

Before undergoing the various procedures of the assisted reproductive techniques, patients should be fully acquainted with the following: how the treatment will be carried out, how long it will take, how effective it will be and what possible complications are, whereat they have to sign their consent for the performance of the treatment. Gamete and embryo donation procedures are absolutely secret, except in rare and legally foreseen cases, while other treatments (in vitro fertilization — IVF, intrauterine insemination of husband's spermatozoa — IUI, intracytoplasmic sperm injection — ICSI) can also be made secret at the explicit request of patients. The Yugoslav Ethical Committee is of the opinion that it is indispensable to establish a center for registration of all donation data (M i l a č i ć, 2000). Legally controlled secrecy and conscious consent of the patient are significant characteristics in the field of reproductive medicine, ensuing from the establishment of the constitutional right to privacy in reproductive treatment, and the reactions of political and moral opponents to the realization of such rights (R o c k e t t et al., 2000).

DONATION OF GENETIC MATERIAL

Genetic material donation has become an integral part of infertility treatment. Donations of spermatozoa, oocytes, embryos and pre-embryos (embryo not older than 14 days) are successful in the medical and technical sense and ethically approved. Medical problems and ethical dilemmas that require understanding and evaluation are: selection of donors, evaluation of recipients, quality control of genetic material, relationship between biological and social parents, and protection of the rights of offsprings through specially legislated decisions. Sperm donation has to be anonymous, and the donors cannot be known donors, friends or relatives.

Oocyte donation is also ethically permissible in specific cases: in patients with premature ovarian failure and regular menopause, in patients with inferior-quality oocytes and in patients after several unsuccessful IVF treatments. In order to avoid a long waiting period, different procedures to recruit oocyte donors are proposed, such as oocyte-sharing (donors share their oocytes with an anonymous recipient and in return, recipients share the costs of treatment of the donor) and the recruitment of a donor by the patients themselves. Attention should be paid to possible psychological consequences of this decision (K a h n et al., 1998, B a e t e n s, 2002).

Indications for embryo donation are women without oocytes and men with azoospermia, in which cases only embryos obtained from spermatozoa and oocytes of mutually unknown donors can be donated. Embryo donation can be achieved in two ways: (I) using a combination of oocytes and sperm donation — such donors should already have been properly counseled; and (II)

using spare cryopreserved embryos from patients who have already been successful and have consented to the donation of their remaining embryos. The standpoint of the Yugoslav Ethical Committee is that embryos have certain moral status, hence they cannot be preserved longer than 14 days (until „primitive streak" appearance), with permission to use the treatments that do not diminish the genetic status of the embryo (embryo defragmentation and ooplasm transfer, Milačić, 2000). Although in the U.S.A. donation of couples' embryos has been permitted, very few couples decide to donate their embryos (a greater percentage preserves the embryos for possible future use), however, as proved in studies, which is interesting and important, transfer of „donated" couples' embryos has resulted in a high percentage of successful pregnancies per treated cycle (50%, Van Voorhis et al., 1999). Pre-embryo banks are to be specifically legalized and issued appropriate individual permits; the concerned professional medical and social institutions would have to take into consideration the interests of infertile couples, but also the interests of the embryo, i.e., of future descendants (Eisenberg, 1998).

CRYOPRESERVATION OF GENETIC MATERIAL

Sperm and embryo cryopreservation is permitted and they can be preserved up to 10 years. Sperm cryopreservation has long been routine and helpful in preserving the fertility potential of many young men treated for iatrogenic sterility or threatened by cancer. Oocyte cryopreservation, however, is not permitted in any of the countries belonging to the International Federation of Fertility Societies (IFFS).

Ovarian tissue cryopreservation is permitted, with significant prospects for clinical use in reproductive medicine and oncology. Ovarian cryopreservation, which lately has been in the focus of experimental research, opens new moral and ethical dilemma, requiring critical consideration for tissue preservation („bank"), and also require working out specific instructions by medical, ethical and legal experts on the criteria for future clinical use and benefits of such procedures (De Wert et al., 2000).

POSTHUMOUS REPRODUCTION

Recent events posing ethical dilemmas relate to posthumous reproduction, pre-implantation genetic diagnosis (PGD) and cloning. They illustrate the difficulties for closed, legally controlled systems to forecast all the possibilities of scientific progress and ethical dilemmas arising from it. The advent of successful techniques of spermatozoon and embryo cryopreservation makes the birth of a child whose genetic father is dead technically possible, following the usual period deemed legally necessary to recognize the paternity of the posthumous child. Most of the centers for infertility treatment in the U. K. support the idea of posthumous reproduction, hence the posthumous treatment is permitted provided and explicit prior written consent has been given after the ga-

mete(s) provider(s) had received counsel. General attitude is that each case should be individually analyzed and approved by a multidisciplinary committee consisting of a gynecologist, a psychiatrist, a sociologist, a clergyman and other appropriate specialists (B e n s h u s h a n et al., 1998).

PREIMPLANTATION GENETIC DIAGNOSIS

Preimplantation genetic diagnosis (PGD) is a result of development and convergence of assisted reproduction techniques and genetic methods, allowing the couples at risk an early diagnosis of hereditary diseases, even before the conception. PGD, however, triggers the fear of potential genetic manipulation and of getting closer to criminal eugenics, and therefore the standpoint of the Yugoslav Ethical Committee is that PGD is justified only in medically indicated cases. Within the framework of infertility treatment, pre-implantation genetic diagnosis is part of a range of potential diagnosis options, which help our patients when making an important decision about screening their future child from serious diseases (P a p p, 2000; M i l a č i ć, 2000).

CLONING

Human reproductive cloning is unjustified and unnatural for it offends human dignity and violates the individual rights to genetic uniqueness. One can consider reproductive cloning of embryos by means of nucleus transplantation or embryo splitting, and the ethical aspects in the context of genetic reproductive techniques are to be evaluated separately. Many countries and institutions have analyzed possibilities of therapeutic cloning when other alternatives are exhausted, as well as the cloning within the framework of genetic engineering with the aim of producing appropriate human proteins.

Therapeutic cloning technology serves to culture stem cells that are genetically identical to those of the patient, with an aim of replacing diseased cells, for example in nerves damaged by neurodegenerative disorders, in the heart muscle affected by infarction, in diabetes or in liver damaged by poisoning. Stem cells may be derived from the embryo (more precisely, from blastocysts), the fetus or the adult. There are several types of embryonic stem (ES) cells: those issued from blastocysts either as supernumerary or created *de novo* and those created by nuclear transfer from somatic cells (SCNT). The latter method is usually referred to as cloning (S h e n f i e l d, 2002).

The final report of the European Group on Ethics (EGE), made public in November 2000, forbids reproductive cloning. It deems ethically unacceptable to create embryos from donated gametes, because supernumerary embryos are an alternative available source. In the case of embryos obtained by SCNT, extreme concern is voiced, despite the awareness that the creation of such embryos may be the most effective way for obtaining pluripotent stem cells genetically identical to the patient's and thus obtaining perfectly compatible tissues with the aim of avoiding rejection after transplantation (2000). Nevertheless, the concerned scientists agree that research should continue with all sources of

stem cells, as we cannot yet know which source — if any — is going to fulfill the therapeutic expectations.

Cloning is due to receive extensive legislation, but is has to be carefully and selectively performed in order to make room for further improvements in this field of research for the benefit of the entire mankind (De Wert, 2000, Lupton, 1999).

CONCLUSION

Sex selection, multiple pregnancies and embryocide, surrogate parentage and treatment of older women open numerous ethical and legal dilemmas and call for multidisciplinary and expert approach to analyzing each individual case, as well as to defining clear ethical and legal regulations, open to correction in respect to further investigative work. Ethical postulates provide unequivocal directions in the creation of new life and resolve all possible ethical dilemmas, protecting the rights of doctors and participant in relevant procedures alike and reasserting the crucial principle — respect of human dignity. Defined legal principles are to be reconciled with the „natural laws" for the sake of protection of the freedom of thought and the right of individual choice and for the realization of the goal aimed at the preservation of life and justification of the purpose of existence.

REFERENCES

Papp, Z., Urbancsek, J. (2000): *Professional and ethical challenges in reproductive biology and genetics at the beginning of the millennium*, Orv Hetil, 141(24): 1327—42.

Human Fertilization and Embryology Act (1990): United Kingdom.

Milačić, D. (2000): *Draft of ethical codex on assisted reproduction* Proceedings of XIV Yugoslav Symposium on Fertility and Sterility, Belgrade, 122—5.

Strong, C. (1998): *Teaching medical ethics and law within medical education: a model for the UK*, J. Med. Ethics, 24(3): 188—92.

Dickens, B. M. (1999): *Reproductive health and legal duties of medical confidentiality*, Med. Law; 18(2—3): 217—23.

Rockett, L. R. (2000): *Legal issues affecting confidentiality and informed consent in reproductive health*, J. Am. Med. Women's Assoc., 55(5): 257—60.

Kahn, J. P. (1998): *The ethics of egg donation*, Minn. Med., 81(10): 12—5.

Baetens, P. (2002): *Oocyte donation.* In: Boivin, J., Kentenich, H. (eds.) Guidelines for Counseling in Infertility. ESHRE Monographs: 33—4.

Van Voorhis, B. J., Grinstead, D. M., Sparks, A. E., et al. (1999): *Establishment of a successful donor embryo program: Medical, ethical, and policy issues, Fertil. Steri.*, 71(4):604—8.

Eisenberg, V. H., Schenker, J. G. (1998): *Pre-embryo donation: Ethical and legal aspects*, Int. J. Gynaaecol. Obstet., 60(1):51—7.

De Wert, G., De Beaufort, I. D. (2000): *Cryopreservation of ovarian tissue open for discussion*, Ned Tijdschr Geneeskd, 1444(15): 692—4.

