Received Date : 04-Feb-2016

Accepted Date : 19-Jul-2016

Article type : Original Article

CITYSCAPE GENETICS: STRUCTURAL VERSUS FUNCTIONAL CONNECTIVITY OF AN URBAN LIZARD POPULATION

Joscha Beninde[1], Stephan Feldmeier[1], Maike Werner[2], Daniel Peroverde[1], Ulrich Schulte[3], Axel Hochkirch[1] & Michael Veith[1]

1. Department of Biogeography, Trier University, Universitätsring 15, 54296 Trier, Germany
2. Zoological Institute & Museum, Ernst-Moritz-Arndt-Universität Greifswald, Germany
3. Federal Agency for Nature Conservation (BfN), Konstantinstr. 110, 53179 Bonn, Germany

Corresponding Author: Joscha Beninde, beninde@uni-trier.de, Tel.: +49-651-2014911

Running title: Cityscape genetics of lizards

ABSTRACT

Functional connectivity is essential for the long-term persistence of populations. However, many studies assess connectivity with a focus on structural connectivity only. Cityscapes, namely urban landscapes, are particularly dynamic and include numerous potential anthropogenic barriers to animal movements, such as roads, traffic or buildings. In order to assess and compare structural connectivity of habitats and functional connectivity of gene flow of an urban lizard, we here combined species distribution models (SDMs) with an individual-based landscape genetic optimization procedure. The most important environmental factors of the SDMs are structural diversity and substrate type, with high and medium levels of structural diversity as well as open and rocky/gravel substrates contributing most to structural connectivity. By contrast, water cover was the best model of all environmental factors following landscape genetic optimization. The river is thus a major barrier to gene flow, while of the typical anthropogenic factors only buildings showed an effect. Nonetheless, using SDMs as a

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/mec.13810

basis for landscape genetic optimization provided the highest ranked model for functional connectivity. Optimizing SDMs in this way can provide a sound basis for models of gene flow of the cityscape, and elsewhere, while presence-only- and presence-absence-modelling approaches showed differences in performance. Additionally, interpretation of results based on SDM factor importance can be misleading, dictating more thorough analyses following optimization of SDMs. Such approaches can be adopted for management strategies, e.g. aiming to connect native common wall lizard populations or disconnect them from non-native introduced populations, which are currently spreading in many cities in Central Europe.

KEYWORDS

biodiversity, conservation, corridor, dispersal, ecology, isolation, management, movement, reptiles, urbanization

INTRODUCTION

Urbanization is a striking phenomenon of the Anthropocene. It entails a substantial, continuous, highly dynamic and usually irreversible land transformation from a previously non-urban environment into a cityscape. However, recent analyses have shown that many native species are able to persist in cities worldwide (Aronson *et al.* 2014; Ives *et al.* 2016). Therefore, and in the light of predictions of soaring global urbanization (Seto *et al.* 2011), urban biodiversity will play an increasingly important role for maintaining ecosystem services, especially cultural ones, generated by human-wildlife interactions.

Although urban areas are still underrepresented in ecological research (Martin *et al.* 2012), the awareness and application of ecological and evolutionary theory to the cityscape is growing rapidly (McDonnell & Hahs 2015). The size of habitats as well as connecting corridors within a cityscape have been identified to best explain intra-urban variation in species richness (Beninde *et al.* 2015). However, ensuring long-term persistence of single species in cities may be particularly difficult (Rézouki *et al.* 2014; Sumasgutner et al. 2014; Björklund *et al.* 2010). Usually, habitat patches in cities are small and isolated, habitat alteration dynamics is high, and disturbance pervasive. Nonetheless, in a review of behavioral responses to urbanization, Sol *et al.* (2013) have shown that many species are able to adjust to the challenges of the cityscape, either through behavioral plasticity (Meillère *et al.* 2015) or through evolutionary adaptation (Mueller *et al.* 2013). Such species can thrive in urban areas and become urban residents (McDonnell & Hahs 2015).

At the same time, it has been shown repeatedly that urban areas represent barriers to gene flow for non-urban species, even highly mobile species, such as pine martens, *Martes martes* (Ruiz-González *et al.* 2014) and mountain lions, *Puma concolor* (Riley *et al.* 2006). It remains open if and to what degree urban residents may also be affected by intra-urban barriers. Barriers could lead to arrays of disjunctive populations within the cityscape. Unfortunately, our knowledge on connectivity of urban areas is scarce and studies within cityscapes are rare (LaPoint *et al.* 2015). Per definition, urban residents find sufficient suitable habitat in cities. However, like non-urban landscapes, a cityscape is a heterogeneous environment with a mosaic of suitable and non-suitable habitats. Dispersal barriers such as traffic arteries, highly disturbed habitats, or vast spaces devoid of vegetation may hamper gene flow among sub-populations. This could lead to genetic drift in isolated sub-populations and reduce the chance of recolonization after local extinction. When exploring connectivity in the landscape, it is important to distinguish 'structural connectivity' from 'functional connectivity' (LaPoint *et al.* 2015). Structural connectivity refers to physical components of the landscape and its habitats. It is often assessed based on habitat suitability maps, used to approximate how suitable the habitat is that connects locations. Functional connectivity, on the other hand, is a measure that has to be viewed from the perspective of the organism under investigation and describes actual gene flow between localities. One approach to quantify functional connectivity is by using landscape genetics, which aims to explain genetic variation in space with landscape features. Thus far, studies employing genetic analyses in cityscapes mainly assessed the long-term viability of populations, with a focus on population-based sampling to assess potential source-sink metapopulation dynamics, while landscape genetic studies are largely lacking. An exception is a study on the white-footed mouse, *Peromyscus leucopus*, by Munshi-South (2012), which identified urban canopy cover as important for gene flow among populations. However, and like most other population genetic research in urban areas (LaPoint *et al.* 2015), a population-based sampling scheme was followed here. Along these lines of research Björklund *et al.* (2010) showed that some populations of great tits, *Parus major*, in green spaces within a city function as 'sink' populations, while others function as 'source' populations. In four butterfly species and a skink dispersal was found not to be impeded significantly across urban areas , with barely detectable population structuring in these species (Angold *et al.* 2006; Brashear *et al.* 2015). Importantly, while all urban genetic studies described the long-term effects of the cityscape on the population genetic structure of species, they did not compare structural and functional connectivity or employ an individual-based sampling scheme, which is important for this purpose (LaPoint *et al.* 2015).

Individual-based sampling schemes makes assignment of individuals to populations obsolete, which is especially useful in continuously distributed species where *a priori* population assignment would be difficult or even impossible (Shirk *et al.* 2010; Landguth & Schwartz 2014). Compared to population-based sampling it has the advantage of allowing detection of population structure where this was not previously known or anticipated (Schwartz & McKelvey 2009). This sampling-scheme was also shown to be especially suitable for landscape genetic questions (Cushman & Landguth 2010; Ruiz-González *et al.* 2014), since it is more representative of the spatial context and allows the identification of population borders at a finer spatial scale.

Adopting an individual-based sampling scheme we here focus on the genetic population structure of the common wall lizard, *Podarcis muralis*, a species typical of anthropogenic habitats (Schulte 2008). The species is native throughout the city of Trier, southwestern Germany, although clumped in distribution along suitable habitat patches, such as railway tracks, urban vineyards as well as ancient Roman sites and other suitable dry stone walls. We here aim to identify those environmental factors of a cityscape that determine structural as well as functional connectivity. We first mapped the distribution of the species throughout the entire city area and composed landscape models of environmental factors. Subsequently, we use these landscape models to develop species distribution models (SDMs) and derive habitat suitability maps of the cityscape. Based on the assumption that areas connected by more suitable habitats provide better connectivity, these habitat suitability maps were used to calculate structural connectivity, as in the pairwise-resistance between individuals. To calculate pairwise genetic distance, we sampled 223 individuals across the city and barcoded and genotyped them at 17 microsatellite loci. Pairwise genetic distances of individuals were used to develop landscape genetic models of gene flow, depicting functional connectivity.

It has been shown previously that populations of the common wall lizard can be strongly structured at small spatial scales (< 2km), despite a continuous distribution along favorable habitat, such as railway tracks (Schulte *et al.* 2013). This raises concerns as to the functional connectivity of populations of this species within the cityscape. We therefore predict this population of common wall lizards to be structured and assume that gene flow can be severely reduced by roads, especially those with high traffic volume, which could lead to a putatively high number of small isolated populations within the cityscape. At the same time we hypothesize that southern aspect and dry stone walls, often found in structurally diverse vineyards, and a rocky/gravel substrate type, associated with railway tracks, will facilitate gene flow, reducing population structure between localities.

MATERIAL & METHODS

Species account

The common wall lizard is a small lacertid lizard with a total length of up to 20 cm and a weight of 4-10 g. The species is distributed from Spain to Turkey and southern Italy to southwestern Germany. It is well adapted to stone walls, and its post-glacial colonization often closely tracked human advances within Europe, such as the vineyards established by Romans on their way to the North into Germany. Here, at its northern range margin the species is mainly found in vineyards, along railway tracks, in quarries as well as at stonewalls. However, it also inhabits urban areas containing these or similar structures. Consequently, the common wall lizard is considered an urban resident, thriving also in frequently disturbed sites, such as along roads and railway tracks with a high traffic volume or near dense human visitor traffic. The species has become invasive in northwestern Europe and North America, with currently more than 100 populations of non-native origin known to exist in Germany alone (Schulte *et al.* 2008; Schulte & Deichsel 2015).

