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Genetic differentiation and diversity of *Lacerta viridis viridis* (Laurenti, 1768) within the northern part of its range: an investigation using mitochondrial haplotypes

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Abstract. The range of *Lacerta viridis viridis* is the result of a rapid postglacial dispersal, followed by range-restriction events leading to the isolation of the present northern relict populations. Current conservation efforts focus on these endangered, northern isolates. An understanding of the genetic diversity of these populations is a prerequisite for any conservation measures. In order to provide such data we analysed mtDNA sequence variation in 57 *L. v. viridis* from the northernmost part of the subspecies range, including representatives of isolated relict populations in Brandenburg (Germany) and northern Bohemia (Czech Republic). We detected a positive correlation between genetic distances of mtDNA sequences and geographic distances, which corresponds well with the migration behaviour of *L. v. viridis*. Furthermore, our data reveal the importance of a comparative analysis for estimating the genetic diversity of an endangered taxon like *Lacerta v. viridis*.

Key words: Reptilia: *Lacerta viridis viridis*; edge population; mitochondrial haplotypes; cytochrome *b*; control region; genetic distance.

Introduction

In modern conservation strategies peripheral populations become more and more important. Although central populations of a species are usually not endangered, the more threatened peripheral populations represent important sources for adaptation and for the evolutionary future of a species (Lesica & Allendorf 1995). Peripheral populations often inhabit atypical, less suitable habitats compared to populations in the central part of the species' range. Consequently, in peripheral populations individuals are often subject to strong environmental pressure, different from the situation in the centre of the species' range. These conditions near the range border are thought to trigger the evolution of genetic and/or morphological distinctiveness (Lesica & Allendorf 1995). Their localisation at the range border and their smaller population size makes these populations vulnerable to founder effects, inbreeding and genetic drift. It is well known that decreasing genetic variation can lead to a higher extinction risk, especially in small populations. These problems are the focus of many recent conservation studies (Prior et al. 1997, Mockford et al. 1999, Edenhamn et al. 2000, Garner et al. 2004).

The Green Lizard *Lacerta viridis* (Laurenti, 1768), is one species out of eight in the genus *Lacerta* sensu stricto (Nettmann 2001). Currently five subspecies of *L. viridis* are recognized (Rykena et al. 2001): *L. v. viridis* (Laurenti, 1768), *L. v. guentherpetersi* (Rykena et al., 2001), *L. v. infrapunctata* (Schmidtler, 1986), *L. v. meridionalis* (Cyren, 1924) and *L. v. paphlagonica* (Schmidtler, 1986). *Lacerta v. viridis* inhabits a wide range, extending from the Balkan Peninsula northwards across the Carpathian Basin to the more isolated edge populations in the Czech Republic and the northernmost populations in eastern Germany. This subspecies is highly endangered in Germany and the Czech

Republic (ELBING 2001a, MIKÁTOVÁ 2001) and many conservation efforts are undertaken to protect populations.

The German populations are situated in two narrow areas: one near Passau (Bavaria) and the other in the Lusatia region (Niederlausitz, Brandenburg). The Brandenburg habitats differ significantly from typical *L. v. viridis* habitats. The isolated Lusatian populations represent relict populations resulting from a rapid range expansion from a southern glacial refuge during the Holocene warming. According to PETERS (1970), the migration of this mesophile lizard to eastern Germany started 8000-7000 years BP in Preboreal times. Natural changes such as postglacial climatic oscillations, vegetation succession and anthropogenic impact on habitats have probably caused an increased habitat fragmentation. This process is leading to the current situation, with patchy localities harbouring only small isolated populations as in eastern Germany (Brandenburg). Due to its rare occurrence in Brandenburg, *L. v. viridis* has provoked attention since the 1930s (HECHT 1930, MERTENS & SCHNURRE 1946, 1949, ELBING 1996, 2001a, b, SCHNEEWEISS et al. 2004). These investigations revealed the collapse of most of the Brandenburg populations during the 20th century. A current conservation programme in Brandenburg monitors the remaining populations and includes a captive breeding group for reintroduction measures in regions where the subspecies has already become extinct (SCHNEEWEISS 2001). Later we will refer to this breeding group as a captive population. Because of the endangered situation in Brandenburg, information on the phylogeographic origin and the genetic variation of the wild populations becomes a necessity. Our investigation on the genetics of *L. v. viridis* is embedded in this program and seeks to compare the genetic variation of Brandenburg Green Lizards with other northern and central populations.

As a genetic marker we use the fast evolving mitochondrial DNA (mtDNA) fragment containing the cytochrome *b* gene (cyt *b*) and the control region (CR).

These DNA fragments have been used as powerful tools for the detection of phylogeographic patterns and Holocene range fluctuations so far (LENK et al. 1999, HARING et al. 2000, SEDDON et al. 2001, BABIK et al. 2004). Furthermore, these mitochondrial fragments yield information about the genetic relationships of populations and their genetic variation even within small regions (GÜBITZ et al. 2000, PAULO et al. 2002, HIROTA et al. 2004).

To test the genetic status of the endangered *L. v. viridis* populations in Brandenburg, we estimated the genetic differentiation of these populations from the geographically closest populations of the continuous range (edge populations) in the Czech Republic. We also estimated genetic differentiation in comparison to the more central populations of the subspecies' range in Slovakia, Austria and Hungary. To obtain more information about the genetic status of the endangered populations within the Brandenburg region, we conducted an analysis of the population differentiation between captive and wild Brandenburg populations. This seems to us a prerequisite for further projects concerning the reintroduction of *L. v. viridis* in Brandenburg.

