

## Conservation genetics of *Lacerta viridis* populations in the Czech Republic (Reptilia: Lacertidae)

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**Abstract.** In order to obtain genetic data for the conservation of populations of Czech *Lacerta viridis* (Laurenti, 1768) the genetic diversity of 12 microsatellite markers was assessed for five Bohemian and three Moravian populations. Comparison of the genetic variation and differentiation between the highly fragmented and isolated Bohemian populations and the Moravian populations connected to the continuous species range revealed a lower level of genetic variation in Bohemian populations. Presence of a genetic split between the Bohemian and Moravian populations indicates that these populations have been isolated from one another for long time and currently there is no gene flow between them. The genetic structures of the populations in both regions are significantly correlated with respective geographic distances and influenced by the low level of habitat connectivity between the populations. Basic implications for the conservation of *L. viridis* in the Czech Republic are suggested.

**Key words.** genetic diversity, population genetic structure, regional historical biogeography, Reptilia, Lacertidae, *Lacerta viridis*, Czech Republic.

### INTRODUCTION

Identification and understanding of the influences of evolutionary, ecological and anthropogenic processes on the genetic status of natural populations are important prerequisites for the short term management and conservation of species (DeSalle & Amato 2004, Palsbøll 2007, Schwartz et al. 2007). Nevertheless, population genetic studies of endangered reptiles (especially European lizards) are still comparatively rare (Gullberg et al. 1997, Madsen et al. 2000, Beebee & Rowe 2001, Petrosyan et al. 2003, Pinho et al. 2004, Le Galliard et al. 2005).

Among other European lacertids the Green Lizard *Lacerta viridis* (Laurenti, 1768) is listed within the FFH directive of the European Union (1992) in annex IV as a species of community interest in need of strict protection. While this species is not endangered within its central and southern range, the populations at the northernmost edge of its range in Germany and the Czech Republic are at great risk of extinction (e.g. Schneeweiss et al. 2004, Zavadil & Moravec 2005).

In the Czech Republic *L. viridis* occurs within two disjunctive areas (Bohemia and Moravia). The Bohemian populations are situated on the northwestern border of the species range and have a relict character. Their occurrence is restricted to three isolated areas: (i) area of the river Labe, northern Bohemia; (ii) area of the river Ohře, northwestern Bohemia; and (iii) area of the rivers Vltava, Berounka and Sázava, central Bohemia. These populations consist of several more or less separated metapopulations. Some display local morphological differences, explained as an effect of possible genetic drift (Šapovaliv 1987). The Bohemian populations show a strong connection with deep cut river valleys, which have optimal microclimatic conditions (Riverine phenomenon; Noll 1878, Ložek 1988, Strödicke 1995). In contrast the Moravian populations are believed to be

connected with the continuous species range and not so closely connected with river valleys (e.g. Nettmann & Rykena 1984, Strödicke 1995, Naulleau 1997, Grillitsch & Cabela 2001, Mikátová et al. 2001a, Böhme et al. 2006).

Regarding their relict and fragmented character, small Bohemian (Hercynian) populations are considered critically endangered, whereas the larger Moravian (Carpathian and Pannonian) populations are classified as endangered (Zavadil & Moravec 2005). Because of the endangered status of the Bohemian and Moravian populations of *L. viridis*, there are several basic studies on the ecology and distribution of this species within the Czech Republic (e.g. Mikátová 2001a, b). However, data about the genetic status of the populations and the genetic differentiation between the two distribution areas are rare (Böhme et al. 2006, 2007a, b). Nevertheless, previous genetic analysis of the Central-European populations of *L. viridis* revealed a positive correlation between genetic distances of mtDNA sequences and geographic distances of the relevant populations. In addition, the populations from Bohemia and eastern Germany (Brandenburg) show remarkably small genetic distances in contrast to the geographically closer related central populations from Moravia, Austria, Slovakia and Hungary (Böhme et al. 2006). These results support the hypothesis of a postglacial migration of *L. viridis* from the territory of the Czech Republic to the area of today's eastern Germany and the former existence of link populations between Bohemia and Moravia.

In respect of the unique character of the Czech populations of *L. viridis*, this study aimed to:

- (i) provide information on nuclear microsatellites needed for a thorough comparison of the ge-

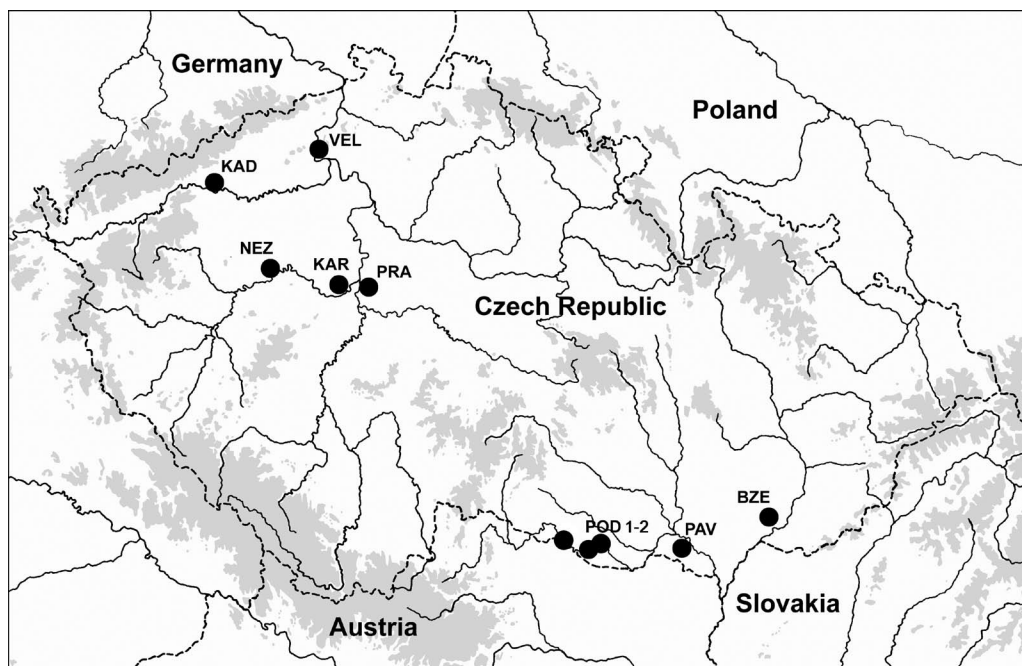


Fig. 1. Geographical distribution of the *Lacerta viridis* populations sampled within the Czech Republic (for explanation of locality abbreviations see Table 1).