Field methods

To best assess Trier's common wall lizard population proportionally to its abundance we conducted field surveys prior to sampling individuals. Field surveys were conducted from March to July 2012 and covered the entire sampling area (Fig. 1) with a total of 24.45 km^2 of Trier's city center and its contiguous residential areas. Based on these observations we established a fine-scale distribution map and noted the spatial extent of presences of lizards. Afterwards, patch specific lizard numbers were estimated in a standardized procedure following the protocol of the German Federal Agency for Nature Conservation, issued for assessments of conservation status of the species protected under the Habitats Directive (BfN 2010). It entails a fixed walking speed for surveys to be conducted early or late during the day, omitting the hot hours of midday. The numbers of individuals counted applying this procedure were compared to describe relative abundance of lizards at surveyed patches. Where areas were inaccessible, potential distribution and abundance were estimated from habitat availability and the presence and abundance in its surroundings. We used this inferred abundance map for the common wall lizard in Trier to select 200 random points for sampling single lizards throughout the city (using the 'create random points' function in ArcGIS) weighted by their abundance. In practice, this implemented a stratified random sampling of individuals throughout the cityscape based on the species' distribution and abundance, which constituted the basis for our individual-based sampling scheme. We sampled 133 individuals from July to September 2012 and another 90 individuals in April and May 2013 (223 individuals in total; 9.1 individuals per km^2 of sampling area). Sampling individuals only once within a sampling period was ensured by marking them with a color code which lasted for ca. 2-3 weeks. Between periods we prevented to sample individuals twice by sampling different areas and by checking photographs taken from all specimens for individual identification. Furthermore, we checked all genotypes for duplicates. We adjusted the number of sampled individuals from 200 to 223 to ensure representative sampling, as we found larger abundances than previously estimated while sampling at two sites.

Molecular genetic analyses

We obtained DNA by buccal swabbing individuals using sterile dry swabs (Copan Diagnostics Inc, 'Sterile R') as described in Schulte *et al.* (2011). Within 12 hours samples were stored at -20°C until DNA extraction, which was done according to the manufacturer's protocol of the Qiagen DNEasy blood and tissue kit (as recommended in the supplementary protocol for buccal swabs ATL buffer was replaced by 400 µl PBS buffer).

To rule out that non-native lineages of the common wall lizard occur in the sampling region, we sequenced a 450 bp fraction of the mitochondrial cytochrome *b* gene (Salvi *et al.* 2013; Schulte *et al.* 2012b; While *et al.* 2015) for all but two individuals (221 out of 223). This was pivotal since secondary contact of lineages with divergent evolutionary origin can profoundly influence population differentiation and inferences thereof. For this species, this was especially important, as non-native common wall lizards have established at over 100 localities in Germany, both accidentally through transport and cargo as well as deliberately by hobbyists (Schulte & Deichsel 2015).

We used 50 µl PCR reactions, containing 0.0625 pmol/µL of the primers LGlulk (5'-AACCGCCTGTTGTCTTCAACTA-3') and HPod (3'-GGTGGAATGGGATTTTGTCTG-5'), 20 µl 5 Prime Mastermix and 25 µl purified water (Schulte *et al*. 2012). PCR settings were 15 min at 95°C, 35 cycles of 30s at 94°C, 30 s at 43°C, 90 s at 72°C and 10 min at 72°C. Sequences were aligned with sequences of known geographic origin and of all lineages known to have established in Germany: Western France AY234155 (Busack *et al.* 2005); Calabria DQ001023, Tuscany DQ001028, Eastern France (native lineage) DQ001029, Venetia DQ001032 (Podnar *et al.* 2007); Central Balkans HQ652887, Romagna HQ652921, Southern Alps HQ652963 (Schulte *et al.* 2012b). A phylogenetic tree was fitted using *P. siculus* and *P. melisellensis* as outgroups

(HQ154646, AY185097, Podnar 2004). We used MEGA6 (Tamura *et al.* 2013) to assign lineages employing the neighbour-joining method with 2,000 bootstrap replicates.

All 223 individuals were genotyped at 17 microsatellite loci, twelve of which have been developed for *Podarcis muralis* (B3, B4, C9: Nembrini & Oppliger 2003; PmurC150, PmurC168, PmurC275-278, PmurC164, PmurC038, PmurC028, PmurC356, PmurC109, PmurC103; Heathcote *et al.* 2014), two for *Zootoca vivipara* (Lv-319 and Lv-472; Boudjemadi *et al.* 1999) and three for *Podarcis bocagei* (Pb10, Pb50, Pb73; Pinho *et al.* 2004). Primers were labelled with FAM, TAMRA or HEX. Multiplex PCR protocols were used with the following annealing temperatures: 57°C for C9, B4, Pb73 and all PmurC-primers; 56°C for B3, Pb10 and Lv319; 53°C for Lv472 and Pb50. Using the HotMasterMix by 5PRIME or Multiplex Mastermix by Qiagen and Multigene Gradient Thermal Cyclers (Labnet) amplifications were conducted as recommended by manufacturers. Multiplex PCRs were performed in 10 µl reaction mix containing: 2-10 ng genomic DNA, 5.0 µl MasterMix, 2.0 µl water and 0.1 µM of each primer. Fragment lengths of PCR products were determined on a MEGABACE 1000 using the software Fragment Profiler 1.2 (Amersham Biosciences).

To test for the occurrence of null alleles we used Micro-Checker (v2.2.3; van Oosterhout *et al.* 2004). FSTAT (v2.9.3.2) was used to test for linkage disequilibria among loci (Goudet 2001). Calculations of population genetic parameters were conducted with GenAlEx v6.5 (Peakall & Smouse 2012). Population structure was inferred using GENELAND (Guillot *et al.* 2005). We ran GENELAND in R 3.0.2 (R Core Team 2013). We calculated Nei's genetic distance (Nei *et al.* 1983) between individuals using Alleles in Space to proxy functional connectivity (Miller 2005). These distances were the basis for a landscape genetic optimization approach in CIRCUITSCAPE (v4.0.5.; McRae 2006; Shah & McRae 2008) in combination with the R-package ResistanceGA (Peterman 2014).

We used GENELAND to assess the spatial borders of sub-populations, based on microsatellite multilocus genotypes and their spatial distribution. We ran 800,000 Markov chain Monte Carlo simulations, with a burn-in of 250,000, for K = 1-10. Furthermore, we used the correlated allele frequency model and the admixture model in STRUCTURE and ran Markov chain Monte Carlo simulations with a burn-in of 100,000 and 1,000,000 simulations thereafter. We ran simulations for K = 1–10 with 10 iterations per K. We used STRUCTURE harvester (Earl & von Holdt 2012) to determine the second-order rate of change (ΔK) as suggested by Evanno *et al*. (2005).

Following a hierarchical approach, we continued exploration within clusters at the highest Δ*K* using the same settings. Results of STRUCTURE were combined in CLUMPP (v1.1.2; Jakobsson & Rosenberg 2007). Using ArcGIS (v10.2.1 ©Esri Inc.), we plotted the results for spatial representation.

Landscape modelling

We digitized the sampling area of the cityscape using the world imagery embedded in ArcGIS (basemap; taken on August 11, 2012; ArcGIS v10.2.1 © Esri Inc.) at a scale of 1:2,000 for twelve environmental factors. For further analyses, we converted the digitized layers of environmental factors into a grid layer using the majority rule in ArcGIS, containing 31,797 grid cells, without "no data" cells. The grid size was set to 25 × 25 meters, with each grid cell covering 625 m^2. This results in a reasonable computation time; it also well represents the area at which wall lizards can be expected to assess habitat quality according to available information on home-range sizes of *Podarcis muralis* of up to 50 m^2, which regularly change between years (Schulte 2008). As recommended by LaPoint *et al.* (2015), we did not limit our environmental factors to those contained in typically available datasets; rather we created 9 of the 12 layers of environmental factors specifically for this study, to encompass all habitat requirements essential for *Podarcis muralis*: (i) aspect grasps temperature differences depending on cardinal point of slopes (8 levels: northern/north-western/north-eastern/western/eastern/south-western/south-eastern/southern); (ii) slope also affects temperature differences independently from aspect, with steeper slopes capturing more solar irradiance then flat areas (continuous); (iii) substrate type determines the absorption and storage capacities of thermal radiation and moisture (4 levels: sealed surfaces/open ground/rocky + gravel/none of these); (iv) trees have negative effects due to shadowing (2 levels: canopy cover/no canopy cover); (v) vegetation height determines the degree of protection from predators as well as the degree of habitat provision for arthropods, i.e. proxy of food source (2 levels: herb/shrub); (vi) vegetation type determines the abundance of arthropods, with less intensively managed and more natural vegetation showing higher abundances (4 levels: planted vegetation/cultivated vegetation/semi-natural vegetation/no vegetation); (vii) structural diversity captures the capacity for escape behavior by the number of hiding places, such as crevices, joints or hollow spaces (4 levels: low/medium/high/none); (viii) south-facing walls represent a preferred habitat type (2 levels: wall yes/no); (ix) buildings have negative effects due to shadowing (2 levels: building yes/no); (x) roads can be barriers to movement (2 levels: road yes/no), potentially mediated by (xi) the volume of traffic (continuous factor scaled to maximum traffic volume) ; (xii) water surfaces represent unsuitable habitat (2 levels: water yes/no). Further coding details are given in the supplements, section 1.

This dataset was analyzed in two different ways: (1) species distribution models (SDMs) were applied as tools to identify the most important environmental factors predicting the presence of lizards and to generate habitat suitability maps from which we inferred structural connectivity; (2) a landscape genetic approach was used with pairwise genetic distances between individuals to analyze the importance of environmental factors as barriers ("resistance") within the landscape models and assess functional connectivity. Artificial boundaries, caused by the extent of the grid, can affect the inference of resistances between individuals if they are too close to this boundary. Potential movements of such individuals can be artificially constrained by the proximity to the grids boundary (Koen *et al.* 2010). For the landscape genetic analysis we therefore expanded, when necessary, the extent of the environmental factor grids by buffers around sample locations of individuals. We chose a buffer distance of 1 km, equaling the maximum distance a wall lizard has been recorded to disperse and strongly exceeding average dispersal distances of <200 m (Schulte 2008). Buffered areas partly extended beyond areas with known data for environmental factors and we filled these areas with random data values, in proportion to values of the study area, i.e. with known data, following Koen *et al.* (2010). Koen *et al.* (2010) showed that this does not lead to overestimates of resistances when compared with true data but alleviates the effects of artificial boundaries. This increased the number of grid cells to 50,896.

(1) Species Distribution Modeling

We used two SDM methods, a presence-only (PO) method (Maxent) and a presence-absence (PA) method (a generalized linear model, GLM), to build SDMs for *Podarcis muralis*. Maxent is a machine learning method following a maximum entropy approach (Phillips *et al.* 2004) implemented in the Maxent software (v3.3.3k; Phillips *et al.* 2006; Phillips & Dudík 2008) Maxent is widely applied for PO data in species distribution modeling and also used to explore and interpret the environmental drivers shaping a species' distribution (Merow *et al.* 2013). Maxent uses presence locations, background points and a set of predictor variables to estimate the probability of presence (logistic output) for each grid cell of the landscape. Circumventing the criticism of interpreting the logistic output in this way (Royle *et al.* 2012; Yackulic *et al.* 2013), it is commonly viewed as a habitat suitability (Elith *et al.* 2011). The background points are taken from the landscape and are used to contrast the conditions at presence sites.