Materials and methods

Taxon sampling and DNA extraction

Blood samples from 57 individuals of *L. v. viridis* from the northern and more central parts of the species' range were collected (Tab. 1). We sampled three isolated relict populations and one captive breeding population from eastern Germany. Furthermore, four populations from the Czech Republic, one population from Slovakia, three populations from the eastern part of Austria and one population from northern Hungary were sampled. Whole blood was obtained by foreleg vein puncture. Blood samples were stored in a special EDTA -Thymol buffer at minus 20 °C. Captured individuals were marked with a dot of nail polish, thereby preventing recapture of the same individuals. Total genomic

Regions	Pop	Location	Sample size
Germany, Brandenburg (D)	**1**	secured	6
	2	secured	3
	3	secured	3
	Cp	Linum	5
Czech Republic, Bohemia (CzB)	**4**	Karlik	4
Czech Republic, Moravia (CzM)	**5**	Podny	2
	6	Pavlov	2
	7	Bzenec	3
Austria, Lower Austria (A)	**8**	Weißenkirchen	3
	9	Hundsheim	2
	10	Gumpoldskirchen	11
Hungary (H)	**11**	Gödöllö	4
Slovakia, Slovak Carst (SK)	**12**	Turna	9

Tab. 1. Sampled regions, populations and individuals (sample size) of the present study. Populations were numbered consecutively (numbers occur in Fig. 6) Because of their high protection status (conservation guidelines) we can not include the exact geographic information of Brandenburg localities. Brandenburg population numbers refer to terminology of ELBING (2000). Cp stands for captive breeding population in Germany (Brandenburg, Linum).

DNA was extracted using the Qiagen Blood Kit, following the manufactures protocol.

Amplification and sequencing

To investigate the regional differentiation within *L. v. viridis*, we amplified an mtDNA fragment encompassing the partial cytochrome *b* gene, tRNA Thr, tRNA Pro and partial control region (CR). This amplification was conducted for 47 individuals with the primers LvF and LvR1 (Fig. 1, Tab. 2) under the following conditions: 1 x buffer (Sigma), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 15 pmol of each primer and 1 U Taq polymerase (Sigma). The PCR was performed on an Eppendorf Mastercycler for 35 cycles with 95 °C for 30 s, 60 °C for 30 s, 72 °C for 3 min. For sequencing, four internal primers (Tab. 2) were designed. This resulted in a fragment of a final length of about 2600 bp. Purified PCR products were used for a cycle sequencing reaction using Terminator Ready Reaction Mix ´Big Dye´ Version 3.1 (Applied Biosystems) following the manufacturer's protocol and were analysed with an ABI 3100 DNA Sequencer. The connection of partial sequences and internal sequences was done by using the program DNASIS v.7.0 (Hitachi Software). Final sequences were checked by visual inspection using the program Bioedit

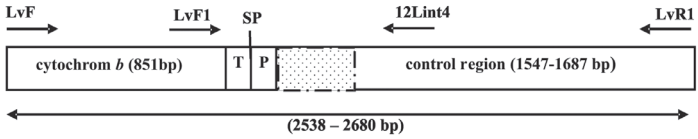

Fig. 1. Composition of the amplified (primers LvF and LvR1) mitochondrial fragment including the partial cytochrome *b* gene, tRNA Threonine (T), 17 bp Spacer (SP), tRNA Proline (P) and a partial fragment of the control region (CR). The dotted area at the 5´end of the CR comprises a length variable repeat region consisting of a 35 bp repeat unit, amplified with primers LvF1 and 12Lint4. Internal Primers (Tab. 2) are not shown.

Primer	Sequence
LvF	5´- CTGCATTTACCTCCATATTGGACG - 3´
LvF1	5´- GCCTATGCAATCCTTCGCTC - 3´
LvF2	5´- CAGTTATGCTATGAGCAAGGGTA - 3´
LvR1	5´- GGCTTTGTAGTTTRATCCTGAC - 3´
12Lint4	5´- TACCCTTGCTCATAGCATAACTG - 3´
LvR3	5´- GGGCGGAATGTTAAGGTCCGTTG - 3´

Tab. 2. Primer used in this study to sequence the mtDNA regions including the cytochrome *b* gene and mitochondrial control region (CR). The Primer 12Lint4 was designed by BREHM et al. (2002), LvF is a modified version of cBL (BREHM et al. 2002). The remaining primers were newly designed for this study.

Version 7.1 (HALL 1999). GenBank accession numbers for nucleotide sequences reported here are AM087227, AM 087228 and AM087289 – AM087330. Final sequence alignment was done using the program Clustal X (THOMPSON et al. 1997).

