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GENETIC DIFFERENTIATION OF THE SAND LIZARD POPULATIONS, *LACERTA AGILIS* LINNAEUS, 1758 (REPTILIA, LACERTIDAE) IN CENTRAL EUROPE*

ZRÓŻNICOWANIE GENETYCZNE JASZCZURKI ZWINKI, *LACERTA AGILIS* LINNAEUS, 1758 (REPTILIA, LACERTIDAE) W EUROPIE ŚRODKOWEJ

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Eight populations of the sand lizard (*Lacerta agilis*) from Poland (allozymes and cytochrome b gene sequences) and 18 populations from Poland, Slovakia, Austria, Hungary, Croatia, Italy, France and Russia (dloop region sequence) were studied. The level of allozyme polymorphism is very low. It was only found in two loci of two enzyme systems (MOD and PGD) among the 8 loci studied and only in two populations from North-Eastern and Central-Eastern Poland. Bayesian Inference and additional phylogenetic analyses basing on cytochrome b gene and dloop region indicate clearly that haplotypes from these two populations form a separate clade. The study confirms the homogenity of sand lizard populations in Central Europe (*L. agilis argus*) except for populations from NE and E of Poland (*L. agilis chersonensis*). Dloop analysis suggests the position of sand lizard from Croatia as *L. agilis bosnica*.

KEY WORDS: *Lacerta agilis*, population genetics, allozymes, mtDNA, cytochrome b, dloop, phylogenetics, Central Europe

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INTRODUCTION

The sand lizard (*Lacerta agilis* Linnaeus, 1758) is an Eurasian species with a very large range. According to the most commonly accepted hypothesis, *Lacerta agilis* as a species first appeared in the Caucasus, an important centre of speciation of palearctic forms, at the end of the miocene or at the beginning of the pliocene (Peters 1958,Yablokov 1976, Yablokov et al. 1980).

According to Bischoff (1988, 1991) 9 recogniseable subspecies exist, which may be divided into 2 groups: western (Balkan) and Eastern (Caucasian). He argues that the differences between these groups are greater than the level of differences traditionally understood to define subspecies. On the other hand, these are not separate species due to the large hybridization zone (occupied by members of *Lacerta agilis chersonensis* and *Lacerta agilis exigua* groups).

The position of Central European and especially Polish populations of sand lizard in this classification has not been clarified yet. The small number of studies on the morphological features of this species have indicated that the populations from the North--East regions of Poland differ from populations from other regions of the country (Bischoff 1970, Maślak 1996). According to Bischoff (1984, 1988), *Lacerta agilis argus* inhabits the vast majority of Central Europe, while *Lacerta agilis chersonensis* appears in East Europe and its range in Poland covers only eastern part of country. The morphological differences which formed the basis of separating *L.a.argus* from the nominative subspecies have been criticised by other authors (Rahmel 1988, Arribas 2001). However, genetic studies have tended to indicate that these forms are separate (Kalyabina et al. 2001, Kalyabina-Hauf and Ananyeva 2004).

The contact zone between *Lacerta agilis argus* and *Lacerta agilis chersonensis* has been given by some of these authors as the North-East of Poland (Yablokov 1976, Yablokov et al. 1980) or the whole of the East of Poland (Bischoff 1970, 1981, 1984, 1988). Obst (1980) argues that *Lacerta agilis chersonensis* appears along the Baltic coast as far as North-East Germany. Unfortunately, these inferences were based on morphological studies involving a small number of specimens (mainly from Mazury and the Białowieża forest). Najbar (1995) states that *Lacerta agilis chersonensis* may appear in South-East Poland, but this hypothesis has never been confirmed. Juszczyk (1987) argues that the nominative subspecies inhabits the whole Polish territory.

The aim of this article is to describe and analyse the differences between sand lizard populations in Central Europe with special emphasis on populations from Poland. Our study based on the analysis of two fragments of mtDNA sequences and the electrophoresis of five allozyme systems.

MATERIALS AND METHODS

Material

Samples from 8 populations from different regions of Poland and 10 populations from other parts of range, mainly from Central Europe, were used (Fig. 1). Sequencing of mtDNA was carried on one specimen from each population and allozyme electrophoresis on 15 specimens from each population (Tab. 1 and 2). The *Lacerta bilineata* from France was used as an outgroup in phylogenetic analyses.

