

Microhabitat preferences in the European green lizard (*Lacerta viridis*): implications for conservation management of isolated populations

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Abstract

The European green lizard (*Lacerta viridis*) populations in Bohemia, Czech Republic, are isolated by more than 150 km from the northern border of the continuous range of the species. These populations are fragmented and further isolated from each other. In this landscape mosaic, they are tied to specific habitats located in deeply incised river valleys (the so-called river phenomenon) and thus may be viewed as stenotopic. The research site is located on the northern edge of the city of Prague. Since 1998, this site has been the subject of long-term conservation management aimed at strengthening and maintaining the abundance of the local *L. viridis* population. To formulate recommendations for the management of other isolated *L. viridis* populations, we performed a spatial analysis of the localities with observed individuals to determine and evaluate the significance of the chosen biotic and abiotic factors for habitat discrimination. We applied principal components and discriminant function analyses and examined the effect of 24 variables on the species' presence. The results revealed the principal role of the presence of rock debris and hiding places for lizard occurrence. The strongest negative predictors were the presence of tall grass and high vegetation coverage. We discuss the applicability of our findings in both the theory and practice of species conservation and population management.

Key Words

climate change, conservation modeling, discriminant function analysis, population characteristics, population ecology, regional stenotopy, spatial analysis

Introduction

Reptile species distribution has been modeled and analyzed on various, but typically large scales – from landscapes to continents (Kaliontzopoulou et al. 2008; Sillero and Carretero 2013; Oraie et al. 2014; Hosseinian Yousefkhani et al. 2015; Wirga and Majtyka 2015; Vargas-Ramírez et al. 2016; Petrosyan et al. 2020; Chmelař et al. 2020, 2023; Srinivasulu et al. 2021). However, due to ecological specifics, reptiles may also show major inter/intraspecific differences in microhabitat preference and usage, which have received less focus. Both microand macro-scales need to be taken into account for effective management of their habitats and populations.

Studies based on positive or negative discrimination of environmental factors are common in botanical works but have been applied just recently to predicting the occurrence of reptiles (Sacchi et al. 2011). A similar analysis can be used to separate sympatrically occurring species according to their ecological demands (Melville and

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Swain 1997; Heltai et al. 2015) or to study preference or avoidance of certain environmental factors, such as invasive plant species (Hacking et al. 2014).

The European green lizard, Lacerta viridis (Laurenti, 1768), is a robust lizard from the family Lacertidae, characterized by distinct sexual dichromatism with males exhibiting a bright blue throat. The species range covers mostly central and eastern Europe, the Balkan peninsula, and the coast of the Black Sea. Czech populations, located on the fringe of the range, are generally isolated and declining due to habitat degradation, making the species' survival in this location uncertain (Baruš et al. 1989, 1992; Mikátová and Nečas 1997; Mikátová 2002; Moravec 2015; Rehák 2015; Mikátová and Jeřábková 2023). All populations in the Czech Republic belong to the nominotypical subspecies L. v. viridis (Böhme et al. 2007b). According to legislative regulations in the Czech Republic (Act no. 114/1992), the European green lizard remains listed among critically endangered species even though the current Czech Red List decreased the category to endangered (Chobot and Němec 2017). The reason for this change is the generally favorable state of the populations in the southeastern Moravia region in contrast to the populations in the Bohemia region. However, the biggest differences are the noticeably lower genetic diversity, heterozygosity rate, and allele richness of the scattered populations in Bohemia (Böhme and Moravec 2011) compared to the populations in the South Moravian region, which are connected to the core area of L. viridis (Nemitz-Kliemchen et al. 2020). This is apparently a consequence of the geographic isolation of the Bohemian populations and represents an important aspect for their conservation and management.

Molecular data confirmed genetic affinities of Bohemian populations to those in neighboring parts of their distribution range in NE Germany (Elbe River) and Moravia (Böhme et al. 2006; Böhme and Moravec 2011). No recent records of L. viridis presence are known from Poland (Skawiński et al. 2019). Moreover, individual relic Bohemian populations are genetically slightly distinct (Böhme et al. 2006; Böhme et al. 2007a; Böhme et al. 2007b; Böhme and Moravec 2011). These populations are ecologically notable as inhabitants of biotopes retaining ancient characteristics, mainly rocky steppes, and these habitats can differ significantly in ecological parameters from the surrounding landscape matrix (Strödicke 1995; Joger et al. 2010; Fischer and Rehák 2010; Blažek 2013) and are in most cases fragmented (Prieto-Ramirez et al. 2018).