Phylogenetic relationships throughout the sampled range

To analyse the phylogenetic relationships and to test whether the populations of *L. v. viridis* in Brandenburg could represent a distinct subspecies as suggested by HECHT (1930), we aligned ten individual *L. v. viridis* mtDNA sequences from all over the sample range, one individual of *Lacerta viridis meridionalis* (AM087227) from Greece and one individual of *Lacerta agilis* (AM087228) from Romania. *Lacerta agilis* was used as the outgroup. For the construction of a Maximum-Likelihood phylogenetic tree (ML) we used PAUP* version 4.0 b10 (SWOFFORD 2002). DNA substitution rate was calculated with a hierarchical likelihood ratio test using Modeltest 3.6 (POSADA & CRANDALL 1998). The selected model was HKY+I+G (HASEGAWA et al. 1985) with base frequencies of A = 0.2946, C = 0.2544, G = 0.1153, T = 0.3357; proportion of invariable sites of I = 0.6708 and a gamma distribution shape parameter of variable sites of G = 0.4713. A Neighbor-Joining tree (NJ) using Tamura-Nei model (TAMURA & NEI 1993) and a Maximum-Parsimony tree (MP) with heuristic search using ten stepwise additions of sequences and TBR branch swapping option was also constructed using PAUP* 4.0 b10. To test the robustness of NJ and MP bifurcations, bootstrap analyses with 2000 replicates were performed.

Genetic differentiation between regions

To estimate the relative migration rates and genetic differentiation between the regions, all aligned individual sequences were assigned to sequence groups representing the six main regions (Tab.1). Geneflow and genetic distances between regions were calculated using DnaSP 4.0 (ROZAS et al. 2003). Significance of population differentiation was tested using Chi-square (NEI 1987, HUDSON et al. 1992) and permutation test (HUDSON et al. 1992) with 999 iterations. To obtain a pattern of the differentiation between the mtDNA haplotypes, the matrix of the pairwise genetic distances (F_{ST}) between regions was analysed using a Neighbor-Joining algorithm (SAITOU & NEI 1987) utilising MEGA3 (KUMAR et al. 2004). Within this analysis, gaps caused by the differing repeat numbers near the CR were excluded.

Intra-populational variation

To estimate the genetic diversity within populations we focussed on the most variable part of the analysed mtDNA fragment: the repeat region of the CR (Fig. 1). The haplotype diversity of a given population corresponded therefore to the number of different

repeats. To increase the data set of this sensitive parameter, we sequenced ten additional individuals for the CR repeat region with the internal primers LvF1 and LvR2 (Fig.1). The rest of the protocol was identical to the analysis of the population differentiation. The analysis comprised twelve different populations and one captive population within the six sampled regions, which were described above (Tab. 1).

Results

The general organisation of the amplified fragment displays the typical vertebrate pattern (Fig. 1, BOORE 1999) consisting of 851 bp of cyt *b*, tRNA Thr (67 bp), tRNA Pro (69 bp) and partial control region (CR, 1528-1670 bp). Both tRNA´s are separated from each other by the insertion (Fig. 1, spacer) of additional seventeen nucleotides. These nucleotides have not been observed in other squamate mtDNA sequences available in the GenBank e.g. *Lacerta dugesii* (BREHM et al. 2003), *Podarcis siculus* (PODNAR et al. unpublished), *Pariocela eregia lividus* (KUMAZAWA & NISHIDA 1999) and *Cordylus warreni* (KUMAZAWA 2004). Therefore, an assignment of these nucleotides to either tRNA Thr or tRNA Pro was impossible. We observed an overall decreased G content in the light strand nucleotide frequencies (A 29.2-29.8 %; G 11.5-12.1 %; T 33.1-33.8 % and C 25.1-25.7 %), which is consistent with the known reptilian pattern so far (MACEY et al. 1997). The organisation of the CR is very similar to the control region of *L. dugesii* (BREHM et al. 2003). However, the mitochondrial genome of *L. v. viridis* incorporates a distinctive and remarkable character: a region consisting of variable number of repeat units next to the tRNA Pro at the 5´end of CR (Fig. 1). The 35 bp motive of this repeat unit is highly conserved within *L. v. viridis*, but differs in repeat number (6-10). This size polymorphism in form of a variable number of tandem repeats (VNTR's, LUNT et al. 1998) results in a varying overall fragment length from 2538 bp to 2680 bp between individuals. Different types of mtDNA molecules within one individual (heteroplasmy) shown in other studies and reviewed in RAND (2001) were not observed in *L. v. viridis* so far. The mentioned VNTR's are often observed in mitochondrial CR (SUMIDA et al. 2000, MUNDY & HELBIG 2004) and are supposed to yield information about population differentiation and biogeography (LUNT et al. 1998).

Phylogenetic relationships throughout the sampled range

All phylogenetic trees (NJ, MP and ML) inferred from aligned complete mtDNA sequences were summarized in Fig. 2. *Lacerta v. viridis* form a monophyletic group distinct from *L. v. meridionalis*, which is supported by bootstrap values of 79 % for NJ, 72 % for MP and 62 % for ML (Fig. 2). This implicates that all sampled regions are inhabited by the same subspecies *L. v. viridis* and that the northernmost populations in Germany (Brandenburg) do not represent a distinct subspecies.