Benshushan, A., Schenker, J. G. (1998): *The right to an heir in the era of assisted reproduction*, Hum. Reprod.: 13(5): 1407—10.
Shenfield, F. (2002): *Cloning: reproductive, therapeutic or not at all?* In: Shenfield, F., Sureau, C. (eds.) Ethical Dilemmas in Reproduction, New York, USA: Parthenon Publishing: 87—97.
The European Group on Ethics and New Technologies to the European Commission (2000): *Adoption of an opinion on ethical aspects of human cell research and use*, Paris, 14 Nov. 2000, revised Jan. 2001, Secretariat of the Group, Brussels.
De Wert, G. M., Geraedts, J. P. (2000): *Cloning: applications in humans. II Ethical considerations*, Ned Tijdschr Geneeskd: 144(20): 926—31.
Lupton, M. L. (1999): *To clone or not to clone — whether the law?* Med. Law: 18(1): 107—23.

ЕТИЧКО ПРАВНЕ ДИЛЕМЕ У ТРЕТМАНУ ИНФЕРТИЛИТЕТА

Светлана Драгојевић-Дикић,[1] Савета Драганић,[2]
Срђан Дикић[3] и Владимир Пилија[4]
[1] Гинеколошко-акушерска клиника „Народни фронт",
Народног Фронта 62, 11000 Београд, Србија и Црна Гора
[2] Институт за ментално здравље, Медицински факултет,
11000 Београд, Србија и Црна Гора
[3] Универзитетска клиника „Бежанијска коса",
Медицински факултет, 11000 Београд
[4] Клинички центар Нови Сад, Институт за судску медицину,
Хајдук Вељкова 7, 21000 Нови Сад

Резиме

Једно од основних обележја новог миленијума је афирмација људских права у свим областима људског битисања са тежњом оживотворења декларативних у реалистичко постулиране односе. Развој савремених асистираних репродуктивних техника (АРТ) у третману инфертилитета отворио је многобројна етичка, правна, религиозна и социјална питања: in vitro фертилизација; донација гамета, ембриона и преембриона; криопрезервација гамета, ембриона, оваријалног и тестикуларног ткива; ембрио трансфер, генетске репродуктивне технике; клонирање и друге софистициране методе у решавању важног и деликатног животног и медицинског питања — инфертилитета, захтевају кооперацију медицинске професије и етичко-правне струке, у циљу повезивања научно-истраживачког рада и одговарајућег, мудрог и индивидуализованог клиничког приступа. Етички аспекти хумане репродукције, као и асистиране фертилизације, заснивају се на поштовању живота јединке и пре успостављања концепције, од преембриоланог стадијума, преко ембриоланог стадијума, стадијума фетуса и новорођеног детета. Овакав став у даљим истраживачким и клиничким подухватима подразумева легализацију свих процедура у оквиру асистираних репродуктивних техника, информисаност и сагласност о њиховом спровођењу, уз поштовање основних етичких принципа: аутономије, користи, правде и опште добробити. Етички постулати у поступцима креирања новог живота помажу својим јасним упутствима у решавању могућих етичких дилема, штитећи права лекара и свих учесника у одговарајућим процедурама, уз уважавање круцијалног принципа — поштовања људског достојанства.

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Mira M. Pucarević, Petar Đ. Sekulić

Institute of Field and Vegetable Crops Novi Sad,
Maksima Gorkog 30, Serbia and Montenegro

POLYCYCLIC AROMATIC HYDROCARBONS AND PESTICIDES IN SOIL OF VOJVODINA

ABSTRACT: The paper deals with several groups of compounds that represent the most frequent pollutants of soil in the world. The paper also reviews results of long-term studies conducted at the Institute of Field and Vegetable Crops in Novi Sad on the residues of pesticides and polycyclic aromatic hydrocarbons (PAHs) in the soil of the Vojvodina Province. The analyzed samples have been found to contain residues of persistent pesticides and their metabolites: lindane and its metabolites 6,20 μg/kg, alachlor 3,56 μg/kg, aldrin 2,3 μg/kg, heptachlor epoxide 0,99 μg/kg, chlordane 3,82 μg/kg, DDT and its metabolites 10,77 μg/kg, dieldrin 2,04 μg/kg, endrin 3,57 μg/kg and endrin aldehyde 1,36 μg/kg. Soil samples from Novi Sad municipality contained 53,69 μg/kg of DDT and its metabolites. The values of atrazine ranged from 0,0005 to 0,8 mg/kg. The values of PAHs were 6,64 mg/kg in industrial soil, 4,93 mg/kg in agricultural soil, and 4,55 mg/kg and 5,48 mg/kg in the Novi Sad municipality. The lowest value, 0.83 mg/kg, was found for nonagricultural/nonindustrial soils.

KEY WORDS: soil, pesticides, polycyclic aromatic hydrocarbons, PAHs

INTRODUCTION

As part of the environment, soil may contain many organic compounds of natural as well as anthropogenic origin. The concentrations and toxicity of organic compounds present in such complicated mixtures range very widely and depend also on possible interactions (synergies) among chemicals.

The development of instrumental analysis techniques and the lowering of the detection limit have made it possible to identify new organic compounds that are present in the soil in very low concentrations. The list of the most commonly studied soil pesticide pollutants has been expanded to include polychlorinated biphenyls (PCBs), alyphatic hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), polychlorinated dibenzo dioxins (PCDDs), polychlorinated dibenzo furans (PCDFs) and polychlorinated naphtalenes (PCNs). The continuous introduction of these persistent compounds into the environment has resulted in their accumulation. Depending on how strong the bond is, organic

compounds adsorbed on soil particles may migrate down to deeper soil layers and then to ground or surface waters, or they may remain in the surface layer of the soil and, in some cases, end up in plants. These compounds also bioaccumulate in fish and other aquatic organisms.

Polychlorinated naphtalenes (PCNs) are a group of 75 compounds with two condensed aromatic rings substituted with one to eight chlorine atoms. They were synthesized back in the 19th century and used as dielectrics under different names (Halovax (USA), Seekay (UK) or Nibren (Germany)). Some of these compounds have similar toxicity to that of dioxins. Polychlorinated naphtalenes are produced by the combustion of substances that contain chlorine, so the most common PCN source are waste incineration plants. They are also present in commercial PCB mixtures as by-products of synthesis (Yamashita et al. 2000). PCN quantities found in urban soils range from 0.1 μg/kg to 15.4 μg/kg, while those found in rural soils range between 0.1 μg/kg and 0.82 μg/kg (Krauss, 2003).

Polychlorinated biphenyls (PCBs) have similar uses and characteristics to PCNs and include a total of 209 compounds in which one to ten chlorine atoms are attached to the biphenyl nucleus (Facchetti, 1993). For the sake of simplicity, each of the 209 compounds (congeners) has been assigned a number from 1 to 209 (so-called Ballschmiter-Zell, or BZ, number). In Germany, seven congeners have been chosen as indicators of pollution by PCBs based on the frequency with which a particular congener appears in commercial PCB mixtures. Their BZ numbers are 28, 52, 101, 118, 138, 153 and 180. PCB concentrations are most oftren correlated with PCN ones, the ratio being 100:1 (Kannan et al., 2000). Commercial PCB mixtures go under a variety of names depending on the manufacturer (Arochlor, Chlorexol, Pyralene, Phenoclor, Clophen, Apirolio, Sovol, Delor, Kanechlor). They are used as dielectrics in transformers, hydraulic oils, pesticide synergists, in nonflammable coatings, and in the manufacture of ink and paper. Because of their widespread use, persistence and often improper disposal, PCBs can now be found in all parts of the ecosystem. The PCB content of Bangkok soils was found to be 0.1—1.2 μg/kg (Müller, 2001).

Polycyclic aromatic hydrocarbons (PAHs) are a group of over 100 compounds made up of condensed benzene rings. They are a product of incomplete combustion of organic substances, coal, gas and wood. Because of their planar structure, these compounds have highly mutagenic and cancerogenic properties. PAH concentrations have often been studied in sediments obtained from ports and near roads. The average PAH content of forest, agricultural and urban soils has been found to 0.05, 0.07 and 1.10 mg/kg, respectively, while that of roadside dust has been determined to be 137 mg/kg (Ayaka et al. 1999). The total PAH content of Bangkok soils has been found to range from 47 to 140 μg/kg (Müller, 2001).

The source whose combustion produced PAHs can be determined by studying PAH residues in soil or some other matrix and by defining relationships among the PAHs concerned (Zou et al. 2003).

PCN, PCB and PAH concentrations in all soil types are interrelated and dependent on human activity. The highest concentrations are those found in

urban areas and gardens. Agricultural soil is polluted by PAHs via particles from the air. Also, the low-molecular-weight fraction of these compounds is more often found in agricultural soils than in urban ones because of greater evaporability and easier transport by air (K r a u s s, 2003).

Pesticides commonly used in plant protection form a large group of diverse compounds (there are 242 active ingredients currently registered in Serbia and Montenegro alone) that come into contact with the soil. Besides the compounds, the products of their decomposition can often be found as well (4,4'-DDT and 4,4'-DDE, 4,4'-DDD, glyphosate and AMPA, atrazine, desethylatrazine, desisopropylatrazins and different hydroxy derivatives of atrazine). DDT (which is no longer in use since the 70s) and its metabolites are very persistent compounds and as such can be found in soil in significant quantities years after they were last used.

The Institute of Field and Vegetable Crops in Novi Sad began monitoring the presence of pesticides (as the first major group of organic compounds) in the soil in 1991, when the Ministry of Science and Technology of the Republic of Serbia and the Fund for Soil Protection. Utilization, Improvement and Management of the Republic of Serbia financed the first such research project. In order to make the initial assessment of the status of soils in the Vojvodina Province, 1,600 soil samples were taken in total, 926 of which were analyzed for the presence of persistent pesticides (15 organochlorine and 4 triazine ones) and products of their degradation.

A study of PAHs was prompted by the NATO bombardment of Serbia and Montenegro that took place in 1999 in which large amounts of oil were set on fire at the Novi Sad oil refinery as a result of repeated bombing. In order to determine if any soil pollution occurred in the area as a result, soil from the Rimski Šančevi Experiment Field of the Institute of Field and Vegetable Crops was studied for the presence of PAHs.

