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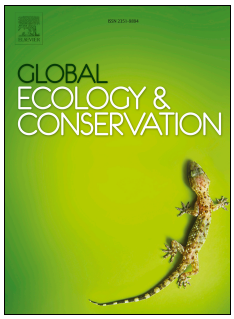


# Spatial and genetic structure of a *Lacerta viridis* metapopulation in a fragmented landscape in Bulgaria

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**Spatial and genetic structure of a *Lacerta viridis* metapopulation in a fragmented landscape in Bulgaria**

**Keywords**

Lacertidae; *Lacerta viridis*; Fragmentation sensitivity; Genetic structure; Isolation-by-distance; Range core

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## Abstract

Numerous studies showed that habitat fragmentation can affect the constitution of species by impairing living conditions, impeding gene flow and thereby reducing genetic variability. However, populations of the same species may react less sensitive to fragmentation in the core than in the periphery of its distribution range. In the core they are assumed to be more euryoecious compared to the periphery, where they are assumed to be stenoecious with lower genetic diversity and higher genetic differentiation. We tested this hypothesis by comparing the genetic variability of 215 individuals of ten populations of *Lacerta viridis* from fragmented habitats within its distribution center in Bulgaria using genotype data of 19 microsatellite loci. We could not detect significant alteration of genetic variation, regardless of patch size and isolation by distance, indicating that fragmentation indeed did not had a strong

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impact on *L. viridis* in the core area of its historical and recent distribution range. We cannot rule out that the time elapsed since habitat fragmentation occurred was too short to yield a genetic response. However, in a similar study on *L. agilis,* which is stenoecious in Bulgaria, all genetic diversity indices declined with patch size. This provides indications that fragmentation at present does not have a strong effect on the genetic variation of Bulgarian *L. viridis* populations.

## 1. Introduction

Genetic differentiation occurs on several hierarchical levels: between individuals, among populations within a common area and between geographic regions. There are numerous studies that have assessed genetic differentiation at a large geographic scale (e.g., Arenas et al., 2012; Dudaniec et al., 2012; Eckert et al., 2008; Vucetich and Waite, 2003). Likewise, many studies addressed the effects of genetic variation at the local scale (e.g. Böhme et al., 2007b; Branch et al., 2003, Delaney et al., 2010; Delicour et al., 2011; Hoehn et al., 2007). In contrast, there are few studies that considered that environmental effects on genetic differentiation among populations at the local scale may depend on large scale geographic factors, such as the position of the populations at the periphery or core of the distribution area of the species (but see e.g. Arenas et al., 2012; Henle et al., 2017).

Studies at a large geographic scale showed that peripheral populations frequently have lower genetic diversities and higher genetic differentiation than populations in the core region (Böhme et al., 2007b; Dudaniec et al., 2012; Eckert et al., 2008) though the speed of range shifts may modify this pattern (Arenas et al., 2012). According to Kühnelt's principle of regional stenoecy, species with a large distribution range tend to be stenoecious at the periphery of their distribution area and more euryoecious in the core, (Böhme, 1978; Prieto Ramirez et al., 2018). For example, the two congeneric lizard species, the sand lizard (*Lacerta agilis*) and the eastern green lizard (*L. viridis*), have low genetic variability and narrower ecological niches at the periphery of their distribution (Bulgaria for *L. agilis*; East Germany for *L. viridis*) but higher variability and broader niches towards the core of their distribution (East Germany for *L. agilis;* Hungary and Bulgaria for *L. viridis*) (Böhme, 1978; Böhme et al., 2007b; Henle et al., 2017, Prieto Ramirez et al., 2018).

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At the local scale, loss and fragmentation of once contiguous habitats is among the most pervasive human driven processes in the Anthropocene that influence the dynamics, genetic variability and differentiation among populations and ultimately persistence (Settele et al., 1996; Hanski & Gilpin 1997; Henle et al. 2004a, Young and Clark, 2000). Nevertheless, species with a broad habitat tolerance or fast adaptation ability can persist in fragmented landscapes without loss of genetic variability even for a long time (Branch et al., 2003; Henle et al., 2017; Meek, 2020).

Commonly, the abundance and dispersal among isolated sites of a species correlate with the quality of the environmental conditions. This creates a tendency in distribution patters of many large populations in the center and progressively less, smaller and more isolated populations towards the periphery (Brussard, 1984; Lawton, 1993; Vucetich and Waite, 2003). Therefore, populations of the same species might react differently to habitat fragmentation depending on their location within the distribution area, an effect well known when comparing species with different degrees of specialization (Henle et al., 2004b; Hoehn et al., 2007; Keinath et al., 2017). Hence, fragmented central populations should experience fewer loss of genetic variation and genetic differentiation following loss and fragmentation of their habitat than peripheral populations, but this prediction has rarely been assessed.

Reptiles are excellent indicators for these processes (Meek, 2020). Due to their limited mobility compared with other taxa like birds or small mammals, lizards are restricted in their ability to migrate from one habitat patch to another through the matrix (Díaz et al., 2000; Hoehn et al., 2007; Jellinek et al., 2004). Therefore, lizards may be particularly sensitive to isolation of suitable habitat areas resulting from fragmentation (Díaz et al., 2000; Sarre et al., 1995).

*Lacerta agilis* is a stenoecious habitat specialist in Bulgaria whereas *L. viridis* is a euryoecious habitat generalist and found very commonly all over the country (Biserkov et al., 2007). Nevertheless, in some regions like the Thracian plain the habitat of the green lizard occurs as more or less fragmented patches within an intensively used agricultural landscape and is further isolated by roads. This might exert a negative pressure upon the genetic variability of the fragmented populations, due to reduced population sizes and a possible reduction in the dispersal of individuals among populations. In a previous study Böhme et al. (2007b) showed that this species shows low genetic variability and high genetic differentiation among

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populations in habitat patches in close proximity at the northern periphery of its distribution area.

This study investigates the potential effects of fragmentation on the genetic structure of *L. viridis* populations in fragmented landscapes in the core area of its distribution range. We hypothesized that, in contrast to *L. agilis,* local *L. viridis* populations were connected by dispersal (e.g. through the matrix) to some extent despite fragmentation of their habitat, thus possessing relatively high genetic diversity and low genetic differentiation. However, populations that were more isolated might have experienced less gene flow than those in closer proximity to each other due to isolation by distance. Furthermore, reduction of habitat size might have influenced genetic diversity, as population size shrank and inbreeding increased.

