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RESEARCH ARTICLE

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*)

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Abstract
Context Species show different sensitivity to habitat loss and fragmentation depending on their specialization. Populations of a species at the range margin are generally assumed to be more stenoecious than populations at the core of the distribution and should therefore be more sensitive to habitat fragmentation.
Objectives We evaluated the hypothesis that fragmentation effects species more strongly at the range periphery of their range compared to the core, resulting in lower genetic variability in comparable patch sizes and lower gene flow among populations.
Methods We compared the genetic diversity and structure of five sand lizard (*Lacerta agilis*) populations at the margin of its range in Bulgaria and of 11 populations at the core of its distribution in Germany. We based the analysis on microsatellites, comprising 15 loci in Bulgaria and 12 in Germany.
Results All diversity indices declined with patch size. For medium-sized patches all diversity indices were lower at the range periphery compared to the core, with two of them being significant. AIC_c based model selection showed strong support for core/periphery and patch size effects for observed and expected heterozygosity but only a patch size effect

Nikolay Tzankov recently deceased

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for allelic richness. There was no isolation-by-distance and each sampled population was allocated to a separate cluster with high probability for both countries, indicating that all populations are (almost) completely isolated.

Conclusion Our study indicates an increased sensitivity of a species to fragmentation at the periphery compared to the core of its distribution. This differential sensitivity should be accounted for when prioritizing species based on their fragmentation sensitivity in landscape management.

Keywords Lacertidae · Fragmentation sensitivity · Genetic variability · Genetic structure · Isolation-by-distance · Patch size · Range core · Range periphery

Introduction

Habitat loss and fragmentation are major drivers of biodiversity loss. Habitat fragmentation can lead to the isolation of formerly interconnected populations. Resulting small population sizes and reduced gene flow can decrease genetic variability through genetic drift and inbreeding (Frankham 2005), which in turn reduce population sizes even further and may ultimately cause extinction (Soulé 1986). At the genetic level, the negative impacts of habitat fragmentation should be reflected by a decline in genetic diversity within reduced patch size and an increased genetic differentiation among them (Young and Clarke 2000).

Not all species show the same sensitivity to fragmentation (Henle et al. 2004). Various traits contribute to sensitivity, with habitat specialization being one of these key traits (Henle et al. 2004; Devictor et al. 2008). Specialist species have a more discontinuous distribution because their niche is less likely to be found across the landscape compared to generalist species (Jellinek et al. 2004). Furthermore, habitat specialists may have a lower dispersal capacity because they are less tolerant to unsuitable habitats in the matrix (Hoehn et al. 2007; Öckinger et al. 2010). They therefore have more difficulties overcoming fragmentation and maintaining functional meta-populations compared to habitat generalists.

The degree of habitat specialization may change within the distribution area of a species. Towards the periphery, populations are expected to be smaller, spatially more isolated, and restricted to particularly favourable habitats. In contrast, the core region is expected to possess the highest abundance due to optimal living conditions and a wider range of suitable habitats (Eckert et al. 2008). Hence, along a continuum, species are often more euryoecious in the core area of their distribution range and more stenoecious at the periphery of their range (Böhme and Rödder 2008). This change is called the Kühnelt principle of regional stenoecy (Böhme and Rödder 2008). Concomitantly, genetic diversity is expected to be lower and genetic differentiation higher in populations at the periphery of their range compared to core populations (Eckert et al. 2008; Dudaniec et al. 2012). Therefore, species should be more sensitive to fragmentation at the periphery compared to the core of their distribution. However, to our knowledge a comparison has not yet been made between the sensitivity of a species to habitat fragmentation at the core and the periphery of its distribution range, in spite of its high relevance for ecological generalizations and for the conservation of species.

According to the abundance centre theory, a higher specialisation at the periphery of the range should lead to lower genetic diversity irrespective of fragmentation (Eckert et al. 2008). Empirical evidence for this theory, however, is equivocal. While some studies have supported this theory (Eckert et al. 2008), others have observed the opposite (e.g., Munwes et al. 2010; Dudaniec et al. 2012). Part of the reason for this inconsistency is that the speed of a range shift, and thus the mobility of a species, is a major determinant of the extent to which a species retains or loses its genetic diversity during its range expansion (Arenas et al. 2012). Thus fragmentation and range shifts may or may not act synergistically in reducing genetic variability at the periphery of the species' range.

The aim of our study was to investigate whether populations of a species located at the periphery of its current distribution range displayed an increased sensitivity to fragmentation at the genetic level compared to populations in the current core of the species' range while accounting for potential effects of a range shift. We chose the sand lizard (*Lacerta agilis*) as a model species because the ecology of *L. agilis* is comparably well studied (Elbing et al. 1996). Although nothing is known about its sensitivity to fragmentation on the periphery of its current southern distribution range in Bulgaria, *L. agilis* is considered to be a rare species in

Bulgaria, with a fragmented distribution pattern due to specific habitat requirements (Stoyanov et al. 2011). By contrast, it is a widespread species in Germany, although classified as endangered or critically endangered due to habitat loss (Elbing et al. 1996).

We hypothesized that genetic diversity would be lower for the equivalent patch size and decline more strongly with patch size at the current periphery (Bulgaria) compared to the current core (Germany) of its distribution range. We also expected a stronger isolation of patches (less dispersal) in Bulgaria compared to Germany. This should lead to higher genetic differentiation among populations in the Bulgarian compared to the German populations. We tested these hypotheses using microsatellite data.

Materials and methods

Study design, and data collection

Because both differential fragmentation sensitivity and historic range expansions may reduce genetic variability, locations of the contemporary periphery and the core must be chosen carefully. Peripheral populations that are more distant to the historic core than current cores are not well suited as the effects of both historic range expansions and differential fragmentation sensitivity on lower genetic variability are indistinguishable. Thus, peripheral populations must be selected that are equidistant or closer to the historic core than to the selected current core in question. Like many European species, *L. agilis* expanded northwards after the last Ice Age from the Balkans as its glacial refuge (Kalyabina et al. 2001; Andres et al. 2014) (Fig. 1). Therefore, we selected Bulgaria as the current periphery and Germany as the current core. This approach accounts for the possibility that the current core (Germany) may have suffered reduced genetic variability due to range expansion but not the current periphery (Bulgaria). As a consequence, genetic diversity should only be lower at the current periphery, if the core/periphery sensitivity effect to fragmentation is stronger than the range shift effect that may have occurred when the species expanded its range from its historical core in the Balkans to the current core (Germany). This makes our approach conservative.