Fig. 1. Map of the sampled localities of the *L. agilis* species. Numbers as explained in Table 1
Rys. 1. Rozmieszczenie badanych populacji *L. agilis*. Numery lokalizacji wyjaśnione w tabeli 1

Table 1
Tabela 1

Localities of the studied samples of *L. agilis* with GenBank accesion numbers. As an outgroup *Lacerta bilineata* was used (GenBank accesion numbers: cytochrome b – AF248006, dloop – EU541212)
Lokalizacje badanych populacji *L. agilis* z numerami akcesji w GenBank'u. Jako grupa zewnętrzna była wykorzystana *Lacerta bilineata* (numer akcesji: cytochrom b – AF248006, dloop – EU541212)

Population No.	Locality	Country	cytochrome b	dloop
1	Świnoujście	Poland	EU497988	EU497976
2	Godziszewo near Gdańsk	Poland	EU497984	EU497972
3	Woszczele near Elk	Poland	EU497985	EU497973
4	Mosina near Poznań	Poland	EU497986	EU497974
5	Międzyrzec Podlaski	Poland	EU497987	EU497975
6	Zgorzelec	Poland	EU497983	EU497971
7	Młynek near Częstochowa	Poland	EU497989	EU497977
8	Ustrzyki Dolne	Poland	EU497990	EU497978
9	Kolonica	Slovakia	–	EU497981
10	Botany	Slovakia	–	EU497980
11	Debrecen	Hungary	–	EU497968
12	Acsalag	Hungary	–	EU497969
13	Podersdorf	Austria	–	EU497982
14	Tarvisio	Italy	–	EU497970
15	Plitvice	Croatia	–	EU497967
16	Paris	France	–	AF290392
17	Rostov-on-Don	Russia	EU497991	EU497979

Table 2
Tabela 2

The studied enzyme systems of *L. agilis*
Systemy enzymatyczne badane u *L. agilis*

Enzyme system		EC#	Skeletal muscle loci*	Allozyme subunits
MOD (ME)	Malic enzyme	1.1.1.40	*Mod-1* *Mod-2*	4
LDH	Lactate dehydrogenase	1.1.1.27	*Ldh-1* *Ldh-2*	4
MDH	Malate dehydrogenase	1.1.1.37	*Mdh-1* *Mdh-2*	2
CK	Creatine kinase	2.7.3.2	*Ck-1*	2
PGD	Phosphoglukonate dehydrogenase	1.1.1.44	*Pgd-1*	2

* The numbers of loci and enzyme subunits have been determined from electrophoretic band patterns
* Liczba loci i podjednostek enzymatycznych została określona na podstawie wzorów prążkowych

Laboratory protocols and phylogenetical analyses of mtDNA

The tail fragments used for DNA sequencing were conserved in 95% ethanol. The extraction and amplification of the DNA was carried out according to the procedure of Estoup et al. (1996). For amplification of cytochrome b we used primers: MVZ 04 (H14542) 5'-GCAGCCCCTCAGAATGATATTTGTCCTC-3' and MVZ 05 (L14115) 5'-CGAAGCTTGATATGAAAAACCATCGTTG-3' (Smith and Patton 1991). Primers for dloop were DL3F 5' GGCCTCTGGTTAATGGGTTAGTTAC-3' andDL4R 5'-AATTGTTGGTAGGGGGGTAGG-3' (Crochet et al. 2004).

DNA was sequenced in both directions using the BigDyeTM kit produced by Perkin-Elmer-Biosystems according to the manufacturer instructions an ABI 310 sequencer (Perkin Elmer Biosystems). Sequences were edited and aligned with the Sequencher software (Gene Codes Corporation). The basic sequence characteristics and statistics were calculated with the SeqState software (Müller 2005) and uncorrected p-distances with help of PAUP 4.0b10 (Swofford 2003).

As the most important phylogenetic method the model based Bayesian Inference was adopted (Huelsenbeck and Ronquist 2001, Huelsenbeck et al. 2002). Data partitions were chosen a priori depending on the gene identity (two partitions: cytb i dloop). The models of nucleotide substitutions for Bayesian analyses were selected individually for each gene partition using MultiPhyl (Keane et al. 2007) and Bayesian Information Criterion (BIC) as well as Akaike Information Criterion. The same models and parameter settings chosen for the individual genes (separate analyses) were also used for these partitions in the analysis of the combined data set (total evidence analysis). For particular partitions the following models suggested by majority of tests: cytb – HKY+G and dloop – TrN+I were chosen. The TrN+I model is not included in MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001) and it was replaced in subsequent analyses with more simple HKY+I model.