Table 1. Population genetic variation and regional means of genetic variation

| locality             | abbr. | n         | heterozygosity         |                        | allelic richness       |                         |
|----------------------|-------|-----------|------------------------|------------------------|------------------------|-------------------------|
|                      |       |           | Ho <sup>a</sup> (± SE) | He <sup>b</sup> (± SE) | Ac <sup>c</sup> (± SE) | PAc <sup>d</sup> (± SE) |
| BOHEMIA              |       |           |                        |                        |                        |                         |
| Nezabudice           | NEZ   | 5         | 0.40 ± 0.09            | 0.50 ± 0.06            | 3.00 ± 0.28            | 0.20 ± 0.11             |
| Karlík               | KAR   | 6         | 0.35 ± 0.08            | 0.51 ± 0.07            | 3.03 ± 0.34            | 0.21 ± 0.11             |
| Velké Žernoseky      | VEL   | 7         | 0.48 ± 0.08            | 0.59 ± 0.05            | 3.64 ± 0.40            | 0.34 ± 0.11             |
| Prague               | PRA   | 11        | 0.64 ± 0.09            | 0.59 ± 0.07            | 3.51 ± 0.39            | 0.42 ± 0.18             |
| Kadaň                | KAD   | 10        | 0.56 ± 0.09            | 0.56 ± 0.06            | 3.36 ± 0.40            | 0.46 ± 0.18             |
| <b>regional mean</b> |       | <b>39</b> | <b>0.52 ± 0.06</b>     | <b>0.70 ± 0.05</b>     | <b>7.50 ± 0.96</b>     | <b>1.97 ± 0.45</b>      |
| MORAVIA              |       |           |                        |                        |                        |                         |
| Bzenec               | BZE   | 7         | 0.58 ± 0.09            | 0.62 ± 0.06            | 4.29 ± 0.50            | 1.10 ± 0.31             |
| NP Podyjí            | POD   | 12        | 0.56 ± 0.07            | 0.76 ± 0.04            | 5.43 ± 0.45            | 1.49 ± 0.28             |
| Pálava               | PAV   | 6         | 0.61 ± 0.09            | 0.63 ± 0.03            | 3.75 ± 0.33            | 0.50 ± 0.21             |
| <b>regional mean</b> |       | <b>25</b> | <b>0.58 ± 0.07</b>     | <b>0.78 ± 0.04</b>     | <b>11.00 ± 1.22</b>    | <b>5.47 ± 0.70</b>      |

<sup>a</sup> observed heterozygosity, <sup>b</sup> expected heterozygosity, <sup>c</sup> number of alleles per locus corrected for sample size, <sup>d</sup> number of private alleles per locus corrected for sample size

netic diversity of the Bohemian and Moravian populations of *L. viridis*, (ii) contribute to a better understanding of the genetic differentiation between Bohemian and Moravian populations, and (iii) provide conservation genetic data needed for the effective conservation and management of the endangered populations of *L. viridis* in the Czech Republic.

## MATERIAL AND METHODS

### Study area and sample sites

The populations of *L. viridis* studied are situated at the northern edge of the range of the subspecies *L. v. viridis*. We sampled populations within two disjunctive distribution areas (Bohemia and Moravia), which are separated by the Českomoravská vrchovina highlands (Fig. 1). The Bohemian samples represent all three isolated Bohemian populations: (i) area of the river Labe (Velké Žernosky [VEL]), (ii) area of the river Ohře (Kadaň [KAD]), (iii) area of the rivers Berounka and Vltava (Karlík in the Český kras protected landscape area [KAR]; Nezabudice in the Krivoklátský protected landscape area [NEZ]; Prague [PRA]). The Moravian populations are located within the National park of Podyjí [POD] (sample sites near Čížov, Šobes and Znojmo), within the Pálava protected landscape area [PAV] and near Bzenec [BZE].

### Genetic analysis

We collected blood samples from 64 *L. viridis* belonging to the above mentioned populations (39 from Bohemia and 25 from Moravia; Table 1). Captured individuals were marked with a dot of nail polish, thereby preventing recapture of the same individuals. Blood samples were stored in a special EDTA-Thymol buffer at –20 °C. Total genomic DNA was extracted using the NucleoSpin Blood Kit (Machery & Nagel), following the manufacturers protocol. We used 12 highly variable microsatellites, previously designed for this subspecies (Böhme et al. 2005), to analyse the genetic diversity. PCR setup and amplification was performed following the standard multiplex protocol for the primers used (Böhme et al. 2007a). Fluorescent PCR fragments were analysed using an ABI Prism 3100 automated sequencer (ABI) following the manufacturers protocol. Individual genotypes were detected by GeneMapper Software v. 3.7 (ABI).