We selected urban and peri-urban populations because the loss of natural habitats is particularly severe in urban landscapes due to high land use pressures (Miller and Hobbs 2002) and because urban habitat fragmentation can have strong genetic effects (Delaney et al. 2010). The study was carried out in and around the cities of Sofia (Bulgaria) and Leipzig (Germany) (Fig. 2). Both cities have been exposed to dramatic structural changes over recent decades leading to the alteration of habitats suitable for lizards.

Sofia experienced considerable growth from the middle of the 20th Century and especially over recent decades, following political changes in Europe, leading to a high fragmentation of the remaining natural habitats (Hirt 2008). Alongside urban development, several large parks were created in the city to provide more favourable living and recreational conditions and to ensure connectivity between the city centre and the surrounding rural areas. A second wave of active building construction has continuously been taking place since the end of the twentieth century. This ongoing construction of new buildings has led to the fragmentation of the city greens and a disconnection of the lizard populations. Sand lizards were still common in the 1990s with many populations occurring in the parks, cemeteries and the greens between the blocks, especially in the periphery of the city. Over the last 10–15 years many green spaces were converted into residential and/or industrial areas. Sand lizard populations taking refuge in small green areas and between houses suffered significantly from the shrinkage of habitats and in many parts of the city the species became locally extinct or was pushed towards its periphery (Tzankov et al. 2015).

In Leipzig, considerable growth took place earlier, from the beginning of the twentieth Century, and following political changes, the city went through a period of suburbanisation and an increase in the built-up area (Grosse 2009; Haase and Nuissl 2010). Nevertheless, approximately 50 % of the area is still green space. However, not all of these green spaces are suitable habitat for *L. agilis* (Grosse 2009). Reptiles have been absent from the city centre and densely built-up suburbs already since the 1870s. In more peripheral city areas and along green belts *L. agilis* was regarded as being widely distributed in the 1920s and 1930s. Up until 1993 the species was still regarded as being rather widespread in peripheral areas and within remaining green belts, occurring primarily along railway

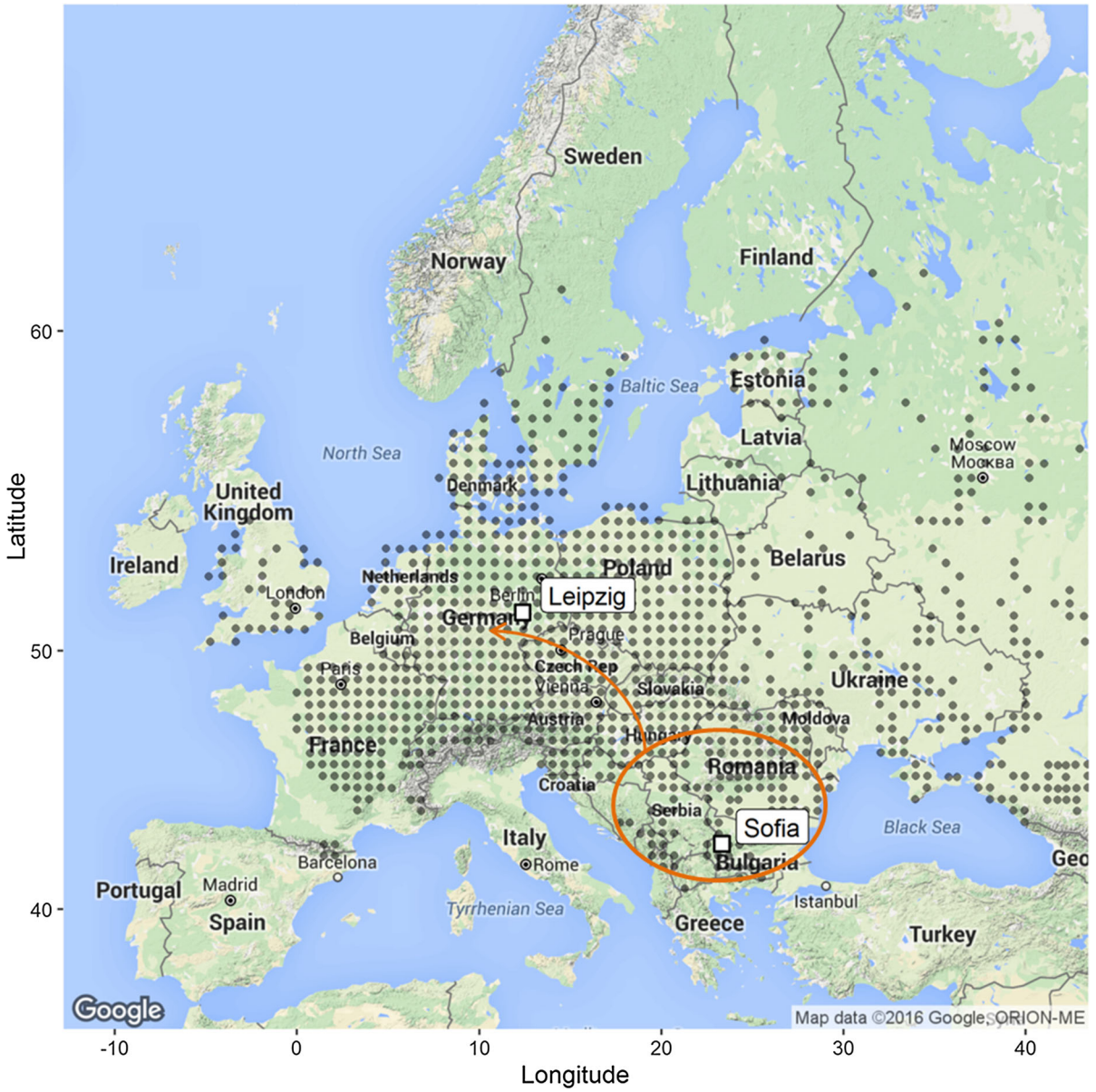

Fig. 1 Location of the study regions (*white squares*), current European distribution (Sillero et al. 2014, data accessed 27.5.2016; *grey dots*) and presumed glacial refuge (*orange circle*) of *Lacerta agilis*. Various authors suggested the Balkan Peninsula and/or the Pannonian Basin as glacial refuge (Andres et al. 2014) but the extent of the refugium or refugia is unknown. The distribution of the species in easternmost Europe and Asia is not well documented and these regions are inhabited by different genetic lineages

embankments and allotments, and populations were thought of as being well connected. The distribution map for the period 1961–2008 shows scattered occurrences, with many neighbouring occurrences at distances of 1–2 km but distance between most clusters of occurrences at 4 km and above (i.e. greater than the maximum dispersal distance reported for the species (Grimm et al. 2014)).