All Bohemian populations are bound to the so-called "river phenomenon" that affects deeply incised river valleys where thermophilous organisms, otherwise absent in the surrounding landscape, inhabit slopes with southern exposure (Ložek 1988). Therefore, the formation of metapopulations is unlikely. This creates the possibility of comparing these individual populations, in terms of morphology, ecology, and ethology. These populations also show regional stenotopy, linked to specific biotopes at the northern limit of the species distribution, and occur sympatrically with other animal and plant species connected with the river phenomenon (Ward 1998; Chmelař et al. 2023). The data on the ecology obtained from these localities are therefore very valuable, as the European green lizards are probably found here at the ecological limits of the species. The Bohemian relict autochthonous populations of *L. viridis* have high scientific and conservation value due to their genetic exceptionality related to isolation, fragmentation, small population size, genetic drift, reduced variability, and the possibility of occurrence of unique genetic variants; therefore, they also require special methods of conservation management (cf. Böhme et al. 2007a; Joger et al. 2010).

The distribution of *L. viridis* in the Czech Republic has already been analyzed, and a model has been developed to identify suitable habitats on a large state-wide (78,870 km²) scale (Chmelař et al. 2020). The aim of the current work is to identify the key factors influencing microhabitat selection of isolated populations of *L. viridis* at the fringe of their distribution, to evaluate if these factors correspond at both micro- and macro-habitat scales, and to contribute to the practice and theory of conservation of isolated populations in general.

Material and methods

The research was carried out along the Únětický stream, otherwise also called Tiché údolí (the silent valley), located on the border of the Prague and the Central Bohemian regions (coordinates: 50.1472°N, 14.3772°E; Fig. 1). The whole sampling area is part of the "Roztocký háj-Tiché údolí" Nature Reserve.

Geologically, the research site falls into the area of the Barrandien Paleozoic, where sedimentary rocks, especially shale and silicite, predominate. The filling of the valley consists of alluvium deposits on sandy gravels (Fediuk 1997). The rock composition is important for the soil characteristics and vegetation species and coverage, thus affecting the thermal and hydrological properties of the surface and thus for the distribution of the lizards. Parts of the area were inaccessible either due to steep rocky slopes or vegetation too dense to pass through.

The location of the European green lizard population is a south-facing slope with an area of 4.2 ha consisting of two abandoned quarries and the slope itself. It mainly consists of fragments of heaths and rocky steppes with native flora on rocky outcrops. Such diverse terrain provides a considerable number of microclimates with relatively high temperature differences. There are frost basins in the area of the valley floor, with a frequent temperature inversion, especially in spring. In contrast, the exposed rock outcrops showed significantly higher temperatures than would be usual for the given time of year when measured by an infrared thermometer. The part of the site is shown in Fig. 2, and a photograph of an individual from the site is shown in Fig. 3.



Figure 1. Map of the research site at "Roztocký háj - Tiché údolí" Nature Reserve, Bohemia, Czech Republic.



Figure 2. Typical habitat of *Lacerta viridis* (maintained by active management) at "Roztocký háj-Tiché údolí" Nature Reserve, Bohemia, Czech Republic.

A linear transect of 1.9 km was laid out through the research site, maximizing the coverage of the area inhabited by lizards and habitat diversity by its effective width. The observer (JC) walked once, alternating starting eastern and western starting points per sampling to avoid double counting of individuals. The location of each lizard visually detected along this transect was recorded by GPS, along with their relative age and sex. Detection was at times aided with binoculars. In case the presence of the observer caused the lizard to move, its original position was recorded. The obtained data were also used for mapping the annual and daily activity, for estimating the size of the population, and as an indicator of the relative composition of the population in terms of gender ratios and age categories (juveniles, subadults, and adults). Presences were recorded between 2011 and 2014 throughout the whole activity period of the



Figure 3. Adult male *Lacerta viridis* from the "Roztocký háj - Tiché údolí" Nature Reserve, Bohemia, Czech Republic.

lizards by the same observer. The observer visited the site 119 times in total, walking the transect 60 times. Only observed presences during the transect sampling are included in the study since many visits were made in periods with low or no lizard activity for confirmation purposes. The transect was sampled during different times during the years, and depending on seasonal and weather conditions, sampling started between 7:00 and 17:00 h and ended between 11:00 and 21:00 h. The sampling lasted between 2 and 4 hours, depending mainly on the number of recorded individuals. Yearly and monthly distribution is summarized in Fig. 4.