As the intensive field surveys for presence of lizards also provides information on species absence, we additionally built a generalized linear model for PA data (GLM, see McCullagh & Nelder 1989), which is also frequently applied in species distribution modeling (Franklin 2010). Instead of background points, absences were used here to estimate the probability of presence or habitat suitability. Pseudo-absences were randomly created in cells without presences

(Barbet-Massin *et al.* 2012), using the 'randomPoints' function of the R-package dismo (Hijmans *et al.* 2016) in R 3.3.0 (R Core Team 2016).

To avoid data collinearity and model overfitting (Burnham & Anderson 2002; Dormann *et al*. 2013) we applied the following procedure to reduce the number of predictors and determine the optimal model complexity: In a first step we checked the pairwise correlations between all twelve environmental factors using SDMtoolbox (Brown 2014) and removed factors with a Pearson correlation coefficient larger than 0.7 (Dormann *et al*. 2013).

PO-Model: We used all 223 presence points of the sampled individuals and the remaining environmental predictors to run Maxent (settings see supplementary materials, section 2). In a stepwise procedure we eliminated the predictor contributing least to the model, using Maxent's own analysis of variable contribution and re-ran Maxent with the reduced predictor set. AICc values were calculated for all models using NicheAnalyst (v3.0; Qiao *et al.* 2015) to determine the best model based on the minimum ΔAICc values (Burnham & Anderson 2002; Warren & Seifert 2011). Because there was more than one equally good model we chose the one with the smallest number of environmental factors as the final predictor set. Although, to our knowledge, the use of almost only categorical predictors is uncommon in this application, Elith & Graham (2009) state that categories are modeled well with Maxent.

PA-Model: To find the best predictor set for the GLM, we chose a similar approach. We also used all 223 presences and started with the full uncorrelated predictor set to build a GLM (settings see supplementary materials, section 2). AICc values were calculated again, this time with the R-package AICcmodavg (Mazerolle 2016). We then explored different predictor combinations, dropping predictors identified as not significant by the GLM, and as above identified the best model via minimum AICc. For the model selection process we used 10,000 pseudo-absences, as for this number no replicates are needed to enhance model quality (Barbet-Massin *et al.* 2012). To identify the most important predictor variables, in turn, we dropped each variable from the full model and calculated the difference in residual deviance between the full and the reduced model. The variable which leads to the largest change in deviance is considered to be the most important one (Leathwick *et al.* 2006; Elith *et al.* 2010).

Final model fit of PO-model: In a last step we ran the final PO-model with 10-fold cross-validation (CV), so AUC (area under the curve of receiver-operator characteristic) values could be calculated on independent test data as a measure of model fit. Although AUC scores for PO data as a measure of performance can be misleading (Lobo *et al.* 2008), Merow *et al.* (2013)

note that AUC is appropriate for high sampling intensities, which is the case in our study. As an additional performance measure we show the omission of test localities (or extrinsic omission error, Anderson *et al.* 2003) with respect to the maximum sum of test sensitivity plus specificity (maxSSS) threshold, which is proposed for PO data (Liu *et al*. 2013, 2016). It is calculated by Maxent and describes the proportion of test localities which fall in areas predicted as unsuitable after thresholding the continuous model output into a binary presence absence map.

Final model fit of PA-model: We also ran the final PA-model with a 10-fold CV. Ten replicates were produced, calculating 1,000 new random pseudo-absences for each replicate (Barbet-Massin *et al.* 2012). The fitted models were predicted to the entire city area to generate suitability maps (using the 'predict.glm' function, stats package). Test AUC and the extrinsic omission error were calculated using the dismo package (using the 'evaluate' and 'threshold' functions).

Structural connectivity was calculated from final mean suitability maps, calculated over all CV folds and replicates generated by both PO- and PA-modelling approaches. Although these are the final mean suitability maps as identified by both methods, we will refer to them as the PO-raw model and the PA-raw model from now onwards for clarity, and to distinguish them from optimized models we created later on, based on these raw models. For evaluation of structural connectivity, we followed a similar approach as for the landscape genetic analysis but excluding the optimization step. We used CIRCUITSCAPE directly to calculate pairwise resistances between individuals based on PO- and PA-raw models and fitted a linear mixed effect model that evaluated their fit to the genetic distances of individuals (these methods are explained in detail below and we only skipped the optimization procedure implemented in ResistanceGA at this stage). To enable ranking and comparison of these PO- and PA-raw models of structural connectivity with landscape genetic models specified below, we calculated AICc values in the same way as for landscape genetic analyses.

(2) Landscape Genetic Analysis

Functional connectivity was assessed using pairwise genetic distances of individuals and the R-package ResistanceGA (Peterman 2014). We refer to this later in the manuscript as an optimization procedure, as ResistanceGA is a novel approach that transforms resistance surfaces to optimally fit genetic data (Richardson *et al.* 2016), circumventing typical issues of subjectivity in assigning resistance values. It also makes a wider parameter space disposable for the process of optimization and, additionally, ResistanceGA accounts for spatial autocorrelation (Peterman *et al.* 2014; Richardson *et al.* 2016). Once pairwise genetic distances and coordinates of sample sites of individuals have been specified, it calls CIRCUITSCAPE (Shah & McRae 2008) to calculate pairwise resistance distances between individuals and employs a genetic algorithm to maximize fit of resistance surfaces to the specified dataset, based on AICc values of linear mixed effect models. Due to small inter-sample distances, we had to thin our samples to 198 individuals for calculations in CIRCUITSCAPE, which allows a maximum of one sample location per grid cell. As recommended by Peterman *et al.* (2014) ResistanceGA was run twice for each environmental factor. The runs were checked for convergence, and AICc values were compared between runs for each landscape model. There were only marginal differences in AICc values between runs and no change in the ranks of the best performing factors, while ranks changed slightly among lower ranked factors (for differences between runs among lower ranked factors see supplementary spreadsheet). This enabled final ranking of landscape models by ΔAICc values.

In addition to environmental factors, we also used the PO- and PA-raw models as a basis for the standard optimization procedure in ResistanceGA, resulting in PO- and PA-optim models, optimized to fit pairwise genetic distances.

Comparing structural and functional connectivity is difficult, especially when using suitability, or conductance values for the former and resistance values for the latter. Nevertheless, CIRCUITSCAPE can perform analyses using both surfaces, allowing to indicate computations to be based on conductance or resistance layers.

To infer which factors contribute most to PO- and PA-raw and PO- and PA-optim models, we correlated environmental factors with these models and also extracted conductance (suitability scores) of PO- and PA-raw models, as well as resistances of PO- and PA-optim models per subcategory of environmental factors (only possible for categorical factors). This was necessary foremost for the optim models, as these went through two independent optimization processes, making interpretation of the contributing environmental factors difficult.

As the best model of gene flow was supported with ΔAICc ≥ 4, we did not start additional runs containing multiple factors simultaneously. Additionally, we incorporated measures of goodness-of-fit of final models using the R^2_{GLMM} function by Nakagawa & Schielzeth (2013), incorporated into the MuMIn-package (Barton 2016).

RESULTS

The cytochrome *b* sequences of all specimens belonged to the Eastern-France lineage, which is native in this region. Thus, a confounding effect of individuals of non-native origin on our results is unlikely.

Due to a high probability of a linkage disequilibrium, we excluded locus Pb73, and analyses were therefore based on 16 microsatellite loci. For five of these loci Micro-Checker detected the possibility of null alleles (PmurC275-278, PmurC164, C9, Lv319 and Lv472). As Oosterhout values were below 0.2 in all cases, we did not exclude any further loci.

The results of GENELAND showed a strong separation of genetic clusters with a steep border along the river Moselle (Fig. 3). Population membership was assigned with probabilities ≥ 99.5 % for all but three individuals of the eastern population (91.9 %, 96.3 % and 64.8 %) which were sampled closest to the river. STRUCTURE results showed the max. ΔK=2 (eastern and western Trier – similar to Geneland), while the likelihood was highest at K=3, suggesting some sub-structure in the eastern part of the city.

The final predictor set used for the PO-raw model consisted of the following environmental factors (listed in order of percent variable contribution; see Tab. 1): substrate type, structural diversity, buildings, vegetation type, trees, water, slope and aspect, while the remaining four factors were not part of the model. Substrate type and structural diversity were the two variables contributing most to the model (Tab. 1). In the PA-raw model, substrate type, structural diversity, roads, buildings, trees, vegetation type, slope, water and traffic were used (listed in order of descending differences in deviance; see Tab. 1). Here, substrate type and structural diversity were also clearly the most important variables. Within the substrate category 'rocky/gravel' was the most suitable subcategory, while 'sealed surfaces' was the least suitable. For structural diversity 'no structural diversity' was the least suitable subcategory, while 'medium structural diversity' was the most suitable. These results were the same for both modelling methods (see supplementary materials, section 2). The mean PO- and PA-raw model habitat suitability maps are shown in Fig. 2. The average AUC of the PO-raw model was 0.852 (± 0.041 SD) and 0.862 (± 0.007 SD) for the PA-raw model. Average test omission with respect to the maxSSS threshold was 0.144 (± 0.085 SD) for the PO-raw model, whereas it was 0.598 (± 0.021 SD) for the PA-raw model, which would assign almost 60% of test presences to unsuitable areas (if the suitability output was converted to a binary map). Structural connectivity calculated directly with CIRCUITSCAPE better explained functional connectivity when the PO-raw model was used, than with the PA-raw model (ΔAICc > 5; Tab. 2).

Among environmental factors, water cover is the highest ranked model following landscape genetic optimization (compared to the respective second best model (slope) with ΔAICc > 20 in both runs; Tab. 2). In a comparison of models of environmental factors with the models of structural connectivity, models of water cover and slope ranked higher than both PO- and PA-based calculations of structural connectivity. The model of the environmental factor structural diversity ranked between both of these calculations of structural connectivity.