Genetic variation and differentiation of regions

Altogether, the genetic variation of the analysed fragment is low. Of 2680 aligned sites only 79 were variable and only 44 sites of these yielded a phylogenetic signal (MEGA3). Generally, the observed differentiation pattern in Fig. 3 fits well to the geographic distribution of the Green Lizards in the studied regions. Pairwise genetic distances F_{ST} (permutation test 999 replicates $p = 0{,}000$; $\chi^2 = 0{,}0003$, $df = 150$) between the regions correlate significantly with their geographic distance and Fig. 4 illustrates this positive relationship of genetic and geographic distance between regions. The regions of Brandenburg (eastern Germany) and Bohemia (northern Czech Republic) displayed the lowest genetic distance (Tab. 3)

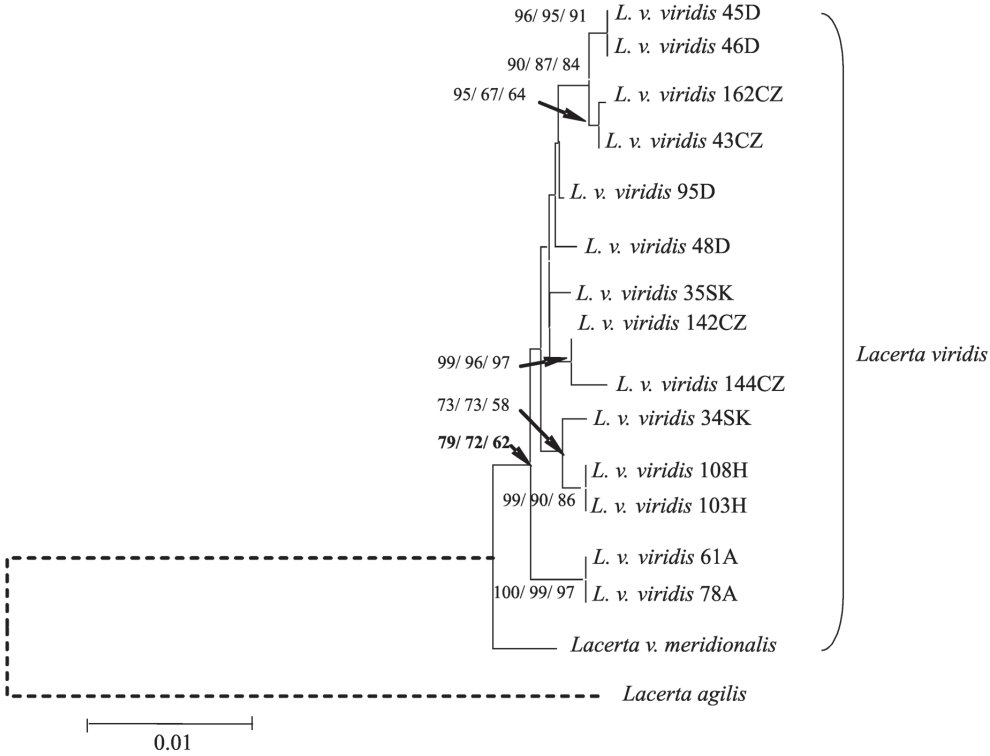

Fig. 2. Neighbor Joining tree (PAUP* 4.0 b10) obtained from 10 *L. v. viridis* mtDNA (cyt *b*, CR) sequences within the sample range (D- Germany (Brandenburg), CZ- Czech Republic, SK- Slovakia, H- Hungary, A- Austria), one *L. v. meridionalis* and the outgroup *Lacerta agilis*. Distances shown are Neighbor-Joining distances. Numbers (NJ/ MP/ML) at the nodes show bootstrap values for NJ analysis (first number) bootstrap values for Maximum Parsimony (second number) and Maximum Likelihood probabilities (> 50 %) resulting from 2000 replications.

although they are not the geographically closest regions within the sampled range. Interestingly, the geographically closer regions of Austria and Moravia (southern Czech Republic) as well as Slovakia and Hungary are genetically more distant to each other (Fig. 3).

Genetic diversity within populations

Despite an observed low overall nucleotide variation within the investigated mtDNA fragment, there appear remarkable variations within the repeat region of the mitochondrial control region (CR, BÖHME et al., unpublished). However, the variation was mostly limited to insertions or deletions of repeat units. This high mutation rate can be traced back to a special D-loop structure and slipped-strand mispairing during mtDNA replication (HOELZEL et al. 1994, SBISA et al. 1997, SAVOLAINEN et al. 2000). Changing repeat number was found across the whole sampled species range but also within populations. An analysis of the variable CR repeat number and the observed haplotype frequency showed five different repeat haplotypes within the investigated populations. Furthermore, we detected that smaller repeat num-

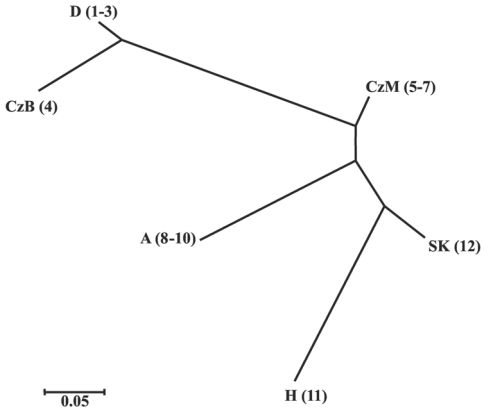

Fig. 3. Neighbor Joining (NJ) distance tree based on pairwise genetic distances (F_{ST}) of the mtDNA haplotypes between six regions within the range of *Lacerta v. viridis*. Letters refer to regions and the numbers in parenthesis refer to numbered populations (Tab. 1, Fig. 6).

