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ORIGINAL PAPER

Small edge populations at risk: genetic diversity of the green lizard (*Lacerta viridis viridis*) in Germany and implications for conservation management

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Abstract Edge and central populations can show great differences regarding their genetic variation and thereby also in their probability of extinction. This fact might be of great importance for the conservation strategies of endangered species. In this study we examine the level of microsatellite variability within three threatened edge populations of the green lizard subspecies *Lacerta viridis viridis* (Laur.) in Brandenburg (Germany) and compare the observed variation to other edge and central populations within the northern species range. We demonstrate that the northernmost edge populations contain less genetic variation in comparison to the central population. However, there were no observable significant differences to the other edge population included in this study. Surprisingly, we observed a high genetic differentiation in a small geographical range between the three endangered populations in Brandenburg, which can be explained by processes like fragmentation, isolation, genetic drift and small individual numbers within these populations. We also detected unique genetic variants (alleles), which only occurred in these populations, despite a low overall genetic variation. This study demonstrates the potential of fast evolving markers assessing the genetic status of endangered populations with a high resolution. It also illustrates the need for a comparative analysis of different regions within the species range, achieving a more exact interpretation of the genetic variation in endangered populations. This will aid future management decisions in the conservation of genetic diversity in threatened species.

Keywords Genetic variation · Microsatellites · Isolated population · *Lacerta viridis*

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Introduction

It has long been recognized that the conservation of biodiversity should entail the protection of genetic diversity (DeSalle and Amato 2004). Especially for endangered species are estimates about the overall level of genetic diversity and its distribution within and among populations of vital importance. Fast evolving markers allow conservation biologists the analysis of current genetic status of populations as well as genetic consequences of possible recent influences, such as founder events or population bottlenecks (Avise 1994; Balloux and Lugon-Moulin 2002; Spencer et al. 2000). Consequently, conservation studies focusing on the genetic status of endangered species have become more and more common. Also the number of studies, which have used microsatellites as a fast evolving nuclear marker has increased. However, many of these studies, especially within vertebrates, focus on mammals, birds and amphibians. A smaller number of studies are available for reptiles and especially for

lizards. Only a few studies on certain European lizards such as *Lacerta agilis* (Gullberg et al. 1997; Ryberg et al. 2004), *Lacerta vivipara* (Le Galliard et al. 2005), *Podarcis bocagei* (Pinho et al. 2004) and some *Darevskia* species (Petrosyan et al. 2003) have been published so far. This is surprising, as almost all of the European lizards are listed as endangered species. This is also the case for another European species, the green lizard *Lacerta viridis* and especially for the nominate form *Lacerta viridis viridis*. This species is listed in the Flora–Fauna-Habitat directive (FFH) of the European Union (1992) in the appendix IV, which contains strictly protected species of public interest. Edge populations of the *L. v. viridis* species range have a high extinction risk and many efforts are undertaken to protect these local populations, especially in Brandenburg, where *L. v. viridis* is highly endangered (Schneeweiß et al. 2004).

On the one hand, it is known that small edge populations of a species are often more vulnerable to effects like isolation, genetic drift and increased inbreeding (Lesica and Allendorf 1995). Therefore, they are supposed to have a reduced genetic diversity and are presumed to have a higher extinction rate (Lande 1988). On the other hand, these populations often comprise genetic variants, which enable them to adapt to changing environments and therefore to different selection pressures. Frequently, these edge populations mark a threshold of environmental variation, beyond which the species can not expand. These edge populations may be subject of intensive selection, which is often reflected in morphological peculiarities of the individuals within these edge populations. Consequently, these populations often exhibit a low degree of genetic variation, but are of great importance for the evolution and long term persistence of the species (Lesica and Allendorf 1995).

The northernmost edge populations of *L. v. viridis* exist within the German federal state Brandenburg. It is well known that these are small isolated populations, which inhabit fragmented and unusual relict habitats (Peters 1970; Elbing 2001a). Because of this situation the Brandenburg populations were studied since early 1930 (Hecht 1930; Mertens and Schnurre 1946, 1949) and a large amount of ecological work and field observation were conducted especially by Peters (1970) and Elbing (2000, 2001a). Today's existence of these fragile *L. v. viridis* populations depends highly on seasonal microclimatic conditions and habitat management. Therefore, a conservation project was initiated (Kirmse 1990, 1994; Elbing 2001b; Schneeweiß 2001), including a breeding program for *L. v. viridis*. This captive breeding population (CP) provides the great opportunity to analyse genetic consequences of a recent founder event for *L. v. viridis*. The founding individuals of this captive population were three autochthonous individuals (Kirmse 1990) from the Brandenburg populations. The two males came from the Brandenburg population B3 (Fig. 1) and the female from an already extinct subpopulation 4 km away.