Using the PO-raw model surface as a basis for optimization in ResistanceGA delivered the best model for functional connectivity (PO-optim model), performing better also than the model of water cover alone, while the PA-optim model ranked lower than water cover but above all other models. Both optim models were transformed with the inverse monomolecular equation (PO-optim model: shape = 0.373, max = 485.8; PA-optim model: shape = 0.069, max = 248.2). Marginal and conditional R^2-values were similar between all models (0.02-0.1 and 0.25-0.33, respectively).

Correlations of all environmental factors with the best performing model, the PO-optim model, showed water to correlate most strongly, followed canopy cover and buildings. An evaluation of the median resistances of PO- and PA-optim models per subcategories within environmental factors showed that area covered by water has the highest resistance, followed by buildings and canopy cover. The median value of all other subcategories approached 1 (lowest resistance; histograms of resistance values per subcategory are supplied in the supplementary materials).

DISCUSSION

Natural *versus* anthropogenic factors

Our landscape genetic analysis shows that the effect of a natural barrier, the river Moselle, dominates the genetic structuring of this urban population of common wall lizards (both as a single factor landscape model as well as by its high weighting in the PO-optim model, the highest ranking model overall), although both riversides are connected by three large stone bridges and one iron bridge. This indicates an isolation-by-barrier scenario. Interestingly, among all prevailing anthropogenic factors in the cityscape, e.g. roads, traffic volume or walls, only resistances of buildings contributed to the best landscape genetic model of functional connectivity. This suggests that for this urban resident typical city features may indeed not represent strong barriers. The river, on the other hand, acts as a strong barrier. Similar results have been found for a variety of other animal species (Eriksson *et al.* 2004; Coulon *et al.* 2006; Marrotte *et al.* 2014), but none of these studies was conducted within city boundaries, and their spatial scales exceed ours by orders of magnitude. The only other intra-urban study of comparable spatial scale revealed no structuring caused by a river (Straub *et al.* 2015). However, focal species of this study was the fire salamander, *Salamandra salamandra*, the larvae of which predominantly live in running waters and eventually may survive even in rivers. Apparently, the four bridges connecting both riversides, one of which also carries railway tracks, do not provide functional connectivity that can negate the resistance of the river for the lizard population investigated here.

It is contrary to our expectations that roads, and especially traffic volume, did not contribute to any of the landscape genetic models. This also contradicts findings in many other species (see Holderegger & Di Giulio 2010), such as a flightless ground beetle (Keller & Largiadèr 2003) or a cricket (Vandergast *et al.* 2009), but also in very mobile species such as the mountain lion, for which freeways have been shown to significantly reduce gene flow (Riley *et al.* 2006). In an urban population of red squirrels, *Sciurus vulgaris*, however, roads changed routine behavior but did not alter frequency of road crossings while dispersing (Fey *et al.* 2015). It may be hypothesized that urban residents are more tolerant to roads than species occurring mainly outside of cities. Even though we regularly found road-kills of lizards during field-work, this must not necessarily prevent dispersal and gene flow, as the relative frequency of such events remains unknown. On the other hand the detectability of roads as barriers might also be influenced by time lags that could mask its effect as a barrier. As it takes time for a population to reach genetic equilibria after the emergence of a new barrier, roads could promote future differentiation of populations although this signal remains undetectable presently (see Epps & Keyghobadi 2015 for a review). This effect was shown indirectly for cities, with the age of urban areas found to impact gene flow most heavily in three reptile species and a bird (Delaney *et al.* 2010). Thus, putative cases of traffic volume impeding gene flow cannot be ruled out for the lizard populations investigated here.

Functional *versus* structural connectivity

Using AICc values to rank models, structural connectivity measured using the PO-raw model ranked lower than the environmental factors water and slope in its prediction for functional connectivity. Structural connectivity measured from the PA-raw model ranked still lower and was additionally outperformed by the environmental factor structural diversity. To identify the most important environmental factors for the PO- or PA -raw model, typically tables of variable importance or contribution are calculated and referred to. The environmental factors structural diversity and substrate type contributed most to both the PO- or PA-raw models while water cover ranked low in both models (see Tab. 2). Although water cover contributes little to these models, the area of the river is nonetheless depicted as unsuitable in habitat suitability maps. This is more pronounced in the PO- than in the PA-raw model, which might also explain differences in rank when using these models for landscape genetic optimization: The best model for functional connectivity was obtained when using the PO-raw model as a basis for optimization, resulting in the PO-optim model. The performance even surpassed the best environmental factor of water cover. The PA-optim model also ranked highly, but below water (ΔAICc>10 between models). Most weight of both PO- and PA-optim model was – contrary to the PO-raw model - attributed to environmental factors of water cover, followed by buildings and canopy cover (Tab. 4). Although structural connectivity assessed from PO- and PA-raw models did not perform badly in predicting functional connectivity, only referring to the variable importance of environmental factors for these models would point away from the river and towards structural diversity and substrate type as most important factors. This means that raw SDMs and the calculated contribution of factors do not predict the underlying environmental factors responsible for functional connectivity. At the same time, these models are a useful basis for an optimization procedure, as implemented in ResistanceGA. This exemplifies the difficulties of inferring environmental factors responsible for functional connectivity from structural connectivity assessments.

Yet, after extraction of values computed for separate subcategories within environmental factors for each of the PO- and PA-raw- and PO-and PA-optim models, we see remarkable congruence across analyses: The three decisive subcategories, enforcing resistance to gene-flow, are water cover, buildings and canopy cover (PO-and PA-optim models) and it is exactly these three subcategories of both PO- and PA-raw models that have received the lowest median conductance scores (Tab. 4.). This might be unique to our dataset, but a comparative assessment of this potentially more prevalent association appear worthwhile.

The inference of functional connectivity in cityscapes, and elsewhere, from suitability maps may, admittedly, have the appeal of being a time-saving alternative to the more time consuming and costly evaluation of functional connectivity via genetic analyses. Nevertheless, our results show that relying on them as the sole basis, without running an optimization procedure and extracting and comparing resistance and conductance scores per subcategories, probably leads to erroneous conclusions when evaluating gene flow. The results of our functional and structural connectivity analyses underpins the need for a cautious interpretation of structural connectivity data with respect to dispersal and gene flow. This, and similar findings by Aavik *et al.* (2014) for the grassland plant species *Lychnis flos-cuculi*, and by Mateo-Sánchez *et al.* (2015) for the brown bear, *Ursus arctos*, reinforces the need to validate functionality of structural connectivity, if not via genetic analyses then through mark-recapture experiments. However, a recent study on leopards, *Panthera pardus*, (Fattebert *et al.* 2015), showed that predictions of structural connectivity can match dispersal routes well, demonstrating that species-specific differences in the importance of structural connectivity for dispersal have to be considered.

Functional connectivity within the cityscape is still not well understood (LaPoint et al. 2015). Potential discrepancies between structural and functional connectivity highlight that conservation measures need to be informed by both. It is important to recall that cityscape and landscape genetic analyses reveal the resistance of environmental factors to gene flow, while a habitat suitability map identifies those environmental factors of greatest importance for the occurrence of individuals at a given site. Consequently, both approaches complement each other (Driscoll *et al.* 2012) and should be used in this fashion when assessing management measures (Neuwald & Templeton 2013).

Implication for lizard conservation in the cityscape and beyond

The river Moselle represents a major barrier, dividing the lizard population of Trier into two separate clusters, with the eastern cluster being further divided into two separate sub-clusters (Fig. 3), although no single landscape factor sufficiently explains this latter separation. The best model for functional connectivity, the PO-optim model, includes more information than just the river and we assume that this additional information pays tribute to the population structure in eastern Trier. Contrary to our assumption, none of the other environmental factors appears to hamper gene flow within the city so strongly, as to further separate individuals into highly isolated clusters. With respect to the wall lizard population in Trier this may relax conservation concerns. The three genetic clusters are unlikely to go extinct in the near future due to genetic stochasticity which can follow complete isolation and small population sizes, a scenario postulated for urban salamander populations (Munshi-South *et al.* 2013). On the contrary, our analyses suggest that urban lizards readily disperse along suitable elements of the cityscape. Interestingly, substrate type did not rank highly, and although railway tracks are strongly associated with our substrate type level rocky/gravel, our results do not support their overarching importance for gene flow. This is contrary to the assumption that common wall lizards readily use railway tracks as dispersal corridors (Schulte *et al.* 2013).

Even though common wall lizards cope well with the challenges of an urban habitat, the long term persistence of native lineages inside German cities may be compromised by the detrimental effect of non-native invasive lineages. With no non-native haplotypes among sampled lizards an effect of non-native introduced individuals is very unlikely. We cannot fully rule out an introduction event of individuals of the native haplotype, which have been detected elsewhere in Germany (Schulte *et al.* 2012a). Foremost though, it is non-native lineages that are rapidly expanding in Germany and can displace native lineages upon secondary contact (Schulte *et al.* 2012b; While *et al.* 2015). Identification of intra-urban environmental factors that may hinder the expansion of non-native lineages, and hence gene flow, is therefore pivotal for developing mitigation strategies for a successful conservation of native lineages. Admittedly, our results do not point at an easy solution.

Conclusion

Our results demonstrate the difficulties of correctly interpreting results from habitat suitability maps and functional connectivity. Depending on a species' ecology the matrix of the cityscape determines specific resistance-levels to gene flow, making assessments of functional connectivity for multi-species assemblages of cities complex. Nevertheless, factors important for intra-urban gene flow have to be subjected to the urban planning process for effective conservation management (Keller *et al.* 2014). There is great potential of such knowledge to improve decision making in conservation management and legislation in the cityscape (Barton *et al.* 2015). Even though SDMs alone are not suitable for assessing functional connectivity, our study also shows that using them as a basis for landscape genetic optimization provides better results than using simple landscape models.

ACKNOWLEDGEMENTS

Permits for catching and sampling of lizards were obtained from the local authority (Struktur- und Genehmigungsdirektion Nord: 425-104.211.1202 & 425-104.211.1303). JB, MV and AH are members of the interdisciplinary research training group "Cooperation of Science and Jurisprudence in Improving Development and Use of Standards for Environmental Protection – Strategies for Risk Assessment and Management" funded by the German Science Foundation (DFG, RTG 1319).

DATA ACCESSIBILITY

Input files for SDMs and ResistanceGA, GPS-coordinates of sampling locations as well as microsatellite genotypes are available on Dryad (doi:10.5061/dryad.31qg7).