We assessed the availability of suitable lizard habitats with Google Earth maps and personal observations in the field. For Leipzig (Germany), we additionally used the list compiled by Grosse (2009)

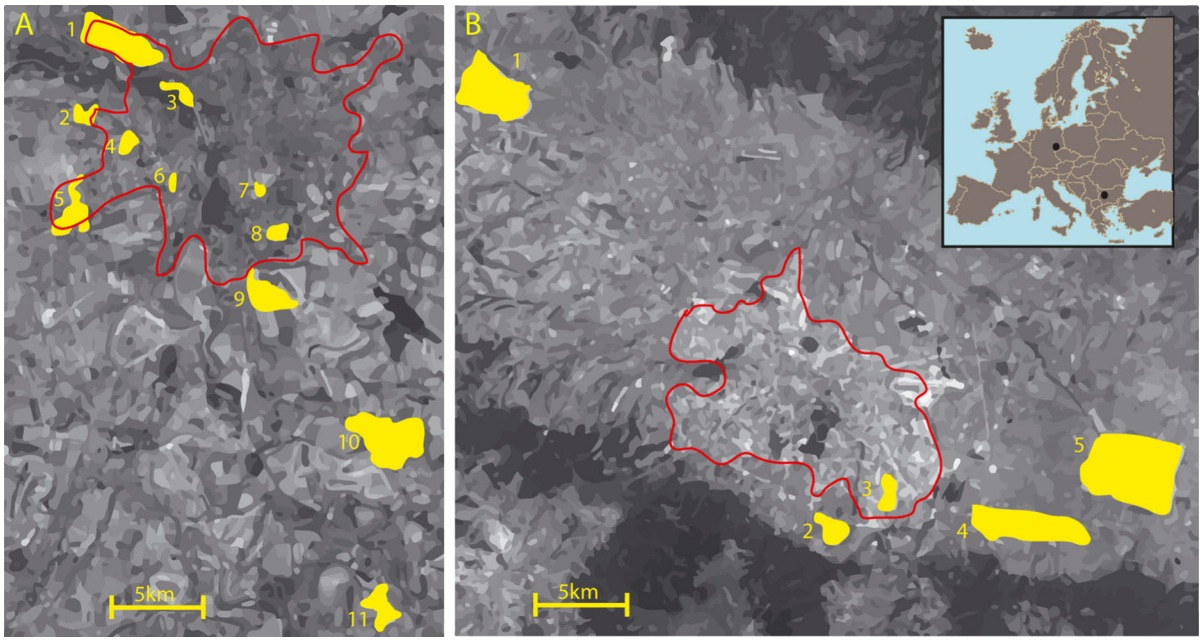

Fig. 2 Numbered sampling sites for *Lacerta agilis* in and around the city of Leipzig, Germany (**a**) and in and around the city of Sofia, Bulgaria (**b**). *Red lines* represent the borders of the cities

on *L. agilis* observations since the 1920s. Subsequently, we randomly selected potentially suitable patches for sampling, excluding inaccessible sites.

We estimated the size and distance of the habitat patches with ArcGIS 10 (ESRI 2011). We judged the suitability of habitats based on the descriptions in Elbing et al. (1996) and Märtens (1999) and on personal observations in the field. Patch size and distance was 8–856 ha and 1.7–36.7 km, respectively, in Germany and 268–3112 ha and 3.3–43 km, respectively, in Bulgaria (Table S1.1). In total, we sampled 11 and 5 populations in Germany and Bulgaria, respectively (Fig. 2).

Patches were sampled by walking crisscross over the entire patch, searching specifically around those structures preferred by lizards (wooden piles, stones, small trees etc.) and avoiding capturing more than one lizard at the same spot. We obtained tissue samples by cutting the tip of the tail and storing them in 99 % ethanol. After disinfecting the surface of the cut, we released the lizards at the sites of capture and took the GPS-coordinates of the exact capture locations. Sample sizes ranged from 19–28 to 17–21 individuals per site for the German and Bulgarian populations, respectively, with 20 being the most frequent sample size (Table S1.1).

DNA extraction, amplification and fragment analysis

We extracted DNA using the NucleoSpin Tissue Kit (Macherey–Nagel) according to the manufacture protocol. We conducted a multilocus microsatellite analysis using primers already established for *L. agilis* (Gullberg et al. 1997; Schwartz and Olsson 2008). We labelled primers fluorescently with either FAM or HEX (Supplementary material Tables S1.2, S1.3). We conducted the microsatellite-PCR in a volume of 25 µl containing 0.2 mM of each dNTP, 2.5 µl of 10× Dream Taq^{TM}-Buffer including 25 mM $MgCl_2$, 1U Dream Taq^{TM} Green DNA polymerase, 0.4 µM for each forward and reverse primer and 0.5 µl DNA-extract. PCR was performed on an Eppendorf Mastercycler under the following conditions: initial denaturation at 95 °C for 15 min, followed by 35 cycles of 30 s at 95 °C, 30 s at the specific annealing temperature and 30 s at 72 °C and eventually the final extension for 10 min at 72 °C. We amplified each microsatellite locus separately (one primer pair per reaction tube). We analysed PCR-products on the ABI PRISM Genetic Analyzer 3100 using *POP-6 Polymer* and *GeneScan ROX 500* as a size standard. We analysed an amplified fragment of a HEX-labelled

locus with the amplified fragment of another FAM-labelled locus in one probe. Only four loci were run separately on the Genetic Analyzer. We scored allele size in *GeneMapper* 3.7.