All analyses were performed using the same logic and procedures of Bayesian Inference as implemented in MrBayes 3.1.2. To reduce the chance of reaching apparent stationarity on local optima, two separate analyses consisting of four Markov chains were performed (in every case three chains were cold and one heated, as a default in MrBayes). Each chain was performed by $2x10^7$ generations and was sampled every 1000 generations. The assumptions were congruent with the default settings. Stationarity and convergence of analyses were estimated by default MrBayes statistics and graphically in Tracer (Rambaut and Drummond 2007). The burn-in trees and parameters were discarded (50000 samples or $5x10^6$ generations in every case) and the remaining trees and associated parameters were saved, with the frequency of clades representing estimation of posterior probabilities on bayesian consensus 50% majority rule tree (BC). The Bayesian analysis was completed for three data matrix variants. The first analysis was based on partitioned matrix from two sequenced fragments, the second spanned only dloop fragment and the third only cytb.

As a supporting phylogenetic analysis the Maximum Parsimony (PAUP4b10) was used. The shortest MP tree (SMP) was calculated with the exact algorithm "branch and bound". The bootstrap 50% majority rule MP tree on the basis of 1000 "branch and bound" pseudoreplications was constructed.

The split network (MPS) using SplitsTree4.8 (consensus network, threshold=0.1, edge weights calculated as means, EqualAngle algorithm) was constructed (Huson and Bryant 2006) to identify the area of phylogenetic uncertainty in the reconstruction.

Allozyme electrophoresis

The skeletal muscles used as samples were stored at a temperature of approximately -35°C. All the allozymes were identified using vertical polyacrylamide gel electrophoresis (PAGE). The experimental protocol followed Pasteur et al. (1988) with substantial modifications.

Eight loci coding for five allozyme systems were selected for final analysis (Tab. 2). The loci and their alleles are identified by symbols in accordance with Shaklee et al. (1990) and Utter and Sebb (1990). The allele with the highest anodal mobility for a given locus was defined to be allele 100. For allozyme systems with more than one locus, the locus with the highest anodal mobility is referred to as locus 1. Calculations were carried out using the TFPGA 1.3 package (Miller 1997). The number of alleles per locus, allele frequencies and genotype frequencies were calculated for each of the populations, together with the observed, expected and unbiased estimator of expected heterozygosity (H_0, H_e, H_u) and the proportions of polymorphic loci $P_{0.95}$ and $P_{0.99}$ (Nei 1987). The mean heterozygosity averaged over all the samples from these populations reflect the heterozygosity of the species and the percentage of polymorphic loci averaged over all these samples measures its degree of polymorphism.

Interpopulation genetic variation was estimated using the Weir-Cockerham method based on all the loci and a reduced set of loci using the jackknife method (Weir and Cockerham 1984). Interpopulation diversity was estimated using Nei's genetic distance (D), using both the original measure and the version for small samples (Nei 1978, 1987), and Rogers' genetic distances (Rogers 1972, Wright 1978). UPGMA dendrograms were constructed from these distances (Sneath and Sokal 1973) using NTSYSpc 2.02 (Rohlf 1998).

RESULTS

mtDNA

Sequences of the fragments of cytochrome b gene of a length of 429 base pairs and dloop of a length of 451 base pairs were obtained. The sequence statistics are compared in Table 3.

Uncorrected p-distance for both studied mtDNA sequences and between majority of Polish populations is equal 0.0. Only for cyt b p-distances between Miedzyrzec or Woszczele and any of remaining Polish populations reach 0.012 - 0.014. Taking into consideration all Central European populations and dloop region the biggest differences are between population from Plitvice (Croatia) and all other populations (Tab. 4).

The BC tree indicates that populations Woszczele and Miedzyrzec form a separate clade (Pp=1) nested altogether with all other Polish populations in substantial polytomy (Pp=0.65) (Fig. 2). The sequential outgroups are: Paris (Pp = 0.99) and Rostov (Pp = 1). The topology of BC tree obtained from analysis of only the dloop matrix and broader taxonomic sample (Fig. 2) is congruent with partitioned analysis in separate position of Rostov population (with weak Pp of 0.53) and in combining all other European populations with only Plitvice population separated from broad polytomy spanning the rest of populations (Pp=0.72). Miedzyrzec and Woszczele populations do not form a monophyletic group (Fig. 2). The topology of BC tree obtained from cyt b matrix is congruent with partitioned analysis in Miedzyrzec and Woszczele forming clade (with weak Pp of 0.57).