### Population assignment

As we were mainly interested in genetic differences between populations or regions it was necessary to test if assumed population structure in the field correlates with the observed genetic population structure. Therefore, we checked individual assignment to the sampled populations by using two different approaches. Based on individual genotypes we ran a Bayesian clustering implemented in Structure v. 2.1 (Pritchard et al. 2000). This analysis uses genotype data of unlinked markers to detect hidden population structure within sampling units. Consequently it can group non differentiated populations into genetic groups. The algorithm thereby assumes Hardy Weinberg equilibrium and linkage equilibrium for these genetic groups. We ran Structure with four iterations for each assembled number of populations K (K 1–15) with an initial “Burn-in” of 30,000 Markov chains Monte Carlo (MCMC) and 70,000 MCMC repeats after this initial phase. As run

parameters we chose the admixture model, assuming that individuals may have a mixed ancestry, and the correlated allele frequencies model with the default settings. The most adequate number of genetic groups for the data set, indicated by the highest log likelihood value  $[-\ln P(X|K)]$  was compared to the population structure based on geographic information. The portion of membership ( $Q$ ), calculated by Structure, was used to define the population structure (genetic populations). The program Distruct (Rosenberg 2004) was used to generate a figure illustrating the revealed structure. To test the robustness of the revealed genetic structure, assignment of individuals to the sampled populations was tested using the assignment test within GeneClass v. 2.0 (Piry et al. 2004). The Bayesian criterion (Rannala & Mountain 1997) and the resampling algorithm of Paetkau et al. (2004) simulating 1000 individuals was used to calculate assignment probabilities for each individual (type one error 0.01).

### Genetic diversity and differentiation

Loci and populations were tested for deviations from Hardy-Weinberg expectations and linkage disequilibrium using exact probability tests with default settings integrated in Genepop v. 3.4 (Raymond & Rousset 1995).

The populations were checked for evidence of recent bottlenecks using Bottleneck v.1.2.01 (Cornuet & Luikart 1996). Simulations with 1000 iterations were done assuming infinite allele model (IAM; Kimura & Crow 1964), two phased model (TPM; Di Rienzo et al. 1994) or stepwise mutation model of microsatellite mutation (SMM; Ohta & Kimura 1973). Significance of the results was tested using the Wilcoxon sign rank test.

Standard values for genetic diversity, like observed and expected heterozygosity, were calculated using GenAlEx v. 6.0 (Peakall & Smouse 2006). The allelic diversity (number of alleles per locus,  $A_c$ ) and private allelic richness (number of private alleles per locus,  $P_{Ac}$ ) were calculated using HP-Rare v. 1.0 (Kalinowski 2005). A rarefaction approach was used to correct the data for unequal sample size between the populations. Differences of mean genetic variation between the populations were tested for significance by an analysis of variance (ANOVA). Significance of the observed regional differences was tested by a nested ANOVA, where all populations were nested within the respective region. For all indices mentioned above, we compared the mean genetic diversity over loci for the two regions (regional mean) and tested for significant differences by using a pair wise t-test or Mann-Whitney test. Furthermore, we tested for differences in genetic variation among regions by calculating the mean for each region over samples and loci for allelic richness ( $R$ ), observed heterozygosity ( $H_o$ ) and gene diversity ( $H_s$ ) by using the approach implemented in Fstat v. 2.9.3.2 (Goudet 1995). The significance of the observed regional differences was tested by a two sided probability test with 1000 permutations of samples within regions (Fstat).

Due to the still ongoing discussion about the most appropriate statistics for detection of genetic differentiation and genetic distances using microsatellite data (Takezaki & Nei 1996; Goldstein & Pollock 1997; Balloux & Lugon-Moulin 2002) we decided to consider both basic models of microsatellite mutation; the infinite allele model (IAM; Kimura & Crow 1964) and the stepwise mutation model (SMM; Ohta & Kimura 1973).

An analysis of molecular variance (AMOVA; Excoffier 1992; Michalakis & Excoffier 1996) implemented in GenAlEx v. 6.0 (Peakall & Smouse 2006) was conducted for an initial comparison of genetic structure between and within regions using the most commonly reported statistics for estimation of population structure:  $F$ -statistics (Weir & Cockerham 1984) and  $R$ -statistics (Slatkin 1995). Statistical significance of the analyses was tested using 999 permutations. Further information about regional differences of inbreeding ( $F_{IS}$ ), genetic differentiation ( $F_{ST}$ ) and relatedness ( $Rel.$ ) were calculated using Fstat.

Pair wise genetic distances ( $R_{ST}$  and  $F_{ST}$ ) between populations were calculated and a test for isolation by distance using Mantel test (Mantel 1967) with 999 permutations was conducted afterwards by using GenAlEx. For this purpose, geographic distance between populations was defined as the simple mean surface distance between the geographic locations.

The relationships between populations were also achieved by reconstructing evolutionary distances between the populations. Goldstein et al. (1995) assume that differences in repeat score between alleles carry information about the amount of time that has passed since they shared a common ancestral allele. Therefore, we used Goldstein distances ( $\delta\mu^2$ ) as a widely used measure of evolutionary genetic distances between the closely related populations. A Neighbour Joining tree (NJ) based on these distances was reconstructed using Populations v. 1.2.28 (<http://www.cnrs-gif.fr/pge/bioinfo>). Support for the revealed relationships was provided by 500 bootstrap replications on individuals.

## RESULTS

### Population assignment

Figure 2 presents the average  $\ln P(D)$  values for each  $K$  simulated (1–15) using the Bayesian approach implemented in the program Structure. For simulations of  $K=8$  to  $K=10$  differences between the likelihood values of  $\ln P(D)$  were very small. The most likely number of genetic groups was calculated for a run of  $K=8$  ( $-\ln/k = 2330.7$ ), which also presented the highest correlation of individual assignment with the population structure observed in the field. With some