AUTHOR CONTRIBUTION

AH, JB, MV and US designed the research. JB, DP and US conducted field and laboratory work. JB, DP and MW prepared the dataset. JB and SF conducted analyses. JB wrote a first draft of the paper and all authors contributed substantially to revisions.

References

Aavik, T., Holderegger, R. & Bolliger, J. (2014). The structural and functional connectivity of the grassland plant Lychnis flos-cuculi. *Heredity*, 112, 471–478.

Angold, P.G., Sadler, J.P., Hill, M.O., Pullin, A., Rushton, S. & Austin, K. *et al.* (2006). Biodiversity in urban habitat patches. *Sci. Total Environ.*, 360, 196–204.

Aronson, M.F.J., La Sorte, F.A., Nilon, C.H., Katti, M., Goddard, M.A. & Lepczyk, C.A. *et al.* (2014). A global analysis of the impacts of urbanization on bird and plant diversity reveals key anthropogenic drivers. *Proceedings of the Royal Society B: Biological Sciences*, 281, 20133330.

Barton, K. (2016). MuMIn: Multi-Model Inference. R package version 1.15.6. [WWW document]. URL https://CRAN.R-project.org/package=MuMIn.

Barton, P.S., Lentini, P.E., Alacs, E., Bau, S., Buckley, Y.M. & Burns, E.L. *et al.* (2015). Guidelines for Using Movement Science to Inform Biodiversity Policy. *Environmental management*, 56, 791–801.

Beninde, J., Veith, M. & Hochkirch, A. (2015). Biodiversity in cities needs space: a meta-analysis of factors determining intra-urban biodiversity variation. *Ecol Lett*, 18, 581–592.

Björklund, M., Ruiz, I. & Senar, J.C. (2010). Genetic differentiation in the urban habitat: the great tits (Parus major) of the parks of Barcelona city. *Biological Journal of the Linnean Society*, 99, 9–19.

Boudjemadi, K., Martin, O., Simon, J.-C. & Estoup, A. (1999). Development and cross-species comparison of microsatellite markers in two lizard species, Lacerta vivipara and Podarcis muralis. *Mol Ecol*, 8, 518–520.

Brashear, W.A., Ammerman, L.K. & Dowler, R.C. (2015). Short-distance dispersal and lack of genetic structure in an urban striped skunk population. *Journal of Mammalogy*, 96, 72–80.

Burnham, K.P. & Anderson, D.R. (2002). Model Selection and Multimodel Inference: A Practical Information-theoretic Approach.

Busack, S., Lawson, R. & Arjo, W. (2005). Mitochondrial DNA, allozymes, morphology and historical biogeography in the Podarcis vaucheri (Lacertidae) species complex. *Amphibia-Reptilia*, 26, 239–256.

Coulon, A., Guillot, G., Cosson, J.-F., Angibault, J.M.A., Aulagnier, S. & Cargnelutti, B. *et al.* (2006). Genetic structure is influenced by landscape features: empirical evidence from a roe deer population. *Molecular ecology*, 15, 1669–1679.

Cushman, S.A. & Landguth, E.L. (2010). Spurious correlations and inference in landscape genetics. *Mol Ecol*, 19, 3592–3602.

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.

Driscoll, D.A., Whitehead, C.A. & Lazzari, J. (2012). Spatial dynamics of the knob-tailed gecko Nephrurus stellatus in a fragmented agricultural landscape. *Landscape Ecol*, 27, 829–841.

Earl, D.A. & vonHoldt, B.M. (2012). STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genet Resour*, 4, 359–361.

Elith, J. & Graham, C.H. (2009). Do they? How do they? Why do they differ? - on finding reasons for differing performances of species distribution models. *Ecography*, 32, 66–77.

Elith, J., Phillips, S.J., Hastie, T., Dudík, M., Chee, Y.E. & Yates, C.J. (2011). A statistical explanation of MaxEnt for ecologists. *Diversity and Distributions*, 17, 43–57.

Epps, C.W. & Keyghobadi, N. (2015). Landscape genetics in a changing world: disentangling historical and contemporary influences and inferring change. *Molecular ecology*, 24, 6021–6040.

Eriksson, J., Hohmann, G., Boesch, C. & Vigilant, L. (2004). Rivers influence the population genetic structure of bonobos (Pan paniscus). *Molecular ecology*, 13, 3425–3435.

Evanno, G., Regnaut, S. & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol Ecol*, 14, 2611–2620.

Fattebert, J., Robinson, H.S., Balme, G., Slotow, R. & Hunter, L. (2015). Structural habitat predicts functional dispersal habitat of a large carnivore. How leopards change spots. *Ecological Applications*, 25, 1911–1921.

Fey, K., Hämäläinen, S. & Selonen, V. (2015). Roads are no barrier for dispersing red squirrels in an urban environment. *BEHECO*, arv215.

Goudet, J. (2001). *FSTAT. a program to estimate and test gene diversities and fixation indices*.

Guillot, G., Mortier, F. & Estoup, A. (2005). GENELAND: a computer package for landscape genetics. *Mol Ecol Notes*, 5, 712–715.

Heathcote, Robert J. P., Dawson, D.A. & Uller, T. (2014). Characterisation of nine European wall lizard (Podarcis muralis) microsatellite loci of utility across sub-species. *Conservation Genet Resour*, 7, 85–87.

Holderegger, R. & Di Giulio, M. (2010). The genetic effects of roads. A review of empirical evidence. *Basic and Applied Ecology*, 11, 522–531.

Ives, C.D., Lentini, P.E., Threlfall, C.G., Ikin, K., Shanahan, D.F. & Garrard, G.E. *et al.* (2016). Cities are hotspots for threatened species. *Global Ecology and Biogeography*, 25, 117–126.

Keller, D., Holderegger, R., van Strien, Maarten J. & Bolliger, J. (2014). How to make landscape genetics beneficial for conservation management? *Conserv Genet*, 16, 503–512.

Keller, I. & Largiadèr, C.R. (2003). Recent habitat fragmentation caused by major roads leads to reduction of gene flow and loss of genetic variability in ground beetles. *Proceedings. Biological sciences / The Royal Society*, 270, 417–423.

Koen, E.L., Garroway, C.J., Wilson, P.J., Bowman, J. & Bersier, L.-F. (2010). The Effect of Map Boundary on Estimates of Landscape Resistance to Animal Movement. *PLoS ONE*, 5, e11785.

Landguth, E.L. & Schwartz, M.K. (2014). Evaluating sample allocation and effort in detecting population differentiation for discrete and continuously distributed individuals. *Conserv Genet*, 15, 981–992.

LaPoint, S., Balkenhol, N., Hale, J., Sadler, J. & van der Ree, Rodney (2015). Ecological Connectivity Research in Urban Areas. *Funct Ecol*, 29, 868–878.

Lobo, J.M., Jiménez-Valverde, A. & Real, R. (2008). AUC. A misleading measure of the performance of predictive distribution models. *Global Ecol Biogeography*, 17, 145–151.

Marrotte, R.R., Gonzalez, A. & Millien, V. (2014). Landscape resistance and habitat combine to provide an optimal model of genetic structure and connectivity at the range margin of a small mammal. *Molecular ecology*, 23, 3983–3998.

Martin, L.J., Blossey, B. & Ellis, E. (2012). Mapping where ecologists work. Biases in the global distribution of terrestrial ecological observations. *Frontiers in Ecology and the Environment*, 10, 195–201.

Mateo-Sánchez, M.C., Balkenhol, N., Cushman, S., Pérez, T., Domínguez, A. & Saura, S. (2015). A comparative framework to infer landscape effects on population genetic structure. Are habitat suitability models effective in explaining gene flow? *Landscape Ecol*, 30, 1405–1420.

McDonnell, M.J. & Hahs, A.K. (2015). Adaptation and Adaptedness of Organisms to Urban Environments. *Annu. Rev. Ecol. Evol. Syst.*, 46, 261–280.

McRae, B.H. (2006). Isolation by Resistance. *Evolution*, 60, 1551–1561.

Meillère, A., Brischoux, F., Parenteau, C. & Angelier, F. (2015). Influence of Urbanization on Body Size, Condition, and Physiology in an Urban Exploiter: A Multi-Component Approach. *PLoS ONE*, 10, e0135685.

Merow, C., Smith, M.J. & Silander, J.A. (2013). A practical guide to MaxEnt for modeling species' distributions. What it does, and why inputs and settings matter. *Ecography*, 36, 1058–1069.

Miller, M.P. (2005). Alleles in space (AIS): computer software for the joint analysis of interindividual spatial and genetic information. *The Journal of heredity*, 96, 722–724.

Mueller, J.C., Partecke, J., Hatchwell, B.J., Gaston, K.J. & Evans, K.L. (2013). Candidate gene polymorphisms for behavioural adaptations during urbanization in blackbirds. *Molecular ecology*, 22, 3629–3637.

Munshi-South, J. (2012). Urban landscape genetics: canopy cover predicts gene flow between white-footed mouse (Peromyscus leucopus) populations in New York City. *Mol Ecol*, 21, 1360–1378.

Nakagawa, S. & Schielzeth, H. (2013). A general and simple method for obtaining R2 from generalized linear mixed-effects models. *Methods Ecol Evol*, 4, 133–142.

Nei, M., Tajima, F. & Tateno, Y. (1983). Accuracy of estimated phylogenetic trees from molecular data. *J Mol Evol*, 19, 153–170.

Nembrini, M. & Oppliger, A. (2003). Characterization of microsatellite loci in the wall lizard Podarcis muralis (Sauria: Lacertidae). *Mol Ecol Notes*, 3, 123–124.

Neuwald, J.L. & Templeton, A.R. (2013). Genetic restoration in the eastern collared lizard under prescribed woodland burning. *Mol Ecol*, 22, 3666–3679.

Peakall, R. & Smouse, P.E. (2012). GenAlEx 6.5: Genetic Analysis in Excel. Population Genetic Software for Teaching and Research—An Update. *Bioinformatics Application Note*, 28, 2537–2539.

Peterman, W.E. (2014). ResistanceGA: An R package for the optimization of resistance surfaces using genetic algorithms.