Table 3
Tabela 3

Basic sequence statistics
Podstawowe statystki uzyskanych sekwencji

Gene	#	mean	S.D.	%D	S.E.	S.E.R	ti/tv	S.E.	S.E.R	%V	%I	%GC	%A	%C	%G	%T
cytb	429	425,73	10,35	3,18	0,44	0,00-12,12	3,42	1,26	2,33-5,00	13,76	2,8	39,08	28,89	27,03	12,04	32,03
dloop	451	449,78	3,12	1,5	0,25	0,00-7,56	2,28	1,28	0,00-5,50	9,76	2,66	30,57	34,45	21,48	9,09	34,98

S.D. – standard deviation, S.E. – standard errors calculated from 1000 bootstrap replicates, ti/tv – transitions to transversions ratio, # – character number (nucleotides), %D – percent of divergence, %V – percent of variable characters, %I – percent of informative characters, S.E.R. – Standard error range
S.D. – odchylenie standardowe, S.E. – błąd standardowy obliczony techniką "bootstrap"z 1000 powtórzeń, ti/tv – stosunek tranzycji do transwersji, # – liczba cech (nukleotydów), %D – procent dywergencji, %V – procent cech zmiennych, %I – procent cech informatywnych, S.E.R. – zakres błędu standardowego

Table 4
Tabela 4

Uncorrected p–distances between studied populations
Niekorygowane dystansy „p" pomiędzy badanymi populacjami

	15	16	11	12	14	6	2	3	4	5	1	7	8	17	10	9
15	–															
16	0,027	–				0,019	0,019	0,023	0,019	0,026	0,019	0,019	0,019	0,035		
11	0,027	0,004	–													
12	0,024	0,002	0,002	–												
14	0,024	0,002	0,002	0	–											
6	0,027	0,004	0	0,002	0,002	–	0	0,012	0	0,014	0	0	0	0,036		
2	0,027	0,004	0	0,002	0,002	0	–	0,012	0	0,014	0	0	0	0,036		
3	0,027	0,004	0	0,002	0,002	0	0	–	0,012	0,002	0,012	0,012	0,012	0,033		
4	0,027	0,004	0	0,002	0,002	0	0	0	–	0,014	0	0	0	0,036		
5	0,027	0,004	0	0,002	0,002	0	0	0	0	–	0,014	0,014	0,014	0,035		
1	0,027	0,004	0	0,002	0,002	0	0	0	0	0	–	0	0	0,036		
7	0,027	0,004	0	0,002	0,002	0	0	0	0	0	0	–	0	0,036		
8	0,027	0,004	0	0,002	0,002	0	0	0	0	0	0	0	–	0,036		
17	0,038	0,035	0,031	0,033	0,033	0,031	0,031	0,029	0,031	0,029	0,031	0,031	0,031	–		
10	0,027	0,004	0	0,002	0,002	0	0	0	0	0	0	0	0	0,031	–	
9	0,027	0,004	0	0,002	0,002	0	0	0	0	0	0	0	0	0,031	0	–
13	0,027	0,004	0	0,002	0,002	0	0	0	0	0	0	0	0	0,031	0	0

Upper right – cytochrome b, lower left – dloop, Woszczele and Miedzyrzec populations shaded
Prawa górna część – cytochrom b, lewa dolna – dloop, populacje Woszczele i Międzyrzec zacieniowane