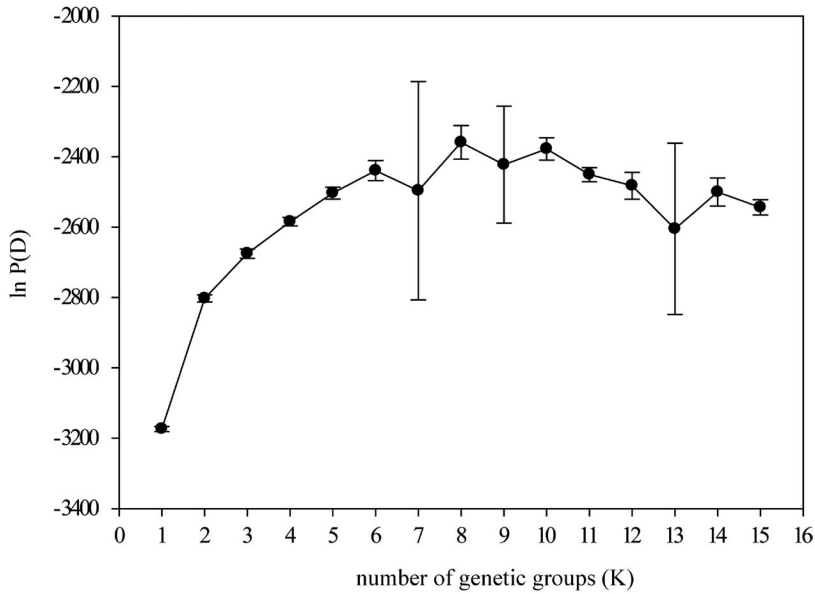


Fig. 2. Average probability,  $\ln P(D)$ , for each genetic cluster  $K$ , simulated using the Bayesian approach implemented in program Structure.

exceptions most of the individuals showed a high portion of membership (Q-values) to their respective sample sites (Fig. 3). The individuals from the Bohemian sample site at Karlík (KAR) showed mixed membership either with the Nezabudice (NEZ) or the Velké Žernoseky (VEL)

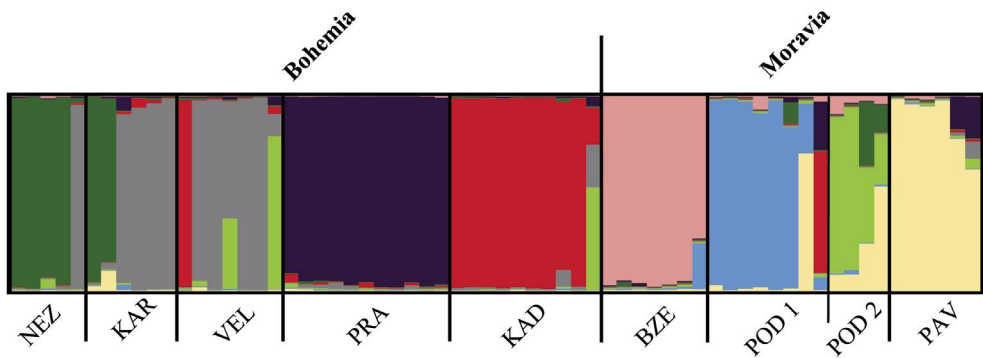


Fig. 3. Individual assignment (Q-matrix) of 64 samples of *Lacerta viridis* from eight Czech populations to genetic clusters inferred from the analysis of 12 microsatellite loci for the Structure run of highest estimated probability. Individual colours represent the revealed genetic clusters ( $K = 8$ ). Abbreviations below the graph indicate the populations sampled (for explanation see Table 1).

Table 2. Assignment of populations based on the Bayesian criterion of Rannala & Mountain (1997). Values indicate the number of assigned individuals based on their genotypes. Probability calculation was performed using the simulation algorithm of Paetkau et al. (2004) and 1000 simulated individuals. Individuals were not assigned if their assignment probability was less than 0.01 (for explanation of locality abbreviations see Table 1)

|          | NEZ      | KAR      | VEL      | PRA       | KAD      | BZE      | POD       | PAL      | correct |
|----------|----------|----------|----------|-----------|----------|----------|-----------|----------|---------|
| NEZ (5)  | <b>3</b> |          | 1        |           |          |          | 1         |          | 60%     |
| KAR (6)  |          | <b>4</b> |          |           |          |          | 2         |          | 67%     |
| VEL (7)  |          |          | <b>6</b> |           | 1        |          |           |          | 86%     |
| PRA (11) |          |          |          | <b>11</b> |          |          |           |          | 100%    |
| KAD (10) |          |          | 1        |           | <b>9</b> |          |           |          | 90%     |
| BZE (7)  |          |          |          |           |          | <b>6</b> | 1         |          | 86%     |
| POD (12) |          |          |          |           |          |          | <b>11</b> |          | 92%     |
| PAL (6)  |          |          |          |           |          |          | 2         | <b>4</b> | 67%     |

population. In contrast, the Podyjí (POD) population consisting of three geographically close sample sites is subdivided into two clusters Podyjí 1 (POD 1) and Podyjí 2 (POD 2, Fig. 3). Individuals of Podyjí 2 form a separate cluster with its own genetic features, which are present also in the Velké Žernoseky and Kadaň populations. In addition, they share genetic features with individuals from Pálava and Nezabudice and to less extent also with individuals from Bzenec. Genetic material predominantly found at Kadaň is also present in single individuals from Podyjí 1 and Velké Žernoseky. Similarly, genetic features typical of lizards from Prague were detected in single individuals from Pálava and Podyjí 1. The assignment test implemented in GeneClass showed that most of the individuals have a high probability of being a resident of the assumed population (60–100%, Table 2). A mixed membership at Karlík or a non-assignment of Podyjí 2 individuals to the Podyjí group was not observed using GeneClass.

### Genetic diversity

The analysis of the 12 highly polymorphic loci revealed a total number of 159 alleles within the eight Czech *L. viridis* populations. Single loci had allele numbers of from five (Lv11) to 21 (Lv17) in all the samples.

Four out of twelve microsatellite loci (Lv17, Lv11, Lv2 and Lv1) showed significant deviations from Hardy-Weinberg expectations after Bonferroni corrections for multiple testing (adjusted  $P$  value  $< 0.006$ ). However, an exclusion of these loci did not affect calculations of genetic diversity or population differentiation (data not shown). After Bonferroni correction two of the sample sites (KAR, POD) were also significantly out of Hardy Weinberg equilibrium (adjusted  $P$  value  $< 0.004$ ). We are aware that testing for Hardy-Weinberg equilibrium is problematic due to the small samples size per population in this study. Nevertheless, global tests applied, showed highly significant evidence that all observed deviations for loci and populations are due to heterozygote deficiency ( $P$  value  $< 0.0005$ ).