The aim of this case study is to provide genetic information for the conservation management of the Brandenburg populations. To achieve this, we analysed the genetic variation of these populations at different levels. First, the genetic structuring of the Brandenburg populations was investigated. Second, we tested if these edge populations do actually show a reduced genetic diversity compared to other northern edge populations and central populations. We also conducted an analysis of the genetic status of the captive population, in order to compare this data with the wild populations in Brandenburg and evaluate the genetic basis for a reintroduction of this species to former habitats (Schneeweiß 2001). To achieve these goals we used newly established microsatellites to estimate the genetic diversity of the Brandenburg populations and of additional edge and central populations from the Czech Republic and Hungary. Here, we present the results of this comparative genetic analysis of this endangered species. We believe that the results of this case study will be useful for the conservation management of other endangered lizard populations living close to the edge of their species range.

Methods

Species and study area

The green lizard *L. viridis* is currently divided into five subspecies while the most widespread subspecies *L. v. viridis* inhabits a wide eastern European range, spanning from western Ukraine and the Balkan Peninsula northwards across the Carpathian Basin to the edge populations in the Czech Republic and the northernmost populations in eastern Germany (Fig. 1). In this study we investigated the highly endangered northwestern populations of this subspecies. These populations are located within the eastern part of Germany in the federal state of Brandenburg in the Lower Lusatia region (Niederlausitz, Fig. 1).

L. v. viridis occurs in this region within three small, isolated relict populations. Within this region we studied these wild populations (Table 1). The populations one (B1) and two (B2) are located in the same

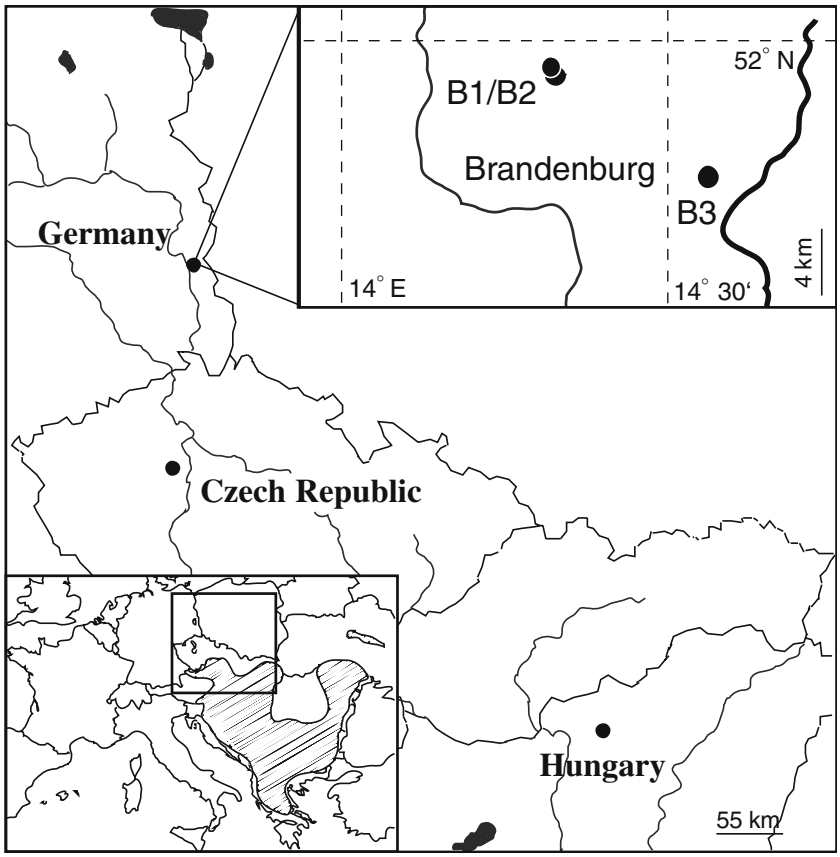

Fig. 1 Sample sites within the northern part of the *Lacerta v. viridis* range. The enlargement shows detailed locations of the three isolated edge populations within the Brandenburg region (Germany)