Statistical analyses

Testing the dataset

We screened all amplified loci for null alleles and scoring errors with MICROCHECKER 2.2.3 (Van Oosterhout et al. 2004) and, if necessary, excluded them from the dataset. We tested the remaining loci pairwise for linkage disequilibrium with GENEPOP 4.1 (Raymond and Rousset 1995) with 1000 batches, 10,000 iterations per batch and 100,000 dememorisation steps. We corrected tests for significance of the calculated linkage disequilibrium with the False Discovery Rate (Pike 2011). We calculated the Hardy–Weinberg-equilibrium (HWE) with ARLEQUIN 3.5.1.2 (Excoffier and Lischer 2010) with 100,000 dememorisation steps and a Markov–Chain Monte Carlo (MCMC) of 10^6 iterations and corrected the results with the False Discovery Rate.

Genetic diversity, patch size, and core/periphery effects

We calculated observed and expected heterozygosity with ARLEQUIN with 100,000 dememorisation steps and a MCMC of 10^6 iterations. We estimated allelic richness and private allelic richness with HP RARE 1.1, and corrected for sample size (Kalinowski 2005). We calculated the heterozygote deficit F_{IS} as an indicator for inbreeding with ARLEQUIN with 10,000 permutations.

We first tested the effect of patch size on the measures of genetic diversity (observed and expected heterozygosity, allelic richness, private alleles) within each country by grouping the patches into large (>1000 ha), medium (250–1000 ha), and small (<250 ha) patches and comparing mean values with a *t* test. We used one-sided tests for patch size because theory predicts a directional effect (diversity decline with patch size). We then used the corrected Akaike's Information Criterion (AIC_c) (Burnham and Anderson 2002) to compare the odds for linear models that assume (1) no patch size effect and no difference in genetic diversity between the core and the periphery, (2) a patch size effect only, (3) a difference between core and periphery with no patch size effect, and (4) an effect of both. We consider models within $\Delta AIC_c < 2$ of the best models as having substantial evidence and models within $2 < \Delta AIC_c < 4$ as having considerably less support. We used cumulative weights to assess the relative importance of the predictor variables. We conducted these analyses using package *AICcmodavg* (Mazerolle 2013) in program R (R Core Team 2015). We excluded private alleles form these comparisons because the probability of obtaining private alleles strongly depends on the number of populations assessed and these differed between the two study regions.

Genetic differentiation and isolation

There are different opinions about which diversity measure should be given preference (Heller and Siegismund 2009). F_{ST} (Wright 1951) is the most common measure but may be inadequate for high diversity systems, as is often the case for microsatellite studies (Jost 2008). A frequently recommended alternative is to calculate both G_{ST} (Nei 1973) and Jost's D_{EST} (Jost 2008), with G_{ST} tending to underestimate and D_{EST} to overestimate the genetic differentiation when high mutation markers are used. We computed all three measures to enable a broader comparison with other publications. We computed Jost's D_{EST} and Nei's G_{ST} using the *DEMEtics* package in R (Gerlach et al. 2010) with a bootstrapping of 1000. We calculated Wright's pairwise distances (F_{ST}) and conducted an AMOVA (Analysis of Molecular Variance) for both data sets separately using ARLEQUIN with a permutation number of 10,000.

We tested whether genetic differentiation among populations declined with geographic distance using a Mantel test with 1000 permutations, 500 bootstraps, an 85 %-bootstrap interval, and a 0.9 bootstrap level. We used one-sided tests to assess the statistical significance of the correlation coefficient in each country as the core/periphery hypothesis predicts a stronger isolation at the periphery of the distribution range compared to its core. To compare the core and the periphery of the distribution range, we used non-overlap of 85 %-confidence-intervals, which is equivalent to a significant difference at the 5 % level (two-sided test). We used Spearman's rank correlation as opposed to Pearson's product-moment correlation

because the former is independent of the specific form of the relationship and allows the same test for all three differentiation parameters. Whereas a model with a logistic transformation is recommended for F_{ST} (Rousset 1997), a linear model without transformation is more frequently applied to G_{ST} data (e.g. Palumbi 2003). The two approaches produced almost identical results; therefore, we only present the results for the linear models. We performed all tests using the R-package *ecodist* (Goslee and Urban 2012).

Population clustering

We performed a Bayesian cluster analysis with the R-package *Geneland* (Guillot et al. 2005). We allowed the number of clusters K to vary between 1 and 15 (for Germany) or between 1 and 8 (for Bulgaria) with a replication rate of ten runs. We used 10^6 iterations and a thinning of 1000 for the MCMC calculation. We then fixed K to the value inferred in the first step and carried out another MCMC simulation with the same setup. To detect migrants, we allowed individuals to originate from a population other than the one where they were caught by setting the uncertainty of coordinates to 5 km. (The maximum reported dispersal distance is 4 km: Grimm et al. 2014.) The allele frequency model used for the estimation was the F-model. We set the maximum rate of the Poisson process to 300 and used 300 nuclei. We recalculated the inferred number of clusters with a burn in of 50, setting the post process burning to 50,000 and to 200 pixels for x and y.

We further assessed population clustering using the program STRUCTURE. The results were internally inconsistent and therefore are not presented (see supplement S3).

Results

Sample sizes and the number of utilizable microsatellite loci

Of the available 21 primer pairs one pair (LA37) did not result in a successful amplification of fragments in the populations from both countries. Three loci (La-1, La-3, LA10) from the German samples and one locus (La-1) from the Bulgarian samples showed illegible stutter peaks in the electropherograms, making it impossible to assign allele sizes correctly. Another three loci in the German samples (LA50, LA58, LA64) and another two (LA47, LA58) in the Bulgarian ones exhibited an excess number of homozygotes (Tables S2.3, S2.4), indicating the presence of null alleles and therefore had to be excluded from the analysis.

The test for linkage disequilibrium was corrected with the FDR-method and revealed for all populations in both countries that two loci (LA12, LA45) were strongly linked with one another (Tables S2.5–S2.8). This linkage was obvious in the electropherograms indicating a high possibility of an artefact. Therefore, both loci were excluded from further statistical analyses. Furthermore, all loci were subjected to an exact test of Hardy–Weinberg-equilibrium (S2.9, S2.10). After FDR-correction four loci in three German populations (population 2: LA01; population 9: LA55; population 4: LA01, LA3E, LA40) were not in Hardy–Weinberg-equilibrium. The Bulgarian dataset showed two loci that were not in Hardy–Weinberg-equilibrium after FDR-correction (La-6 in populations 2, 3, 4, and 5 and LA64 in population 5). With 11 patches and 12 loci for Germany and 5 patches and 14 loci for Bulgaria, these analyses comprised 202 individual tests. At a 5 % significance level, ten significant results are expected to occur purely by chance. As we obtained ten significant results, there is no indication of a systematic deviation from the Hardy–Weinberg-equilibrium. Furthermore, the F_{IS} values revealed no signs of a heterozygote deficit. Therefore, we retained these loci for further analyses.

