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Agricultural intensification alters marbled newt genetic diversity and gene flow through density and dispersal reduction

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Abstract

Recent agricultural intensification threatens global biodiversity with amphibians being one of the most impacted groups. Because of their biphasic life cycle, amphibians are particularly vulnerable to habitat loss and fragmentation that often result in small, isolated populations and loss of genetic diversity. Here, we studied how landscape heterogeneity affects genetic diversity, gene flow and demographic parameters in the marbled newt, *Triturus marmoratus*, over a hedgerow network landscape in Western France. While the northern part of the study area consists of preserved hedged farmland, the southern part was more profoundly converted for intensive arable crops production after WWII. Based on 67 sampled ponds and 10 microsatellite loci, we characterized regional population genetic structure and evaluated the correlation between landscape variables and (i) local genetic diversity using mixed models and (ii) genetic distance using multiple regression methods and commonality analysis. We identified a single genetic population characterized by a spatially heterogeneous isolation-by-distance pattern. Pond density in the surrounding landscape positively affected local genetic diversity while arable crop land cover negatively affected gene flow and connectivity. We used demographic inferences to quantitatively assess differences in effective population density and dispersal between the contrasted landscapes characterizing the northern and southern parts of the study area. Altogether, results suggest recent land conversion affected *T. marmoratus* through reduction in both effective population density and dispersal due to habitat loss and reduced connectivity.

KEYWORDS
demographic inferences, dispersal, genetic diversity, landscape genetics, microsatellites, *Triturus marmoratus*

1 | INTRODUCTION

Habitat loss and fragmentation induced by human activities is a major threat to global biodiversity (Fisher & Lindenmayer, 2007; Foley et al., 2005). This often results in small, isolated populations that are more vulnerable to loss of genetic diversity and fitness decrease through inbreeding and fixation of deleterious alleles (Frankham, 2005). Patterns of neutral genetic diversity mainly depend on the balance between genetic drift and gene flow (Hutchison & Templeton, 1999). While decrease in effective population size results in lower genetic diversity due to increased genetic drift, gene flow is a source of genetic variability. Gene flow homogenizes allele frequencies and maintains population connectivity, which buffers against the negative effects of isolation and inbreeding, and allows for future responses to environmental change (Frankham et al., 2017). Understanding how genetic diversity and genetic differentiation respond to current landscape structure and past land conversion is critically important for conservation decisions and could allow robust predictions about species responses to global change (Palsboll et al.,2007; Scoble & Lowe, 2010; Sgrò et al.,2011).

In European agricultural hedgerow landscapes, land conversion has caused loss of permanent habitats and their connection in space (Benton et al., 2003). While land conversion threatens all taxa, amphibians constitute one of the most impacted ones (Ceballos et al., 2020; Crawford et al., 2016; Cushman, 2006; Hof et al., 2011; McCartney-Melstad & Shaffer, 2015). This sensitivity is primarily due to (i) their specific habitat requirements and complex life cycle involving spatially distinct breeding and foraging habitats (Karraker & Gibbs, 2009; Sztatecsny et al., 2004), and (ii) their low dispersal capacity (Hillman et al., 2014; Smith & Green, 2005). Among amphibians, urodeles (newts and salamanders) have lower mobility (Smith & Green, 2005), and are particularly vulnerable to temperature and water constraints (Riddell et al.,2019; Riddell & Sears, 2020). Most European newt species live in water during their larval stage and for reproduction but they are generally terrestrial for the rest of the year. Newts mainly reach breeding ponds following corridors connecting ponds with woodlands, and avoid arable crops (Marty et al., 2005). Therefore, land conversion for intensive agriculture, including the loss of hedgerows, shelters and corridors, could affect populations by decreasing effective population size and connectivity, both resulting in loss of genetic diversity and gene flow.