regional area with only 2 km apart, whereas the population three (B3) is a very isolated, small one with a distance of about 14 km to the other Brandenburg populations. For a better assessment of the observed genetic diversity we compared the genetic variation of the Brandenburg populations to a northern edge population in the Czech Republic (Bohemia, Fig. 1) and a central population in Hungary (Böhme et al. 2005, Fig. 1). As mentioned before we integrated a captive population (CP) in this study, in order to assess the genetic basis for further reintroduction projects.

Table 1 Populations, approximate population size, size of habitat area and number of individuals analysed in this study

Population	Approx. population size	Area in ha	Sample size
Edge population Germany			
Brandenburg 1 (B1)	125	8.3	17
Brandenburg 2 (B2)	35	5.8	8
Brandenburg 3 (B3)	35	3.9	8
captive population (CP)	35	–[a]	8
Edge population			
Czech Republic (CZ)	<80	3.5	6
Central population			
Hungary (H)	>400	3.0	27

[a] no natural habitat

Genetic analysis

We collected blood samples from 33 individuals of the three wild Brandenburg populations and from eight individuals from the captive population of the breeding program in Brandenburg (Table 1). Furthermore, we collected six individuals from a Czech population (CZ) and 27 individuals from a Hungarian population (H). Blood samples were stored in a special EDTA–Thymol buffer at –20°C. Captured individuals were marked with a dot of nail polish, thereby preventing recapture of the same individuals. Total genomic DNA was extracted using the NucleoSpin Blood Kit (Machery & Nagel), following the manufacturers protocol. Amplification of the 12 microsatellite loci was done with primers already published for this species (Böhme et al. 2005). A multiplex amplification was performed with four sets of primers except Locus Lvir18, which had to be amplified separately. Primer set one (set 1) included the primers for amplification of the loci Lvir16, Lvir1, Lvir17; set 2 amplified the loci Lvir10 and Lvir11; set 3 amplified the loci Lvir2, Lvir4, Lvir14

and set 4 comprised primers for loci Lvir1, Lvir6 and Lvir9. The PCR composition for multiplexing in a total volume of 25 µl was 1 × buffer (Qiagen), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 10 pmol of each primer and 1 U Taq polymerase (Qiagen). Multiplex PCR was performed on an Eppendorf Mastercycler using the following profile: initial denaturation at 95°C for 1 min; 30 cycles at 94°C for 30 s, 55°C for 30 s and 72°C for 30 s. Fluorescent PCR fragments were analysed with an ABI Prism 3100 automated sequencer (ABI) following the manufacturers protocol. Individual genotypes were detected by GeneMapper Software v. 3.7 (ABI).

Data analysis

Genetic diversity and genetic subdivision

All loci were tested over all populations for deviations from Hardy–Weinberg expectations and linkage disequilibrium using the probability test integrated in the program GENEPOP v. 3.4 (Raymond and Rousset 1995). The first step to analyse genetic diversity of the wild Brandenburg populations was to ensure that the genetic data mirrored the observed population structure in the field. Therefore, we tested the individual assignment quality to the predefined populations by using the program STRUCTURE v. 2.1 (Pritchard et al. 2000). After this assignment an analysis of genetic diversity was carried out using the program GENALEX v. 6.0 (Peakall and Smouse 2006). Genetic diversity within a population was assessed by expected (H_E), observed heterozygosity (H_O, Nei 1987) and the percentage of polymorphic loci (L_{POL}). The number of alleles (A) and number of private alleles (A_P), which occur only in one single population, were corrected for sample size by rarefaction using the program HP-RARE 1.0 (Kalinowski 2005). Differences of genetic variation between the populations were tested for significance by an analysis of variance (ANOVA), assuming a statistical independence of all the loci between populations. If no homogeneity of variances was detected, we performed a Kruskal–Wallis test (H-test). Additionally we performed a two factorial ANOVA, with loci and populations as factors. Furthermore, we checked the analysed populations for evidences of recent bottlenecks using the program BOTTLENECK (Cornuet and Luikart 1996).