In summary, the number of utilizable loci differed between the German (12 loci) and the Bulgarian (14 loci) populations. The number of alleles per locus varied among loci between 1 and 23 (Table S1.4). The original multi-loci genotype data can be found in Tables S2.1 and S2.2.

Genetic diversity

All four diversity measures (observed and expected heterozygosity, allelic richness, private alleles) were higher in the larger compared to the medium-sized patches (Bulgaria) and likewise in the medium-sized patches compared to the small-sized patches (Germany) (Table 1—see Table S1.5 for site specific data). These differences were significant for observed heterozygosity (H_O) and allelic richness (AR) in Bulgaria and for expected heterozygosity (H_E) and

AR in Germany. Likewise, for medium-sized patches, H_O, H_E, and AR were lower at the periphery (Bulgaria) than the core of the distribution area (Germany); with the difference being significant at $\alpha < 0.05$ for H_O and H_E. Moreover, for the large patches in Bulgaria H_O and H_E were even as low as for the small-sized patches in Germany.

Comparing the odds of alternative models regarding the effects of patch size, core/periphery, or their combination on H_O based on AIC_c, the best models ($\Delta AIC_c = 0.10$) were those assuming that either core/periphery or both factors influenced H_O (Table 2a). The model including both factors was statistically significant (ANOVA, $\alpha = 0.014$). The support for the covariate core/periphery (78 %) was almost twice the support for the covariate patch size (44 %). Likewise, the best model that explained H_E included both covariates, patch size and core/periphery, with all the other models only receiving weak support ($\Delta AIC_c \geq 3.40$, Table 2b). Both covariates had equal support (0.97 and 0.84 for core/periphery and patch size, respectively) and the model that included both factors was significant (ANOVA, $\alpha < 0.001$). Figure 3a, b show that both observed and expected heterozygosity were indeed remarkably lower at the periphery of the distribution area than at the core for comparable patch sizes.

In terms of allelic richness, the model that only included a patch-size effect was the best, with the combined weights of all models with a patch-size effect at 79 % (Table 2b). This model was significant (ANOVA, $\alpha = 0.011$), whereas all other models had only weak support ($\Delta AIC_c \geq 3.04$). The combined support of models with a core/periphery effect (20 %) was about one quarter of the support of patch size. Figure 3c shows almost no difference in allelic richness between core/periphery for comparable patch sizes.

Genetic differentiation

For both countries the AMOVA revealed that most of the genetic variation was found at the individual level (Table 3). The population level only accounted for 10.2 % (Germany) and 3.6 % (Bulgaria) of the genetic variation. The estimated pairwise genetic distances of the Bulgarian populations ranged from 0.013 to 0.028 for G_{ST} (Table 4a), from 0.028 to 0.048 for F_{ST} (Table S1.6) and from 0.094 to 0.125 for D_{EST} (Table S1.7). For the German populations the range of the estimated pairwise distances among populations was 0.025–0.097 for G_{ST} (Table 4b), 0.049–0.178 for F_{ST} (Table S1.8) and 0.137–0.449 for D_{EST} (Table S1.9). There was no overlap of the D_{EST}-values for Bulgaria and Germany and only a marginal overlap for F_{ST} and G_{ST} (Fig. 4), with the values being significantly smaller for Bulgaria than for Germany (Tukey's (1959) quick test, unequal sample sizes: $T_{adjusted} > 13$; $\alpha < 0.001$ for all three parameters).

In Germany, the genetic distance was not correlated to the geographical distance for any of the three distance measures used (Table 5; Fig. S1.1). In Bulgaria, the correlation approached significance for only one genetic distance measure (G_{ST}) (Table 5; Fig. S1.1). For F_{ST} and G_{ST} the correlation was significantly different at the 5 % level between the two countries (non-overlap of the 85 %-confidence interval; two-sided test).

Population clustering

The analyses using *Geneland* resulted in five clusters in five out of ten replicated runs for Bulgaria and in six clusters in the remaining five runs. However, no individual was assigned to this sixth cluster in any of

Table 1 Diversity measures (mean ± one standard deviation) for different patch size classes in Bulgaria and Germany

Parameter	Bulgaria-large	Bulgaria-medium	Germany-medium	Germany-small
H_o	0.61 ± 0.01*	0.58 ± 0.01*,†	0.67 ± 0.03†	0.63 ± 0.05
H_e	0.63 ± 0.02	0.61 ± 0.01†	0.69 ± 0.04*,†	0.65 ± 0.02*
AR	6.48 ± 0.30*	5.73 ± 0.27*	6.25 ± 0.72*	5.55 ± 0.32*
AP	0.68 ± 0.26	0.35 ± 0.24	0.24 ± 0.11	0.23 ± 0.14

H_O observed heterozygosity, *H_E* expected heterozygosity, *AR* allelic richness, *AP* percentage of private alleles

* Significant at $\alpha < 0.05$ for within country comparisons (1-sided tests)

† Difference between the countries significant at $\alpha < 0.05$ for within patch-size class comparisons (2-sided tests)

Table 2 Model evaluation metrics for (a) observed heterozygosity, and (b) expected heterozygosity, and (c) allelic richness

Model	# Parameters	AIC_c	ΔAIC_c	AIC_c weights
(a) Observed heterozygosity				
Core/periphery	2	−53.92	0.00	0.38
Patch size + core/periphery	3	−53.82	0.10	0.36
Intercept only	1	−52.44	1.48	0.18
Patch size × core/periphery	4	−49.57	4.35	0.04
Patch size	2	−49.37	4.55	0.04
(b) Expected heterozygosity				
Patch size + core/periphery	3	−65.21	0.00	0.74
Core/periphery	2	−61.80	3.40	0.14
Patch size × core/periphery	4	−60.93	4.28	0.09
Intercept only	1	−58.49	6.71	0.03
Patch size	2	−55.42	9.78	0.01
(c) Allelic richness				
Patch size	2	28.19	0.00	0.66
Intercept only	1	31.23	3.04	0.14
Patch size + core/periphery	3	31.78	3.59	0.11
Core/periphery	2	32.60	4.40	0.07
Patch size × core/periphery	4	35.40	7.20	0.02

the five runs. Hence, this sixth cluster was an empty inferred cluster (i.e. a so-called ghost cluster). The problem of ghost clusters is well known from other studies (Fontaine et al. 2007; Guillot 2008). As ghost clusters do not represent existing populations, five clusters were assumed and the second run with a fixed K was carried out for $K = 5$. For the German data set, eleven clusters were inferred in all ten runs. Thus, for both locations the number of detected clusters equalled the number of sampled populations. The calculated posterior probabilities of genetic group membership clearly assigned all sampled individuals to their sampling site in both cities with values higher than 0.9 or equal to 1. This can also be seen in the maps of population membership, where each population was assigned to only one colour (Fig. 5).