Fisher's exact test for genotypic disequilibrium between each pair of loci demonstrated that the microsatellite loci used were not linked. Applying a test for recent bottlenecks we found no significant evidence for a bottleneck effect in any of the populations sampled (Hex, one tailed Wilcoxon test  $P > 0.05$ ).

The statistics for genetic diversity within each population are summarized in Table 1. Mean heterozygosity of the populations ranged between 0.35 and 0.64 for observed heterozygosity ( $H_o$ ) and between 0.50 and 0.76 for expected heterozygosity ( $H_E$ ). Overall the Moravian populations displayed a marginally higher level of heterozygosity than the Bohemian populations. Althou-

gh the observed differences were not significant in a multiple comparison between all pairs of populations (ANOVA,  $H_0$ ,  $df = 7$ ,  $P = 0.156$ ;  $H_E$ ,  $df = 7$ ,  $P = 0.052$ ), a nested ANOVA revealed significant differences for  $H_E$  between the regions Moravia and Bohemia ( $H_0$ ,  $P = 0.105$ ,  $df = 1$ ;  $H_E$ ,  $P = 0.004$ ,  $df = 1$ ).

After correction for unequal sample size (HP-rare), mean allelic richness ( $A_c$ ) ranged from 3.00 to 5.43 and the number of private alleles per locus (PAC) ranged from 0.20 to 1.49 (Table 1). The highest number of alleles for both indices was detected within the Moravian populations. In a multiple comparison between all pairs of populations only the Moravian populations, Bzenec and Podyji, showed significant differences (ANOVA,  $A_c$ ,  $P = 0.0005$ ,  $df = 7$ ; H-test,  $Pac$ ,  $P = 0.0005$ ,  $df = 7$ ). Comparing mean allelic diversity of populations between the regions we found a significantly higher allelic diversity in Moravia (nested ANOVA,  $A_c$ ,  $P < 0.0001$ ,  $df = 1$ ;  $Pac$ ,  $P < 0.0001$ ,  $df = 1$ ).

To obtain the degree of regional differences in genetic variation the regional means of all indices were calculated (Table 1). The level of heterozygosity was not significantly different between the regions (pairwise t-test,  $P > 0.05$ ) but the number of alleles ( $A_c$ ) and private alleles (PAC) was again significantly higher in Moravia (Mann-Whitney test,  $A_c$ ,  $P < 0.001$ ; pairwise t-test,  $Pac$ ,  $P < 0.001$ ). These findings were supported also by the results of the Fstat analysis (Table 3), as significant differences were observed in the mean allelic richness ( $R$ ) and gene diversity ( $H_s$ ) between Bohemia and Moravia, whereas the level of observed heterozygosity was not significantly different between the regions.

### Genetic differentiation

Results of AMOVA differed depending on the statistics used (F- or R-statistics).

For both statistics most of the genetic diversity within the dataset was significantly explained by differences among individuals within populations (72%,  $F_{ST} = 0.276$ ,  $P < 0.001$ ; 67%,  $R_{ST} = 0.327$ ,  $P < 0.001$ ). Among populations within the region we found 15% of the overall variation explained by F-statistics ( $F_{SR} = 0.170$ ,  $P < 0.001$ ) and only 9% by R-statistics ( $R_{SR} = 0.115$ ,  $P < 0.001$ ). The most striking differences between the statistics used were found at the level of regional differentiation (13%,  $F_{RT} = 0.128$ ,  $P < 0.001$ ; 24%,  $R_{RT} = 0.239$ ,  $P < 0.001$ ).

As expected, pairwise genetic distances averaged across all loci for all population pairs were relatively high and ranged from 0.102 to 0.344 for  $F_{ST}$  and from 0.014 to 0.459 for  $R_{ST}$  (Table 4 and 5). Thereby, both genetic distances increased significantly ( $P < 0.001$ ) with increasing geographic distance between the populations (Fig. 4). In concordance with the large geographical and possible long temporal separation the highest genetic distances were observed between Bohemian and Moravian populations.

Genetic distances between Bohemian populations varied between 0.137 and 0.287 for  $F_{ST}$  and between 0.014 and 0.221 for  $R_{ST}$ . While differentiation between all Bohemian populations was

Table 3. Genetic differentiation between populations in Bohemia and Moravia (Fstat).  $P$ -values are for a two sided test based on 1000 permutations

| region     | $R^a$ | $H_o^b$ | $H_s^c$ | $F_{is}^d$ | $F_{st}^e$ | $Rel^f$ |
|------------|-------|---------|---------|------------|------------|---------|
| Bohemia    | 3.310 | 0.515   | 0.599   | 0.140      | 0.190      | 0.292   |
| Moravia    | 4.491 | 0.580   | 0.740   | 0.216      | 0.114      | 0.174   |
| $P$ -value | 0.005 | 0.311   | 0.023   | 0.607      | 0.163      | 0.251   |

<sup>a</sup> allelic richness, <sup>b</sup> observed heterozygosity, <sup>c</sup> genetic diversity, <sup>d</sup> inbreeding coefficient

<sup>e</sup> differentiation among populations within region under IAM model, <sup>f</sup> relatedness