As it is known that the highest PAH concentrations are found in urban areas and near roads, gardens in the city of Novi Sad were studied for PAH presence during 2000 and 2001. Almost all of these compounds were found to be present, especially in suburban areas and on the outskirts of the city, where conventional heating methods are the most common source of PAHs. The total PAH content was somewhat higher in 2001 than in 2000.

In soil studies conducted in 2001, which were financed by the Executive Council of the Vojvodina Province, 50 samples of agricultural soils of Vojvodina were investigated. In 2002, 11 additional samples of soils near industrial sites were studied.

MATERIALS AND METHODS

Pesticide extraction was done using supercritical extraction accompanied by gas chromatography (V e r e š b a r a n j i et al., 1993), while soil PAH extraction was performed by the US EPA 3540C and 3630C method. The extraction of 16 PAHs from the soil was carried out by a glass apparatus according to Soxhlet in the following manner: soil and sediment were extracted

with and 100 ml methylene-chloride for 24 hours. The extract was reduced to 2 ml and dissolved in cyclohexane to 5 ml volume. The resulting extract was purified on a silica gel column. PAHs were eluted from the silica gel column with 25 ml of methylene/pentane mixture (2:3), reduced till dry and dissolved for analysis in acetonitrile. The acetonitrile extract was analyzed by gas chromatography (HP1100) with peak identification using a diode array detector (DAD). The C-18 column was used with acetonitrile/water gradient.

RESULTS AND DISCUSSION

Pesticides

Table 1 shows the results of the study of organochlorine pesticide residues found in the 926 soil samples from the Vojvodina Province and 19 soil samples from the Novi Sad municipality. As we can see, the average contents determined in the study were much lower than those reported in the foreign and domestic literature, i.e. than the threshold values (Šovljanski et al. 1989), Table 2.

Tab. 1. Residues of pesticides and their metabolites in soils, μg/kg d.w.

Pesticide/metha-bolite	alfa-HCH	beta-HCH	Lindan	Alahlor	Heptahlor	Hlorpirifos	Aldrin	Heptahlor-epoksid	Hlordan	4,4-DDE	Dieldrin	4,4-DDD	Endrin-aldehid	4,4-DDT	Endrin
926 soil samples from Vojvodina Province, 1991															
Mean	0,99	1,09	4,12	3,56	n.d.	n.d.	2,3	0,99	3,82	2,59	2,04	2,47	1,36	5,71	3,57
Max.	16.4	47	48,12	0,02	n.d.	n.d.	96	48,91	109,5	105,2	96	120,5	68,78	302,1	248,05
Min.	0,29	0,01	0,55	467,7	n.d.	n.d.	0,01	0,15	0,02	0,29	0,01	0,01	0,01	1,4	0,01
19 soil samples from Novi Sad municipality, 2001. year															
Mean	0.22	0.20	6.61	1.91	n.d.	n.d.	3.08	n.d.	n.d.	25.2	7.58	4.09	7.53	24.4	n.d.
Max.	0.22	0.56	19.54	2.45	n.d.	n.d.	5.5	n.d.	n.d.	128.5	13.9	13.45	24.53	133.0	n.d.
Min.	0.22	0.15	0.83	1.3	n.d.	n.d.	0.61	n.d.	n.d.	0.55	1.24	0.36	0.95	0.56	n.d.

n. d. — not detected

The level of DDT and its metabolite was found to be higher in the urban soils of Novi Sad (19 samples, DDT + metabolites = 53.69 μg/kg) than in the agricultural soils (926 samples, DDT + metabolites = 10.77 μg/kg) (Table 1). This is probably a result of actions by uninformed individuals who continued to use DDT in their home gardens in excessive doses even after the substance was banned.

Tab. 2. Proposed maximum residue levels for DDT and Lindane in soil

Pesticide	Proposed MRL mg/kg
Lindane + metabolites	60
DDT + metabolites	100

Among the triazine herbicides that were studied, the most important were atrazine residues in the soil. Atrazine residues are analyzed on a regular basis at the Laboratory for Agroecology. Depending on precipitation and temperatures, the atrazine used for maize protection may be retained in the surface layer of the soil in a quantity phytotoxic to the following crop. The atrazine tests are necessary in droughty years and may prevent major losses. Table 3 shows the results of multiyear studies of atrazine residues in the soil. By comparing the results from Table 3 with the maximum tolerable levels for sensitive crops shown in Table 4, we can see that atrazine is very often present in our soils in phytotoxic concentrations.

Tab. 3. Atrazine residues content of soil

Year	Number of soil samples	Atrazine concentration
		mg/kg soil
1991	926	0.002—0.570
2000	4	0,03—0,08
2001	7	0,0005—0,018
2002	15	0,022—0,8

Tab. 4. Maximum residue levels for atrazine in soil for sensitive crops (Official Herald of RS 11, 239, 1990)

Crop	mg/kg soil
Alfalfa, rapeseed and sugarbeet	0.06—0.09
Oat, soybean, barley and cucumber	0.15—0.20
Sunflower	0.20—0.25
Wheat and rye	0.25—0.30
Potato, flax and onion	0.30—0.40
Brussels sprouts	1

Polycyclic aromatic hydrocarbons (PAHs)

The results of our study of PAH content at the Institute's experiment fields have been already published (P u c a r e v i ć et al., 2000). A total of 42 soil samples were studied. The average PAH content was 0.173 mg/kg, ranging from 0.056 to 1.022 mg/kg. Of the 16 PAHs analyzed in total, only four were found to be present — naphthalene, chrisene, fluorene and pyrene. The general conclusion was that no accumulation of by-products of combustion occurred at the Institute's fields due to favorable winds.

The results of the next PAH study are shown in Table 5. Analysed in the study was the PAH content of nonagricultural soils, soils in the municipality of Novi Sad (two-year results), and soils located near industrial facilities.

Tab. 5. Average total PAH content of the soil

Origin of soils	Year	Number of samples	Mean	Max.	Min.
			mg/kg		
Nonagricultural soils	2002	37	**0,83**	0,09	3,57
Urban soils of Novi Sad	2000	18	**4,55**	8,79	2,25
Agricultural soils	2001	50	**4,93**	7,89	1,85
Urban soils of Novi Sad	2001	19	**5,48**	8,26	2,44
Industrial soils	2002	11	**6,64**	37,05	0,89

The average PAH levels are presented in Table 5 in ascending order. As we can see, the levels of these compounds are the lowest in soils that are neither agricultural nor industrial. As the samples of nonagricultural soils were taken from nature reserves, where human activity is minimal, the PAH content of those soils was minimal as well. The agricultural soils (50 samples) tested in 2001 had more total PAHs than the soil from trial fields analyzed in 2000. This may have been a result of the use of agricultural machinery that requires large amounts of oil to run, but it also may have been due to the fact that the plots studied in 2001 were in closer proximity to roads. The results for the soils in the vicinity of industrial facilities indicate that industry is a major source of PAHs and that it contributes to soil pollution by these compounds. The highest PAH content among the industrial soils was found near an accumulator-manufacturing plant in Sombor.

Our study of soil PAH content continued as part of the national biotechnology program under the auspices of the Ministry of Science, Technology and Development of the Republic of Serbia as part of a project entitled *A Program of Soil Protection. Utilization and Management* lasting from 2000 to 2003. During the first year of research, agricultural soils of Vojvodina were studied. Table 6 shows the results of PAH content study for the purpose of characterization and management of soils to be used for the production of safe food.

Tab. 5. Average total PAH content in soil intended for safe food production

	Year	Area, ha	Number of samples	Mean	Max.	Min.
					mg/kg	
Potato	2002.	240	45	0,43	0,82	0,16
Vegetable crops		1200	199	0,50	1,23	0,16
Corn and wheat		370	159	0,51	0,70	0,36
Soybean and sunflower		316	49	0,40	0,70	0,35
MRL Guidelines for organic production, mg/kg soil Official herald of SRJ 51/2002				1		

The results of these studies showed that most of the soils were suitable for the production of safe food in accordance with organic production guidelines (Official herald of SRJ 51/2002). The only exceptions were three soil samples taken from sites near Novi Kneževac, which had increased total PAH levels.

CONCLUSION

— The levels of residues of persistent organochlorine pesticides and their metabolites found in the soil under study are such that they require no special measures to be taken

— The study of soil pesticide residues should be expanded to include study of the presence of new persistent compounds that are used in plant protection or in large quantities.

— The maximum atrazine concentrations found in the soil in our study were within the limits of phytotoxicity to sensitive crops, ranging from 0.0005 to 0.8 mg/kg.

— The level of DDT and its metabolite was found to be higher in the urban soils of Novi Sad (53.69 μg/kg) than in the agricultural soils (10.77 μg/kg).

— Total PAH content was lower in the nonagricultural soils (0.83 mg/kg) and higher in the urban soils (4.55 mg/kg and 5.48 mg/kg) and soils situated near industrial facilities and roads (6.64 mg/kg)

— Almost all of the PAHs tested for were found in the soils from the municipality of Novi Sad. In suburban areas and on the outskirts of the city, the PAH content was higher because of the use of conventional heating methods, which are the most common source of PAHs. The total PAH content was somewhat higher in 2001 than in 2000.

— The study of soil pesticide residues should be expanded to include study of the presence of PCBs, PCNs, PCDDs and PCDBs, since these compounds are present in the environment according to the literature.

REFERENCES

Ayaka, U., Queency, L. (1999): *Http:/qlink.queensu.cal/-4mql/PAH.html*, Queen's University, Kingston, ON, Canada.

Facchetti, S. (1993): *Mass spectrometry in the analysis of polychlorinated biphenyls*, Mass Spectrometry Reviews, 12, pp. 173—203.

Kannan, K., Yamashita, N., Imagawa, T., Decoen, W., Khim, S. J., Day, M. R., Summer, L. C. and Giesy, P. J. (2000): *Polychorinated Naphtahalenes and Polychlorinated Biphenyls in Fishes from Michigan Waters Including the Great Leaks*, Environ. Sci. Technol. 34, pp. 566—572.