Discussion

Theory predicts (Wright 1978; Frankham 2005; Mona et al. 2014) and many empirical studies show (Young and Clarke 2000; Hoehn et al. 2007; Delaney et al. 2010) that genetic diversity should decrease with decreasing population (remnant habitat) size. We hypothesized that according to Kühnelt's principle of regional stenoecy (Böhme and Rödder 2008) this effect should be stronger at the periphery of the distribution range of a species rather than at its core. We further hypothesized that dispersal should be lower at the margin of its distribution range compared to populations in the centre of a species' distribution range. Together, these processes should lead to a stronger genetic differentiation at the periphery compared to the core of the distribution range. As predicted, our results supported a patch size effect for all diversity parameters (except for the percentage of private alleles) (Table 1). There was also strong support for a core/range effect on observed and expected heterozygosity but for allelic richness only a patch size effect was observed (Table 1; Fig. 3). H_O and H_E in small and medium-sized patches in Germany were even as high as for large patches in Bulgaria. In both countries patches were (almost) completely isolated and genetic differentiation of the populations was stronger at the core of the distribution area in Germany (Fig. 4).

Theory also predicts that the rate at which rare alleles are lost is much faster than the rate at which more common alleles decline in frequency (Wright 1978); i.e., allelic richness should decline more rapidly with patch size than heterozygosity. Our results conform with this prediction (Fig. 3). Similarly, in fragmented populations of the arboreal gecko species

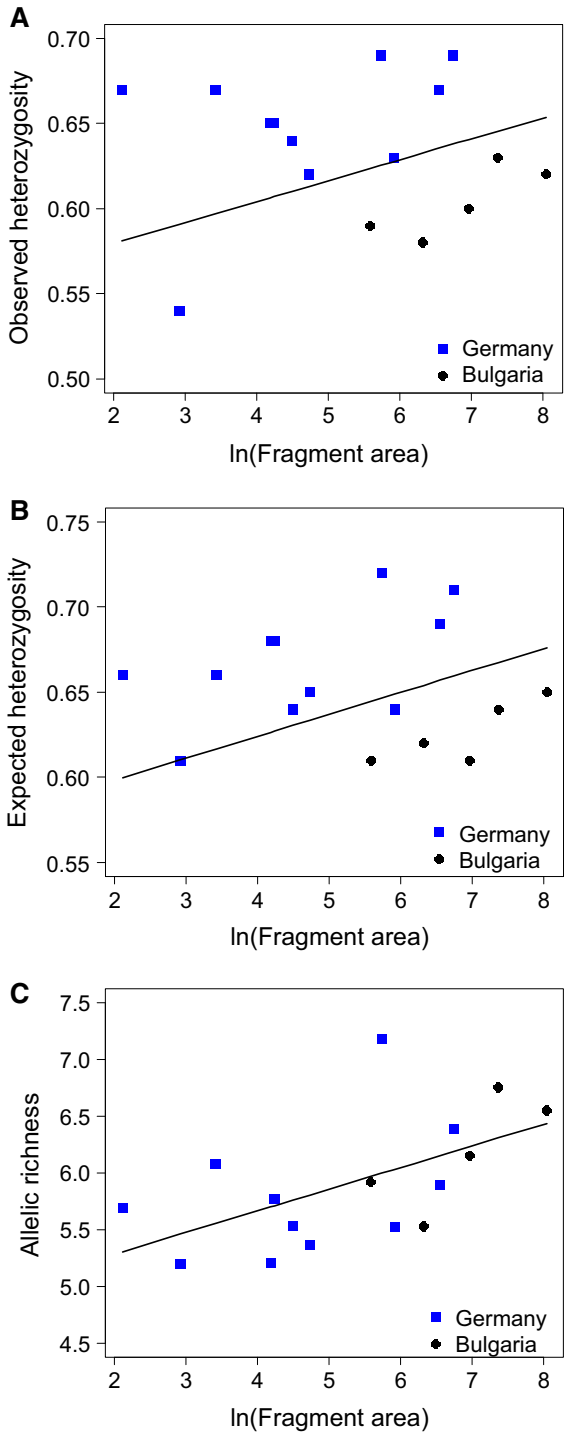

Fig. 3 Relationship between patch area and **a** observed heterozygosity (H_o), **b** expected heterozygosity (H_E), and **c** allelic richness at the core (Germany) and the periphery (Bulgaria) of the distribution range

Gehyra variegata and *Oedura reticulata* allelic richness was lost quicker than heterozygosity (Hoehn et al. 2007). Likewise, the decrease in both diversity measures was stronger in the latter species, which is a habitat specialist whereas the former is a habitat generalist. This higher sensitivity is expected, because the more specialized or stenoecious a species is the larger and better connected the habitat patches have to be to increase the probability of successfully dispersing among patches and thus creating a viable metapopulation (Henle et al. 2004; Öckinger et al. 2010). At the margin of the distribution range, species are frequently more stenoecious compared to in the core area of their distribution (Böhme and Rödder 2008). Thus, they should also be more sensitive to habitat loss and fragmentation at the margin of their distribution range rather than in its core. As expected, observed and expected heterozygosity—but not allelic richness—in our study were lower at the range periphery than in the core when accounting for different patch size (Table 2). The absence of a statistical core/periphery effect on allelic richness does not necessarily mean the absence of such an effect, as our study design is conservative meaning that a core/periphery effect will only be detected if this effect is stronger than the reduction in genetic variability due to the range expansion from the historic core in the Balkan to the current core in Germany. Our results are thus very similar to those that have already been observed when comparing less and more specialized species.