Peterman, W.E., Connette, G.M., Semlitsch, R.D. & Eggert, L.S. (2014). Ecological resistance surfaces predict fine-scale genetic differentiation in a terrestrial woodland salamander. *Mol Ecol*, 23, 2402–2413.

Phillips, S.J., Anderson, R.P. & Schapire, R.E. (2006). Maximum entropy modeling of species geographic distributions. *Ecological Modelling*, 190, 231–259.

Phillips, S.J. & Dudík, M. (2008). Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. *Ecography*, 31, 161–175.

Phillips, S.J., Dudík, M. & Schapire, R.E. (2004). A Maximum Entropy Approach to Species Distribution Modeling. In: *Proceedings of the Twenty-First International Conference on Machine Learning,* pp. 655–662.

Pinho, C., Sequeira, F., Godinho, R., Harris, D.J. & Ferrand, N. (2004). Isolation and characterization of nine microsatellite loci in Podarcis bocagei (Squamata: Lacertidae). *Mol Ecol Notes*, 4, 286–288.

Podnar, M. (2004). Mitochondrial phylogeography of the Dalmatian wall lizard, (Lacertidae). *Organisms Diversity & Evolution*, 4, 307–317.

Podnar, M., Haring, E., Pinsker, W. & Mayer, W. (2007). Unusual origin of a nuclear pseudogene in the Italian wall lizard: intergenomic and interspecific transfer of a large section of the mitochondrial genome in the genus Podarcis (Lacertidae). *Journal of molecular evolution*, 64, 308–320.

Qiao, H., Peterson, A.T., Campbell, L.P., Soberón, J., Ji, L. & Escobar, L.E. (2015). NicheA. Creating Virtual Species and Ecological Niches in Multivariate Environmental Scenarios. *Ecography*, 39, 1–9.

Rézouki, C., Dozières, A., Le Cœur, C., Thibault, S., Pisanu, B. & Chapuis, J.-L. *et al.* (2014). A viable population of the European red squirrel in an urban park. *PLoS ONE*, 9, e105111.

Richardson, J.L., Brady, S.P., Wang, I.J. & Spear, S.F. (2016). Navigating the pitfalls and promise of landscape genetics. *Molecular ecology*.

Riley, S.P.D., Pollinger, J.P., Sauvajot, R.M., York, E.C., Bromley, C. & Fuller, T.K. *et al.* (2006). A southern California freeway is a physical and social barrier to gene flow in carnivores. *Mol Ecol*, 15, 1733–1741.

Ruiz-González, A., Gurrutxaga, M., Cushman, S.A., Madeira, M.J., Randi, E. & Gómez-Moliner, B.J. (2014). Landscape Genetics for the Empirical Assessment of Resistance Surfaces: The European Pine Marten (Martes martes) as a Target-Species of a Regional Ecological Network. *PLoS ONE*, 9, e110552.

Salvi, D., Harris, D.J., Kaliontzopoulou, A., Carretero, M.A. & Pinho, C. (2013). Persistence across Pleistocene ice ages in Mediterranean and extra-Mediterranean refugia: phylogeographic insights from the common wall lizard. *BMC Evol. Biol.*, 147.

Schulte, U. (2008). *Die Mauereidechse. erfolgreich im Schlepptau des Menschen.* Laurenti-Verlag, Bielefeld.

Schulte, U. & Deichsel, G. (2015). Eingeschleppte Mauereidechsen in Deutschland – ein Überblick mit Empfehlungen zum naturschutzfachlichen Umgang. In: *Verbreitung, Biologie und Schutz der Mauereidechse Podarcis muralis (LAURENTI, 1768)* (ed. Laufer, H. & Schulte, U.), Mannheim, pp. 74–85.

Schulte, U., Gebhard, F., Heinz, L., Veith, M. & Hochkirch, A. (2011). Buccal swabs as a reliable non-invasive tissue sampling method for DNA analysis in the lacertid lizard Podarcis muralis. *N. West. J. Zool.*, 7, 325–328.

Schulte, U., Hochkirch, A., Lötters, S., Rödder, D., Schweiger, S. & Weimann, T. *et al.* (2012a). Cryptic niche conservatism among evolutionary lineages of an invasive lizard. *Global Ecology and Biogeography*, 21, 198–211.

Schulte, U., Thiesmeier, B., Mayer, W. & Schweiger, S. (2008). Allochthone Vorkommen der Mauereidechse (Podarcis muralis) in Deutschland. *Zeitschrift für Feldherpetologie*, 15, 139–156.

Schulte, U., Veith, M. & Hochkirch, A. (2012b). Rapid genetic assimilation of native wall lizard populations (Podarcis muralis) through extensive hybridization with introduced lineages. *Mol Ecol*, 21, 4313–4326.

Schulte, U., Veith, M., Mingo, V., Modica, C. & Hochkirch, A. (2013). Strong genetic differentiation due to multiple founder events during a recent range expansion of an introduced wall lizard population. *Biol Invasions*, 15, 2639–2649.

Schwartz, M.K. & McKelvey, K.S. (2009). Why sampling scheme matters. The effect of sampling scheme on landscape genetic results. *Conserv Genet*, 10, 441–452.

Seto, K.C., Fragkias, M., Güneralp, B. & Reilly, M.K. (2011). A Meta-Analysis of Global Urban Land Expansion. *PLoS ONE*, 6, e23777.

Shah, V.B. & McRae, B.H. (2008). Circuitscape: A Tool for Landscape Ecology. In: *Proceeding of the 7th Python on Science Conference* (ed. Varoquaux, G., Vaught, T. & Millman, J.), pp. 62–66.

Shirk, A.J., Wallin, D.O., Cushman, S.A., Rice, C.G. & Warheit, K.I. (2010). Inferring landscape effects on gene flow: a new model selection framework. *Mol Ecol*, 19, 3603–3619.

Sol, D., Lapiedra, O. & González-Lagos, C. (2013). Behavioural adjustments for a life in the city. *Animal Behaviour*, 85, 1101–1112.

Straub, C., Pichlmüller, F. & Helfer, V. (2015). Population genetics of fire salamanders in a pre-Alpine urbanized area (Salzburg, Austria). *Salamandra*, 51, 245–251.

Sumasgutner et al. (2014). Hard times in the city – attractive nest sites but insufficient food supply lead to low reproduction rates in a bird of prey, 11, 48.

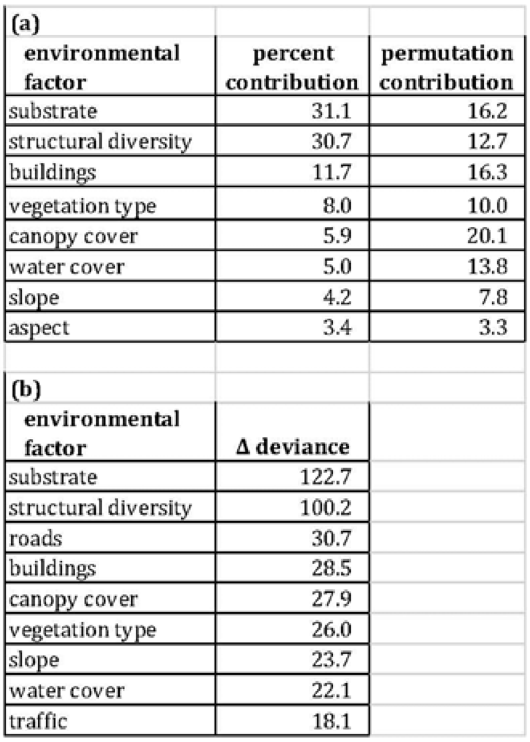
van Oosterhout, C., Hutchinson, W.F., Wills, D. & Shipley, P. (2004). micro-checker: software for identifying and correcting genotyping errors in microsatellite data. *Mol Ecol Notes*, 4, 535–538.

Vandergast, A.G., Lewallen, E.A., Deas, J., Bohonak, A.J., Weissman, D.B. & Fisher, R.N. (2009). Loss of genetic connectivity and diversity in urban microreserves in a southern California endemic Jerusalem cricket (Orthoptera. Stenopelmatidae: Stenopelmatus n. sp. “santa monica”). *J Insect Conserv*, 13, 329–345.

While, G.M., Michaelides, S., Heathcote, Robert J. P., MacGregor, H.E.A., Zajac, N. & Beninde, J. *et al.* (2015). Sexual selection drives asymmetric introgression in wall lizards. *Ecology letters*, 18, 1366–1375.

Yackulic CB, Chandler R, Zipkin EF et al. (2013) Presence-only modelling using MAXENT. When can we trust the inferences? Methods in Ecology and Evolution, doi: 10.1111/2041-210x.12004.

Table 1: Importance of environmental factors to the final SDMs assessed by Δ deviance for PO-raw model (a) and factor contribution to PA-raw model (b). Environmental Factors differ between models due to model-specific variable selection.