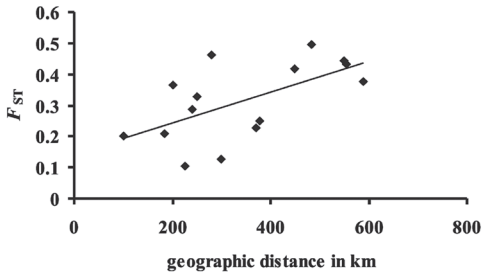

Fig. 4. Correlation of pairwise genetic distance (F_{ST}) and geographic distance between different regions within the range of *Lacerta v. viridis*.

bers (r6-r7) show a higher overall haplotype frequency (Fig. 5) than larger repeat numbers (r8-r10). Within populations, considerable differences occur in the repeat haplotype distribution (Fig. 6). More centrally located populations, like population 9 (East Austria) and 12 (Slovakia), contain private repeat haplotypes, haplotypes which only occur in one population, e.g. r9 and r10. Interestingly, the most northern populations 1-3 (Germany) and 4 (Czech Republic) contain no private repeat haplotypes.

Region	D	CzB	CzM	H	SK	A
D		0.106	0.251	0.444	0.376	0.416
CzB	226		0.327	0.496	0.433	0.461
CzM	395	250		0.288	0.126	0.200
H	608	483	247		0.208	0.226
SK	590	554	334	183		0.365
A	458	258	102	248	374	

Tab. 3. Genetic differentiation between different regions of the northern *Lacerta v. viridis* subspecies' range. Values in the upper part display pairwise genetic distances (F_{ST}) between regions, whereas the values in the lower part display approximate geographic distance in km between regions. Regions: D – Germany (Brandenburg), CzB – Czech Republic (Bohemia), CzM – Czech Republic (Moravia), H – Hungary, SK – Slovakia, A – Austria.

Situation in Brandenburg

To obtain more information about the endangered and isolated populations in Brandenburg, twelve individuals from three wild populations (Pop1, Pop2, Pop3) and five individuals of the captive population were analysed. Within the 2680 aligned base pairs we found 22 variable sites between the individuals of the wild populations; of these, sixteen yielded phylogenetic information. Within the captive population, we found only four variable sites with no phylogenetic information. In comparison to the wild populations in Brandenburg (Fig. 7) the captive population displayed the strongest relationship to the population Pop3 (F_{ST} 0,067), whereas the genetic distance to Pop1 and Pop2 is higher (F_{ST} 0,241 / 0,584). Overall haplotype frequency of the repeat number in Brandenburg populations was low (Fig. 6); only two different repeat haplotypes were observed (r6 and r8). Like in most of the sampled Brandenburg populations, only one repeat haplotype (r6) occurred in the captive population.

Discussion

A genetic analysis of all mtDNA haplotypes revealed no strong nucleotide differences

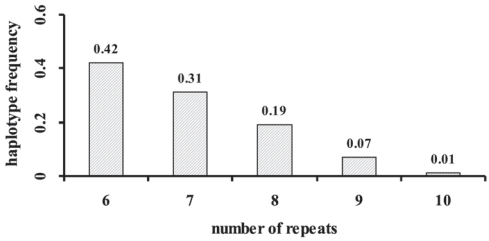

Fig. 5. Overall haplotype frequency of repeat numbers within the mitochondrial control region (CR) of *Lacerta v. viridis*.

between the northern edge or isolated populations and the central populations in the observed subspecies' range (Fig. 2) of *Lacerta v. viridis*. Our results of a positive correlation between genetic and geographic distance (Fig. 4) suggest a stepwise dispersal of individuals over short distances. This fits to the hypothetical postglacial migration behaviour of *L. v. viridis* (NOLL 1878, PETERS 1970), with a strong relationship to microclimatic favourable regions, following rivers and the southern slopes of hills. Nevertheless, the genetic differentiation between Brandenburg (eastern Germany) and Bohemia (Czech Republic) needs to be discussed in detail (Fig. 3). There is a remarkably small genetic distance between Bohemia (CzB) and Brandenburg (DD) in contrast to the geographically closer related region pairs (Tab. 3) such as Austria (A) and Moravia (CzM) or Slovakia (SK) and Hungary (H). This result seems to yield information for the reconstruction of postglacial migration routes. The common hypothesis (PETERS 1970) assumes a Holocene immigration of *L. v. viridis* from the Czech Republic (Bohemia or Moravia) to eastern Germany. Our results support this hypothesis but also favour a route from Bohemia, along the valleys of rivers Vltava (Moldau) and Labe (Elbe).

Generally, in contrast to other vertebrate studies which do not focus at the subspecies level (GÜBITZ et al. 2000, PAULO et al. 2002,

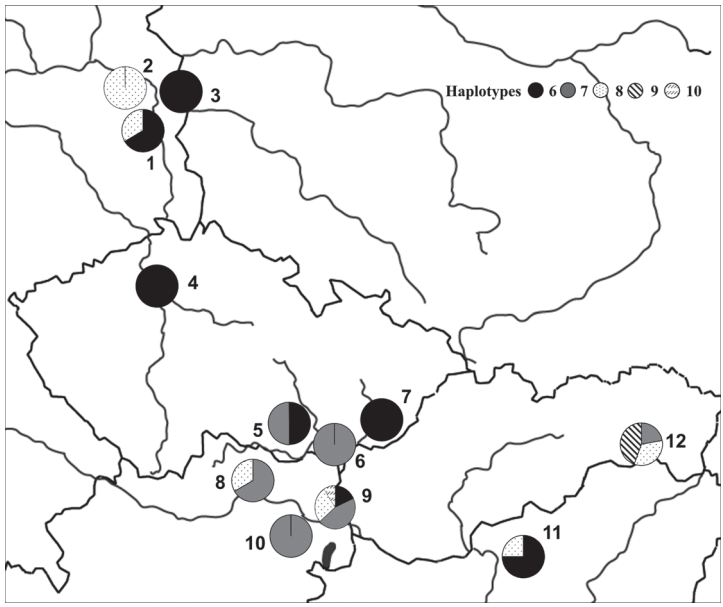

Fig. 6. Haplotype pattern of the variable number of repeats (VNTR's) within populations of *Lacerta v. viridis* in different regions of its northern range. Circles display haplotypic frequencies within the sampled populations. Population numbers refer to Table 1. Populations 1-3 Germany (Brandenburg), 4 Czech Republic (Bohemia), 5-7 Czech Republic (Moravia), 8-10 Austria, 11 Hungary, 12 Slovakia.