The coordinates of the observations were obtained by a Trimble GeoXT GeoExplorer 2005 hand-held GPS receiver. The accuracy of the positions was further enhanced by geodetical software Leica GNSS SPIDER V4. The post-processed accuracy of the recorded points was within 50 cm in 87.6% of cases, within 1 m in 9%, and only 3.4% of the measurements had a deviation between 1 and 2 m.



Figure 4. Sampling distribution in consecutive years.

These recorded locations were used to perform the spatial analysis. The mapping was carried out during June and July 2014 in order to minimize seasonal differences in microhabitat layout. We did not notice substantial changes in microhabitat layout between the consecutive years of the study. Also, all the variables were selected with minimizing the effect of seasonal change in mind. Especially in the case of variables related to vegetation, we focused on the percentage of their coverage and/or number rather than the exact height or the degree of shading of the surface. We standardized the environmental variables according to their assumed ecological function, not according to systematics. Variables were visually assessed in the field within a radius of 0.5 and 2.5 m from the location of each lizard's point of presence and are summarized in Table 1. The distance of 2.5 m was chosen as it is the approximate maximum distance that an adult individual was able to run without stopping. In this environment, we assumed discrimination based on the presence of a potential long-term shelter.

For comparison with the presence records, we created 200 random points within the same area along the transect using QGIS software version 2.2.0. Variables were recorded for these points using the same method as above. We calculated the Haversine distance in order to measure the minimum distances between real presence and random points.

Prior to further analyses, environmental variables were screened for spatial correlation using the Mantel test, and variables with a significant correlation of r > 0.2 were not included in further analyses. Principal component analysis (PCA) was performed to identify ordination axes in the factor plane. Points of occurrence and random points were then compared using discriminant function analysis (DFA) in the STATISTICA software version 9.0, using the presence as the grouping variable (value of 0 for a random point and 1 for a point of recorded presence). The final model was constructed by a method of backwards stepwise variable elimination. Other DFAs were performed afterwards with the age category (adult, subadult, juvenile) and sex of the adults as a grouping variable in order to identify possible differences in their microhabitat structure. Again, backwards stepwise variable elimination was used.

Results

A total of 403 presences were recorded during 60 samplings. On average, 6.7 individuals were recorded along the transect per visit (=1–16, SD = 3.7). The highest monthly (?) average numbers of recorded individuals were in May (9.7) and June (9.6) and the lowest in September (4.3). Of all the observed individuals, 99 were males, 70 females, 93 unidentified adults, 42 subadults, and 103 juveniles.

The calculated Haversine distance (shortest distances of real presence and random points) was 6.9 m (0.16–21.1 m, median = 6.2 m). The unreduced model shows significant differences between random and recorded presence points (Wilks' Lambda: 0.577, $F_{(24.578)} = 17.69$, p < 0.0001).

Using the Mantel test to identify correlating variables, only the percentage of soil (at 0.5 and 2.5 m) from the presence record exceeded the predetermined correlation value of r > 0.2 and thus were not included in further analyses. The reduced number of variables in the model was therefore 9: scree percentage (0.5 m), grass percentage

Table 1. Measured variables within 0.5 and 2.5 radius of presence and random points.

Name	Description	Measured as	Removed due to correlation
Scree	Continuous scree coverage	%	Retained
Grass	Herbs shorter than 30 cm	%	Removed
Soil	Exposed soil	%	Retained
Tall vegetation	Herbs higher than 30 cm	%	Retained
Raised rock	Min. length 30 cm, min. elevation 15 cm	%	Retained
Stump	Stump or fallen log	%	Retained
Leaves	Fallen leaves coverage	%	Retained
Branches	Mounds of cut or fallen branches	%	Retained
Bush	Woody plant up to 2 m tall, sprouting close to surface	count	Retained
Thornbush	Woody thorny plant up to 2 m tall, sprouting close to surface	count	Retained
Tree	Woody plants higher than 2 m with branches high above the ground	count	Retained
Raised rock	Min. length 30 cm, min. elevation 15 cm	count	Retained
Stump	Stump or fallen log	count	Retained
Shelter	Subsurface space large enough to hide	count	Retained
Deep shelter	Usable for over-wintering or laying eggs	count	Retained

(0.5 m), high vegetation (0.5 m), branches percentage (0.5 m and 2.5 m), elevated rock/stump percentage (0.5 m), number of trees (0.5 m), number of shelters (2.5 m), number of deep shelters (2.5 m).