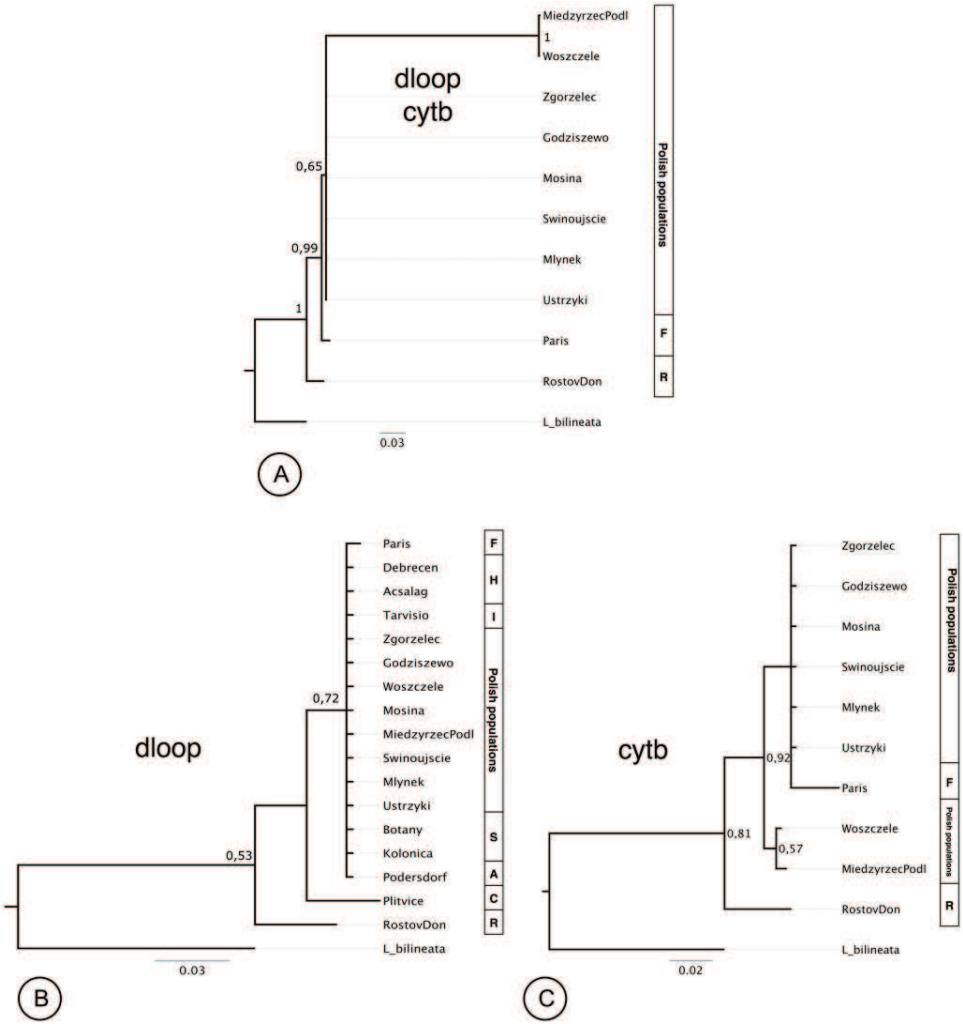

Fig. 2. The trees obtained in Bayesian Analyses: (a) – Bayesian 50% majority-rule consensus tree from partitioned analysis (cytb and dloop), (b) – Bayesian 50% majority-rule consensus tree from separate analysis (dloop), (c) – Bayesian 50% majority-rule consensus tree from separate analysis (cytb). Abbreviations: A – Austria, C – Croatia, F – France, H – Hungary, I – Italy, R – Russia, S – Slovakia

Rys. 2. Drzewa uzyskane w analizie bayesowskiej: (a) – bayesowskie drzewo większościowe (50%) z analizy dwóch partycji łącznie, (b) – bayesowskie drzewo większościowe (50%) z analizy partycji dloop, (c) – bayesowskie drzewo większościowe (50%) z analizy partycji cytb. Skróty: A – Austria, C – Chorwacja, F – Francja, H – Węgry, I – Włochy, R – Rosja, S – Słowacja

The other Polish populations are placed in broad polytomy altogether with Paris population (Fig. 2). The Rostov population is separated from all other European populations. The results of Bayesian Analyses indicate clearly that two populations from CE and NE Poland form monophyletic group with substantial posterior probability (Pp=1). The dloop analysis did not reject the group combining Woszczele and Miedzyrzec but only place these populations in broad polytomy.