Significance of genetic differences between the Brandenburg region and the edge and central populations was tested by a nested ANOVA, where all Brandenburg populations were nested within one region. Genetic subdivision (F_{ST}) within Brandenburg and between Brandenburg, Czech and Hungarian populations was calculated by an analysis of molecular variance (AMOVA, Excoffier et al. 1992) using GENALEX. Statistical significance associated with population structure was estimated using 999 permutations.

Sex biased dispersal

Using the program FSTAT version 2.9.3 (Goudet 1995) different tests evaluating potential sex biases in dispersal in this species were performed. For a better evaluation we calculated different assignment indices: the fitting to Hardy–Weinberg expectations (F_{IS}), the portion of total genetic variance within the sample (F_{ST}), the relatedness (Rel.) and the within group diversity (H_S). Because the power of the F_{ST} test (Hartl and Clark 1997) for sex biased dispersal becomes weaker when strong isolation by distance prevails (Goudet et al. 2002) we additionally performed the corrected assignment index test (AIc, Favre et al. 1997). This test should not be sensitive regarding isolation by distance processes. We applied sex biased dispersal tests only for the populations B1 and B2 because of the usually limited dispersal propensity within this species over distances greater than 5 km (Peters 1970; Elbing 2001a; M. Stein, pers. commun.). In summary, we tested 16 females and 9 males. This female biased sampling reflects the real situation observed within the Brandenburg populations (Elbing 2001a) containing a larger proportion of females in all populations B1 (1:1.4) B2 (1:2.0) and B3 (1:3.3). The relatively small sample used for this test was caused by the difficulties to catch enough adult individuals within the small populations.

Results

A total number of 74 lizards were captured from six populations (Table 1). We analysed three wild populations from Brandenburg (Germany), one captive population from Germany, one edge population from Czech Republic (Bohemia) and one central population from Hungary. Altogether 12 microsatellite loci were amplified from all individuals. All analysed loci were in linkage disequilibrium. After Bonferroni correction for multiple testing of the data set two of the used loci showed deviations from Hardy–Weinberg equilibrium (adjusted to critical P-value of $P = 0.008$). Within each population almost all loci were polymorphic (Table 2).

Genetic diversity and structuring within Brandenburg populations

Heterozygosity

Observed heterozygosity was similar between B1, B2, B3 and CP (Table 2). Looking at the expected heterozygosity, the population B1 showed the highest value, whereas the captive population (CP) showed a decrease of H_E in comparison to all wild populations.

However, the observed differences were only small and not significant between the four Brandenburg populations (B1, B2, B3 and CP; ANOVA, df = 3, $P > 0.05$, Table 2), also the analysis with a two factorial ANOVA did not detect significant differences between these populations (two way ANOVA, factor 1 df = 3, $P > 0.05$).

Surprisingly, we observed more heterozygotes within the populations B2 and CP as expected from predictions of the Hardy–Weinberg equilibrium (Table 2.) However, this heterozygote excess was only significant for the population B2 ($P < 0.05$, probability test GENEPOP).

Polymorphic status and allele frequencies

In contrast to the pattern of heterozygosity, the calculated percentage of polymorphic loci (% L_{POL}), the number of alleles (A) and the proportion of private alleles (A_P) showed a more diverse pattern between the studied populations (Table 2). However, concerning the number of alleles (A) and the portion of private alleles (A_P) we only found moderate, nonsignificant differences between the Brandenburg populations (B1, B2, B3 and CP; A, ANOVA, df = 3, $P > 0.05$; A_P, H-test, df = 3, $P > 0.05$). Again also the analysis with a two factorial ANOVA did not detect significant differences between these populations (two way ANOVA, A, factor 1, df = 3, $P > 0.05$; A_P, factor 1, df = 3, $P > 0.05$). Altogether the population B1 showed higher values of absolute alleles and private alleles. For the population B2 we detected the same amount of polymorphic loci as in B1, but the number of alleles and private alleles was smaller than in population B1 and B3. The captive population CP showed the lowest values for all these genetic diversity indices within the Brandenburg samples. To test if the reduced allelic diversity in these populations is due to recent bottlenecks, we conducted a further analysis using the program BOTTLENECK (Cornuet and Luikart 1996). However, we could not detect any significant gene diversity excess (two phased model, Wilcoxon test), which would indicate a demographic bottleneck in these populations.