Müller, S., Wilcke, W., Kanchanakool, N. (2001): *Polyciclic aromatic hydrocarbons (PAH) and polychlorinated biphenyls (PCB) in density fractions of urban soils in Bangkok, Thailand*, Soil Science 166, pp. 672—680.

Official Herald of RS 11, 239, 1990.

Official herald of SRJ 51/2002.

Pucarević, M., Hadžić, V., Sekulić, P. (2000): *Uticaj gorenja rafinerije Novi Sad na zemljište, II Policiklični aromatični ugljovodonici*, Eko-konferencija 2000, Novi Sad, pp. 123—128.

Šovljanski, R., Ostojić, Z. (1989): *Studija o jedinstvenim kriterijumima za registrovanje zagađivača i zagađenja voda, vazduha i zemljišta na području SAP Vojvodine i potrebne hitne mere zaštite na mestima sa najizraženijim zagađenjem. I Etapa, II faza, Deo zemljište*, Nosilac: Prof dr Radmila Šovljanski; PIV, Novi Sad, 1—37.

Verešbaranji, I., Šovljanski, R., Pucarević, M., Kastori, R., (1993): *Zagađenost zemljišta Vojvodine pesticidima i njihovim metabolitima. Monografija, Kastori R.,: Teški metali i pesticidi u zemljištu*, Poljoprivredni fakultet Institut za ratarstvo i povrtarstvo Novi Sad. str. 223—258.

Yamashita, N., Kannan, K., Imagawa, T., Miyazaki, A., Giesy, J. (2000): *Concentrations and Profiles of Polychlorinated naphthalene Congeners in Eighteen Technical Polychlorinated Bipheny Preparations*, Environ. Sci. Technol., 34, pp. 4236—4241.

Zou, Y. L., Zhan, W., Atkinskon, S. (2003): *The characterisation of polycyclic aromatic hydrocarbons emissions from burning of different firewood species in Australia*, Environmental pollution, 124. pp. 283—589.

ПОЛИЦИКЛИЧНИ АРОМАТИЧНИ УГЉОВОДОНИЦИ И ПЕСТИЦИДИ У ЗЕМЉИШТУ ВОЈВОДИНЕ

Мира М. Пуцаревић, Петар Ђ. Секулић
Научни институт за ратарство и повртарство Нови Сад,
Максима Горког 30, Србија и Црна Гора

Резиме

Органска једињења која се могу наћи у земљишту приказана су у овом раду. Такође је приказан и део вишегодишњих испитивања садржаја остатака пестицида и полицикличних ароматичних угљоводоника у земљишту изведених у Институту за ратарство и повртарство у Новом Саду. Испитано земљиште са територије Војводине садржи остатке перзистентних пестицида и њихових метаболита: линдан заједно са метаболитима 6,20 μg/kg, алахлор 3,56 μg/kg, алдрин 2,3 μg/kg, хептахлор епоксид 0,99 μg/kg, хлордан 3,82 μg/kg, ДДТ заједно са метаболитима 10,77 μg/kg, диелдрин 2,04 μg/kg, ендрин 3,57 μg/kg и ендрин алдехид 1,36 μg/kg. Земљиште са територије општине Нови Сад садржи остатке DDT-а заједно са метаболитима у количини од 53,69 μg/kg. Током вишегодишњих испитивања садржаја атразина у земљишту нађене вредности су се кретале у опсегу од 0,0005 mg/kg до 0,8 mg/kg. Нађени садржај полицикличних ароматичних угљоводоника је у индустријском земљишту 6,64 mg/kg, у пољопривредном земљишту 4,93 mg/kg, у земљишту на територији општине Нови Сад 4,55 mg/kg и 5,48 mg/kg, док је садржај на непољопривредно/неиндустријском земљишту најнижи и износи 0,83 mg/kg.

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Leka G. Mandić, Dragutin A. Đukić, Vladeta I. Stevović

Faculty of Agronomy Čačak, Cara Dusana 34,
32000 Čačak, Serbia and Montenegro

THE SOIL PROTEOLYTIC ACTIVITY AND ORGANIC PRODUCTION OF CORN UNDER THE CONDITIONS OF APPLYING DIFFERENT NUTRITION SYSTEMS

ABSTRACT: The effects of increasing amounts of mineral nitrogen (90, 120, 150 kg·ha[1]), liquid (80 t·ha^{-1}) and solid manure (45 t·ha^{-1}) those of inoculation with *Azotobacter chroococcum* (the strain 84) on the soil proteolytic activity and corn yield grown in monoculture were surveyed over the 3-year-long research work.

The research results indicated that proteolytic activity and corn yield depended highly on the fertiliser sorts, amounts and on the research year, too.

Over the first research year, the highest proteolytic activity was recorded on the variants with organic fertilisers, whereas the fertiliser stimulating effect markedly decreased, particularly with liquid manure over the following two years of the research. Mineral fertilisers were also found to remarkably stimulate the biological parameters we have been concerned above, particularly the mean N amount (120 kg · ha^{-1}). While the highest corn yield could be obtained using 120 kg N · ha^{-1} throughout all the research years, its insignificant rise and barely altered soil proteolytic activity using corn seed inoculation with 84 *Azotobacter chroococcum* could be noticed.

KEY WORDS: Azotobacter, corn, yield, liquid manure, mineral fertilisers, proteinase activity, soil, solid manure

INTRODUCTION

Of the whole array of agrotechnical steps taken (from the conventionally to the currently utilised ones) the timely and appropriately used fertilisation systems deserve mention for enabling their effects to come into being fully. The research trends in this field mainly tend to raise the yield of agricultural crops, whereas the basis of their cumulative impact (the changes in biological and in chemical properties of the soil) are often disregarded. Therefore, a rational and an efficient application of the mineral (particularly nitrogen) and organic fertilisers may be attained only if they are approached holistically with a significant role of the microbiological studies taken into consideration, too.

The number and the activity of the soil microorganisms, as the significant indices of the biological soil productivity, may indicate an economical justifiability of using differing fertiliser sorts and amounts (Đukić, Mandić, 2000, Acosta-Martinez and Tabatabai, 2000).

Narrowing the ratio C: N by introducing nitrogen fertilisers may exert and favour mineralization processes, thereby suiting the soil proteolytic activity as well as the amount of the available nitrogen compounds (Solovева et al., 2001). The level and the value of these processes primarily depends on the amount and the type of nitrogen fertilisers. However, the increased hydrolityc ability of the soil, brought about by N amount, may result in weakening of the soil physico-chemical properties, thereby leading to an array of serious environmental disruptions (Gostkowska et al., 1998), which suggests that such dimension of fertiliser application should be regarded with utmost care.

The issues put forth may be overcome by partial replacement of these fertilisers with the microbiological and organic ones. This will help improve the physico-chemical and biological soil properties, introduce certain amounts of other nutritional elements, phytohormones, enzymes and some useful microorganisms (Šlimek et al., 1999). In contrast, an uncontrollable utilisation of organic fertilisers, particularly that of liquid manure, may even exhibit certain undesirable effects on the biocenosis and on cultivated plants, as well (Đukić and Mandić, 1993).

The objective of the paper was, aided by a continual surveilance of the proteolytic activity, to establish the most optimal N fertiliser amounts as well as the possibilities of their replacement with the organic and microbiological fertilisers.

MATERIALS AND METHOD

The 3-year-long research was performed at the trial field of the Faculty of Agronomy, Cacak on the smonitza type of soil with its chemical characters outlined in tab.1.

Tab. 1. Agrochemical characteristics of the studied soil

Depth	pH		Humus %	N %	mg / 100 g	
	H_2O	nKCl			P_2O_5	K_2O
0—20 cm	6.12	5.01	2.68	0.134	2.9	26.4

Environmental characteristics of the area under way as being over the research period are given in Tabs. 2 and 3.

Tab. 2. Precipitation sum (l/m^2) over the period 1996—1998

Month	Year			$\overline{X}$ 1965—1994
	1996	1997	1998	
I	14,2	20,5	61,5	50,2
II	63,5	35,1	38,5	44,8
III	50,5	54,7	28,6	53,8
IV	61,4	64,8	34,0	57,8
V	138,3	45,8	68,8	88,6
VI (I faza)	46,5	22,9	58,8	98,2
VII	10,2	129,9	44,6	76,0
VIII (II faza)	38,8	116,3	45,4	59,5
IX	141,4	32,9	85,7	56,5
X (III faza)	54,2	111,7	112,9	47,8
XI	42,1	13,2	84,8	58,6
XII	99,7	80,3	42,7	57,6
Total	760,8	728,1	715,8	749,4

Tab. 3. Average monthly air temperatures (°C) over the period 1996—1998

Month	Year			$\overline{X}$ 1965—1994
	1996	1997	1998	
I	0,0	0,7	0,2	–3,3
II	–0,9	4,0	4,7	2,4
III	1,9	5,6	4,3	6,4
IV	11,2	6,7	13,6	11,5
V	17,5	17,0	15,5	16,2
VI	20,4	21,2	21,4	19,5
VII	21,4	20,9	23,0	20,9
VIII	21,7	19,6	22,6	20,5
IX	13,9	15,6	16,5	16,9
X	11,5	8,4	12,7	11,8
XI	8,2	7,4	3,9	5,8
XII	0,8	2,8	–3,0	1,5
Average	10,6	10,8	11,3	11,1

The trial was set up following the random-split-block design with four replications. The elementary plot amounted to 21.25 m^2, the inter-row 0.5 m and each block of 1 m spacing.

The corn hybrid NSSC-640, cultivated in the 3-year long monoculture, was utilised as a test plant over the research work.

The following fertilisation variants were surveyed: N1PK (90:75,60 kg · ha^{-1}), N_3PK (150:75:60 kg · ha^{-1}), solid manure (45 t · ha^{-1}), liquid manure (80 t · ha^{-1}) and inoculaation with *Azotobacter chroococcum* (the strain 84) — 1l/10 kg of seed. This strain of Azotobacter was obtained from the microorganism collec-

tion of the Microbiology laboratory of the Agriculture faculty in Novi Sad. The cell titration in the inoculum amounted to $40 \cdot 10^6$/ml.