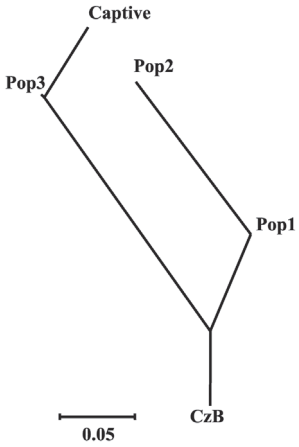

Fig. 7. NJ tree based on pairwise genetic distances (F_{ST}) of mtDNA haplotypes between Czech (Bohemian) and German wild populations of *Lacerta v. viridis* and the captive population in Brandenburg.

Brehm et al. 2003, Hirota et al. 2004), the mitochondrial DNA (mtDNA) fragment including the cytochrome *b* gene and the control region (CR) displayed a low genetic variability within *L. v. viridis*. It yields not enough phylogenetic information to obtain a phylogeny based on individual haplotypes. The genetic variation within populations, analysed using the repeat haplotype frequency of the CR (Fig. 6), does not show a higher genetic variation within the central populations in contrast to the northern, more isolated populations. There is an overall small variation of only five different CR repeat haplotypes (Fig. 5). Shorter repeat numbers (r6/r7) seem to be the main repeat haplotypes whereas longer repeat numbers are more uncommon. Furthermore, we detected private repeat haplotypes only in the more central populations for example in Pop9 and Pop12. Because of the chosen amplification conditions we can exclude a skewed distribution to smaller size classes due to a laboratory artefact as mentioned in Lunt et al. (1998). Despite the well known high variability of the CR, this marker system could not resolve the genetic variation of the populations satisfactorily. Further information of nuclear microsatellite data (Böhme et al. 2005) is needed to clarify this genetic situation (work in progress). However it is important to note that due to the comparison of the endangered populations in Brandenburg with the central populations in Slovakia and Hungary, the low variability of the CR was revealed. A single analysis which would have focussed only at the endangered populations would have led to the false conclusion that the genetic diversity of these populations is strongly reduced whereas in reality the molecular marker itself displays a low variation. Therefore this is an important example that genetic markers need to be „calibrated", before they can be used to estimate the genetic diversity of endangered populations.

Captive and Brandenburg populations

We observed an overall low genetic mtDNA variation in the captive population and the Brandenburg populations and no pronounced differences with other northern populations within the subspecies range of *L. v. viridis*. Therefore, genetic data (Fig. 2) confirm the morphological findings of Mertens & Schnurre (1946, 1949), that the Brandenburg populations represent the subspecies *L. v. viridis* and do not represent a distinct subspecies as suggested by Hecht (1930). Also the CR repeat data (Fig. 6) support this conclusion.

Within the Brandenburg populations our data (Fig. 7) show a strong genetic link between the captive population and Pop3. This is expected, as the founder individuals of the captured population originated from this population (Kirmse 1990, 1994). Consequently, we are confident that the genetic methods employed by us are appropriate to analyse the genetic relationships between populations in *L. v. viridis*. There is also a surprising genetic differentiation between the three wild populations (Pop1/2/3), suggesting that within a small geographic distance of only 2-14 km, genetic differentiation of populations

is possible. Maybe the small individual numbers, the low population density (NETTMANN & RYKENA 1984, ELBING 2001b) and the strong microrelief (wall) in some localities will enforce this effect.

Acknowledgements

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References

BABIK, W., W. BRANICKI, M. SANDERA, S. LITVINCHUK, L.J. BORKIN, J.T. IRWIN & J. RAFINSKI (2004): Mitochondrial phylogeography of the moor frog, *Rana arvalis*. – Molecular Ecology, **13**(6): 1469-1480.

BOORE, J.L. (1999): Animal mitochondrial genomes. – Nucleic Acids Research, **27**(8): 1767-1780.

BÖHME, M.U., T.U. BERENDONK & M. SCHLEGEL (2005): Isolation of new microsatellite loci from the Green Lizard (*Lacerta viridis viridis*). – Molecular Ecology Notes, **5**: 45-47.

BREHM, A., A.D. JAMES HARRIS, C.D. ALVES, J.D. JESUS, F.D. THOMARAT & L.D. VICENTE (2003): Structure and evolution of the mitochondrial DNA complete control region in the lizard *Lacerta dugesii* (Lacertidae, Sauria). – Journal of Molecular Evolution, **56**(1): 46-53.

EDENHAMN, P., M. HOGGREN & A. CARLSON (2000): Genetic diversity and fitness in peripheral and central populations of the european tree frog *Hyla arborea*. – Hereditas, **133**(2): 115-122.