Population structure

All individuals of the wild Brandenburg populations were checked for their assignment to the sampled population (STRUCTURE v. 2.1). The result of the analysis showed that the observed population structure in the field was well reflected by the genetic data. Three populations (B1-3) were identified, although population B1 contained a slight substructure caused by unique allele combinations of three individuals. However, these individuals clustered clearly within the population B1. This way the autonomous status of all three sampled populations was validated by the genetic analyses. To detect population differentiation of the wild Brandenburg populations we calculated pairwise genetic distances (F_{ST}) of observed allele frequencies assuming the infinite allele model (IAM, Kimura and Crow 1964). Calculated F_{ST} distances between the populations are shown in Table 3. We were able to detect a genetic differentiation of over 0.05 between the populations B1 and B2, which is surprisingly high

Table 2 Summarized genetic features of the studied populations

Populations	$H_E^a \pm$ SE	$H_O^b \pm$ SE	% L_{POL}^c	$A^d \pm$ SE	$A_P^e \pm$ SE
Brandenburg 1	0.541 ± 0.079	0.514 ± 0.074	91.60	3.63 ± 0.44	0.55 ± 0.17
Brandenburg 2	0.449 ± 0.067	0.531 ± 0.091	91.60	2.76 ± 0.35	0.34 ± 0.13
Brandenburg 3	0.430 ± 0.077	0.427 ± 0.065	83.33	2.89 ± 0.48	0.50 ± 0.21
Captive population	0.406 ± 0.081	0.437 ± 0.101	75.00	2.55 ± 0.35	0.15 ± 0.08
Czech Republic	0.506 ± 0.066	0.347 ± 0.083	91.60	3.14 ± 0.36	0.72 ± 0.17
Hungary	0.728 ± 0.055	0.669 ± 0.052	100.00	5.56 ± 0.67	2.10 ± 0.39

[a] Expected heterozygosity and standard error

[b] Observed heterozygosity and standard error

[c] Percentage of polymorphic loci

[d] Number of alleles and standard error within the population corrected for unequal sample size

[e] Number of private alleles per locus and standard error within populations corrected for unequal sample size

Table 3 Estimates of pairwise genetic distances (F_{ST}, $P < 0.001$) between the sampled populations (below diagonal) and corresponding geographic distances in km (above diagonal)

	B1	B2	B3	CP	CZ	H
Brandenburg 1 (B1)	–	2.0	14.0	0[a]	223	602
Brandenburg 2 (B2)	0.076	–	14.0	0[a]	223	602
Brandenburg 3 (B3)	0.127	0.167	–	0[a]	217	584
Captive pop. (CP)	0.162	0.173	0.134	–	0[a]	0[a]
Czech Rep. (CZ)	0.278	0.296	0.345	0.338	–	457
Hungary (H)	0.137	0.181	0.191	0.234	0.233	–

[a] no natural habitat

for this small geographic distance of about 2 km (Table 3). Between each of the two populations B1/B2 and the population B3 we detected a higher differentiation: in one case of over 0.15 (14 km distance, Table 3). Nearly the same level of differentiation was shown for the relationships between the wild populations and the captive population (CP, Table 3).

Sex biased dispersal

Testing 16 females and 9 males of the populations B1 and B2 for sex biased dispersal we were able to show a trend to a male biased dispersal for all indices (Table 4). We observed a strong negative mean assignment index (mAIc) and a much higher variance of assignment index (vAIc) within the males. Also the relationships of female to male values of F_{IS}, F_{ST}, Relatedness and H_S gave evidence for male biased dispersal. However, none of the differences were significant after Bonferroni correction for multiple testing of the data set (adjusted critical P-value = 0.01).