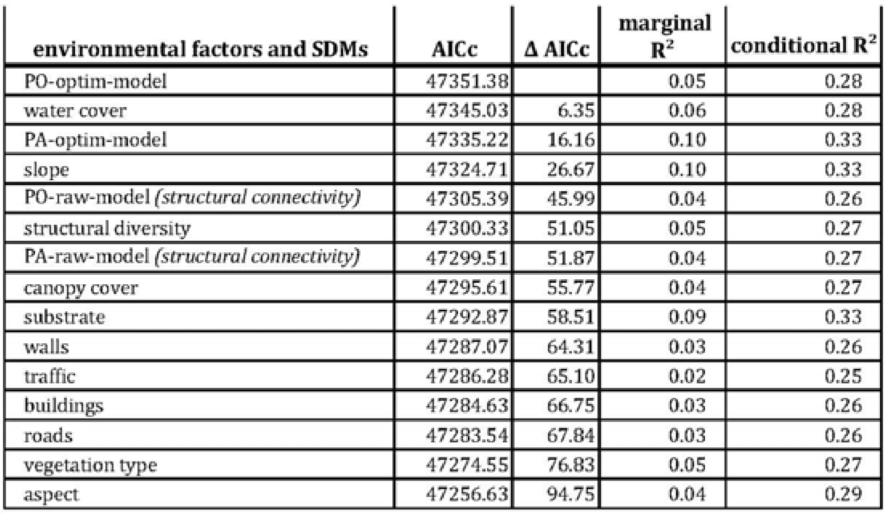
(a)

environmental factor	percent contribution	permutation contribution
substrate	31.1	16.2
structural diversity	30.7	12.7
buildings	11.7	16.3
vegetation type	8.0	10.0
canopy cover	5.9	20.1
water cover	5.0	13.8
slope	4.2	7.8
aspect	3.4	3.3

(b)

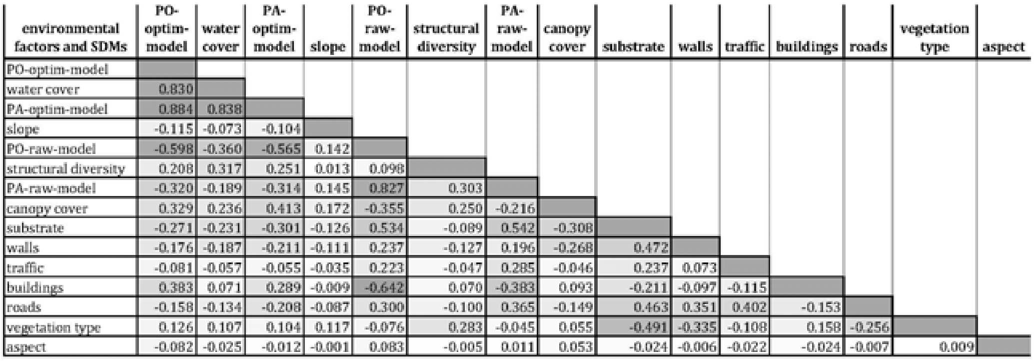
environmental factor	Δ deviance
substrate	122.7
structural diversity	100.2
roads	30.7
buildings	28.5
canopy cover	27.9
vegetation type	26.0
slope	23.7
water cover	22.1
traffic	18.1

Table 2: Results of the landscape genetic analyses showing model rank and fit of environmental factors and SDMs.



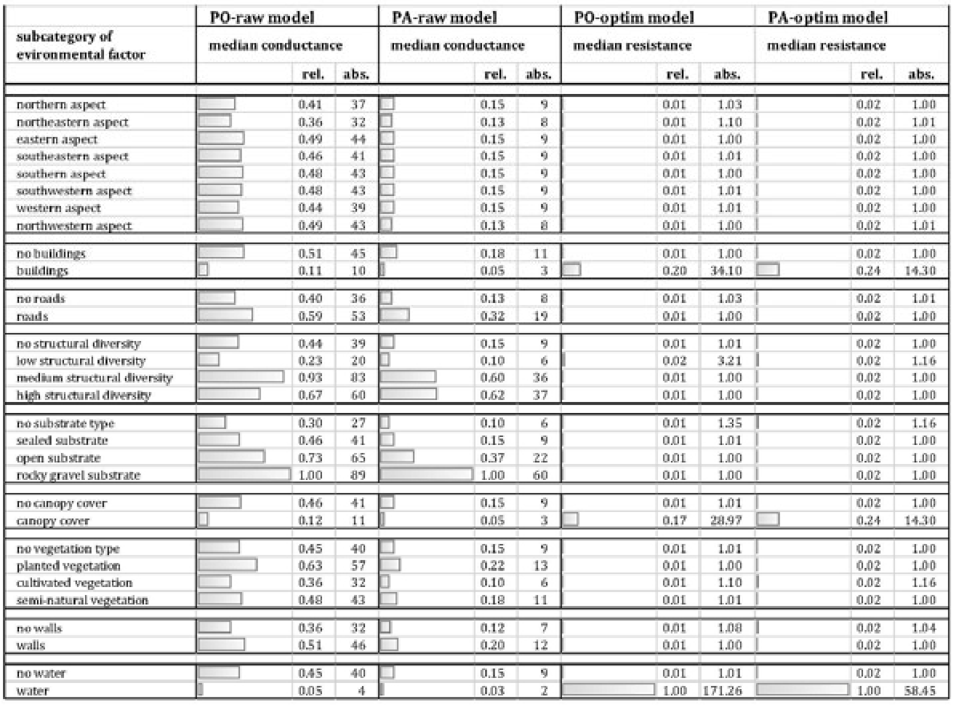
environmental factors and SDMs	AICc	Δ AICc	marginal R^2	conditional R^2
PO-optim-model	47351.38		0.05	0.28
water cover	47345.03	6.35	0.06	0.28
PA-optim-model	47335.22	16.16	0.10	0.33
slope	47324.71	26.67	0.10	0.33
PO-raw-model *(structural connectivity)*	47305.39	45.99	0.04	0.26
structural diversity	47300.33	51.05	0.05	0.27
PA-raw-model *(structural connectivity)*	47299.51	51.87	0.04	0.27
canopy cover	47295.61	55.77	0.04	0.27
substrate	47292.87	58.51	0.09	0.33
walls	47287.07	64.31	0.03	0.26
traffic	47286.28	65.10	0.02	0.25
buildings	47284.63	66.75	0.03	0.26
roads	47283.54	67.84	0.03	0.26
vegetation type	47274.55	76.83	0.05	0.27
aspect	47256.63	94.75	0.04	0.29

Table 3: Correlation matrix of all environmental factors included in analyses and the raw as well as optimized SDMs. Structural connectivity was measured as the pairwise resistances between individuals on the basis of PO- and PA-raw-models using CIRCUITSCAPE. The PA- and PO-optim-models were optimized to fit genetic distances of individuals using ResistanceGA.

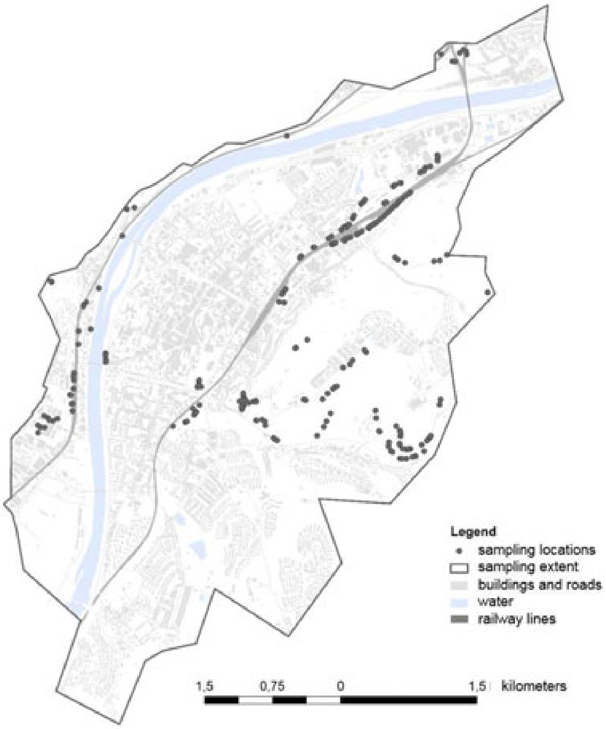


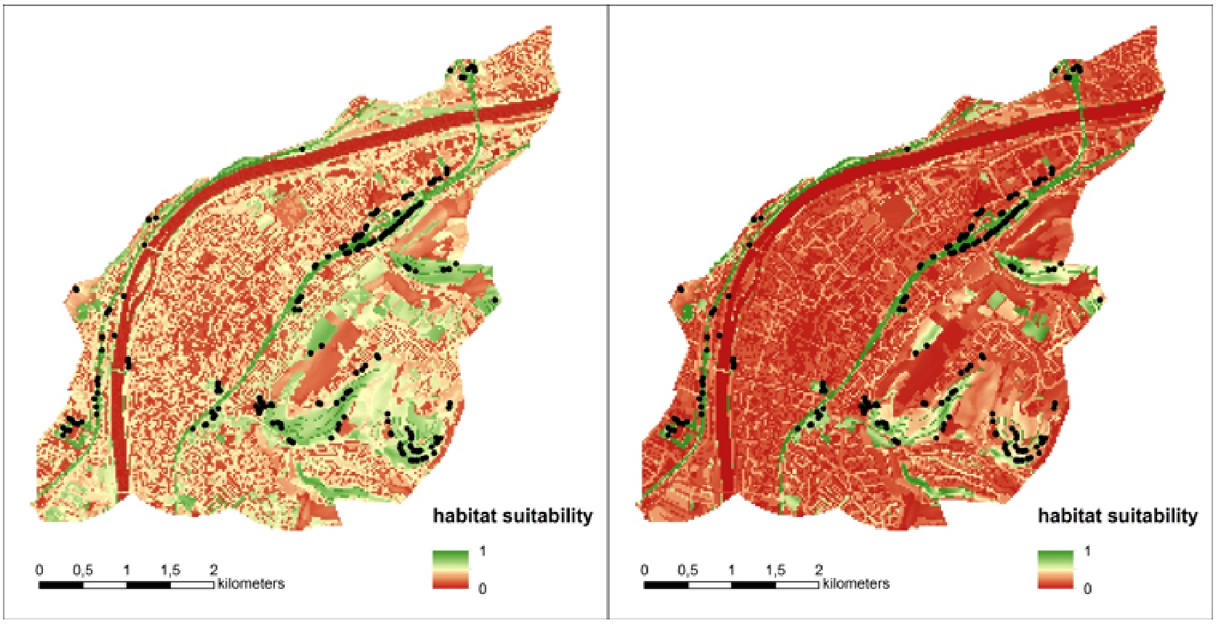
environmental factors and SDMs	PO-optim-model	water cover	PA-optim-model	slope	PO-raw-model	structural diversity	PA-raw-model	canopy cover	substrate	walls	traffic	buildings	roads	vegetation type	aspect
PO-optim-model															
water cover	0.830														
PA-optim-model	0.884	0.838													
slope	-0.115	-0.073	-0.104												
PO-raw-model	-0.598	-0.360	-0.565	0.142											
structural diversity	0.208	0.317	0.251	0.013	0.098										
PA-raw-model	-0.320	-0.189	-0.314	0.145	0.827	0.303									
canopy cover	0.329	0.236	0.413	0.172	-0.355	0.250	-0.216								
substrate	-0.271	-0.231	-0.301	-0.126	0.534	-0.089	0.542	-0.308							
walls	-0.176	-0.187	-0.211	-0.111	0.237	-0.127	0.196	-0.268	0.472						
traffic	-0.081	-0.057	-0.055	-0.035	0.223	-0.047	0.285	-0.046	0.237	0.073					
buildings	0.383	0.071	0.289	-0.009	-0.642	0.070	-0.383	0.093	-0.211	-0.097	-0.115				
roads	-0.158	-0.134	-0.208	-0.087	0.300	-0.100	0.365	-0.149	0.463	0.351	0.402	-0.153			
vegetation type	0.126	0.107	0.104	0.117	-0.076	0.283	-0.045	0.055	-0.491	-0.335	-0.108	0.158	-0.256		
aspect	-0.082	-0.025	-0.012	-0.001	0.083	-0.005	0.011	0.053	-0.024	-0.006	-0.022	-0.024	-0.007	0.009	