These amounts of N as well as those of P and K and inoculum were added in the pre-sowing stage each year, while the organic fertilisers were introduced in the first research year with the aim of surveying their extended effects.

Proteolytic activity (R o m e i k o, 1969) was determined in edaphosphere over the three different corn growth vegetation stages (the intensive plant growth, milk-waxy and full maturity of corn).

Corn yield was determined in the stage of its full maturity, calculated to have 14% moisture.

The obtained results were worked out through the three-factorial trial analysis $7 \times 2 \times 3$ (fertiliser x sampling zone x vegetation stage) — for the purpose of microbiological analyses, i.e., the two-factorial trial 7×3 (agent × year) — for corn green matter.

RESULTS AND DISCUSSION

Analysing the experimental data (tab 4, 5 and 6), the proteolytic activity was found to highly significantly statistically depend on both, fertiliser sorts and on their concentrations (A), on the soil zone used for taking samples intended for analysis (B) and on the corn vegetation period (C).

In the first year of studies, the proteolytic activity increased throughout corn vegetation, which was in agreement with results of numerous authors (Kandeler, et al., 1990). This finding suggested higher dependence of the soil proteolytic activity on the plant root metabolitic activity and its lower dependence on the environmental factors existing over the plant vegetation.

The lower doses of the mineral (90 and 120 kg $N \cdot ha^{-1}$) and particularly organic fertilisers notably increased proteolytic activity, whereas the effect of the high N amount (150 kg $\cdot$ ha^{-1}) compared to its previously used amounts seemed to be insignificant, statistically (Tab. 4). This is in full agreement with results obtained by other authors, who emphasized a positive impact of both, organic and lower mineral fertiliser amounts on the majority of hydrolytic soil enzymes (G o m o r o v a, 1986, B l e c h a r c z y k et al., 1993, B e l i n s k a, 1999).

The corn seed inoculated with *Azotobacter chroococcum* (the strain 84) didn't significantly alter the soil proteolytic activity.

Further, over the whole research period, the activity of enzymes proved to be highly higher in the rhizosphere soil rather than being in the edaphosphere of the corn (tab. 4). Fertilisers causing no changes in differences level being reinstated. The obtained results infer metabolites to be of the plant and microbiological origin, showing higher dominance in the root system zone and visibly exerting the production and activity of the extra-cellular enzymes of the protease type (J a r a k et al., 1991).

Tab. 4. The average proteolytic activity (gel. units g^{-1} of soil) during 1996

A		Control		Azoto-bacter		N_1PK		N_2PK		N_3PK		Solid manure		Liquid manure		$\overline{X}$
B		Ed.	Rh.	Ed..	Rh.	Ed..	Rh.	Ed.	Rh.	Ed.	Rh.	Ed.	Rh.	Ed.	Rh.	
Periods I	I	24.0	26.0	24.3	26.0	29.0	29.3	30.0	40.0	35.0	41.2	40.0	42.0	28.0	30.5	31.81
	II	34.6	40.0	35.0	37.5	33.0	40.0	36.0	41.0	36.5	45.0	62.5	68.3	57.5	66.0	45.21
	III	60.0	70.5	59.0	70.0	76.0	95.0	72.8	75.0	70.0	71.3	85.0	90.0	80.0	81.5	75.43
$\overline{X}$		42.53		41.97		50.39		49.14		49.75		64.64		57.25		
$\overline{X}$	Edaphosphere									48.49						
	Rhizosphere									54.10						

L s d

Lsd	A	B	C	A×B	A×C	B×C	A×B×C
0.05	2.19	1.17	1.44	3.13	3.82	2.04	5.41
0.01	2.93	1.55	1.91	4.14	5.05	2.70	7.15

Ed. — Edaphosphere; Rh — Rhizosphere
A — fertilizers applied; B — sampling zone; C — vegetation period

Over the second year, the vegetation growth trend of proteolytic activity was established only in the first two periods of studies (Tab. 5), its fall recorded in the final vegetation phases of corn, as the consequence of an extreme soil humidity over that stage, which, on behalf of anaerobity, visibly decreased the number of microorganisms responsible for the production of numerous enzymes and subsequently of the proteolytic ones, too (E m t s e v and Đ u k i ć, 2000).

Tab. 5. The average proteolytic activity (gel. units g^{-1} of soil) during 1997

A		Control		Azoto-bacter		N_1PK		N_2PK		N_3PK		Solid manure		Liquid manure		$\overline{X}$
B		Ed.	Rh.	Ed.	Rh.	Ed.	Rh.	Ed.	Rh.	Ed.	Rh.	Ed.	Rh.	Ed.	Rh.	
Periods (C)	I	62.5	62.5	60.0	60.3	57.5	60.0	57.5	60.0	58.0	59.3	70.3	75.3	60.0	65.0	62.01
	II	50.0	70.3	67.6	65.3	67.5	68.0	70.0	73.0	62.3	68.0	70.3	75.0	52.5	65.3	66.07
	III	62.5	65.3	67.5	60.3	52.8	60.0	55.0	57.5	58.3	63.5	65.3	72.5	65.0	70.0	62.53
$\overline{X}$		62.19		61.86		61.21		62.16		61.61		71.47		62.97		
$\overline{X}$	Edaphosphere									60.68						
	Rhizosphere									64.93						

L s d

Lsd	A	B	C	A×B	A×C	B×C	A×B×C
0.05	1.01	0.53	0.65	1.45	1.76	0.93	2.49
0.01	1.34	0.71	0.86	1.91	2.33	1.23	3.30

Ed. — Edaphosphere; Rh — Rhizosphere
A — fertilizers applied; B — sampling zone; C — vegetation period

Unlike being in the previous year, in 1997, neither the three N amounts contained in N fertilisers nor the liquid manure had any effects on the soil activity. The results of some authors (Šćerbakova, 1983) indicated that, in conditions of high soil humidity and somewhat lower temperatures over the summer months, the application of mineral fertilisers was not always favoring soil biological activity. Compared with the preceding research year, the decline in proteolytic activity on the variant with liquid manure could be attributed to rather swift mineralisation processes having taken place in the first year of its introducing (Stevanović and Rakočević-Bošković, 2001). Additionally, the effect of corn seed initially inoculated with the strain 84 *Azotobacter chroococcum* was, similarly to the preceding year, insignificant statistically.

Excepting the variants with organic fertilisers, the last research year confirmed the soil proteolytic activity to be very much the same that recorded in 1996 (Tab. 6.). The fall in proteinase on the variants with organic fertilisers could be expected allowing for their remarkably lower value and amount compared with the year of their introducing. Accordingly, Šćerbakova (1983) pointed out that over the third year upon manure introducing, the enzyme processes proceeded rather slowly, which was clear-cut only in the variant with manure applied in large amounts (above 80 $t \cdot ha^{-1}$).

Tab. 6. The average proteolytic activity (gel. units g^{-1} of soil) during 1998

	A	Control		Azoto-bacter		N_1PK		N_2PK		N_3PK		Solid manure		Liquid manure		$\overline{X}$
	B	Ed.	Rh.	Ed.	Rh.	Ed.	Rh.	Ed.	Rh.	Ed.	Rh.	Ed.	Rh.	Ed.	Rh.	
Periods (C)	I	35.0	36.0	35.5	37.0	40.0	42.5	47.3	48.0	38.0	41.3	39.0	43.6	33.0	36.3	39.41
	II	41.0	43.6	39.8	47.0	52.3	52.0	50.2	51.5	40.2	43.3	40.6	44.0	48.6	43.6	45.58
	III	57.0	56.5	55.0	57.3	60.0	61.5	57.5	58.5	50.0	55.5	58.3	58.6	51.0	56.3	56.63
$\overline{X}$		44.86		45.22		51.33		52.08		44.67		47.50		44.77		
$\overline{X}$		Edaphosphere								46.14						
		Rhizosphere								48.28						

L s d

Lsd	A	B	C	A×B	A×C	B×C	A×B×C
0.05	1.98	0.99	1.31	2.81	3.46	1.84	4.89
0.01	2.62	1.31	1.73	3.72	4.58	2.44	6.47

Ed. — Edaphosphere; Rh — Rhizosphere
A — fertilizers applied; B — sampling zone; C — vegetation period

Statistically highly significant effect of the fertiliser types and amounts applied was manifested on the corn yield, too (Tab. 7).

Tab. 7. Maize corn yield (t·ha^{-1}) as affected by the fertilizers applied (1996—1998)

Fertilizers (A)	Year (B)			$\overline{X}$
	1996	1997	1998	
Control	6.69	13.76	6.59	9.01
Azotobacter	6.81	13.94	6.86	9.20
N_1	8.19	16.73	7.13	10.68
N_2	8.70	17.48	7.65	11.27
N_3	9.27	18.61	10.67	12.85
Solid manure	8.32	14.00	6.96	9.76
Liquid manure	8.57	13.91	6.97	9.82
$\overline{Y}$	8.08	15.49	7.55	10.37

Lsd	A	B	AB
0.05	0.74	0.52	1.32
0.01	0.99	0.69	1.78

A — fertilizers applied; B — sampling zone; C — vegetation period

The high amounts of N fertilisers used during 1996 brought about a pronounced increase in the corn yield compared with the control variant, as well as with that of 90 kg · ha^{-1} N, with no statistically significant differences found between N_2 and N_3. Also, in the same year and on the comparatively same level was exhibited the statistically highly higher yield on the variants with organic fertilisers. The achieved yields approximated that on the variants with mineral fertlisers.

Similarly to the previous year, during 1997, the highest yield was achieved on the N_3 variant, with no statistically significant differences found between the two nearly identical N concentrations, or none of them manifested among organic fertilisers, the use of which barely increased yield in relation to the control variant.

No significant impact of organic fertilisers was prolonged over 1998 just as being with their lower rates being utilised. Fairly high N amounts applied to this year, characteristic of the dry year conditions.