## 2. Methods

### 2.1 Study area and field sampling

We focused our study on the Thracian plain in Bulgaria. Bulgaria has undergone an intensive period of urbanization and modification over the last 65 years (Ganev, 1989), resulting in highly fragmented landscapes. The area of interest included populations within the city of Plovdiv and populations in surrounding areas (Mollov, 2011).

Potentially suitable lizard habitats were identified based on Google Earth maps and on personal observation in the field, following the habitat description reported in the literature. Subsequently, patches with suitable habitat were surveyed for lizards and final sampling sites were chosen based on abundance of individuals and spatial distribution of patches. Thus, an appropriate sample size per patch was assured and the extent of the study area was covered as much as possible (Fig. 1).

Figure 1: Sample sites of the 10 investigated populations. Light brown outlines illustrate the city boundary of Plovdiv (Map data © OpenStreetMap contributors). For abbreviations of sampling sites see Table 1.


Overall, 215 individuals from 10 populations were sampled. The populations “Kapitan Dimitrievo” and “Gorno Brystovo” served as unfragmented controls as the surrounding landscapes consisted of suitable habitat with more than 6 $km^2$ in size. All other habitat patches were considered as fragmented. Five populations were sampled in 2010, another six populations in 2015. The habitat fragment at Trilistnik (TN) was sampled in both years; the sampled areas overlapped substantially, and the samples of the two years did not differ significantly in any genetic parameter (see results). Therefore, we combined the two samples for comparisons with other populations.

The size of each habitat patch and geographic distances between sampling sites were calculated using ArcGIS 10 (ESRI, Redlands, CA). Degree of isolation was measured as the distance between sampling areas and the next patch of suitable habitat within the landscape. In case of strong barriers within a suitable habitat area, such as rivers or major roads, we considered habitats on both sides of the barrier as separate patches. Sampling areas differed in size between 0.1 $km^2$ and 9.5 $km^2$ with isolation ranging from 8 m to 714 m (Tab. 1).

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Table 1: Abbreviations of the site names for the investigated populations in Bulgaria, sample areas, patch size, degree of isolation to the next suitable area of habitat measure edge-to-edge and geographic coordinates averaged for the sampled individuals.
(*) For non-fragmented control populations, that belong to the same continuous habitat only sample area was estimated. (**) Corresponds to isolation distance of a population surrounded by a river, which represents a strong barrier.

| Population ID/ name | Sample size | Latitude | Longitude | Patch size ($km^2$) | Isolation (m) |
|---|---|---|---|---|---|
| **TN (Trilistnik North)** | 42 | 42.225583N | 24.853214E | 5.27 | 347 |
| **VI (Vinitsa)** | 20 | 42.145567N | 25.140667E | 3.75 | 206 |
| **GR ( Gradina)** | 19 | 42.145533N | 25.183450E | 4.87 | 526 |
| **PC (Plovdiv Center)** | 16 | 42.156056N | 24.763611E | 0.10 | 714 |
| **PW (Plovdiv West)** | 19 | 42.139513N | 24.695156E | 1.71 | 339 |
| **RO (Rogosh)** | 19 | 42.153294N | 24.885640E | 0.74 | 18 |
| **PE (Plovdiv East)** | 17 | 42.162449N | 24.797865E | 0.46 | 8** |
| **YA ( Yagodowo)** | 22 | 42.138650N | 24.869379E | 0.40 | 27 |
| **KD (Kapitan Dimitrievo)*** | 21 | 42.087033N | 24.335733E | 6.55 | |
| **GB (Gorno Brystovo)*** | 20 | 41.930450N | 25.314217E | 9.49 | |

Animals were captured with permits of the issuing authorities (please refer to the Acknowledgements) by hand or with a fishing rod and handled according to the guidelines of the Herpetological Animal Care and Use Committee of the American Society of Ichthyologists and Herpetologists. GPS coordinates and elevation records in situ (reference system WGS 84) were taken for each lizard sampled.
A small tissue sample was taken from the tail tip and afterwards the wound was disinfected. Samples were stored in 98% ethanol at -20°C.

### 2.2 DNA extraction, amplification and fragment analysis

DNA was isolated using “NucleoSpin® Tissue” Kit (Macherey-Nagel) following the manufacturer’s protocol. Microsatellite loci were amplified with polymerase chain reaction (PCR) (Mullis and Faloona, 1987) using previously established primers of Laube and Kuehn (2006) and Böhme et al. (2005) (Appendix II, Tab. A2.1). In total 24 loci were amplified. Forward primers were fluorescently labelled with FAM, HEX, NED or Atto550. Microsatellite PCR was conducted in a total volume of 25µl containing 0.2mM of each dNTP, 2.5µl of 10x Dream Taq$^{TM}$-Buffer including 25mM $MgCl_2$, 1U Dream Taq$^{TM}$ Green DNA polymerase, 0.4µM for each forward and reverse primer and 0.5µl DNA-extract on an Eppendorf Mastercycler under the

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following conditions: initial denaturation at 95°C for 15 min, followed by 35 cycles of 30s denaturation at 95°C, 30s annealing phase at primer specific temperatures and 30s extension at 72°C, the final elongation step lasted for 10 min at 72°C. Each microsatellite locus was amplified separately (one primer pair per reaction).

Amplified products with different labels and non-overlapping size ranges were multiplexed and electrophoresed on the ABI 3130xl at the Interdisciplinary Centre for Clinical Research of the Leipzig University. Allele sizes were scored against the internal size standard GeneScan$^{TM}$ 500 ROX$^{TM}$ using Peak Scanner$^{TM}$ v1.0 (Applied Biosystems, USA). Missing data ranged from 0.5% to 2.3% per locus. At least 14 loci (>73%) could be genotyped for each sample. All individuals were included in the subsequent analyses (n=215).

### 2.3 Statistical Analyses

Microsatellite data were screened for the presence of null alleles, allelic dropout and stutter bands using the software Micro-Checker v2.2 (Van Oosterhout et al., 2004). Loci fulfilling at least one of these criteria were excluded from the data set. The remaining loci were tested for Hardy-Weinberg-Equilibrium and pairwise linkage equilibrium using Genepop v4.2 (Raymond and Rousset, 1995) with default parameter values. P-values of linkage disequilibrium were corrected using the FDR-method (Benjamini and Hochberg, 1995).