The matrix may also impart dispersal more for habitat specialists than for habitat generalists (Hoehn et al. 2007). Our results indicate that dispersal is very low in both study regions. The *Geneland* analysis did not reveal any migrants and genetically all individuals could be allocated with high certainty to their population of origin with no signs of first generation migration. Likewise, there was no isolation by distance (Table 5). The absence of a correlation between genetic and geographic distance could either be explained by an almost complete isolation of the patches and random genetic drift in each of them or by the presence of matrix structures that allowed dispersal irrespective of geographic distance. Since only the closest neighbouring patches are just within the maximum dispersal distance reported for the species

Table 3 Analysis of molecular variance for *Lacerta agilis* in Germany and Bulgaria

Source of variation	Percentage variation Bulgaria	Percentage variation Germany
Among populations	3.55	10.19
Among individuals within populations	4.08	2.79
Within individuals	92.37	87.02
Total	100.00	100.00

Table 4 Pairwise genetic (G_{ST}, bottom part) and geographic distances (in km; upper part) of all (a) Bulgarian and (b) German *Lacerta agilis* populations

	B1	B2	B3	B4	B5
(a) Bulgaria					
B1	–	31.2	31.5	36.8	43
B2	0.017	–	3.3	9.2	19.6
B3	0.028	0.013	–	6	16.8
B4	0.027	0.015	0.018	–	11.2
B5	0.022	0.016	0.018	0.026	–

	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11
(b) Germany											
D1	–	5.3	5.3	6.5	10.4	9.1	11.9	14.6	17.9	29.7	36.7
D2	0.084	–	6.2	3.2	5.3	6.1	10.6	13	15.2	26.9	32.9
D3	0.059	0.087	–	4	8.9	5.1	6.8	9.8	13.2	24.3	31.9
D4	0.054	0.088	0.042	–	4.9	3.1	7.5	9.6	12.1	23.9	30.5
D5	0.056	0.090	0.060	0.025	–	5.7	10.6	1.7	12	23.9	28.6
D6	0.055	0.098	0.068	0.061	0.074	–	5.0	6.7	9.1	20.9	27.2
D7	0.065	0.072	0.026	0.034	0.045	0.075	–	2.7	6.5	17.6	25.4
D8	0.047	0.097	0.045	0.039	0.046	0.052	0.037	–	4.2	14.9	22.8
D9	0.054	0.084	0.049	0.044	0.032	0.077	0.045	0.048	–	11.6	18.9
D10	0.068	0.085	0.067	0.053	0.059	0.052	0.068	0.055	0.046	–	9.6
D11	0.033	0.072	0.040	0.072	0.028	0.060	0.030	0.026	0.030	0.040	–

See Table S1.1 for identity of populations

(Grimm et al. 2014), the latter explanation would require a net of stepping stones and corridors between distant sites that excludes some of the more proximate sites. Therefore, random drift in highly isolated sites is the more likely explanation for the lack of a correlation between genetic and geographic distance.

Since our results showed that heterozygosity was lower and fragmentation sensitivity was higher at the margin of the distribution range compared to at the core, why then were the genetic distances among the populations lower in Bulgaria than in Germany? The average patch size was much larger in the Bulgarian than in the German study area. Larger patches translate into larger populations and genetic drift proceeds much slower in larger compared to smaller populations (Wright 1978). In addition, the major urban expansion took place earlier in Leipzig (at the beginning of the twentieth century) compared to Sofia (in the middle of the twentieth century) (Hirt 2008; Haase and Nuissl 2010; Tzankov et al. 2015), with the Simeonovo population only recently being affected and now almost exterminated by large shopping centres. Delaney et al. (2010) showed that genetic distance among populations increased with time since