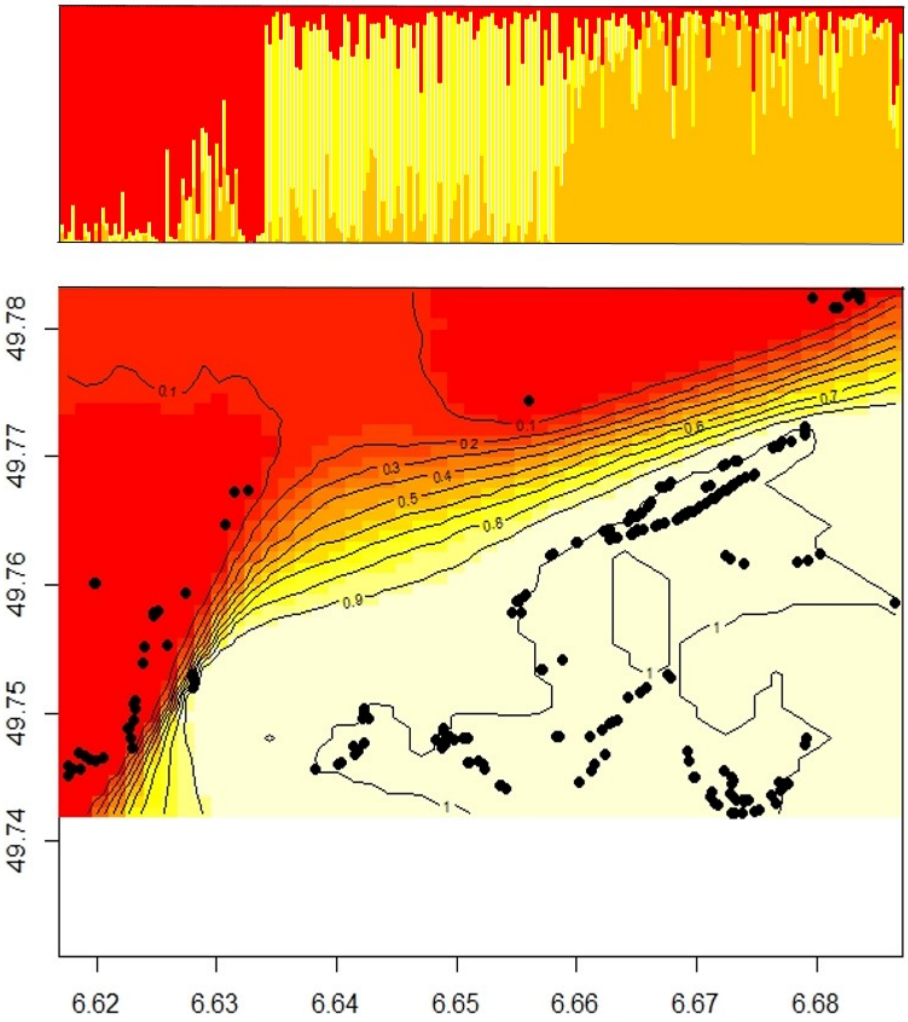
Table 4: Median resistance of optimized PO- and PA-optim models, as well as median conductance of PO- and PA-raw models per subcategories of environmental factors. Absolute values are given as well as relative values, scaled to one to enable comparison.

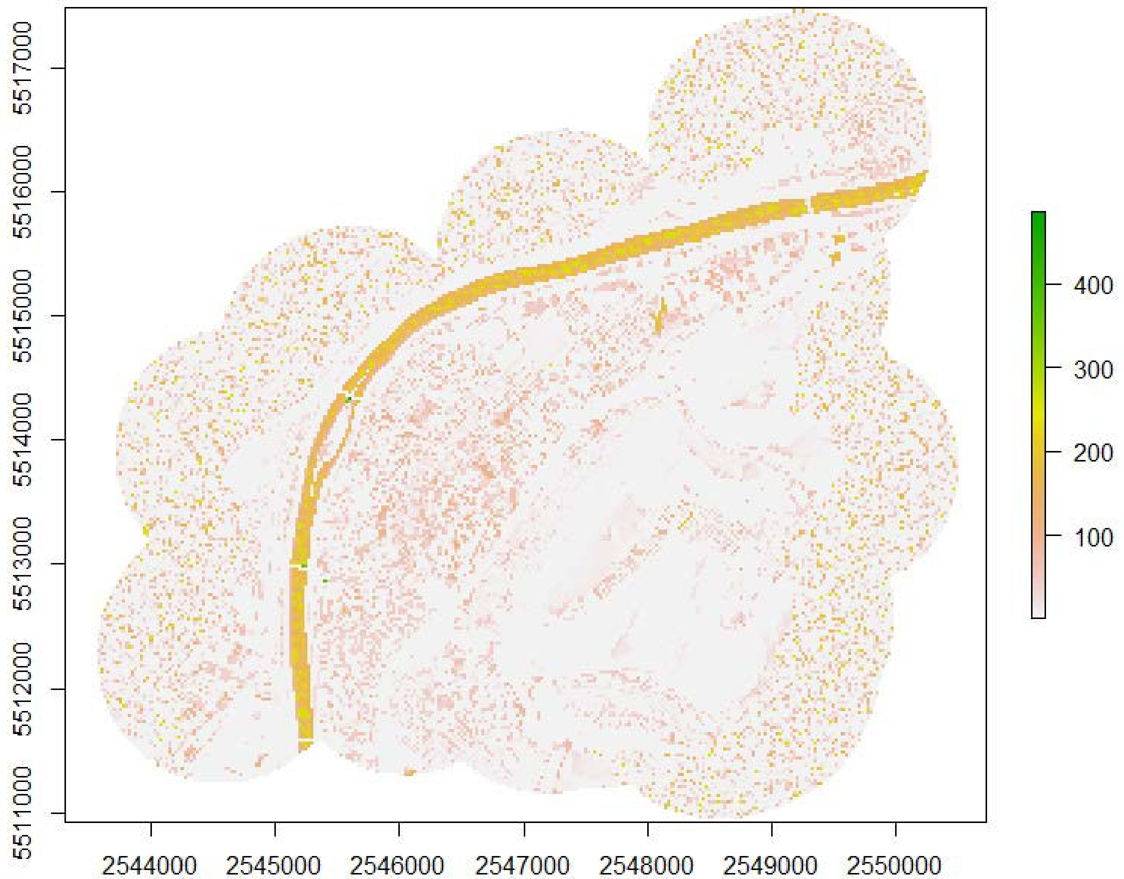


subcategory of evironmental factor	PO-raw model		PA-raw model		PO-optim model		PA-optim model	
	median conductance		median conductance		median resistance		median resistance	
	rel.	abs.	rel.	abs.	rel.	abs.	rel.	abs.
northern aspect	0.41	37	0.15	9	0.01	1.03	0.02	1.00
northeastern aspect	0.36	32	0.13	8	0.01	1.10	0.02	1.01
eastern aspect	0.49	44	0.15	9	0.01	1.00	0.02	1.00
southeastern aspect	0.46	41	0.15	9	0.01	1.01	0.02	1.00
southern aspect	0.48	43	0.15	9	0.01	1.00	0.02	1.00
southwestern aspect	0.48	43	0.15	9	0.01	1.01	0.02	1.00
western aspect	0.44	39	0.15	9	0.01	1.01	0.02	1.00
northwestern aspect	0.49	43	0.13	8	0.01	1.00	0.02	1.01
no buildings	0.51	45	0.18	11	0.01	1.00	0.02	1.00
buildings	0.11	10	0.05	3	0.20	34.10	0.24	14.30
no roads	0.40	36	0.13	8	0.01	1.03	0.02	1.01
roads	0.59	53	0.32	19	0.01	1.00	0.02	1.00
no structural diversity	0.44	39	0.15	9	0.01	1.01	0.02	1.00
low structural diversity	0.23	20	0.10	6	0.02	3.21	0.02	1.16
medium structural diversity	0.93	83	0.60	36	0.01	1.00	0.02	1.00
high structural diversity	0.67	60	0.62	37	0.01	1.00	0.02	1.00
no substrate type	0.30	27	0.10	6	0.01	1.35	0.02	1.16
sealed substrate	0.46	41	0.15	9	0.01	1.01	0.02	1.00
open substrate	0.73	65	0.37	22	0.01	1.00	0.02	1.00
rocky gravel substrate	1.00	89	1.00	60	0.01	1.00	0.02	1.00
no canopy cover	0.46	41	0.15	9	0.01	1.01	0.02	1.00
canopy cover	0.12	11	0.05	3	0.17	28.97	0.24	14.30
no vegetation type	0.45	40	0.15	9	0.01	1.01	0.02	1.00
planted vegetation	0.63	57	0.22	13	0.01	1.00	0.02	1.00
cultivated vegetation	0.36	32	0.10	6	0.01	1.10	0.02	1.16
semi-natural vegetation	0.48	43	0.18	11	0.01	1.01	0.02	1.00
no walls	0.36	32	0.12	7	0.01	1.08	0.02	1.04
walls	0.51	46	0.20	12	0.01	1.00	0.02	1.00
no water	0.45	40	0.15	9	0.01	1.01	0.02	1.00
water	0.05	4	0.03	2	1.00	171.26	1.00	58.45




habitat suitability
1
0
0 0,5 1 1,5 2 kilometers
habitat suitability
1
0
0 0,5 1 1,5 2 kilometers


49.78
49.77
49.76
49.75
49.74
6.62
6.63
6.64
6.65
6.66
6.67
6.68
0.1
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
1
1
1


5511000
5512000
5513000
5514000
5515000
5516000
5517000
2544000
2545000
2546000
2547000
2548000
2549000
2550000
400
300
200
100