Table 4 Results and probability values for testing sex biased dispersal in the Brandenburg *Lacerta v. viridis* populations

	F_{IS}[a]	F_{ST}[b]	Rel.[c]	H_S[d]	mAIc[e]	vAIc[f]
Females (16)	– 0.017	0.087	0.162	0.512	0.639	4.844
Males (9)	0.108	0.025	0.044	0.581	– 1.136	20.129
P-value	0.182	0.217	0.202	0.049[g]	0.129	0.096

[a] Fitting to Hardy–Weinberg expectation

[b] Portion of total genetic variance among populations

[c] Relatedness

[d] Diversity within group

[e] Mean assignment index

[f] Variance of assignment index

[g] Not significant after Bonferroni correction

Comparison of Brandenburg populations to other edge and central populations

Heterozygosity

The three wild populations in Brandenburg (B1-3) showed no reduced expected (H_E) and observed (H_O) heterozygosity compared to the other edge population in the Czech Republic (CZ, Table 2). Compared to the Hungarian population (H), which represents a population of the central continuous area of *L. v. viridis*, the Brandenburg populations showed a significant reduction of H_E (nested ANOVA, df = 2, $P = 0.0086$) and of H_O (nested ANOVA, df = 2, $P = 0.0229$). The excess of heterozygotes observed in the Brandenburg populations B2 and CP was not detected within the edge population CZ or the central population H (Table 2).

Polymorphic status and allele frequencies

Compared to the edge population in the Czech Republic only the Brandenburg populations B3 and CP contained a smaller percentage of polymorphic loci (Table 2), but almost all Brandenburg populations exhibited a lower proportion of alleles and private alleles than the Czech population. Compared to the central population (H), all Brandenburg populations showed smaller percentage of polymorphic loci and a significant smaller number of alleles (nested ANOVA, df = 2, $P = 0.0001$) and private alleles (nested ANOVA, df = 2, $P = 0.0001$).

Regional differentiation

To compare the observed regional differentiation within Brandenburg to the overall differentiation between the regions Brandenburg, Czech Republic and Hungary, we performed an AMOVA (Table 5) summarizing all wild Brandenburg populations in one region and compared this region to the other edge and central populations.

The results showed that the mean of the differentiation between the local populations within Brandenburg was lower than the differentiation between the

Table 5 AMOVA statistics of regional genetic differentiation between wild Brandenburg, Czech and Hungarian *Lacerta v. viridis* populations

	Statistic	Value	Probability (P)
Among regions	F_{RT}	0.125	0.001
Among populations	F_{SR}	0.092	0.001
Among ind/within pop	F_{ST}	0.205	0.001

Brandenburg region and the populations in the Czech Republic and Hungary. In detail, the AMOVA revealed that: 80% of the molecular variance was explained by differentiation between individuals within the populations, 8% by the differentiation between the populations within the Brandenburg region, whereas 12% of the variance could be explained by the differentiation between the Brandenburg region and the other populations.

Discussion

The endangered populations in Brandenburg are supposed to represent relict populations resulting from a rapid range expansion from a southern glacial refuge during the Holocene warming (Peters 1970). Currently, they are endangered due to increasing fragmentation caused by diverse anthropogenic influences. Considering the high differences in census population size between the populations (Table 1), we were surprised that the analysed microsatellite data revealed no significant differences in the heterozygosity level between the populations in Brandenburg (Table 2). The only conspicuous feature within the pattern of heterozygosity was the significant excess of heterozygotes under Hardy–Weinberg predictions ($H_O > H_E$, Table 2) within the population B2. This could be explained by the small population size and a consequent nonrandom mating within this particular population.

The analysis of the allelic richness revealed also slight differences in the number of private alleles between the local populations within the Brandenburg region. Focusing on the genetic diversity of the populations in Brandenburg, it seems that the genetic diversity of the largest wild population B1 is marginally higher, especially in allelic richness, than the other wild populations B2 and B3. This result is not very surprising as it is known that allele number is more strongly affected than heterozygosity after a population bottleneck or due to genetic drift and is therefore more sensitive to such processes (Spencer et al. 2000; Amos and Balmford 2001; Garza and Williamson 2001). However, due to the small sample size, we could not detect a significant excess of gene diversity (BOTTLENECK, two phased model of mutation) and therefore could not provide a statistical evidence for bottleneck effects.