Heterozygosity and the number of alleles per locus ($N_A$) were calculated using Cervus v3.0.7 (Kalinowski et al., 2007). The allelic richness and number of private alleles were determined with the rarefaction method in HP RARE v1.1 (Kalinowski, 2005), normalized to the least number of diploid individuals per population carrying genetic information. $F_{IS}$ values (Weir and Cockerham, 1984) were estimated using FSTAT v2.9.3 (Goudet, 1995).

An analysis of variance (ANOVA) was conducted in PAST v3.16 (Hammer et al., 2001) to examine differences in heterozygosity, allelic richness and private allelic richness among populations and between the groups of populations (fragmented vs. non-fragmented). When Levene's test of homogeneity was significant ($p < 0.05$), the Welch F-Test was used.

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$F_{ST}$ values were calculated with FreeNA (Chapuis and Estoup, 2007) both with and without using the ENA method of Chapuis and Estoup (2007) to exclude excessive null alleles with the implemented Expectation-Maximization algorithm of Dempster et al. (1977) and a bootstrapping of 10,000 (confidence interval of 95%). Pairwise $F_{ST}$ as values for genetic distance (linear transformation) and geographic distance (edge-to-edge of fragmented sampling sites) were correlated with IBD v1.52 (Bohonak, 2002), using reduced major axis (RMA) regression and the Mantel test. Implications of patch size (only fragmented areas) and isolation on genetic diversity (heterozygosity, allelic richness, $F_{IS}$) were analyzed by linear regression models with the package *stats* in RStudio v1.2.1335 (R Core Team, 2019). The graphical output for all correlation models was generated in R with the packages *ggplot2* (Wickham et al., 2016) and *gridExtra* (Auguie, 2017).
An analysis of molecular variance (AMOVA) was carried out with Arlequin v3.5.2 (Excoffier and Lischer, 2010).

To assess genetic structuring among the populations we carried out a principal component analysis (PCA) on allele frequencies using the package *adegenet* v2.1.2 (Jombart, 2008; Jombart and Ahmed, 2011) in RStudio v1.2.1335 (R Core Team, 2019).
In addition, we performed a Bayesian cluster analyses with STRUCTURE v2.3.4 (Pritchard et al., 2010) but because of the low genetic variability explained by the population level (see results) and thus uncertainty whether the results reflect primarily the genetic variation or the geographic location of individuals, we provide the analysis, for comparative purposes, only in Appendix I.

## 3. Results

We detected allelic variation at 21 out of 24 loci and compared it for 215 individuals of 10 populations. Three loci (Lvir6, Lvir14 and LacVirK) exhibited different fragment lengths in 2010 and 2015 and therefore were excluded from the analyses. One locus (Lvir11) showed an excess of homozygotes indicating the presence of null alleles and was omitted from subsequent analyses. One pair of loci (LacVirS, LacVirSY) deviated from linkage disequilibrium despite FDR correction. Consequently, we also

excluded locus LacVirSY from the dataset. The final data set contained 19 microsatellite loci (Appendix II, Tab. A2.1).

### 3.1 Comparison of samples collected in different years

Individuals from a population near Trilistnik North were sampled in 2010 and again in 2015. The samples lacked significant differences in all diversity parameters [two sample t-tests, using PAST v3.16 (Hammer et al., 2001)]: $H_{Obs}$: p = 0.99, $H_{Exp}$: p = 0.63, AR: p = 0.08, $F_{IS}$: p = 0.55.

### 3.2 Comparison of fragmented with control areas

Based on patch sizes we divided the investigated populations into fragmented (< 6 $km^2$) and non-fragmented populations (> 6 $km^2$). Although diversity parameters were larger and $F_{IS}$ values were smaller for the control areas than for the fragmented areas, ANOVA revealed no significant differences between these groups regarding heterozygosity, allelic richness and $F_{IS}$ (Appendix II Fig. A2.1, Tab. A2.2).

### 3.3 Genetic differentiation

The lowest genetic differentiation ($F_{ST}$ = 0.015) could be observed between population GR and population KD (Tab. 2; for locations see Fig. 1). Population PC and PW differed the most with $F_{ST}$ = 0.088. Overall population PC was most different from all other populations, with $F_{ST}$ ranging from 0.052 to 0.088. Despite the fact that the two control populations KD and GB were both located within the same large area of continuous suitable habitat, they were separated by 100.45 km (not included in Tab. 2) and treated as individual populations. They differed genetically with a $F_{ST}$ = 0.028. For comparison, the samples collected five years apart in the population Trilistnik North showed only minimal differentiation ($F_{ST}$ = 0.012).

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Table 2: Geographic and genetic distances. In the lower triangle, the genetic distances using $F_{ST}$ and the ENA method are shown. In the upper triangle, pairwise geographic distances between the populations are shown in kilometers, measured from edge-to-edge of each sampling area. For the control populations KD and GB no distances could be determined as surrounding landscape represents large area of suitable habitat without clear boundaries.

| **Fragmented populations** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **TN** | **VI** | **GR** | **PC** | **PW** | **RO** | **PE** | **YA** | |
| **TN** | - | 30.49 | 33.69 | 13.63 | 17.02 | 9.60 | 10.94 | 10.09 | |
| **VI** | 0.020 | - | 2.50 | 40.00 | 44.16 | 25.61 | 28.00 | 36.22 | |
| **GR** | 0.025 | 0.017 | - | 43.96 | 48.13 | 29.57 | 32.05 | 40.16 | |
| **PC** | 0.083 | 0.081 | 0.086 | - | 3.28 | 7.92 | 11.45 | 2.10 | |
| **PW** | 0.039 | 0.042 | 0.055 | 0.088 | - | 12.02 | 15.45 | 6.10 | |
| **RO** | 0.026 | 0.023 | 0.026 | 0.052 | 0.033 | - | 0.12 | 4.45 | |
| **PE** | 0.043 | 0.058 | 0.052 | 0.061 | 0.054 | 0.023 | - | 8.08 | |
| **YA** | 0.036 | 0.037 | 0.041 | 0.087 | 0.051 | 0.020 | 0.049 | - | |
| **Non-fragmented populations** | | | | | | | | | |
| | **TN** | **VI** | **GR** | **PC** | **PW** | **RO** | **PE** | **YA** | **KD** |
| **KD** | 0.024 | 0.027 | 0.015 | 0.073 | 0.042 | 0.025 | 0.043 | 0.040 | - |
| **GB** | 0.023 | 0.022 | 0.025 | 0.086 | 0.055 | 0.026 | 0.052 | 0.042 | 0.028 |

The Mantel test revealed no significant correlation between the geographic distances and genetic distances (ENA: Z = 26037.61, r = 0.01, p ≤ 0.40; no ENA: Z = 26082.81, r = 0.6e-3, p ≤ 0.43, Fig. A2.2). An additional Mantel test without site PC, to prevent bias by this very small and isolated population, also lacked significance (ENA: Z = 16862.06, r = 0.23, p ≤ 0.17; no ENA: Z = 16762.36, r = 0.20, p ≤ 0.21).