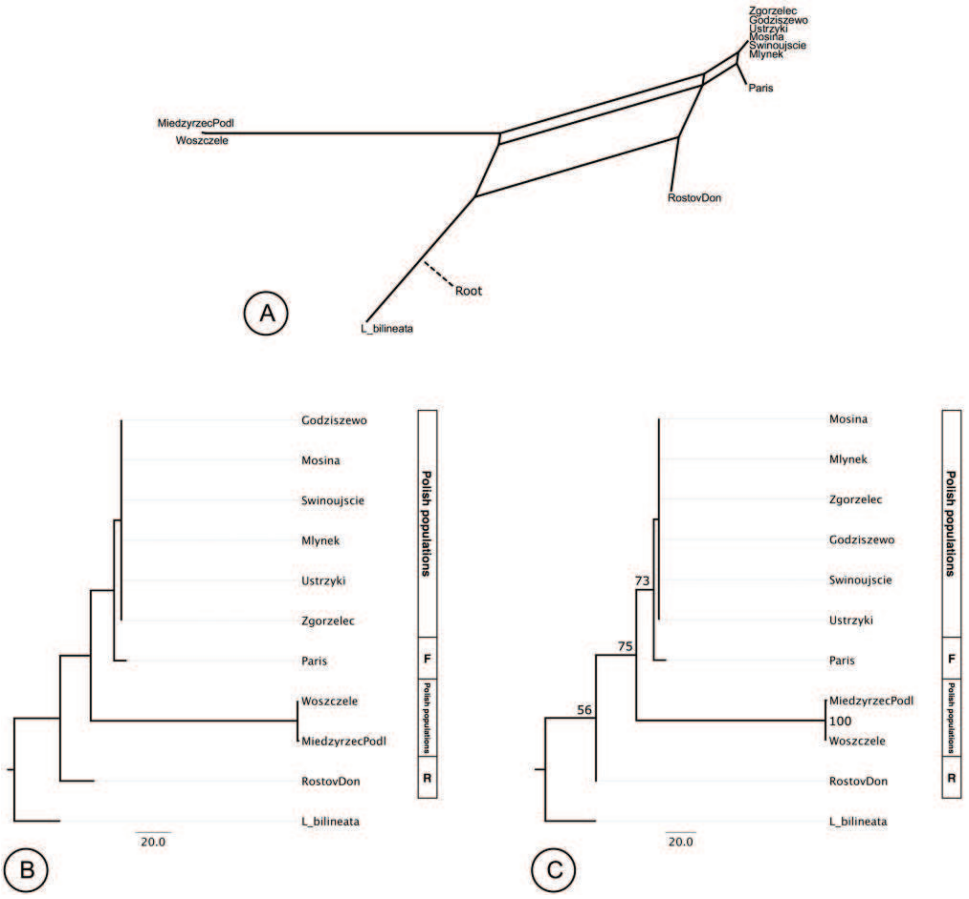

Fig. 3. The reconstructions obtained in Maximum Parsimony analyses: (a) – consensus split network, (b) – the shortest tree, (c) – 50% majority-rule consensus tree with bootstrap supports for nodes

Rys. 3. Rekonstrukcje uzyskane w analizie metodą maksymalnej parsymonii: (a) – konsensus uzyskany metodą „split network", (b) – najkrótsze drzewo, (c) – drzewo większościowe (50%) ze wsparciem dla węzłów uzyskanym metodą „bootstrap"

Fig. 4. Allozyme UPGMA dendrograms from: (a) – Nei's genetic distance (1978), (b) – Roger's genetic distance (1972)

Rys. 4. Dendrogramy UPGMA uzyskane z analizy allozymów: (a) – genetyczny dystans Nei'ego (1978), (b) – genetyczny dystans Roger'a (1972)

Table 5
Tabela 5

Genetics of the allozyme polymorphic populations of *Lacerta agilis,* P0.95 – polymorphism at 0.95, P0.99 – polymorphism at 0.99, Ho – observed heterozygosity, He – expected heterozygosity, Hu – unbiased heterozygosity

Genetyka populacji *L. agilis*, polimorficznych pod względem loci allozymatycznych, P0.95 – polimorfizm 0.95, P0.99 – polimorfizm 0.99, Ho –heterozygotyczność obserwowana, He – heterozygotyczność oczekiwana, Hu – heterozygotyczność nieobciążona

Enzyme system	Locus	alleles	Woszczele					Miedzyrzec Podlaski				
			H_e	H_o	H_u	$P_{0.95}$	$P_{0.99}$	H_e	H_o	H_u	$P_{0.95}$	$P_{0.99}$
MOD	sMOD-1	2	0,12	0,13	0,13			0,18	0,2	0,19		
	sMOD-2	1	0	0	0			0	0	0		
LDH	sLDH-1	1	0	0	0			0	0	0		
	sLDH-2	1	0	0	0			0	0	0		
MDH	sMDH-1	1	0	0	0			0	0	0		
	sMDH-2	1	0	0	0			0	0	0		
CK	sCK-1	1	0	0	0			0	0	0		
PGD	sPGD-1	2	0,18	0,2	0,19			0,06	0,07	0,07		
M*			0,04	0,04	0,04	25	25	0,03	0,03	0,31	12,5	25