ELBING, K. (1996): Zur Situation der östlichen Smaragdeidechse (*Lacerta viridis*) in ihren Niederlausitzer Reliktvorkommen. – Naturschutz und Landschaftspflege in Brandenburg, **3**: 34-37.

ELBING, K. (2000): Fortpflanzungsbiologie und Populationsökologie der Smaragdeidechse (*Lacerta viridis*, LAURENTI, 1768) in ihren brandenburgischen Reliktvorkommen. – Dissertation Universität Bremen.

ELBING, K. (2001a): Das Artenschutzprogramm "Smaragdeidechse" *Lacerta viridis* (LAURENTI, 1768) des Landes Brandenburg. – Mertensiella, **13**: 269-278.

ELBING, K. (2001b): Die Smaragdeidechsen – zwei (un)gleiche Schwestern. – Bochum (Laurenti Verlag), 143 pp.

GARNER, T.W., P.B. PEARMAN & S. ANGELONE (2004): Genetic diversity across a vertebrate species' range: a test of the central-peripheral hypothesis. – Molecular Ecology, **13**(5): 1047-1053.

GÜBITZ, T., R.S. THORPE & A. MALHOTRA (2000): Phylogeography and natural selection in the Tenerife gecko *Tarentola delalandii*: testing historical and adaptive hypotheses. – Molecular Ecology, **9**(9): 1213-1221.

HALL, T.A. (1999): BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. – Nucleic Acids Symposium Series, **41**: 95.98.

HARING, E., B. HERZIG-STRASCHIL & F. SPITZENBERGER (2000): Phylogenetic analysis of alpine voles of the *Microtus multiplex* complex using the mitochondrial control region. – Journal of Zoological Systematics and Evolutionary Research, **38**: 231-238.

HASEGAWA, M., H. KISHONO & K. YANO (1985): Dating of the human ape splitting by a molecular clock of mitochondrial DNA. – Journal of Molecular Evolution, **22**: 160-174

HECHT, G. (1930): Die märkische Smaragdeidechse, *Lacerta viridis* (Laur.) subspec. *brandenburgensis* subspec. nov. – Das Aquarium, **1930**: 62.

HIROTA, T., T. HIROHATA, H. MASHIMA, T. SATOH & Y. OBARA (2004): Population structure of the large Japanese field mouse, *Apodemus speciosus* (Rodentia: Muridae), in suburban landscape, based on mitochondrial D-loop sequences. – Molecular Ecology, **13**(11): 3275-3282.

HOELZEL, A.R., J.V. LOPEZ, G.A. DOVER & S.J. O'BRIEN (1994): Rapid evolution of a heteroplasmic repetitive sequence in the mitochondrial DNA control region of carnivores. – Journal of Molecular Evolution, **39**(2): 191-199.

KIRMSE, W. (1990): Die Smaragdeidechse in Brandenburg: Bestand und Schutzmaßnahmen. – Die Eidechse, **1**: 10-12.

KIRMSE, W. (1994): Zur aktuellen Situation der brandenburgischen Smaragdeidechse (*Lacerta v. viridis*). – Die Eidechse, **5**(11): 2-4.

Hudson, R.R., D.D. Boos & N.L. Kaplan (1992): A statistical test for detecting population subdivision. – Molecular Biology and Evolution, **9**: 138-151.

Kumar, S., K. Tamura & M. Nei (2004): MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. – Briefings in Bioinformatics, **5**: 150-163.

Kumazawa, Y. (2004): Mitochondrial DNA sequences of five squamates: phylogenetic affiliation of snakes. – DNA Research, **11**: 137-144.

Kumazawa, Y. & M. Nishida (1999): Complete mitochondrial DNA sequences of the green turtle and blue-tailed mole skink: statistical evidence for archosaurian affinity of turtles. – Molecular Biology and Evolution, **16**(6): 784-792.

Lenk, P., U. Fritz, U. Joger & M. Wink (1999): Mitochondrial phylogeography of the European pond turtle, *Emys orbicularis* (Linnaeus, 1758). – Molecular Ecolology, **8**: 1911-1922.

Lesica, P. & F.W. Allendorf (1995): When are pheripheral populations valuable for conservation. – Conservation Biology, **9**(4): 753-760.

Lunt, D.H., L.E. Whipple & B.C. Hyman (1998): Mitochondrial DNA variable number tandem repeats (VNTRs): utility and problems in molecular ecology. – Molecular Ecology, **7**(11): 1441-1455.

Macey, J.R., A. Larson, N.B. Ananjeva, Z. Fang & T.J. Papenfuss (1997): Two novel gene orders and the role of light-strand replication in rearrangement of the vertebrate mitochondrial genome. – Molecular Biology and Evolution, **14**(1): 91-104.

Mertens, R. & O. Schnurre (1946): Zur Eidonomie, Taxonomie und Ökologie der norddeutschen Smaragdeidechse. – Senkenbergia, **27**(1/3): 25-52.

Mertens, R. & O. Schnurre (1949): Eidonomische und ökologische Studien an Smaragdeidechsen Deutschlands. – Abhandlungen der Senckenbergischen Naturforschenden Gesellschaft, **481**: 1-28.

Mikátová, B. (2001): The green lizard, *Lacerta viridis* (Laurenti, 1768), in the Czech Republic: distribution, ecology and conservation aspects. – Mertensiella, **13**: 138-149.