The AMOVA revealed that most of the genetic variation could be found within individuals over all populations (92.83%; Sum of squares: 1408.50, Variance components: 6.60). The remaining variance was roughly similar within populations (3.13%; Sum of squares: 1433.35, Variance components: 0.22) and among populations (4.04%; Sum of squares: 172.22, Variance components: 0.29).

### 3.4 Genetic diversity

The values of expected heterozygosity ranged between 0.64 and 0.75, while the observed heterozygosity varied between 0.57 and 0.75 (Tab. 3). Both values did not differ significantly among populations (ANOVA: $H_{Obs}$: F = 1.02, $df_1$ = 9, $df_2$ = 180, p = 0.42; $H_{Exp}$: F = 0.62, $df_1$ = 9, $df_2$ = 180, p = 0.78). Overall the mean values of heterozygosity were relatively high, except those of population PC, which exhibited a

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smaller number of heterozygous individuals ($H_{Obs}$ = 0.57). $F_{IS}$ values ranged between -0.04 in population VI and 0.10 in population PC. A Wilcoxon Signed Rank Test revealed that only the calculated $F_{IS}$ of population PC differed significantly from 0 (p = 0.04). Furthermore, the ANOVA showed no significant differences among populations (F = 1.42, $df_1$ = 9, $df_2$ = 72.53, p = 0.19). The allelic richness, averaged over all loci, varied from 5.86 to 8.11, with a different number of private alleles ranging from 0.18 to 0.73. Population PC showed overall the lowest values of heterozygosity and allelic richness as well as the highest value of $F_{IS}$.

Table 3: Measurements of genetic diversity per population for all loci with standard deviation. Populations KD and GB are considered non-fragmented (marked with *). Pop. – Abbreviations for sampled population, $H_{Obs}$ - observed heterozygosity, $H_{Exp}$ - expected heterozygosity, AR - allelic richness, ARP – percentage of private alleles, $F_{IS}$ Inbreeding coefficient by Weir and Cockerham (1984).

| Pop. | $H_{Obs}$ | $H_{Exp}$ | AR | ARP | $F_{IS}$ |
|---|---|---|---|---|---|
| **TN** | 0.70 ± 0.19 | 0.73 ± 0.18 | 8.11 ± 2.97 | 0.73 ± 0.57 | 0.04 ± 0.09 |
| **VI** | 0.75 ± 0.18 | 0.72 ± 0.18 | 7.41 ± 3.17 | 0.18 ± 0.31 | -0. 04 ± 0.13 |
| **GR** | 0.70 ± 0.22 | 0.71 ± 0.22 | 7.54 ± 3.42 | 0.45 ± 0.53 | 0.02 ± 0.11 |
| **PC** | 0.57 ± 0.28 | 0.64 ± 0.22 | 5.86 ± 2.36 | 0.28 ± 0.44 | 0.10 ± 1.15 |
| **PW** | 0.66 ± 0.22 | 0.67 ± 0.21 | 7.56 ± 3.52 | 0.35 ± 0.41 | 0.02 ± 0.16 |
| **RO** | 0.69 ± 0.21 | 0.73 ± 0.18 | 7.59 ± 3.73 | 0.52 ± 0.94 | 0.06 ± 0.14 |
| **PE** | 0.68 ± 0.25 | 0.70 ± 0.21 | 6.67 ± 2.52 | 0.35 ± 0.79 | 0.00 ± 0.12 |
| **YA** | 0.72 ± 0.20 | 0.75 ± 0.15 | 7.70 ± 3.25 | 0.23 ± 0.32 | 0.03 ± 0.15 |
| **KD*** | 0.71 ± 0.14 | 0.75 ± 0.15 | 7.75 ± 3.02 | 0.46 ± 0.40 | 0.05 ± 0.14 |
| **GB*** | 0.72 ± 0.21 | 0.72 ± 0.20 | 7.68 ± 3.44 | 0.35 ± 0.39 | 0.00 ± 0.17 |

Regression models revealed that observed and expected heterozygosity decreased significantly with isolation distance to the next putative habitat whereas observed heterozygosity and allelic richness increased with patch size (Tab. 4, Fig. A2.3). Expected heterozygosity also decreased with isolation distance but increased only marginally with patch size. Neither the number of private alleles nor $F_{IS}$ were influenced by the degree of isolation or by patch size.

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Table 4: Regression of heterozygosity, $F_{IS}$, Allelic Richness and Private Allelic Richness to the habitat size (area) in $km^2$ and degree of isolation in m. $H_{Obs}$ – observed heterozygosity, $H_{Exp}$ – expected heterozygosity, AR – Allelic Richness, ARP – Private Allelic Richness, df – degrees of freedom. Significance codes: ***: < 0.001; **: < 0.01, *: < 0.05

| | **Estimate** | **Standard Error** | **t-value** | **Pr(>\|t\|)** | |
|---|---|---|---|---|---|
| **$H_{Obs}$ ~ Isolation + Area** | | | | | |
| *Intercept* | 0.70 | 1.50E-2 | 46.44 | 8.74E-8 | *** |
| *Isolation* | -1.74E-4 | 3.79E-5 | -4.58 | 0.006 | ** |
| *Area* | 1.69E-8 | 4.59E-9 | 3.68 | 0.014 | * |
| *Residual standard error* | 0.03 on 5 df | | | | |
| **$H_{Exp}$~Isolation + Area** | | | | | |
| *Intercept* | 0.72 | 1.20E-2 | 60.18 | 2.4E-8 | *** |
| *Isolation* | -1.22E-4 | 3.04E-5 | -4.00 | 0.010 | * |
| *Area* | 8.68E-9 | 3.68E-9 | 2.36 | 0.065 | |
| *Residual standard error* | 0.02 on 5 df | | | | |
| **AR ~ Area + Isolation** | | | | | |
| *Intercept* | 7.23 | 0.28 | 25.62 | 1.69E-6 | *** |
| *Area* | 2.51E-7 | 8.66E-8 | 2.90 | 0.034 | * |
| *Isolation* | -1.69E-3 | 7.15E-4 | -2.37 | 0.064 | |
| *Residual standard error* | 0.47 on 5 df | | | | |
| **ARP ~ Area + Isolation** | | | | | |
| *Intercept* | 0.31 | 0.11 | 2.78 | 0.039 | * |
| *Area* | 4.26E-8 | 3.40E-8 | 1.25 | 0.266 | |
| *Isolation* | -4.89E-5 | 2.81E-4 | -0.17 | 0.869 | |
| *Residual standard error* | 0.18 on 5 df | | | | |
| **$F_{IS}$ ~ Area + Isolation** | | | | | |
| *Intercept* | 3.91E-2 | 2.05E-2 | 1.91 | 0.115 | |
| *Area* | -1.32E-8 | 6.29E-9 | -2.10 | 0.090 | |
| *Isolation* | 9.11E-5 | 5.19E-5 | 1.80 | 0.140 | |
| *Residual standard error* | 0.03 on 5 df | | | | |