* Mean for all loci in each population
* Średnie dla wszystkich loci w każdej z populacji

The Maximum Parsimony analyses were consistent with the results of Bayesian analyses. The SMP and BMP trees have completely congruent topologies (Fig. 3). All Polish populations, except clade grouping Woszczele and Miedzyrzec (bootstrap support – 100), form polytomy sister to Paris population (bootstrap support = 73). The succeding outgroup is Rostov population (bootstrap support = 56). The MPS network (Fig. 3) identifies the phylogenetic uncertainty in placing of Woszczele + Miedzyrzec clade and Rostov population on the tree. The Woszczele + Miedzyrzec clade or the Rostov population form the most outer outgroup. According to MP analyses the Woszczele + Miedzyrzec clade is more separated from all other Polish populations as compared to Bayesian reconstructions.

Allozymes

Interpopulational genetic variation

Wright's F coefficients for the set of loci studied are: F_{IT}= -0.0096, F_{ST}=0.0465, F_{IS}=-0.0588. The mean jack-knifing values are: F_{IT}=-0.0100, F_{ST}=0.0464, F_{IS}=-0.0592. The range observed for Nei's genetic distance was 0.0-0.0017 (original matrix) and 0.0005–0.001 (unbiased matrix). Nei's coefficients of similarity, calculated according Nei (1972), were in the range 0.9983–1.00 and calculated according to Nei (1978) ranged from 0.9990 to 1.0005. Roger's genetic distances covered the range 0.0167–0.0125. The UPGMA dendrograms based on Nei's and Roger's distances are very similar and congruent in clustering Woszczele and Miedzyrzec as separate group (Fig. 4).

Intrapopulational genetic variation

Two of the eight loci (MOD, PGD) were polymorphic using both the 0.95 and 0.99 polymorphism criteria. A total of 10 alleles were found with a maximum of two per locus (sMOD-1 and sPGD-1). The only polymorphic populations were Miedzyrzec and Woszczele. The 25% polymorphism was observed in the both populations according to the 0.99 criterion. According to the 0.95 criterion, the polymorphism was 25% in the Woszczele population and 12.5% in the Miedzyrzec population. The mean level of heterozygosity averaged over all the populations is 0.01 with a range of 0.00–0.004 (Tab. 5).

DISCUSSION

Intrapopulational differentiation

The values of polymorphism obtained in our study are similar to those characterizing the populations of other lizard species (e.g. Bezy et al. 1977, Hall and Selander 1973, Sá-Sousa et al. 1999, Soliman et al. 1994). If the number of loci investigated is small, the sampling error of the proportion of polymorphic loci may be large (Nei 1987). Due to the small number of individuals and loci studied, estimation of this parameter of genetic variation within a population should be treated with extreme care. The heterozygosity calculated for vertebrate species varies greatly (Page and Holmes 1998). The low heterozygosity is surprising for a relatively large population. This low mean heterozygosity and the fact that observed heterozygosity is lower than expected heterozygosity for supposed neutral alleles may well result from a population bottleneck during the last ice age (Nei and Graur 1984). Such a bottleneck may result in a very low level of heterozygosity even hundreds of thousands of years after the population returns to its previous size (Nei 1987). It is also possible that the low level of heterozygosity results from the small number of enzyme systems studied (Gorman and Renzi 1979, Nei 1987).

Interpopulational differentiation

The fixation index, F_{ST}, is an important measure for the assessment of the genetic variation between populations. It can be useful in assessing the structure of the metapopulation, the degree to which the level of heterozygosity has fallen due to genetic drift, or the level of gene flow.

The index obtained in this study (F_{ST}=0.0465) is much lower than index calcuated for Swedish populations of *Lacerta agilis* (on the basis minisatelite mean value=0.322, microsatelite 0.299), where populations are separated by the existence of barriers in the form of large areas of man-made monoculture forestry (Gullberg et al. 1999, Andrén et al. 1988, Berglind 1996). It suggests high level of gene flow between populations (Page and Holmes 1998).