The most surprising result was the level of genetic differentiation between the local populations in Brandenburg (Table 3). We found a moderate level of genetic differentiation ($F_{ST} > 0.05$) within a 2 km range between populations B1 and B2 and a higher differentiation within a 14 km distance ($F_{ST} > 0.15$) between the populations B1/2 and B3. The high degree of this genetic differentiation is illustrated by the fact that the level of differentiation between the populations within Brandenburg was almost as high as between the Brandenburg region and the central population in Hungary (Table 5). Additionally, this data showed that within a rather small geographical range (2–14 km) it is possible to accumulate population specific genetic features. This correlates well with the assumed low individual dispersal capacity of *L. v. viridis* and is maybe also caused by the small census population size (Table 1), which will not force the individuals to emigrate. To test if the dispersal behaviour differs between sexes we analysed allele frequencies of males and females between population B1 and B2. The data showed that the occurring dispersal of individuals seems to be male biased within *L. v. viridis* (Table 4). All analysed indices showed a strong tendency to male biased dispersal. However, this trend is not significant, which was probably due to the small sample size used in the test. The observed male tendency correlates well with the behavioural data (Peters 1970; Elbing 2001a). These authors found that migrating males mate with several sedentary females. It is conceivable that this behavioural feature had an additional influence on the observed population differentiation between the Brandenburg populations.

For a better evaluation of the detected diversity in Brandenburg we compared this data to other edge (CZ) and central populations (H) of the *L. v. viridis* species range. The main result was that the genetic diversity of the studied edge populations in Germany (Brandenburg) and Czech Republic (Bohemia) is reduced in comparison to the central population of *L. v. viridis* in Hungary. From other studies (e.g. Hoffman and Blows 1994; Frankham 1995) we expected to detect a low genetic diversity within the isolated edge populations of *L. v. viridis* in Brandenburg. Between the edge populations in Brandenburg and in the Czech Republic (Bohemia) the data showed no significant differences in genetic diversity. Compared to the central population in Hungary, we detected a significant decrease of heterozygosity, polymorphic loci and allelic richness in both edge populations. Especially, the number of private alleles was much lower in the edge populations than in the central population. This illustrates that the Brandenburg *L. v. viridis* populations show the typical genetic pattern of a northern edge population within a species range (e.g. Lammi et al. 1999; Edenhamn et al. 2000; Hewitt 2001). The cause for the reduced genetic diversity of these edge populations may be simply explained by their small census

size, or by more complex processes such as isolation or genetic drift, further investigations will clarify the possible mechanisms reducing the genetic diversity of the *L. v. viridis* edge populations. The fact, that despite the lower genetic diversity, private alleles occur within the Brandenburg populations, illustrates the importance of these edge populations for the overall genetic diversity of this species. This clearly underlines the significance of these populations for conservation strategies (Lesica and Allendorf 1995; Moritz 1999). Because of the fact that the wild populations are at risk, especially after cold, humid years with low reproduction rates the genetic status of the captive population becomes a focus of interest. In our analysis we showed that this population is not significantly affected by a ''founder event'' (Tables 2 and 3). The captive population showed a drop in the proportion of polymorphic loci and allelic richness in comparison to the population B3, which is the source of two of the founder individuals. These results suggest that such breeding programs are suitable to at least conserve the genetic diversity over relatively short time periods. For a sustainable conservation of genetic diversity of the Brandenburg populations, we would propose to include some new, wild Brandenburg individuals in the breeding program. For example a first measure could be to include individuals of the populations B1 and B2 into the current breeding program, as this study showed that these populations exhibit a higher genetic variation. This seems especially relevant in respect to further reintroduction projects to formerly populated habitats in this region. Considering the long term conservation of this region, the data call for urgent measures to facilitate migration between the local Brandenburg populations, otherwise inbreeding effects will decrease the already comparatively low genetic variation of this endangered region (Keller and Waller 2002). The current work of the management project to improve the habitat structure of the populations and the open space between the populations B1/2 is a good way to reach this goal.

We summarize that this study demonstrated the suitability of a fast evolving genetic marker to estimate the genetic diversity of endangered populations. Our study clearly shows that edge populations of *L. v. viridis* have a reduced genetic diversity. Therefore they are extremely vulnerable to additional processes that may further decrease the genetic diversity and ultimately lead to the extinction of these populations, which despite their lower genetic diversity harbour distinct genetic variants of the species *L. v. viridis*.

Furthermore, these results call for caution in the analysis of edge populations of threatened species. If the genetic analysis of those populations lacks the appropriate resolution and comparative analysis, the unique genetic variants (e.g. alleles) may be underestimated. This in turn would lead to false conclusions concerning the contribution of these populations to the overall genetic diversity of the species, which may result in incorrect management decisions regarding the maintenance of genetic diversity in endangered species.

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