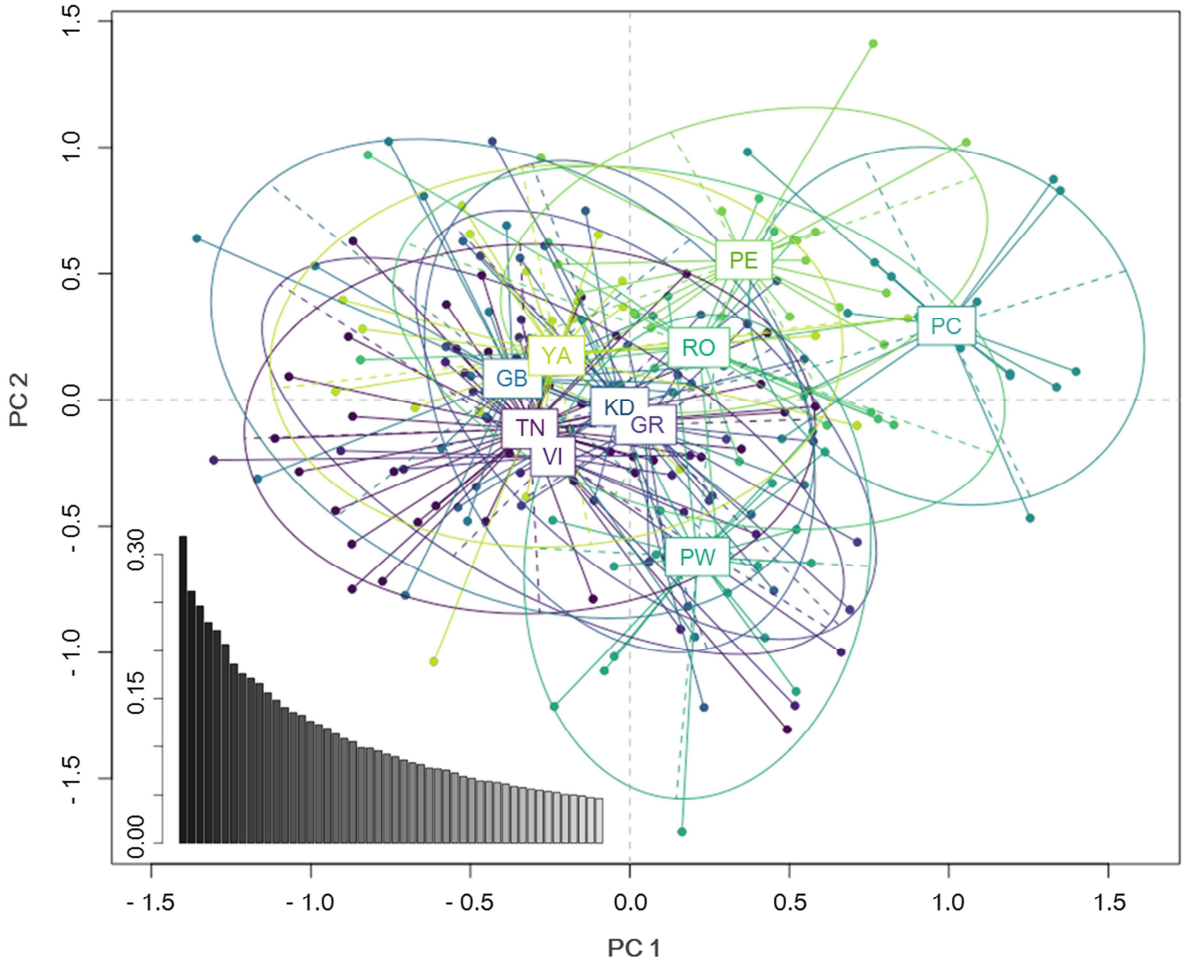
### 3.5 Population structure

The PCA reflected only minor proportions of variation in the microsatellite data set. The first and second component accounted for less than 8% of variation (PC1: 4.27%, PC2: 3.50%). This is in line with the results from the AMOVA that indicated the highest variation between individuals within populations and low genetic differentiation among populations.

Although distinct clusters are lacking in the scatterplots, the PCA results suggest that the population located in the center of the city of Plovdiv (PC) slightly differed genetically from the populations of the surrounding landscape (Fig. 2). Populations from the rural landscape (TN, GR, RO, VI, YA) showed very similar allelic patterns to the unfragmented control populations (KD, GB). The analyses with program STRUCTURE also indicated that the population PC diverged most from other populations but grouped the remaining two urban populations (PW, PE) with two rural

populations downstream the river Mariza (see Appendix I, Fig. A1.2), which is inconsistent with the PCA analyses (Fig. 2).

Fig: 2 Principal component analysis (PCA) of microsatellite frequency data. PCA based on allele frequencies from 10 populations. Populations are color-coded according to the colors used in Fig. 1. The bar plot represents the distribution of the calculated Eigenvalues per component 1 to 50.