In general, the increase in N amounts up to the level of 150 kg · ha^{-1} cannot be considered to be purposeful economically and environmentally, particularly with respect to the microbiological characterization of soil (S t e v o v i ć, 2001). The impact of organic fertilisers on the attained corn yield was fully expected, as corroborated by the results of numerous authors (K a r l e n and C a m p, 1985).

In contrast to the treatments we have been concerned above, the pre-sowing inoculation of the corn seed with the strain 84 *Azotobacter chroococcum* was found to scarcely statistically exert corn yield increase all the three research years round unlike the results obtained by some other authors (G o v e d a r i c a et al., 1997). Such an effect can be attributed to the acid soil reaction, which inhibited Azotobacter growth to a lesser degree, but significantly reduced its energetic metabolism and nitrogenous activity, thereby affecting the corn green matter yield (W e r n e r, 1995).

CONCLUSION

The results of the 3-year-long researches conducted on the impact differing fertiliser amounts and sorts had on the soil proteolytic activity and corn yield infer the following conclusions:

— the soil proteolytic activity and corn yield were found to depend on the fertiliser rate and type being used, on the research year as well as on the soil zone used for sampling;

— over the first research year, proteolytic activity was the highest in the variants with organic fertilisers, whereas their stimulating effect visibly decreased over the second year, particularly with liquid manure;

— mineral fertilisers, particularly the mean N amount (120 kg · ha^{-1}) also benefited the activity of enzymes;

— corn seed inoculation with the *Azotobacter chroococcum* strain 84, didn't noticeably alter the soil proteolytic activity;

— the corn rhizosphere soil had a higher beogenity compared with that featuring the edaphosphere one all the three years round;

— the medium N amount (120 kg · ha^{-1}) rendered the most significant corn yield increase all throughout the three research years just as the organic fertilisers did over the first research year;

— statistically, the Azotobacter strain gave rise to no remarkable increase in corn yield;

— to end up with, under the existing environmental conditions, nitrogen use in the amount of 120 kg · ha^{-1} seemed to be the most appropriate rate used, which, along with solid manure may strongly be recommended for corn cultivation.

REFERENCES

Acosta-Martinez, V., Tabatabai, M. A. (2000): *Enzyme activities in a limed agricultural soil*, Biology and Fertility of Soils 31: 85—91.

Belinska, E. J. (1999): *Enzymatic activity and organic carbon content in orchard soil*, Humic substances in the environment 1 (3/4): 9—14.

Blecharczyk, A., Skrzypczak, G., Malecka, I. (1993): *Effect of long-term organic and mineral fertilization on chemical properties and enzymatic activity of soil. Long-term static fertilization experiments*, Warszawa — Krakow, 167—176.

Đukić, D., Mandić, L. (1993): *Uticaj tečnog svinjskog stajnjaka na mikrobiološku i enzimsku aktivnost zemljišta*, Savremena poljoprivreda, vol. 1, br. 6, 291—293.

Đukić, D., Mandić, L. (2000): *Microorganisms and Tehnogenic Pollution of Agroecosystem*, Acta Agriculturae Serbica, vol. 5, br. 9: 23—46.

Emtsev, V. T., Đukić, D. (2000): *Đubrenje i mikrobiološki procesi u zemljištu*, Mikrobiologija, Vojno izdavački zavod, Beograd, 517—527.

Gomorova, N. F., Zenova, G. M., Pivovarov, G. E., Širokaja, G. M. (1986): *Intensivnost mikrobiologičeskih processov v počve i urožajnost seljsko — hozjajstvennih kuljtur pri dliteljnom primenenii mineraljnih udobrenij*. Tez. respubl. konferencii „Mikrobiologičeskie processi v počvah i urožajnost seljsko-hozjastvennih kuljtur", Viljnus, 83 — 85.

Gostowska, K., Furczak, J., Domzal, H., Belinska, E. J. (1998): *Suitability of some biochemical and microbiological tests for the evaluation of the degradation degree of podzolic soil on the background of it differentiated usage*, Polish J. Soil Sci. 31 (2): 69—68.

Govedarica, M., Milošević, N., Jarak, M. (1997): *Biološka azotofiksacija u poljoprivredi: mogućnosti primene i perspektive*, Zbornik radova Naučnog instituta za ratarstvo i povrtarstvo, XXXI Seminar agronoma, 35—43, Novi Sad.

Jarak, M., Milošević, N., Govedarica, M., Jeličić, Z., Milutinović, V., Rekanović, M. (1991/92): *Aktivnost proteaze i ureaze u aluvijumu (fluvisol) i gajnači (eutrični kambisol)*, Arhiv za poljoprivredne nauke 52, 186, 115—122.

Kandeler, E., Tscherko, D., Spiegel, H. (1999): *Long-term monitoring of microbial biomass, mineralization and enzyme activities of a chernozem under different tillage menagment*, Biology ang Fertility of Soils, 28: 343—351.

Karlen, D. L., Camp, C. R. (1985): *Plant density, distribution and fertilizer effects on yield and quality of irrigated corn silage*, Commun. soil sci. and Plant Anal, 16, No1: 55—70.

Romejko, I. N. (1969): *Proteolitičeskaja aktivnost dernogo — podzolistoj počvi pri raznih sposobah vspaški*, Počvovedenij, 10: 87—90.

Soloveva, G. K., Pronko, V. V., Klimova, N. F., Jarošenko, T. M. (2001): *Aktivizacija fermentativnoj aktivnosti južnogo černozema mineralnim i bakterialnim udobrenijami*, Selskohozjajstvenaja mikrobiologija v XIX—XXI vekah, 14—19. 06. S. Peterburg, 37.

Stevanović, D., Bošković-Rakočević, Lj. (2001): *Aktuelni problemi i moguća rešenja u oblasti mineralne ishrane biljaka, kao nezaobilazne osnove u strategiji razvoja poljoprivrede*, Zimska škola za agronome, Vol. 5, br. 5: 3—10.

Šćerbakova, T. A. (1983): *Fermentativnaja aktivnost počv i transformacija organičeskogo veščestva*, Minsk, „Nauka i tehnika", 221 s.

Šlimek, M., Hopkins, D. W., Kalčik, J., Piecek, T., Šantručkova, H., Stana, J., Travnik, K. (1999): *Biological and chemical properties of arable soils affected by long-term organic and inorganic fertilizer applications*, Biology and Fertility of Soils, 29: 300—308.

Stevović, V. (2001): *Uticaj načina gajenja kukuruza, krmnog graška i ovsa na prinos i kvalitet krme i zrna*, Doktorska disertacija. Univerzitet u Kragujevcu, Agronomski fakultet Čačak, 133 s.

Werner, D. (1995): *Ecology and agricultural application of nitrogen fixing systems: crops and sciences involved*, Proc. 10th Int. Congr. On Nitrogen Fixation. St. Petersburg, May 28, June 3, 620—621.

ПРОТЕОЛИТСКА АКТИВНОСТ ЗЕМЉИШТА И ОРГАНСКА ПРОДУКЦИЈА КУКУРУЗА У УСЛОВИМА ПРИМЕНЕ РАЗЛИЧИТИХ СИСТЕМА ИСХРАНЕ

Лека Г. Мандић, Драгутин А. Ђукић, Владета И. Стевовић
Агрономски факултет Чачак, Цара Душана 34,
32000 Чачак, Србија и Црна Гора

Резиме

Током трогодишњих испитивања праћен је утицај растућих доза минералног азота (90; 120; 150 kg · ha^{-1}), течног (80 t · ha^{-1}) и чврстог стајњака (45 t · ha^{-1}) и инокулације са *Azotobacter chroococcum* (сој 84) на протеолитску активност земљишта и принос зрна кукуруза гајеног у монокултури.

Резултати истраживања показују да су протеолитска активност и принос зрна кукуруза значајно зависили од примењених врста и доза ђубрива, као и од године истраживања.

Највећа активност протеаза, током прве године истраживања, забележена је на варијантама са органским ђубривима, док се у друге две године стимулативни ефекат ових ђубрива значајно снижава, што се посебно односи на течни стајњак. Високу стимулацију наведених биолошких показатеља остварила су и минерална ђубрива, посебно средња доза азота (120 kg · ha^{-1}). У свим годинама истраживања, најзначајније повећање приноса кукуруза добијено је применом 120 kg N · ha^{-1}. Инокулација семена кукуруза са *Azotobacter chroococcum* сој 84, није значајно утицала на промену протеолитске активности земљишта и принос зрна кукуруза.

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Rudolf R. Kastori

Faculty of Agriculture, Trg Dositeja Obradovića 8,
Novi Sad, Serbia and Montenegro
Institute of Field and Vegetable Crops, Maksima Gorkog 30,
Novi Sad, Serbia and Montenegro

NITROGEN VOLATILIZATION FROM PLANTS

ABSTRACT: In plant nitrogen metabolism, a significant role is played not only by the uptake of nitrogen compounds but also by their release into the environment. One of the ways in which plant nitrogen is released is the volatilization of reduced and oxidized nitrogen forms through the above-ground plant organs. During the growing season, depending on plant species, genotype and environmental conditions, plants may release by volatilization a significant portion of their nitrogen uptake and up to 80 kg of ammonia per hectare. Besides releasing ammonia into the atmosphere, plants also take it up from the atmosphere and thus partially compensate for their ammonia losses by volatilization. These losses can be reduced by plant breeding by developing genotypes with reduced ammonia releases and a more effective reassimilation of the ammonia quantities released. Attempts have been made to reduce the volatilization of nitrogen compounds from the above-ground plant parts by applying physiologically active substances.