Values of Nei's genetic distance close to zero (as obtained in our study) suggest that the Woszczele and Miedzyrzec populations diverged from the remaining populations in the recent past and/or there is a high level of gene flow between the populations. According to Gorman et al. (1975) a genetic distance (D) of between 0 and 0.1 characterizes variation between lizards populations, whereas values above 0.2 characterize differences between subspecies. On the other hand, Guillaume and Cirer (1985) argue that a value of D less than or equal to 0.2 corresponds to variation within a species, while values of D between 0.3 and 0.5 correspond to related species. Values of D greater or equal to 0.9 can indicate that the species are not from the same genus.

Nei's genetic distance (1972) between the population from Woszczele and the other Polish populations (excluding the Miedzyrzec population) and between the Miedzyrzec population and the other Polish populations (excluding the Woszczele population) are 0.0017 and 0.0013, respectively. In the light of the opinions considered above, these differences are much too small to talk about different subspecies. But even in the case of isolated populations, where we expect the genetic distance to be high, the actual distance may sometimes be relatively small. For example the values given by Gorman et al. (1975) were only partially confirmed by his studies. He obtained values of Nei's genetic differences between 0 and 0.116 for separate populations of *Podarcis melisellensis*, described as subspecies. For various subspecies of *Podarcis sicula* he obtained values between 0.001 and 0.063, which are below his threshold for defining a subspecies.

It should be noted also that in many cases where electrophoresis of allozymes does not indicate a large genetic distance between species of vertebrates, there may be morphological differences and even reproductive isolation . The range of genetic distance calculated for the several species of reptiles was between 0 and 0.09 (Adest 1977, Gartside et al. 1977, Murphy et al. 1984). These results confirm the view, expressed by Nei himself (1987), that Nei's distance should not be the only criterion for defining subspecies. The differences indicated between populations in our allozyme studies seem not be enough to come to a conclusion, that we deal with two separate subspecies.

The wide-range phylogeographical study carried out by Kalyabina-Hauf and Ananyeva (2004) based on cytochrome b indicated that range of p-distances – 0.007 to 0.073 corresponds to the subspecies level in *L. agilis* group and *L.a.argus* and *L.a.chersonensis* are very similar to each other (p-distance = 0.031). Interpopulational level of divergence varies for particular subspecies beetwen 0.001 and 0.008. In our study distances between Miedzyrzec or Woszczele and any of remaining Polish populations reach 0.012 – 0.014. This suggests that the divergence is intermediate between interpopulational or subspecies level. Separate position of haplotype from Croatia (Plitvice) in our dloop

analysis and 0.024 – 0.027 p-distances beetwen Plitvice and all other populations indicate that it can represent *L.a.bosnica* subspecies.

Our results of mtDNA phylogenetic analyses confirms that populations from NE and Central Eastern part of Poland can represent separate subspecies. However, we cannot exclude possible introgresion of genes from populations occurred in Belarus and Russia. It seems indeterminable without genetical analysis of samples from those eastern, "pure" *Lacerta a. chersonensis* populations.

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ZRÓŻNICOWANIE GENETYCZNE JASZCZURKI ZWINKI, *LACERTA AGILIS* LINNAEUS, 1758 (REPTILIA, LACERTIDAE) W EUROPIE ŚRODKOWEJ

S t r e s z c z e n i e

Wykonano badania zróżnicowania genetycznego ośmiu populacji jaszczurki zwinki z terenu Polski (allozymy i sekwencje genu cytochromu b) oraz 18 populacji z Polski, Słowacji, Austrii, Węgier, Chorwacji, Włoch, Francji i Rosji (sekwencje fragmentu d-loop). Stwierdzony poziom polimorfizmu allozymów był bardzo niski. Spośród ośmiu studiowanych loci polimorfizm znaleziono tylko w dwóch loci dwóch systemów enzymatycznych (MOD i PGD). Dotyczył on populacji z północno- i środkowo-wschodniej Polski. Analiza bayesowska oraz inne zastosowane metody filogenetyczne oparte na sekwencjach cytochromu B oraz regionu d-loop wskazują, że haplotypy ze wschodniej Polski tworzą oddzielny klad. Badania potwierdzają jednorodność genetyczną badanych populacji z Europy Środkowej (L. agilis argus) z wyjątkiem populacji z północno-wschodniej i środkowo-wschodniej części Polski oraz populacji chorwackiej (*L. a. bosnica*).

SŁOWA KLUCZOWE: *Lacerta agilis*, genetyka populacji, allozymy, mtDNA, cytochrom b, dloop, filogenetyka, Europa Środkowa

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