## 4. Discussion

The present study aimed to investigate the effects of fragmentation on genetic diversity and variation of *Lacerta viridis* populations in the core of its distribution range in Bulgaria. As hypothesized all investigated populations showed relatively high genetic diversity, similar to a *L. viridis* population in Hungary (Böhme et al., 2007b) and *L. agilis* populations in Germany (Henle et al., 2017) but in contrast to fragmented populations at the periphery of the distribution range of *L. viridis* in

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Germany and the Czech Republic (Böhme et al., 2007b). Furthermore, nine of the ten Bulgarian populations showed $F_{IS}$ close to 0, which implies absence of inbreeding. Despite the limited dispersal capacity of *L. viridis* (Grimm et al., 2014; Schneeweiss, 2001) and distances between the populations of up to 48 km, our results indicate high genetic variability within (93%) and low differentiation among populations (4%). Large population sizes limit genetic drift and can prevent genetic isolation and differentiation (Frankham, 2005). Alternatively, genetic similarity implies widespread gene flow between the populations that are part of a larger metapopulation. Interconnecting populations that were not included in this study may facilitate gene flow within this population network. Suitable structures and possible matrix permeability may enable migration between habitat patches independent of geographic distance (Henle et al., 2017) as no evidence for isolation by distance could be found. Moreover, *L. viridis* populations in Bulgaria inhabit a greater variety of habitat types than those in the periphery of its distribution range (Prieto-Ramirez et al., 2018). Consequently, individuals in the core are considered euryoecious and may be able to cross areas of inappropriate vegetation (Öckinger et al., 2010; Prieto-Ramirez et al., 2018).

The selected sampling areas varied considerably in size and surrounding landscape intentionally to investigate the implication of the degree of isolation and habitat size on population structure. Fragmented populations showed decreased heterozygosity with increasing distance to surrounding suitable habitat. A reduced distance between potential habitat patches may facilitate migration and hence gene flow (Hanski & Gilpin, 1997; Jellinek et al., 2014). The patch size was positively correlated with heterozygosity and allelic richness of the remnant populations. Despite these significant relationships, populations in fragments did not differ significantly from populations in unfragmented control sites in any genetic parameter. With only two control areas available in the study region, the resolution power may have been insufficient to provide significance for small differences between control areas and fragmented areas. The similarity of samples collected in 2010 and 2015 near Trilistnik North, which did not differ significantly between sampling years in any genetic parameter, indicates that the process of differentiation proceeds at slow pace. The strong overlap of the populations on the first and second PCA axes also indicates that the process of loss of genetic variability and genetic differentiation is

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still at an early stage despite the study region having experienced an intensive period of urbanization and modification over the last 65 years (Ganev, 1989), resulting in highly fragmented landscapes. Nonetheless, some of the chosen fragmented patches presumably are still large enough to provide resources like non-fragmented habitats for an euryoecious species like *L. viridis* in the core of its distribution area. However, in the smallest one, the population in Plovdiv Centre (PC), limited inbreeding has been revealed, diversity diminished, and differentiation to other populations is already appearing. Small populations are often more vulnerable to effects of inbreeding or genetic drift (Lesica and Allendorf, 1995). Furthermore, the population's genetic exchange with other populations may be weakened due to its location on an island within the river Mariza and dense buildings in most of the surroundings. This may also explain why this population is the most differentiated one, though the differentiation remained small.

Böhme et al. (2007b) investigated *L. viridis* edge populations in Brandenburg (Germany) and the Czech Republic. All of the edge populations exhibited reduced genetic diversity, higher genetic differentiation and reduced genetic variance on individual level compared to their central population in Hungary and our investigated populations in Bulgaria. These differences between regions may result from their location in the distribution range. As living conditions diminish from the center to the edge of the range, also matrix conditions become subsequently more hostile. Reduced patch sizes and stronger separation of suitable habitat may have stronger implications on peripheral populations, which can lead to a reduced gene flow (Eckert et al., 2008; Franklin et al., 2002; Henle et al., 2017). Furthermore, they experience more rapid cycles of extinction and recolonization accompanied by bottlenecks or founder effects (Eckert et al., 2008). Central populations inhabit areas with better environmental conditions and a variety of suitable habitats. Thus, it is not surprising that the *L. viridis* populations studied by us in Bulgaria exhibited higher genetic diversity and lower differentiation and are less sensitive to fragmentation compared to the edge populations studied by Böhme et al. (2007b).

During glacial periods in Europe, both *L. agilis* and *L. viridis* had refugia in the Balkans (Böhme et al., 2007a; Kalyabina et al., 2013). Whereas *L. agilis* shifted its range and Bulgaria became the periphery, *L. viridis* retained the core of its

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distribution range in Bulgaria. Therefore, populations in Bulgaria of both species should not have experienced a reduction in genetic variation due to historical range expansion. Remarkably, Henle et al. (2017) showed that peripheral *L. agilis* populations in Bulgaria are more vulnerable to habitat fragmentation than populations in the central regions of their recent distribution range in Central Europe, despite the fact that their historical distribution center was also located in the Balkans. Albeit the opposite dispersal history, these contrasting sensitivities to habitat fragmentation of the two species in Bulgaria indicate that fragmentation of habitats may affect the genetic variation of populations substantially differently depending on whether it occurs at the edge or the core of the extant distribution area. This different sensitivity needs to be assessed in a large number of species and be accounted for in conservation strategies.

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## References

Arenas M, Ray N, Currat M, Excoffier L, 2012. Consequences of range contractions and range shifts on molecular diversity. Molecular Biology and Evolution 29, 207–218.

Auguie, B., 2017. GridExtra: miscellaneous functions for “grid” graphics. R package version 2.3.

Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: A practical and powerful approach to multiple testing. Journal of the Royal Statistical Society. Series B (Methodological) 57, 289–300.

Biserkov, V., Naumov, B., Tzankov, N., Stoyanov, A., Petrov, B., Dobrev, D., Stoev, P., 2007. A field guide to amphibians and reptiles of Bulgaria.

Böhme, M.U., Berendonk, T.U., Schlegel, M., 2005. Isolation of new microsatellite loci from the Green Lizard (*Lacerta viridis viridis*). Molecular Ecology Resources 5, 45–47. https://doi.org/10.1111/j.1471-8286.2004.00825.x

Böhme, M.U., Fritz, U., Kotenko, T., Džukić, G., Ljubisavljević, K., Tzankov, N., Berendonk, T.U., 2007a. Phylogeography and cryptic variation within the *Lacerta viridis* complex (Lacertidae, Reptilia). Zoologica Scripta 36, 119–131. https://doi.org/10.1111/j.1463-6409.2006.00262.x

Böhme, M.U., Schneeweiß, N., Fritz, U., Schlegel, M., Berendonk, T.U., 2007b. Small edge populations at risk: genetic diversity of the green lizard (*Lacerta viridis viridis*) in Germany and implications for conservation management. Conservation Genetics 8, 555–563. https://doi.org/10.1007/s10592-006-9191-0

Böhme, W., 1978. Das Kühnelt’sche Prinzip der regionalen Stenözie und seine Bedeutung für das Subspezies-Problem: ein theoretischer Ansatz. Zeitschrift für Zoologische Systematik und Evolutionsforschung 9, 256-266.