Table 4. Pair wise genetic distances ( $F_{ST}$ , below diagonal) assuming stepwise mutation model (IAM) and associated  $P$ -values calculated using 999 permutations (above diagonal) (for explanation of locality abbreviations see Table 1)

|     | NEZ   | KAR   | KAD   | PRA   | VEL   | BZE   | POD   | PAL   |
|-----|-------|-------|-------|-------|-------|-------|-------|-------|
| NEZ | —     | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |
| KAR | 0.211 | —     | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |
| KAD | 0.274 | 0.209 | —     | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |
| PRA | 0.287 | 0.232 | 0.185 | —     | 0.001 | 0.001 | 0.001 | 0.001 |
| VEL | 0.177 | 0.149 | 0.137 | 0.132 | —     | 0.001 | 0.001 | 0.001 |
| BZE | 0.252 | 0.335 | 0.344 | 0.305 | 0.291 | —     | 0.001 | 0.001 |
| POD | 0.190 | 0.243 | 0.257 | 0.244 | 0.205 | 0.102 | —     | 0.001 |
| PAL | 0.282 | 0.253 | 0.312 | 0.276 | 0.260 | 0.208 | 0.104 | —     |

significant for  $F_{ST}$  ( $P < 0.001$ ) they were not significantly differentiated using other R-statistics. In contrast, Moravian populations were significantly differentiated by both statistics ( $F_{ST}$  0.102–0.208,  $P < 0.001$ ;  $R_{ST}$  0.089–0.216,  $P < 0.05$ ). A comparison of relatedness (Rel.), inbreeding ( $F_{is}$ ) and population differentiation ( $F_{ST}$ ) between Bohemia and Moravia using Fstat detected no significant differences (Table 3). Also the mean level of  $R_{ST}$  (Bohemia 0.076, Moravia 0.106) did not differ significantly between the regions (Mann-Whitney test,  $P > 0.05$ ).

In a final approach a Neighbour Joining distance tree (Fig. 5) was reconstructed from Goldstein distances,  $\delta\mu^2$ , between populations. This analysis showed that populations are more closely rela-

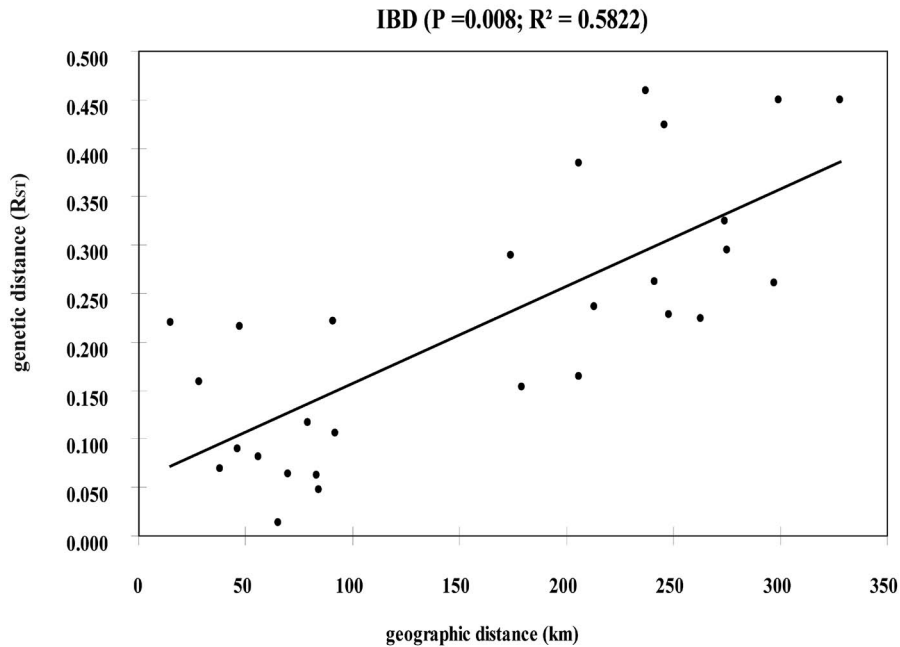


Fig. 4. Plot indicating the pattern of isolation based on distance for the eight Czech populations of *Lacerta viridis*. As an example pair wise geographic distance (km) was plotted against pair wise genetic distance ( $R_{ST}$ ).

Table 5. Pair wise genetic distances ( $R_{ST}$ , below diagonal) assuming stepwise mutation model (SMM) and associated  $P$ -values calculated using 999 permutations (above diagonal) (for explanation of locality abbreviations see Table 1)

|     | NEZ   | KAR   | KAD   | PRA   | VEL   | BZE   | POD   | PAL   |
|-----|-------|-------|-------|-------|-------|-------|-------|-------|
| NEZ | —     | 0.003 | 0.084 | 0.062 | 0.163 | 0.001 | 0.007 | 0.002 |
| KAR | 0.159 | —     | 0.144 | 0.001 | 0.101 | 0.001 | 0.003 | 0.001 |
| KAD | 0.082 | 0.048 | —     | 0.001 | 0.259 | 0.001 | 0.001 | 0.001 |
| PRA | 0.070 | 0.220 | 0.221 | —     | 0.008 | 0.001 | 0.001 | 0.001 |
| VEL | 0.063 | 0.062 | 0.014 | 0.117 | —     | 0.001 | 0.002 | 0.001 |
| BZE | 0.324 | 0.424 | 0.450 | 0.459 | 0.450 | —     | 0.013 | 0.003 |
| POD | 0.165 | 0.154 | 0.224 | 0.289 | 0.228 | 0.106 | —     | 0.022 |
| PAL | 0.262 | 0.237 | 0.261 | 0.384 | 0.295 | 0.216 | 0.089 | —     |

ted to populations in the same region (Bohemia or Moravia). We found that the genetic distance between Bohemia and Moravia exceeded the genetic distances between populations within the regions. Using Goldstein distances the Moravian populations displayed higher genetic distances to each other than the populations within the Bohemian region. The distances between the Moravian populations Pálava and Bzenec even exceeded the distance between Moravia and Bohemia.