KEY WORDS: Nitrogen compounds, volatilization from plants, ammonia absorption from atmosphere, ecological factors

INTRODUCTION

Nitrogen is a highly important biogenic element for all living organisms. In most plants, it has the fourth highest contribution of all the chemical elements. The amount of nitrogen taken up by plants in the course of the growing season is higher than that found accumulated in their organs at the end of the season. It is therefore incorrect to equalize nitrogen amounts accumulated in biological yield with the total amount of nitrogen taken up during the growing season. Plants have long been known to take up as well as release (lose) nitrogen during growth and development. Nitrogen losses occur in a variety of ways: by the release of organic and inorganic compounds though the root (Egeraat, 1975; Janzen, 1990; Jenson, 1996), leaching from the above-ground plant parts by rain or dew (Tanaka and Navasero, 1964), guttation (Goatley and Lewis, 1966), shedding and dying of plant organs (Viets, 1965), or by volatilization from the surface of above-ground

plant parts into the atmosphere (Sharpe and Harper, 1997). The extent to which each of these methods of nitrogen release will contribute to total nitrogen losses depends primarily on plant species and environmental conditions. Data on nitrogen release by volatilization that can be found in the literature vary greatly. According to Manheim et al. (1997), values of nitrogen releases reported in the literature range from 0 to 81 kg per hectare during the growing season. In light of the fact that, depending on species, yield level and nitrogen content, plants accumulate in their biological yield between 100 and 150 kg N/ha on average, it becomes apparent that nitrogen losses by volatilization may account for a major portion of total nitrogen metabolism in cultivated plants. Francis et al. (1993) report that 30—40% of total nitrogen in maize is lost through ammonia volatilization. It is therefore understandable that this problem has been receiving a great deal of attention from the physiological, production and environmental points of view alike.

PHYSIOLOGICAL ASPECTS OF NITROGEN VOLATILIZATION

Through their above-ground organs, plants may release nitrogen into the atmosphere in the form of various gases, such as ammonia, amines, N, NO, N_2O, NO_2, and HCN (Feil, 1998). Besides this, nitrogen losses may also occur as a result of emission of other gases, such as acetaldehyde oxime, for instance (Mulvaney and Hageman, 1984). The results of previous studies indicate that nitrogen is released by volatilization primarily in the oxidized and reduced form. The amount of chemically bound nitrogen released by volatilization has been determined by different procedures, such as using water transpiration (Stutte et al., 1979), using ^{15}N (Sharpe and Harper, 1997), ammonia absorption using an impregnated filter with oxalic acid (Francis et al., 1997), and others. Release of ammonia has been studied in most detail. Ammonia release by plants is thought to be connected with reactions producing NH_3. The most important plant processes producing NH_3 are NO_3^- reduction, NH_4^+ uptake by root, photorespiration, deaminization and protein metabolism (Holtam-Hartwig and Bockman, 1994). According to Yamaya and Oaks (1987), plant C_3 photorespiration produces up to 10 times as much ammonia as nitrate reduction. The dependence of ammonia release on light and O_2 concentration attests to the great role photorespiration and the glycolate cycle play in ammonia release by leaves (Weinland and Sutte, 1985; Holtam-Hartwig and Bockman, 1994). Ammonia emission can be increased if enzymes catalyzing the reassimilation of ammonia that has been released are inhibited. Based on this, it can be surmised that the efficacy of ammonia reassimilation plays the key role in nitrogen losses by volatilization. Schjorring et al. (1993) found that ammonia release in C_3 plants had a diurnal character, as it peaked around noon and reached a low or completely ceased at night. In C_4 plants, in which photorespiration plays a subsidiary role compared with other processes producing NH_4, NH_3 emission does not have a strictly diurnal nature, since it only increases slightly around midday (Francis et al., 1997).

Key roles in ammonia assimilation in C_3 and C_4 plants are played by glutamine synthetase (GS) and glutamate synthase (GOGAT). The GS-GOGAT cycle can be found in chloroplasts too. Ammonia found in the chloroplast stroma is thought to arrive there by diffusion through chloroplast membrane without the participation of the membrane translocator (Baron et al., 1994). The partial pressure of ammonia in cell organelles depends on processes by which it is produced and changes with ammonia concentration in cell sap. This enables diffusion from organelles. If there is no rapid reassimilation of ammonia produced by the GS-GOGAT cycle, the ammonia diffuses into intercellular spaces and is released into the atmosphere. The possibility that plants release ammonia along with water released by transpiration cannot be excluded, either (Weinland and Stutte, 1980). In an earlier paper, the same authors reported that temperature had different effect on transpiration intensity and nitrogen losses, which led them to conclude that the two processes took place independently (Stutte and Weiland, 1978).

IMPORTANCE OF EXTERNAL FACTORS

The intensity of volatilization of nitrogen compounds from above-ground plant parts depends on environmental factors as well. Temperature in particular has an especially large influence. Stutte and Silva (1981) found that nitrogen volatilization in rice rose significantly when temperature increased from 30 to 35°C, especially in genotypes sensitive to temperature changes. Those authors assumed that nitrogen loss by volatilization was a form of defense mechanism by which plants protected themselves under stress conditions from the toxic effects of accumulated ammonia. Temperature stress in plants reduces protein synthesis and increases protein decomposition, thereby increasing the rate of ammonia formation. It also reduces nitrate accumulation and increases the accumulation of reduced nitrogen compounds (Kastori and Petrović, 2003). Their transformation into amides and amino acids and the reoxidation of the reduced forms of nitrogen are further mechanisms besides volatilization for the release of accumulated ammonia. Increased temperature significantly increased nitrogen release by volatilization in soybean too (Weiland and Stutte, 1980).

Ammonia volatilization also depends on plant nitrogen supply. When plant nitrogen supply is good, and especially when it is particularly abundant, ammonia release increases (Holtan-Hartwig and Bockman, 1994). According to Silva and Stutte (1980), increased nitrogen concentration in the nutrient solution did not increase nitrogen loss per leaf unit area in the newly fully developed leaves but only in the older ones. The reduced nitrogen losses by volatilization in plants with insufficient nitrogen supply is attributed first and foremost to the reduced activity of nitrogen metabolism enzymes (Petrović and Kastori, 1990).

It can be rightly assumed that stress conditions, water deficits, deficits of biogenic elements, and, especially, toxic concentrations of heavy metals as well as other factors, all of which affect nitrogen metabolism directly or indi-

rectly, also have an effect on nitrogen volatilization through the above-ground plant parts.

Nitrogen volatilization in plants depends also on a number of internal factors, most notably plant species and genotype. Stutte and Weinland (1978) studied volatilization in four cultivated and seven weed species and found volatilization and transpiration to have been higher in the former than in the latter. It has been determined that soybean releases at least 45 kg N/ha during its growing season. Harper and Sharpe (1995) found very little ammonia volatilization in maize (only 4 kg N/ha), while Francis et al. (1993) reported finding a significantly higher value — 23 kg N/ha released during the growing season.

Stutte and Silva (1981) found significant differences in nitrogen volatilization among rice cultivars. Cultivars with a short growing season released less N per dm^2 leaf area than those with a growing season of medium length. Husted et al. (1996) reported finding significant differences in ammonia volatilization among barley genotypes.

Ammonia volatilization occurs in all stages of plant growth and development. It has been reported during vegetative growth (Harper and Sharpe, 1995), at the reproductive stage (Morgan and Parton, 1989), and at the end of the growing season (Schjoring, 1991). In most cultivated plants, volatilization of nitrogen compounds is at its most intensive during the reproductive stage of plant growth and development (Harper et al., 1987; Holtan-Hartwig and Bockman, 1994).

UPTAKE OF ATMOSPHERIC AMMONIA

Plants release ammonia into the atmosphere, but they take it up from the atmosphere as well (Hutchinson et al., 1972; Denmead et al., 1976; Lemon and Van Houtte, 1980). The uptake of atmospheric ammonia helps plants compensate for some of the ammonia they release in the course of the growing season (Sharpe and Harper, 1997). According to Harper et al. (1987 and 1996), the uptake of atmospheric nitrogen by the above-ground plant parts is higher when plant nitrogen supply is insufficient and there is a high concentration of ammonia in the atmosphere. Denmead et al. (1976) found that ammonia uptake by a grass-clover mixture increased when the source of ammonia was close to or within the surface layer of the soil. Francis et al. (1997) suggest that there is an active exchange of ammonia between maize crops and the atmosphere during the reproductive stage. Based on this, it has been assumed that free ammonia cycling is an major component of metabolic processes taking place in maize plants during the reproductive stage of plant growth and development. The same authors underscore plant life's significant potential in the release of ammonia into the atmosphere and its uptake from the atmosphere. Denmead et al. (1978) report there is significant descendent transport of ammonia into maize crops.

The intensity of ammonia uptake by plants depends on the season as well. When environmental conditions are favorable for crop growth and deve-

lopment, the uptake of ammonia is greater (Harper et al., 1983). Ammonia uptake is therefore at its most intensive in the spring and summer.

A lot is still unclear about the mechanism of ammonia uptake by the above-ground plant parts. According to Cowan and Milthorpe (1968), ammonia uptake during the night surpasses ammonia release, in spite of the stomata being closed. Ammonia has been found to keep the stomata open and reduce stomatal resistance (Rogers and Aneja, 1980). The pH value is considered to play an important role in ammonia absorption and desorption from the liquid phase (Vlek and Stumpe, 1978).

CONCLUSIONS

For maximum utilization of the genetic potential for yield of a genotype, it is important to know its requirement for biogenic elements. What needs to be understood is that plants parallelly take up biogenic elements and release them into the environment, so their quantity accumulated in biological yield is smaller than the overall amount taken up during the growing season. Plants release nitrogen into the environment in the form of ions and organic nitrogen compounds and by volatilization in oxidized and reduced forms. Knowledge of plant nitrogen volatilization is equally important from the physiological, environmental and economic points of view, since this form of nitrogen loss during the growing season may account for a significant portion of the total nitrogen uptake. Values reported in the literature range from 0 to 81 kg N per hectare during the growing season. This also suggests that nitrogen volatilization may considerably alter the picture of how nitrogen from nitrogen fertilizers is utilized, especially if nitrogen utilization is calculated based on the total amount of nitrogen accumulated in biological yield. The problem becomes much more complex if we take into account the fact that plants take up atmospheric ammonia and thus reintroduce into their metabolism some of the nitrogen they have released.