Journal Pre-proof

Bohonak, A.J., 2002. IBD (Isolation by Distance): A program for analyses of isolation by distance. Journal of Heredity 93, 153–154. https://doi.org/10.1093/jhered/93.2.153

Branch, L.C., Clark, A.-M., Moler, P.E., Bowen, B.W., 2003. Fragmented landscapes, habitat specificity, and conservation genetics of three lizards in Florida scrub. Conservation Genetics 4, 199–212. https://doi.org/10.1023/A:1023398908793

Brussard, P.F., 1984. Geographic patterns and environmental gradients: the central-marginal model in Drosophila revisited. Annual Review of Ecology and Systematics 15, 25–64. https://doi.org/10.1146/annurev.es.15.110184.000325

Chapuis, M.-P., Estoup, A., 2007. Microsatellite null alleles and estimation of population differentiation. Molecular Biology and Evolution 24, 621–631. https://doi.org/10.1093/molbev/msl191

Delaney, K.S., Riley, S.P.D., Fisher, R.N., 2010. A rapid, strong, and convergent genetic response to urban habitat fragmentation in four divergent and widespread vertebrates. PLOS ONE 5, e12767. https://doi.org/10.1371/journal.pone.0012767

Dempster, A.P., Laird, N.M., Rubin, D.B., 1977. Maximum Likelihood from incomplete data via the EM algorithm. Journal of the Royal Statistical Society. Series B (Methodological) 39, 1–38. https://doi.org/10.2307/2984875

Díaz, J.A., Carbonell, R., Virgós, E., Santos, T., Tellería, J.L., 2000. Effects of forest fragmentation on the distribution of the lizard *Psammodromus algirus*. Animal Conservation 3, 235–240. https://doi.org/10.1111/j.1469-1795.2000.tb00108.x

Dudaniec, R.Y., Spear, S.F., Richardson, J.S., Storfer, A., 2012. Current and historical drivers of landscape genetic structure differ in core and peripheral salamander populations. PLoS One 7. https://doi.org/10.1371/journal.pone.0036769

Eckert, C.G., Samis, K.E., Lougheed, S.C., 2008. Genetic variation across species' geographical ranges: The central–marginal hypothesis and beyond. Molecular Ecology 17, 1170–1188. https://doi.org/10.1111/j.1365-294X.2007.03659.x

Excoffier, L., Smouse, P.E., Quattro, J.M., 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. Genetics 131, 479–491.

Frankham, R., 2005. Genetics and extinction. Biological Conservation 126, 131–140. https://doi.org/10.1016/j.biocon.2005.05.002

Journal Pre-proof

Franklin, A.B., Noon, B.R., George, T.L., 2002. What is habitat fragmentation? Studies in Avian Biology 203–220.

Ganev, C., 1989. The urban process and the appearance of agglomerations in Bulgaria. Socio-Economic Planning Sciences 23, 17–22. https://doi.org/10.1016/0038-0121(89)90039-6

Goudet, J., 1995. FSTAT (Version 1.2): A computer program to calculate F-statistics. Journal of Heredity 86, 485–486. https://doi.org/10.1093/oxfordjournals.jhered.a111627

Grimm, A., Ramírez, A.M.P., Moulherat, S., Reynaud, J., Henle, K., 2014. Life-history trait database of European reptile species. Nature Conservation 9, 45–67. https://doi.org/10.3897/natureconservation.9.8908

Hammer, O., Harper, D., Ryan, P., 2001. PAST: Paleontological Statistics Software Package for Education and Data Analysis.

Hanski, I.A.A., Gilpin, M.E., 1997. Metapopulation biology. Academic Press, San Diego.

Henle, K., Andres, C., Bernhard, D., Grimm, A., Stoev, P., Tzankov, N., Schlegel, M., 2017. Are species genetically more sensitive to habitat fragmentation on the periphery of their range compared to the core? A case study on the sand lizard (*Lacerta agilis*). Landscape Ecology 32, 131–145. https://doi.org/10.1007/s10980-016-0418-2

Henle, K., Lindenmayer, D.B., Margules, C.R., Saunders, D.A., Wissel, C., 2004a. Species survival in fragmented landscapes: where are we now? Biodiversity and Conservation 13: 1-8.

Henle, K., Davies, K.F., Kleyer, M., Margules, C., Settele, J., 2004b. Predictors of species sensitivity to fragmentation. Biodiversity and Conservation 13, 207–251. https://doi.org/10.1023/B:BIOC.0000004319.91643.9e

Hoehn, M., Sarre, S.D., Henle, K., 2007. The tales of two geckos: does dispersal prevent extinction in recently fragmented populations? Molecular Ecology 16, 3299–3312. https://doi.org/10.1111/j.1365-294X.2007.03352.x

Jellinek, S., Driscoll, D.A., Kirkpatrick, J.B., 2004. Environmental and vegetation variables have a greater influence than habitat fragmentation in structuring lizard communities in remnant urban bushland. Austral Ecology 29, 294–304. https://doi.org/10.1111/j.1442-9993.2004.01366.x

Journal Pre-proof

Jellinek, S., Parris, K.M., McCarthy, M.A., Wintle, B.A., Driscoll, D.A., 2014. Reptiles in restored agricultural landscapes: The value of linear strips, patches and habitat condition: Reptiles in restored agricultural landscapes. Animal Conservation 17, 544–554. https://doi.org/10.1111/acv.12121

Jombart, T., 2008. Adegenet: A R package for the multivariate analysis of genetic markers. Bioinformatics 24, 1403–1405. https://doi.org/10.1093/bioinformatics/btn129

Jombart, T., Ahmed, I., 2011. Adegenet 1.3-1: New tools for the analysis of genome-wide SNP data. Bioinformatics 27, 3070–3071. https://doi.org/10.1093/bioinformatics/btr521

Kalinowski, S.T., 2005. Hp-rare 1.0: A computer program for performing rarefaction on measures of allelic richness. Molecular Ecology Notes 5, 187–189. https://doi.org/10.1111/j.1471-8286.2004.00845.x

Kalinowski, S.T., Taper, M.L., Marshall, T.C., 2007. Revising how the computer program cervus accommodates genotyping error increases success in paternity assignment. Molecular Ecology 16, 1099–1106. https://doi.org/10.1111/j.1365-294X.2007.03089.x

Kalyabina, S.A., Milto, K.D., Ananjeva, N.B., Legal, L., Joger, U., Wink, M., 2013. Phylogeography and systematics of *Lacerta agilis* based on mitochondrial cytochrome b gene sequences: First results. Russian Journal of Herpetology 8, 149–158.