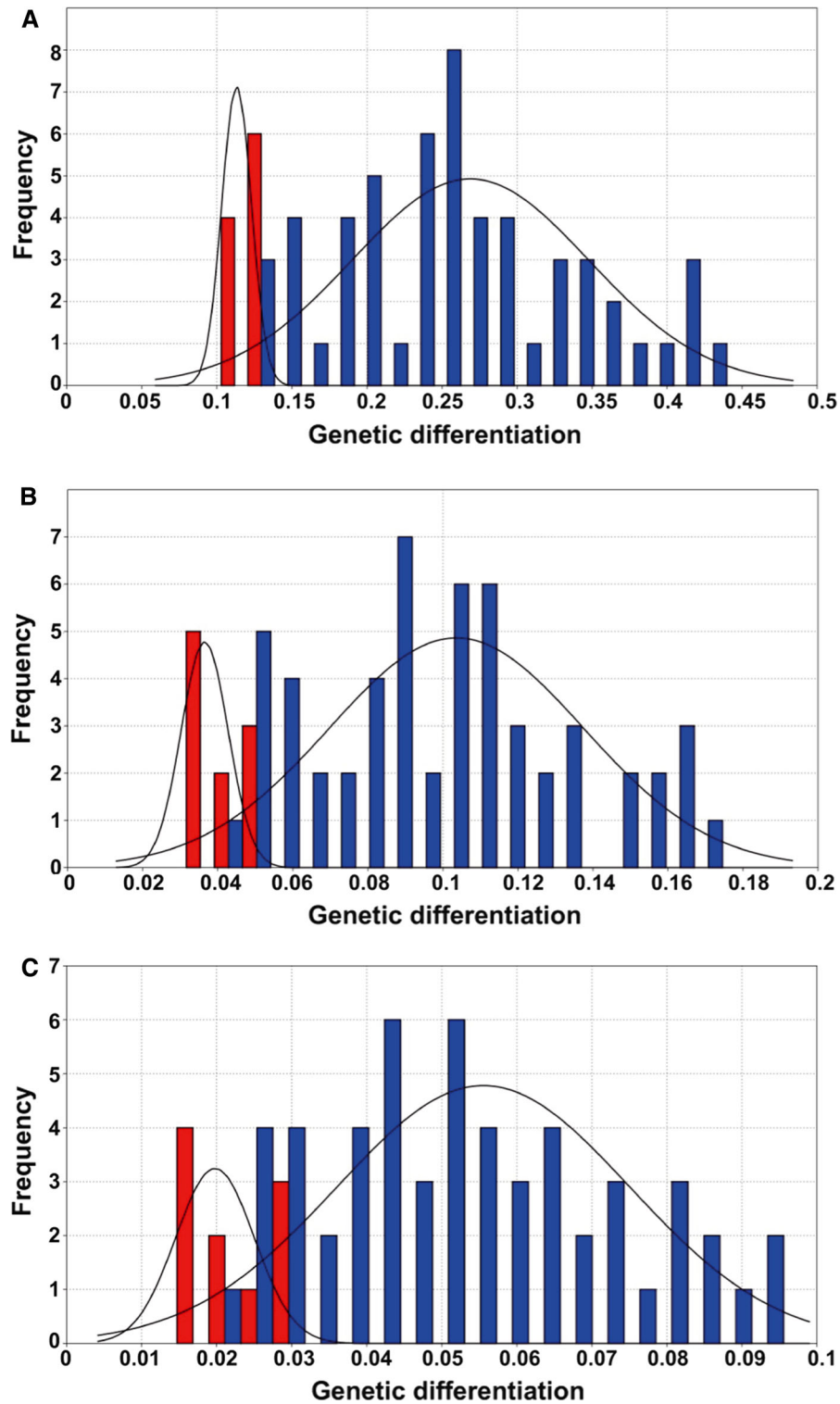

Fig. 4 Frequency distribution of pairwise genetic distances for *Lacerta agilis* populations in Germany (*blue*) and Bulgaria (*red*) for D_{EST} (**a**), F_{ST} (**b**) and G_{ST} (**c**)

Table 5 Correlation between genetic (D_{EST}, F_{ST} and G_{ST}) and geographic distance and their significance level (α)

Distance measure	Country	Spearman r	α one-sided	85 %-CI lower	85 %-CI upper
F_{ST}	Germany	−0.156	0.748	−0.269	−0.014*
G_{ST}	Germany	−0.102	0.642	−0.215	0.072†
D_{EST}	Germany	−0.092	0.621	−0.224	0.157
F_{ST}	Bulgaria	0.425	0.234	0.102*	0.776
G_{ST}	Bulgaria	0.632	0.055	0.326†	0.795
D_{EST}	Bulgaria	0.162	0.408	−0.327	0.677

For country comparisons: *,† Non-overlap of 85 %-confidence intervals (CI) of genetic distance measures, i.e., significantly different at $\alpha < 0.05$ (two-sided)

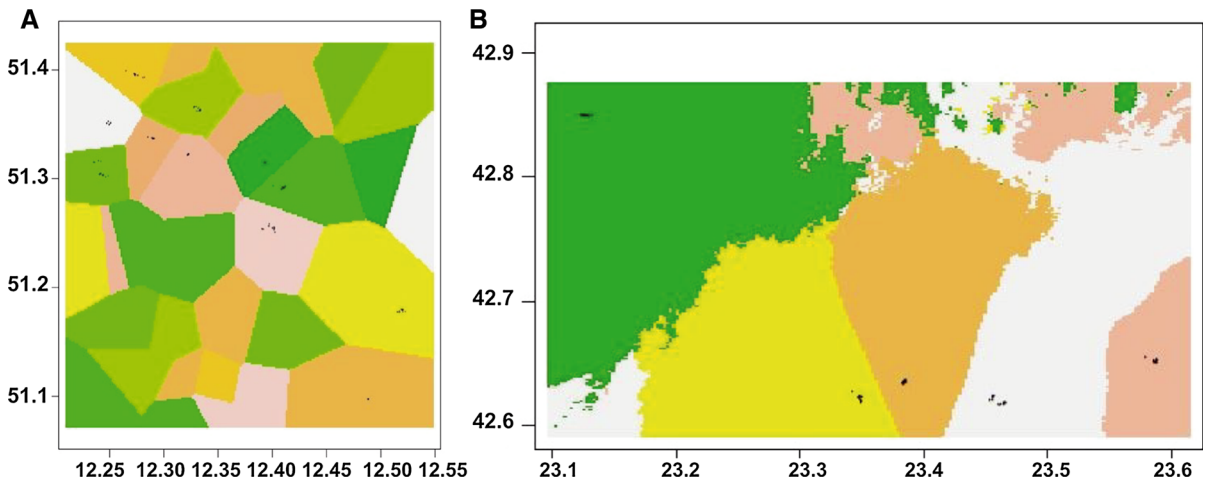

Fig. 5 Map of population membership for *Lacerta agilis* in Germany (**a**) and Bulgaria (**b**) depicting membership to one of the inferred clusters for each pixel. The number of colours is equivalent to the number of estimated clusters; landscape fractions assigned to the same population have the same colour; *black dots* represent the GPS position of the individuals sampled, the *x*-axis represents longitude, and the *y*-axis represents latitude

isolation in three lizard and one bird species in a peri-urban landscape in California. In conclusion, the larger patch sizes and the more recent fragmentation together can explain the lower genetic divergence of the Bulgarian populations despite their higher fragmentation sensitivity.

Conclusion

At the genetic level, our results indicated that in addition to patch size effects on genetic diversity there is a higher sensitivity to habitat fragmentation for populations of *L. agilis* located at the periphery of the distribution range compared to contemporary core populations. Patch sizes resulting from fragmentation may differ between the periphery and the core of a species' distribution, as in our study. Therefore, patch size effects can mask the influence of a higher specialization at the margin of the species' range compared to its core and may lead to results that seem to be inconsistent with a higher sensitivity at the margin of the species' range, unless patch size is accounted for. Likewise, if isolation is almost complete as indicated by our results for both study regions, there cannot be any effect of the location at the periphery or the core of the distribution area on dispersal and genetic differentiation among patches. Populations will then diverge by random drift, with the speed of divergence depending on the time since isolation and population size. Still, this may differ between the periphery and the core of the distribution

range because the relationship between patch size and population size may differ between the core and the edge of the distribution. Generally speaking, separating the effects of the different factors is easier when the study sites are as similar as possible in terms of remnant patch size as well as the degree of and time since fragmentation while still allowing limited dispersal among patches but this is challenging in real landscapes. As species with a wide distribution are often more specialized at the range margin compared to the core, one should expect that their sensitivity to fragmentation will increase at the edge of their distribution and consequently require special conservation attention. Independent of core/periphery related sensitivity, if isolation among patches is almost complete, as in our study, urban planning should strive for providing dispersal corridors for threatened species.

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