Wilks' Lambda of the reduced model (0.598) remained significant ($F_{(9,593)} = 44.24$, p < 0.0001). The model was able to classify the random points correctly in 82.5 percent of cases (165 out of 200). The classification success rate of points of presence was 79.9% (322 out of 403).

DFA of age categories: the unreduced model (Wilks' Lambda: 0.75, $F_{(48,754)} = 2.392618$, p < 0.00001) shows significant differences in classification between points of presence of adults and juveniles (Table 2) (Squared Mahalanobis distance = 1.25, F = 3.52, p < 0.00001) and between points of presence of juveniles and sub-adults (Squared Mahalanobis distance = 1.60, F = 1,65, p = 0.03). The subsequent canonical analysis of age categories showed no distinguishable clusters.

The PCA scree plot identified 5 factors that explained a

Table 2. Classification matrix of DFA analysis with age category as grouping variable. Rows: Observed classifications, Columns: Predicted classifications.

	Percent	а	S	j
a	88.4	236	4	27
\$	13.5	27	5	5
j	35.4	64	0	35
Total	68.5	327	9	67

significant percentage of variability. The first two factors were selected as determinants. The first factor explained 20.13% of the internal variability, while the second factor explained 13%. Four main vectors of synchronous variables can be identified by projecting the variables onto the plane (Fig. 5).



Figure 5. Projection of PCA into factor plane.

Regardless of the chosen statistical method, all the analysis results show that the distribution of individuals in the study site was not random.

The discriminant analysis shows that the created model is reliably able to distinguish a point of presence from a randomly selected point. However, it cannot reliably classify the presence points of individuals into the correct age category. There was a significant discrimination between juveniles and adults and between juveniles and subadults, but the classification success rate was relatively small. The sex of adult individuals was not evaluated in the analysis due to a non-significant difference in the success of the classification of presence points of males and females in preliminary analyses.

Discussion

Previously, we identified four factors that showed positive influence on the species distribution in the Czech Republic: annual precipitation up to 600 mm, slope inclination between 5–25°, mean temperature of the warmest quarter up to 20 °C, and precipitation in the coldest quarter above 150 mm (Chmelař et al. 2020). These factors seem to describe well the preferred habitats that can support the *L. viridis* populations, including our chosen research site.

According to the results of the reduced DFA model, several variables are key to characterizing the localities with lizards present: scree percentage, grass percentage, high vegetation, branches percentage, raised rock/stump percentage, number of trees, proportion of debris, grasses, and tall vegetation within 0.5 m and branches percentage, number of shelters, and number of deep shelters within 2.5 m. It is precisely these variables that are able to distinguish multidimensional groups of objects, in this case localities within the study site. However, these results should be interpreted with great caution. The European green lizard is a relatively large and very mobile species. A circle with a radius of 0.5 m from the place of observation would therefore show which microhabitats are used by individuals. Habitat characteristics at this distance will be important mainly in terms of thermoregulation and passive antipredation. From the point of view of prey accessibility, both these distances are important. The availability of a main shelter is essential in respect to active predation avoidance (escape, temporary cover, vantage point), even at a greater distance.

From the PCA visualization (Fig. 5), four vectors can be recognized.

 The first vector (corresponding with the direction of recorded presence) contains mainly variables of elevated rock and shelter, both within 0.5 m and 2.5 m from the point of observation, and includes variables directly linked to anti-predatory/thermoregulatory function. An elevated position provides a basking opportunity and a vantage point to see potential predators or competitors, while the availability of shelter in the immediate vicinity is necessary to avoid predation (Majláth and Majláthová 2009; Fischer and Rehák 2010).

- 2. The second vector consists of bush and thorny bush variables, which we interpret as mainly anti-predatory. The vector is directed roughly in accordance with the recorded presence, which corresponds with data from similar studies (Heltai et al. 2015). Lizards have been frequently observed running first into a nearby shrub if disturbed and only if pursued further, seeking a refuge in subterranean shelter. This interpretation can also be supported by the fact that 95% of recorded observation points had at least one shrub or shelter within 0.5 m and 99% had at least one shrub or shelter within 2.5 m. We found no difference between the usage of thorny or not thorny shrubs.
- 3. The third identified vector contains grass and high vegetation variables and does not correspond with the direction of recorded presence. Our preferred explanation is that high vegetation and grass provide shade and thus lower the temperature of surfaces covered by them. Also, these objects limit the lizard's field of view without providing substantial cover from the predators, posing a potential risk. Of course, during periods of supra-optimal temperatures, individuals have been observed seeking lower temperatures in shade, but mostly preferring a shade provided by shrubs or seeking a subterranean shelter.
- 4. The last identified vector consists only of the scree coverage (both within 0.5 and 2.5 m) and does not correspond to the presence of lizards, but neither goes in the opposite direction. This is interpreted as mainly related to thermoregulatory effects since the screed can be very easily warmed by the sun, but these surface temperatures can easily reach 60 °C in warmer periods of direct sunlight (measured on site with an infrared thermometer), which makes them unusable. The gaps between scree are sufficient for juveniles and most subadults to be used as cover from predators, but larger adults are not able to fit in most of them. The rising percentage of warmer days in the area (Zahradníček et al. 2020) could also lead to a switch in both microhabitat and habitat usage in the future (Rehák et al. 2022). Thus, we consider scree to be the preferred surface only under very specific circumstances.