Keinath, D.A., Doak, D.F., Hodges, K.E., Prugh, L.R., Fagan, W., Sekercioglu, C.H., Buchart, S.H.M., Kauffman, M., 2017. A global analysis of traits predicting species sensitivity to habitat fragmentation. Global Ecology and Biogeography 26, 115–127. https://doi.org/10.1111/geb.12509

Kopelman, N.M., Mayzel, J., Jakobsson, M., Rosenberg, N.A., Mayrose, I., 2015. Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. Molecular Ecology Resources 15, 1179–1191. https://doi.org/10.1111/1755-0998.12387

Laube, M., Kuehn, R., 2006. Development of microsatellite markers for noninvasive DNA samples of the eastern green lizard *Lacerta viridis*. Molecular Ecology Resources 6, 731–733. https://doi.org/10.1111/j.1471-8286.2006.01324.x

Journal Pre-proof

Lawton, J.H., 1993. Range, population abundance and conservation. Trends in Ecology and Evolution 8, 409–413. https://doi.org/10.1016/0169-5347(93)90043-O

Lesica, P., Allendorf, F.W., 1995. When are peripheral populations valuable for conservation? Conservation Biology 9, 753–760. https://doi.org/10.1046/j.1523-1739.1995.09040753.x

Meek, R., 2020. Temporal trends in *Podarcis muralis* and *Lacerta bilineata* populations in a fragmented landscape in western France: Results from a 14 year time series. The Herpetological Journal 30, 20–26.

Mollov, I., 2011. Habitat distribution of the amphibians and reptiles in the city of Plovdiv, Bulgaria. Biharean Biologist 5, 25–31.

Mullis, K.B., Faloona, F.A., 1987. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction, in: Methods in Enzymology. Elsevier, pp. 335–350. https://doi.org/10.1016/0076-6879(87)55023-6

Öckinger, E., Schweiger, O., Crist, T.O., Debinski, D.M., Krauss, J., Kuussaari, M., Petersen, J.D., Pöyry, J., Settele, J., Summerville, K.S., Bommarco, R., 2010. Life‐history traits predict species responses to habitat area and isolation: a cross‐continental synthesis. Ecology Letters 13, 969–979. https://doi.org/10.1111/j.1461-0248.2010.01487.x

Pérez-Tris, J., Llanos-Garrido, A., Bloor, P., Carbonell, R., Tellería, J.L., Santos, T., Díaz, J.A., 2019. Increased individual homozygosity is correlated with low fitness in a fragmented lizard population. Biological Journal of the Linnean Society 128, 952–962. https://doi.org/10.1093/biolinnean/blz144

Prieto‐Ramirez, A.M., Pe’er, G., Rödder, D., Henle, K., 2018. Realized niche and microhabitat selection of the eastern green lizard (*Lacerta viridis*) at the core and periphery of its distribution range. Ecology and Evolution 8, 11322‐11336. https://doi.org/10.1002/ece3.4612

Pritchard, J.K., Wen, X., Falush, D., 2010. Documentation for structure software : Version 2.3.

R Core Team, 2019. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Raymond, M., Rousset, F., 1995. GENEPOP (Version 1.2): Population genetics software for exact tests and ecumenicism. Journal of Heredity 86, 248–249. https://doi.org/10.1093/oxfordjournals.jhered.a111573

Journal Pre-proof

Sarre, S., 1995. Size and structure of populations of *Oedura reticulata* (Reptilia: Gekkonidae) in woodland remnants: Implications for the future regional distribution of a currently common species. Australian Journal of Ecology 20, 288–298. https://doi.org/10.1111/j.1442-9993.1995.tb00541.x

Schneeweiss, N., 2001. Aspekte der Entwicklung und des Ausbreitungsverhaltens von Smargdeidechsen (*Lacerta viridis viridis*) in einem Ansiedlungsversuch in Brandenburg, in: Elbing, K., Nettmann, H.-K. (Eds.), Beiträge zur Naturgeschichte und zum Schutz der Smaragdeidechsen (*Lacerta* s. str.). Contributions to the natural history and conservation of the Green Lizards (*Lacerta* s. str.), Mertensiella. DGHT, Rheinbach, pp. 229–240.

Settele, J., Margules, C.R., Poschlod, P., Henle, K., 1996. Species Survival in Fragmented Landscapes. Kluwer, Dordrecht.

Van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M., Shipley, P., 2004. Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4, 535–538. https://doi.org/10.1111/j.1471-8286.2004.00684.x

Vucetich, J.A., Waite, T.A., 2003. Spatial patterns of demography and genetic processes across the species' range: Null hypotheses for landscape conservation genetics. Conservation Genetics 4, 639–645. https://doi.org/10.1023/A:1025671831349

Weir, B.S., Cockerham, C.C., 1984. Estimating F-statistics for the analysis of population structure. Evolution 38, 1358–1370. https://doi.org/10.2307/2408641

Wickham, H., Chang, W., RStudio, 2016. Ggplot2: Create elegant data visualisations using the grammar of graphics.

Young, A.G., Clarke, G.M., 2000. Genetics, demography and viability of fragmented populations. Cambridge University Press, Cambridge.

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**Figures and Tables** (included in the main part)

Figure 1: Sample sites of the 10 investigated populations (**1.5 column**)

Figure 2: Scatterplot of the Principal component analysis (PCA) of microsatellite frequency data.

Table 1: Sample name and size of the investigated populations in Bulgaria

Table 2: Geographic and genetic distances

Table 3: Measurements of genetic diversity per population for all loci

Table 4: Regression of parameters of genetic diversity to the habitat size (area) and degree of isolation

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## Highlights

- Lacerta viridis populations in core range show high genetic diversity
- Low differentiation between core populations
- Areas of suitable habitat or vegetation may serve as migration corridors
- Populations are probably part of a larger metapopulation network

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**Conflict of interest statement**

The authors declare no conflicts of interest.

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