Mockford, S.W., M. Snyder & T.B. Herman (1999): A preliminary examination of genetic variation in a peripheral population of Blanding's turtle, *Emydoidea blandingii*. – Molecular Ecology, **8**(2): 323-327.

Mundy, N.I. & A.J. Helbig (2004): Origin and evolution of tandem repeats in the mitochondrial DNA control region of shrikes (*Lanius* spp.). – Journal of Molecular Evolution, **59**(2): 250-257.

Nei, M. (1987): Molecular Evolutionary Genetics. New York (Columbia Univ. Press).

Nettmann, H.-K. (2001): Die Smaragdeidechsen (*Lacerta* s. str.) – Eine Übersicht über Verwandtschaft und Formenvielfalt. – Mertensiella, **13**: 11-32.

Nettmann, H.-K. & S. Rykena (1984): *Lacerta viridis* (Laurenti, 1768) – Smaragdeidechse. – pp. 129-181 in Böhme, W. (ed.): Handbuch der Reptilien und Amphibien Europas. – Wiesbaden (Aula Verlag).

Noll, F.C. (1878): Einige dem Rheinthale von Bingen bis Coblenz eigenthümliche Pflanzen und Thiere mit Rücksicht auf ihre Verbreitung und die Art ihrer Einwanderung. – Jahresber. der Frankf. Ver. Geogr. Statist., **1878**: 1-66.

Paulo, O.S., W.C. Jordan, M.W. Bruford & R.A. Nichols (2002): Using nested clade analysis to assess the history of colonization and the persistence of populations of an Iberian Lizard. – Molecular Ecology, **11**(4): 809-819.

Peters, G. (1970): Studien zur Taxonomie, Verbreitung und Ökologie der Smaragdeidechsen IV. Zur Ökologie und Geschichte der Populationen von *Lacerta v. viridis* (Laurenti) im mitteleuropäischen Flachland. – Veröffentlichungen des Bezirksheimatmuseums Potsdam, **21**: 49-119.

Posada, D. & K.A. Crandal (1998): MODELTEST: testing the model of DNA substitution. – Bioinformatics, **14**(9): 817-818.

Prior, K.A., H.L. Gibbs & P.J. Weatherhead (1997): Population genetic structure in the black rat snake: implications for management. – Conservation Biology, **11**(5): 1147-1158.

Rand, D.M. (2001): The units of selection on mitochondrial DNA. – Annual Review of Ecology and Systematics, **32**: 415-448.

Rozas, J., J.C. Sanchez-DelBarrio, X. Messeguer & R. Rozas (2003): DnaSP, DNA polymorphism analyses by the coalescent and other methods. – Bioinformatics, **19**: 2496-2497.

Rykena, S., H.-K. Nettmann & W. Mayer (2001): *Lacerta viridis guentherpetersi* ssp. nov., eine neue Unterart der Smaragdeidechse aus Griechenland. – Mertensiella, **13**: 89-97.

Saitou, N. & M. Nei (1987): The neighbor-joining method: a new method for reconstructing phylogenetic trees. – Molecular Biology and Evolution, **4**: 406-425.

Savolainen, P., L. Arvestad & J. Lundeberg (2000): mtDNA tandem repeats in domestic

dogs and wolves: mutation mechanism studied by analysis of the sequence of imperfect repeats. – Molecular Biology and Evolution, **17**(4): 474-488.

SBISA, E., F. TANZARIELLO, A. REYES, G. PESOLE & C. SACCONE (1997): Mammalian mitochondrial D-loop region structural analysis: identification of new conserved sequences and their functional and evolutionary implications. – Gene, **205**(1-2): 125-140.

SCHNEEWEISS, N. (2001): Aspekte der Entwicklung und des Ausbreitungsverhaltens von Smaragdeidechsen (*Lacerta viridis viridis*) in einem Ansiedlungsversuch in Brandenburg. – Mertensiella, **13**: 229-240.

SCHNEEWEISS, N., M. BÖHME, M. STEIN, V. ZAVADIL & J. KAUTMAN (2004): Populationsgenetische Studie als Beitrag zum Artenschutzprojekt Smaragdeidechse (*Lacerta viridis*) in Brandenburg. – Elaphe, **12**(2): 65-67.

SEDDON, J.M., F. SANTUCCI, N.J. REEVE & G.M. HEWITT (2001): DNA footprints of European hedgehogs, *Erinaceus europaeus* and *E. concolor*: Pleistocene refugia, postglacial expansion and colonization routes. – Molecular Ecology, **10**(9): 2187-2198.

SUMIDA, M., H. KANEDA, Y. KATO, Y. KANAMORI, H. YONEKAWA & M. NISHIOKA (2000): Sequence variation and structural conservation in the D-loop region and flanking genes of mitochondrial DNA from Japanese pond frogs. – Genes & Genetic Systematics, **75**(2): 79-92.

SWOFFORD, D.L. (2002): PAUP*. Phylogenetic analysis using parsimony (*and other methods), Version 4.0. Sunderland, MA (SinauerAssociates).

TAMURA, K. & M. NEI (1993): Estimation of the number of nucleotide substitutions of the control region of mitochondrial DNA in human and chimpanzees. – Molecular Biology and Evolution, 10: 512-526.

THOMPSON, J.D., T.J. GIBSON, F. PLEWNIAK, F. JEANMOUGIN & D.G. HIGGINS (1997): The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. – Nucleic Acids Research, **24**: 4876-4882.

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