Previous studies have shown that the intensity of nitrogen volatilization varies according to plant species and genotype and can be significantly influenced by environmental factors. It is thought that nitrogen losses by volatilization can be reduced by breeding, specifically by developing genotypes with diminished ammonia release and a more effective reassimilation of the ammonia emitted. This would improve the utilization of nitrogen uptake and hence favorably affect not only yield levels but also the protein contribution in the product, as nitrogen volatilization is particularly intensive at the yield formation stage.

In view of the fact that the amount of plant nitrogen released by volatilization is often greater than expected and may represent a major item in total plant nitrogen exchange, this problem should in the future receive more attention, especially in the sense of developing genotypes that are better able to utilize their nitrogen uptakes.

REFERENCES

Baron, A. C., Tobin, T. H., Wallsgrove, R. M., Tobin, A. K. (1994): *A metabolic control analysis of the glutamine synthetase-glutamate synthase cycl in isolated barley chloroplasts*, Plant Physiol., 105, 415—424.

Cowan, I. R., Milthorpe, P. L. (1968): *Plant factors influencing the water status of plant tissues*. I. Koylowski (ed.) *Water deficits and plant growth*. Academic Press, New York.

Denmead, O. T., Freney, J. R., Simpson, J. R. (1976): *A closed ammonia cycle within a plant canopy*, Soil Biol. Biochem., 8, 161—164.

Denmead, O. T., Nulsen, R., Thurtell, G. W. (1978): *Ammonia exchange over corn crop*, Soil. Sci. Soc. Am. J., 42, 840—842.

Egeraat, A. W. (1975): *Exudation of ninhydrinpositive compounds by pea-seedling roots. A study of the sites of exudation and of the composition of the exudate*, Plant Soil. 42, 37—47.

Feil, B. (1998): *Physiologische und pflanzenbauliche Aspekte der inversen Beziehung zwischen Ertrag und Proteinkonzentration bei Getreidesorten. Ein Übrsicht*, Pflanzenbauwissenschaften, 2, 37—46.

Francis, D. D., Schepers, J. S., Sims, A. L. (1997): *Ammonia Exchange from Corn Foliage during Reproductive Growth*, Agron. J., 89, 941—946.

Francis, D. D., Schepers, J. S., Vigil, M. F. (1993): *Postanthesis nitrogen losses from corn*, Agron, J., 85, 659—663.

Francis, D. D., Schepers, J. S., Vigil, M. F. (1997): *Post-anthesis nitrogen loss from corn*, Agron. J., 85, 659—663.

Goatley, J. L., Lewis, R. W. (1966): *Composition of Guttation Fluid from Rye, Wheat and Barley Seedlings*, Plant Physiol. 11, 373—375.

Harper, L. A., Catchpoole, V. R., Davis, R., Weier, K. L. (1983): *Ammonia volatilization. Soil, plant, and microclimate effects on diurnal and seasonal fluctuations*, Agron. J., 75, 212—218.

Harper, L. A., Bussing, D. W., van der Meer, H. G., Corre, H. J. (1996): *Ammonia transport in a temperate grassland. I. Seasonal transport in relation to soil fertility and crop management*, Agron. J., 88, 614—621.

Harper, L. A., Sharpe, R. R. (1995): *Nitrogen dynamics in irrigated corn. Soil--plant nitrogen and atmospheric ammonia transport*, Agron. J., 87, 669—675.

Harper, L. A., Sharpe, R. R., Langdale, G. W., Giddens, J. E. (1987): *Nitrogen cycling in a wheat crop. Soil, plant and aerial nitrogen transport*, Agron. J., 79, 965—973.

Holtman-Hartwig, L., Bockman, O. C. (1994): *Ammonia exchange between crops and air*, Norw. J. Aric. Sic., Suppl. 14.

Husted, S. M., Mattson, M., Schjoerring, J. K. (1996): *Ammonia compensation points in two cultivars of Hordeum vulgare L. during vegetative and generative growth*, Plant Cell, Environm., 19, 1299—1306.

Hutchinson, G. L., Millington, R. J., Peters, D. B. (1972): *Atmospheric ammonia. Absorption by plant leaves*, Science, 175, 771—772.

Janzen, H. H. (1990): *Deposition of nitrogen into the rizosphere by wheat roots*, Soil Biol. Biochem., 22, 1155—1160.

Jenson, E. S. (1996): *Rhizodeposition of N by pea and barley and its effect of soil N dynamics*, Soil Biol. Biochem. 28, 65—71.

Kastori, R., Petrović, N. (2003): *Nitrati u povrću — fiziološki, ekološki i agrotehnički aspekti*, Naučni institut za ratarstvo i povrtarstvo, Novi Sad.
Lemon, E. R., Van Houtte, R. (1980): *Ammonia exchange at the land surface*, Agron. J., 72, 876—883.
Mannheim, T., Braschkat, J., Marschner, H. (1997): *Ammoniakemissionen aus alternden Pflanzen und bei der Zersetzung von Ernterückstaenden*, Z. Pflanzenernaer. Bodenk. 160, 125—132.
Morgan, J. A., Parton, W. J. (1989): *Characteristics of ammonia from spring wheat*, Crop Sci., 28, 726—731.
Mulvaney, C. S., Hageman, R. H. (1984): *Acetaldehyde oxime, a product formed during the in vivo nitrate reduktase assay of soybean leaves*, Plant Physiol., 76, 118—124.
Petrović, N., Kastori, R. (1990): *Nitrat reductase in sugar beet genotypes suplied with different nitrate levels*. 51—55. In: Bassam, N. E. L. et al. (eds.) *Genetics aspects of plant mineral nutrition*. Kulwer Academic Publishers, Netherlands.
Rogers, H. H., Aneja, V. P. (1980): *Uptake of atmospheric ammonia by selected plant species*, Environ. Exp. Bot., 20, 251—257.
Schjorring, J. K. (1991): *Ammonia emissions from the foliage of growing plants*, 267—292. In: Sharkey, T. D. et al. (eds.) Trace gas emissions by plants. Academic Press, San Diego.
Schjorring, J. K., Kyllingsbaek, A., Mortensen, J. V., Byskov--Nielsen, S. (1993): *Field investigations of ammonia exchange between barley plants and the atmosphere. I Concentration profiles and flux densities of ammonia*, Plant Cell Environ., 16, 161—167.
Schjorring, J. K., Kyllingsbaek, A., Mortensen, J. V., Byskov--Nielsen, S. (1993): *Field investigations of ammonia exchange between barley plants and the atmosphere. I Concentration profiles and flux densities of ammonia*, Plant Cell Environ., 16, 161—167.
Sharpe, R. R., Harper, L. A. (1997): *Apparent Atmospheric Nitrogen Loss from Hydroponically Grown Corn*, Agron. J., 89, 605—609.
Silva, P. R. F., Stutte, C. A. (1980): *Nitrogen loss in conjunction with transpiration from rice leaves as influenced by growth stage, leaf position, and N supply*, Agron, J., 73, 38—42.
Stutte, C. A., Weiland, R. T. (1978): *Gaseous Nitrogen Loss and Transpiration of Several Crop and Weed Species Crop Science*, 18, 887—889.
Stutte, C. A., Weiland, R. T. (1978): *Gaseous Nitrogen Loss and Transpiration of Several Crop and Weed Species Crop Science*, 18, 887—889.
Stutte, C. A., Weiland, R. T., Blem, A. R. (1979): *Gaseous nitrogen loss from soybean foliage*, Agron. J., 71, 95—97.
Stutte, C. A., Da Silva, P. R. F. (1981): *Nitrogen volatilization from rice leaves*, I. Effects of genotype and air temperature, Crop Sci., 21, 596—600.
Tanaka, A., Navasero, S. A. (1964): *Loss of nitrogen from the rice plant through rain or dew*, Soil. Sci. Plant Nutr., 10, 36—39.
Viets, F. G. Jr. (1965): *The plants need for and use of nitrogen*. In: Bartholomew, W. V. and Clark, F. E. (eds) Soil nitrogen. Agronomy, 10 503—549, Am. Soc. Agron Madison, Wis.

V l e k, P. L. G., S t u m p e, J. M. (1978): *Effects of solution chemistry and environmental conditions on ammonia volatilization losses from aqueous systems*. Soil Sci. Soc. Am. J., 42, 416—421.
W e i l a n d, R. T., S t u t t e, C. A. (1980): *Concomitant determination of foliar nitrogen loss, net carbon dioxide uptake, and transpiration*, Plant Physiol., 65, 403—406.
W e i n l a n d, R. T., S t u t t e, C. A. (1985): *Oxygen influence on foliar nitrogen loss from soybean and sorghum plants*, Ann. Bot., 55, 279—282.
Y a m a y a, T., O a k s, A. (1987): *Synthesis of glutamate by mitochondria. An anaplerotic for glutamate dehydrogenase*, Physiol Plant., 70, 749—756.

ВОЛАТИЛИЗАЦИЈА АЗОТА КОД БИЉАКА

Рудолф Р. Кастори
Пољопривредни факултет, Трг Доситеја Обрадовића 8,
Нови Сад, Србија и Црна Гора
Научни институт за ратарство и повртарство,
Максима Горког 30, Нови Сад, Србија и Црна Гора

Резиме

У промету азота биљака поред усвајања значајну улогу има и одавање азотних једињења у спољашњу средину. Један од видова одавања је волатилизација редукованих и оксидисаних облика азота преко надземних органа. Зависно од биљне врсте, генотипа и еколошких услова волатилизацијом биљке у току вегетације могу да одају значајни део усвојене количине азота, и до 80 kg амонијака/ha. Биљке поред тога што одају амонијак у атмосферу истовремено из атмосфере усвајају и тиме делимично надокнађују губитак настао волатализацијом. Губици азота волатализацијом могу се смањити оплемењивањем, стварањем генотипова код којих је одавање амонијака мање, а реасимилација одатог амонијака ефикаснија. Постоје покушаји да се волатализација једињења азота са надземних органа биљака смањи применом физиолошки активних материја.