The nature of the site must also be considered when interpreting the results. The random points were plotted into the polygon covering the site (4.2 ha) and did not include areas inaccessible to lizards. There was therefore no risk that a random point would be placed, for example, in the middle of a stream or beyond the boundary of the site. Due to the relatively small size of the polygon, it was therefore unavoidable that a significant portion of the random points was located in the immediate vicinity of the points of recorded presence, as confirmed by the Haversine distances. Despite this, the analysis was able to distinguish the vast majority of random points from real observation locations.

The observed lizards were not individually identified, and the dataset thus contains some recordings of the same individuals multiple times. This problem could not be avoided but was minimized by using the line transect method, thus significantly lowering the probability of repeated records of the same individuals during the same visit. We were unable to avoid recording the same individual in different visits. The time delay between individual site visits lowered the risk of dependence on subsequent records even more. Nevertheless, we reasonably expected that repeated records of the same individual would be spatially clustered, and thus we tested the dataset for spatial autocorrelation.

The change of habitat preference by *L. viridis* during ontogeny is widely recognized and supported by the published data from many populations (e.g., Fischer and Rehák 2010; Harta et al. 2017). In spite of this, we failed to prove such an ontogenetic change. Concerning differences of microhabitat preferences among adult males and females, our results do not show any, and neither we found any evidence in literature.

Multivariate statistical models are rarely used in the study of reptile habitats. This method is widespread, especially in botany and invertebrate zoology; specifically, it is often used to predict the occurrence of selected species. However, in studies focused on aspects of species protection, these are very valuable methods, the outputs of which can have direct application. For example, the discrimination of a certain type of habitat in reptiles (Hacking et al. 2014) using the MANOVA method. Their results demonstrated that Schmeltz's skink (Carlia schmeltzii) avoids microhabitats with a high proportion of invasive grasses. A similar method was used in a study of the local population of Lacerta bilineata in northern Italy (Sacchi et al. 2011). According to the authors, individuals in the monitored population purposefully seek out ecotones for their microhabitat; however, they do not discriminate based on the specific composition of these ecotones. Other authors also mention the importance of ecotones in L. viridis microhabitat usage (Harta et al. 2017).

The research site has been subject to active management since 2000. By 2013, both the population density and area usage significantly increased in comparison to 1995–1997 (Fischer and Rehák 2010), with the density and abundance corresponding to populations in similar habitats (Prieto-Ramírez 2023). Still, the isolation of the population means a high risk to its long-term survivability (Böhme et al. 2007b), and small isolated populations are presumed to be most threatened by habitat erosion due to climate change (Sinervo et al. 2010). The management measures were focused on keeping the landscape mosaic by retaining key microhabitat elements while avoiding excessive growth of vegetation coverage, ideally by combining grazing and cutting (Fischer and Rehák 2010; Rehák 2015; Fischer et al. 2016, 2023; Mizsei et al. 2023). Our study has identified some of these microhabitat elements and their combinations, which should be taken into account when planning management measures in similar areas. Most notably, we recommend maintaining the maximum distance between potential shelters (provided by vegetation or terrain) at 5 m and to keep the landscape mosaic heterogeneous. Cover against predation from above, mostly provided by shrubs, is also important, though impossible to test from our dataset. Significant avoidance of places covered by high vegetation also suggests that overgrowth of habitat needs to be prevented.

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Supplementary material 1

DFA dataset

- Authors: Jan Chmelař, Daniel Frynta, Veronika Rudolfová, David Fischer, Ivan Rehák
- Data type: xlsx
- Explanation note: Primary dataset for DFA containing age categories and variable values for points of recorded presence and random points.
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- Link: https://doi.org/10.3897/herpetozoa.37.e120806.suppl1

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