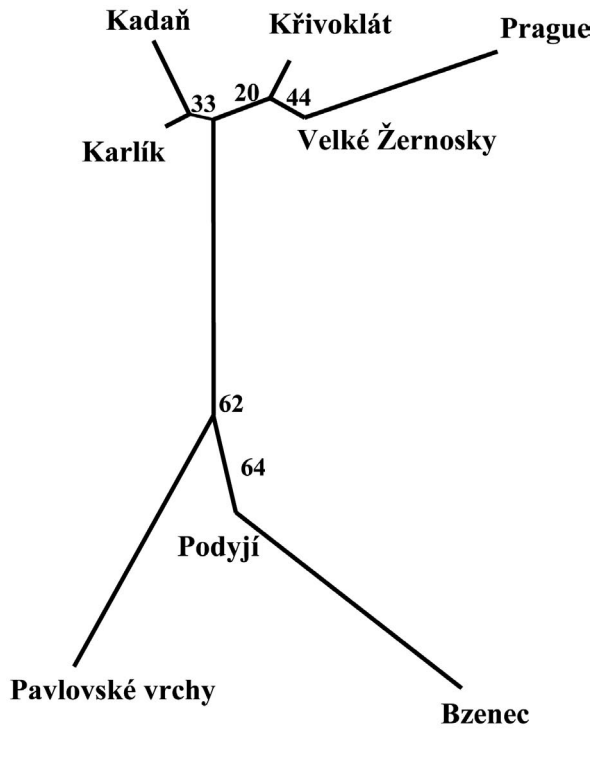


Fig. 5. Unrooted Neighbour Joining distance tree based on pairwise  $(\delta\mu)^2$  distances between eight Czech localities of *Lacerta viridis*. Percentage bootstrap estimates were obtained for 500 replicates of individuals.

## DISCUSSION

### Population assignment and genetic diversity

Current and historical data on the distribution of *L. viridis* in the Czech Republic led to the assumption that due to missing link populations the Bohemian and Moravian populations were geographically and genetically isolated from each other (Štěpánek 1949, Opatrný 1986, Šapovaliv 1987, Kminiak 1992, Mikátová et al. 1989, Mikátová 2001a, b, Zavadil & Moravec 2005). Furthermore, it was suggested that the Bohemian populations in particular have been isolated for a long time and are highly fragmented relict populations, whereas the Moravian population has remained in contact with the continuous species range in the Carpathian and Pannonian region. In accordance with the above assumptions we detected a regional genetic split between the Bohemian and Moravian populations and furthermore a clear difference in genetic structure between the populations within both regions.

On the one hand the used assignment methods (Fig. 3, Table 2) indicated that most of the sample sites (populations) represent genetic units or even comprise an interior genetic substructure (Podyjí, Fig. 3). Individuals showed only low values of admixture between the populations within and between both regions. On the other hand, some of the assumed populations (POD, KAR) show significant deviations from Hardy Weinberg equilibrium due to heterozygote deficiency. Different processes like inbreeding, selection, presence of null alleles or the Wahlund effect (Wahlund 1928) can cause heterozygote deficiency. Assignment tests indicate that the data presented for the National park Podyjí (POD) may be best explained by the Wahlund effect. The Podyjí population has a strong substructure represented by two groups (Fig. 3).

There have been several introductions (including even Slovakian and Bulgarian lizards) into central Bohemian populations during the last decades (reviewed in Mikátová 2001a, b). Such introductions are likely to have affected the Karlík population, which is not represented by a discrete genetic group by the Structure analysis (Fig. 3). Individuals from Karlík grouped either with the lizards from Nezabudice or those from Velké Žernoseky. This is supported by reports of introductions of individuals from the Křivoklátská area (NEZ) and of an unknown origin close to Karlík in 1950–1960 and 1980–1988 (Mikátová 2001a, b). Nevertheless, we found no evidence for such a mixed origin of the Karlík individuals using the assignment algorithm in GeneClass (Table 2). Also the fact, that the genetic features of the individual Moravian populations were detected in single individuals of Bohemian populations (or vice versa; see the chapter Population assignment) may indicate human manipulation.

Despite the potential effect of the introductions, this study revealed significant regional differences in the distribution of genetic variation between the disjunctive Czech populations of *L. viridis*. Using expected heterozygosity ( $H_E$ ) as a measure of genetic diversity we can conclude that the values within Czech populations are comparable to those recorded in other studies on edge and central populations of *L. viridis* ( $H_E$  0.43–0.73, e.g. Böhme et al. 2005, 2007a, Laube & Kuehn 2006).

The lowest genetic diversity was detected within the critically endangered populations in the Bohemian region (Table 1). The populations at Karlík and Nezabudice, which we assume to be the smallest (Karlík < 80 adult individuals; Nezabudice < 100 adult individuals) also displayed the lowest values of heterozygosity and allelic richness (Table 1). Nevertheless, their genetic variation is still comparable with that found in the isolated northernmost populations of *L. viridis* in Germany (Böhme et al. 2007a). Though the other Bohemian populations did not show such low levels of heterozygosity they also had fewer alleles and private alleles compared to the Moravian populations. Overall, significantly higher values of allelic richness and gene diversity were recorded in the Moravian than the Bohemian region (Table 3). Especially the Moravian

populations Bzenec and Podyjí had a significantly higher number of alleles and private alleles (Table 1). These observations accord well with the predictions of the central peripheral theory that populations at the margins of ranges suffer from smaller census size and higher habitat fragmentation and are therefore more affected by processes such as random genetic drift, restricted gene flow or inbreeding (e.g. Rosenzweig 1995, Dias 1996, Goldstein et al. 2000, Hanski 2001). Therefore, these populations show a reduced genetic variation compared to populations at the center of a species range (e.g. Lammi et al. 1999, Schwartz et al. 2003). Hence, we suggest that the low level of genetic variation within the Bohemian populations of *L. viridis* is a result of such edge effects: the small sizes of these populations, the high level of habitat fragmentation at their localities and the long time they have been isolated from the Moravian populations connected with the continuous species range. This suggestion is further supported by the fact that the Moravian populations contain a significantly higher number of private alleles, which were not recorded in Bohemian populations (Table 1).

### Genetic differentiation

In addition to the differences in genetic variation we also observed differences in population differentiation between the Bohemia and Moravia regions. However, the estimate of the genetic structure was dependent on the statistic used ( $F$ -statistics or  $R$ -statistics), which differs in the underlying model of microsatellite mutation (IAM, Kimura & Crow 1964 or SMM, Ohta & Kimura 1973). In contrast to  $F$ -statistics (Weir & Cockerham 1984), calculations based on the stepwise mutation model, like  $R$ -statistics (Slatkin 1995) also consider differences in allele length between individuals, populations and regions.

For both statistics most of the variation was explained by differences among individuals within populations (72%,  $F_{ST} = 0.276$ ; 67%,  $R_{ST} = 0.327$ ). However, a significantly higher level of variation was explained by differentiation between regions using  $R$ -statistics than using  $F$ -statistics (24%,  $R_{RT} = 0.239$ ; 13%  $F_{RT} = 0.128$ ). By using  $R$ -statistics, the differentiation between regions even exceeds that among populations within regions. As  $R$ -statistics consider variance in allele size and therefore mutational processes the greater amount of regional differentiation ( $R_{RT}$ ) support the assumption that differentiation between regions is affected by the length of time for which the populations have been separated (review in Balloux & Lugon-Moulin 2002).

Measurement of pair wise genetic distances ( $F_{ST}$  and  $R_{ST}$ ) between populations showed that genetic distances were influenced by a significant isolation by distance pattern (Fig. 4). This pattern indicates a stepwise dispersal over short distances by individuals of *L. viridis*. The high genetic distances recorded between Bohemian and Moravian populations reflect the large geographical distance separating and also the likelihood that these populations have been separated for a long time. While  $R_{ST}$ -values were only significant for differentiation between the Moravian populations,  $F_{ST}$ -values indicated significant differentiation between populations within both regions. This picture would lead to the assumption that genetic distances between populations within the Czech Republic are more influenced by differences in allele frequencies ( $F_{ST}$ ). This indicates that differentiation between populations is mainly affected by restricted migration of individuals, lower gene flow and higher genetic drift due to fragmentation. Longer evolutionary divergence such as indicated by length mutations of alleles ( $R_{ST}$ ) seems to be less relevant for the development of genetic distances between populations within both these areas. Further support for this comes from the values of mean population differentiation ( $F_{ST}$ ), relatedness and inbreeding coefficient within the regions Bohemia and Moravia not differing significantly (Table 3). At first view this seems surprising as we assumed that habitat fragmentation and isolation of populations within Bohemia is much higher than within Moravia. However, other studies (Böhme et al. 2007a, b) also reveal large genetic distances within small geographic ranges (4–15 km) for *L. viridis* due

to the low tendency of individuals to disperse, especially when population density is low. Also field observations indicate that individuals of this species only disperse over short distances (max. 5 km; Schneeweiss 2001). Therefore, we suggest that despite the generally larger census size and higher connectivity of populations within the Moravian region geographic distances between the sample sites are too large for frequent migration of individuals between the populations. Moreover, the possibility of migration is decreased by the absence of suitable corridors between most of the Moravian populations indicating that these populations of *L. viridis* are fragmented.

### **Population history**

Despite the fact that regional genetic differentiation seems to be mainly affected by restricted gene flow, populations within the Moravian region were also significantly differentiated based on  $R_{ST}$ -values (Table 4). This finding indicates that Moravian populations are evolutionary more distinct from each other than the populations in Bohemia. This picture is supported by the reconstruction of evolutionary distances between populations using Goldstein distances (Fig. 5). The revealed distances ( $\delta\mu^2$ ) are expected to increase linearly with time. Therefore, the observed relationships support the assumption of the existence of former link populations between the main regions (Böhme et al. 2006). However, they also indicate that the evolutionary split between the Bohemian and Moravian regions is older than the evolutionary distances between populations within the regions. The Bohemian populations show lower genetic distances between each other than the Moravian populations. Considering population history, the resulting pattern would lead to the assumption of a single colonization event for the Bohemian region with a subsequent distribution of *L. viridis* along the river systems of central and northern Bohemia. Hence, the larger distances between the Moravian populations may indicate different colonization events within this region from the central species range or of a still ongoing immigration of alleles from the central species range.

### **Conservation genetics and implications for conservation**

In summary, despite the rather small sample sizes for some localities, this study provides useful preliminary information on the genetic diversity of Czech populations of *L. viridis*. Our results support the assumption that the Bohemian and Moravian populations have been isolated from each other for a long time and that there is no evidence for current natural gene flow between these regions. Furthermore, we have to consider that most populations within these regions show moderate or high genetic distances between each other, which significantly correlates with the value of respective geographic distances. With respect to conservation of *L. viridis* in the Czech Republic we have to consider that genetic diversity of populations depends on their position within the species range. The results accord with the current finding that the Moravian populations are connected with the central species range (e.g. Grillitsch & Cabela 2001, Mikátová et al. 2001a, Böhme et al. 2006) and that the Bohemian populations suffer because they are smaller, their habitat is more highly fragmented and isolated in the less optimal peripheral regions of the species range (e.g. Zavadil & Moravec 2005). The Bohemian populations at Karlík and Nezabudice, especially, show a reduced genetic diversity compared to other populations in this region, indicating that a small or reduced population size has an important influence on genetic diversity. It is well known that decreasing genetic variation can lead to a higher risk of extinction (Prior et al. 1997, Mockford et al. 1999, Edenhamn et al. 2000, Garner et al. 2004) and that the most threatened small peripheral populations are sources of adaptations important for the evolutionary future of a species (Lesica & Allendorf 1995). Therefore, the conservation of *L. viridis* in the Czech Republic should pay more attention to the fragmented relict Bohemian populations. The conservation efforts should focus on the maintenance and restoration of suitable habitats with a view to increasing the size

of individual metapopulations of *L. viridis*. In some areas (e.g. valleys of the rivers Vltava and Berounka) some of the neighbouring metapopulations are isolated by relatively narrow areas of unsuitable anthropogenic habitats (e.g. fields, coniferous forests, maintained gardens, zones of weekend houses, communications, etc.). Therefore, despite the low tendency of *L. viridis* to disperse, the building of natural biocorridors through such artificial barriers might facilitate a natural gene flow among neighbouring metapopulations. As the current status of the Bohemian populations of *L. viridis* does not indicate any need for support by translocating *L. viridis* from other Czech localities, future conservation programmes should be aimed at maximizing the genetic diversity of *L. viridis* in the Czech Republic.

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