Landscape genetics combines tools from population genetics, spatial statistics and landscape ecology to relate landscape features directly to population structure, genetic diversity, and gene flow (Manel & Holderegger, 2013; Manel et al., 2003; Storfer et al., 2007). Detection of barriers and genetic clusters is a first step in landscape genetics but, because many populations are continuously distributed, barriers may only exist at large biogeographic scales. However, at smaller spatial scales, gene flow and functional connectivity might be gradually modulated by landscape and environmental heterogeneity (isolation by resistance, IBR, McRae, 2006). In addition, because species dispersal abilities are generally limited, geographic distance is expected to play a significant role in the explanation of genetic differentiation (isolation by distance, IBD, Rousset, 1997; Wright, 1943). IBD is generally considered as the null model in landscape genetics and methods such as causal modelling are employed to compare different models of landscape resistance and IBD (Cushman et al., 2006). More recently, multiple regression methods are becoming more popular as they allow to identify landscape variables influencing genetic differentiation in a more robust statistical framework (Shirk et al., 2017; Wang, 2013), and rank them according to their importance in shaping genetic structure while accounting for correlations among them (Prunier et al., 2015). While matrix-based analyses (i.e., relating genetic differentiation to landscape structure) are the most popular in landscape genetics, node-based analyses (i.e., relating local genetic diversity to surrounding landscape structure) might provide complementary information on landscape genetics relationships (Flavenot et al., 2015). However, both approaches are mostly correlative and only allow identifying landscape genetics relationships without assessing the demographic parameters (e.g., effective population size and dispersal) by which landscape influences genetic patterns. This is particularly important regarding two related issues. First, landscape genetics is mostly used in conservation studies where populations are rarely at a demographic equilibrium (Segelbacher et al., 2010). Furthermore, genetic patterns at a large spatial scale might be integrative of many generations that are likely to have experienced numerous changes in population size and dispersal. It is thus challenging to assess whether landscape genetic patterns result from variations in effective population size or changes in dispersal (Richardson et al., 2016). Landscape genetics studies often interpret increases in measures of genetic differentiation in terms of reduced gene flow and loss functional connectivity, neglecting the effect of local genetic drift on genetic structure. Thus, demographic inferences of population parameters, i.e. effective population size and dispersal rate and distance, in a spatially explicit framework are required to improve the landscape genetics toolbox. Indeed, methods based on models specifying population size and dispersal function might allow inferring whether genetic diversity (or genetic differentiation) is affected by variations in population size, variations in dispersal or both. The second issue is methodological. There is actually a large body of methods in population genetics producing demographic inferences on N_e and dispersal (migration) rate (m) but they are based on the simple, not spatially explicit, island model, while most natural populations (and landscape genetics approaches) follow the IBD model. There is a real lack of use of demographic inference methods based on the IBD model, including methods within the ABC framework or likelihood based methods (Bertorelle et al., 2010; Rousset & Leblois, 2012). To investigate landscape genetics effects, comparative approaches involving multiple landscapes or gradients of landscape composition are recommended (Goldberg & Waits, 2010). These approaches should be particularly appropriate to infer and compare demographic parameter estimates in contrasted landscapes, which can provide further information for conservation efforts (Beebee & Griffiths, 2005).

In this study, we investigated the effect of landscape composition and land conversion on genetic diversity, genetic structure and demographic parameters in the marbled newt, *Triturus marmoratus* (Latreille 1800). This endangered species (listed on Annex III of the Bern Convention and Annex IV of the European Habitats Directive) is distributed over the north Iberian Peninsula and western France. It prefers areas with bushes, hedgerows and trees, and avoids pastures and open areas (Jehle, 2000; Jehle & Arntzen, 2000; Trochet et al., 2017). In contrast to those sampled in agricultural lands, newts in woodlands moves greater distances (Trochet et al., 2017). Therefore, *T. marmoratus* might be particularly impacted by agricultural intensification. More specifically, we hypothesize a negative influence of habitat loss with the conversion of permanent grasslands and meadows to arable crops on *T. marmoratus* population size and connectivity as recently suggested (Costanzi et al., 2018). Determining which landscape features influence *T. marmoratus* population genetic patterns would help to understand population connectivity and to identify key habitats for preserving genetic variation.

We studied *T. marmoratus* in a 6,000 km^2 agricultural landscape in Western France. The study area is heterogeneous with preserved hedgerow farmland landscape with permanent grasslands and meadows (hereafter grasslands) in the northern part, while recently opened landscape dedicated to arable crops (mostly cereals) dominate the southern part (see Figure 1). We sampled 67 ponds distributed in these two contexts. Previous studies showed that pond disappearance was associated with a decrease in grasslands and an increase in arable crop land covers (Curado et al., 2011). In the study area, ponds were initially created to water the livestock and are thus associated to grasslands. When grasslands are converted to arable crops ponds are usually drained to increase cultivated land (Arntzen et al., 2017). We characterized landscape changes (hedgerow loss) over the last 60 years in the study area using historical landscape data and analysed genetic data collected in order to address the following goals. (i) To identify the effect of landscape composition at various spatial scales on within pond genetic diversity. We expected genetic diversity to increase with pond density and forested land cover (woodlands and hedgerows) in the local landscape and to decrease with arable crop land cover. (ii) Characterize population genetic structure and test whether it was homogeneous across the study area. In particular, we expected the northern and southern parts of the study area to show contrasted levels of genetic structuring with stronger IBD in the southern part. (iii) Identify landscape features that affect genetic differentiation. We expected roads, arable crops and urbanized land cover to negatively affect gene flow while the opposite was expected for pond density and forested land cover. (iv) Infer population demographic parameters, including effective population density (equivalent to effective population size in the continuous population model), dispersal rate and the shape of dispersal distribution between contrasted landscape contexts. Enhanced dispersal and larger population effective densities were expected in more connected areas.

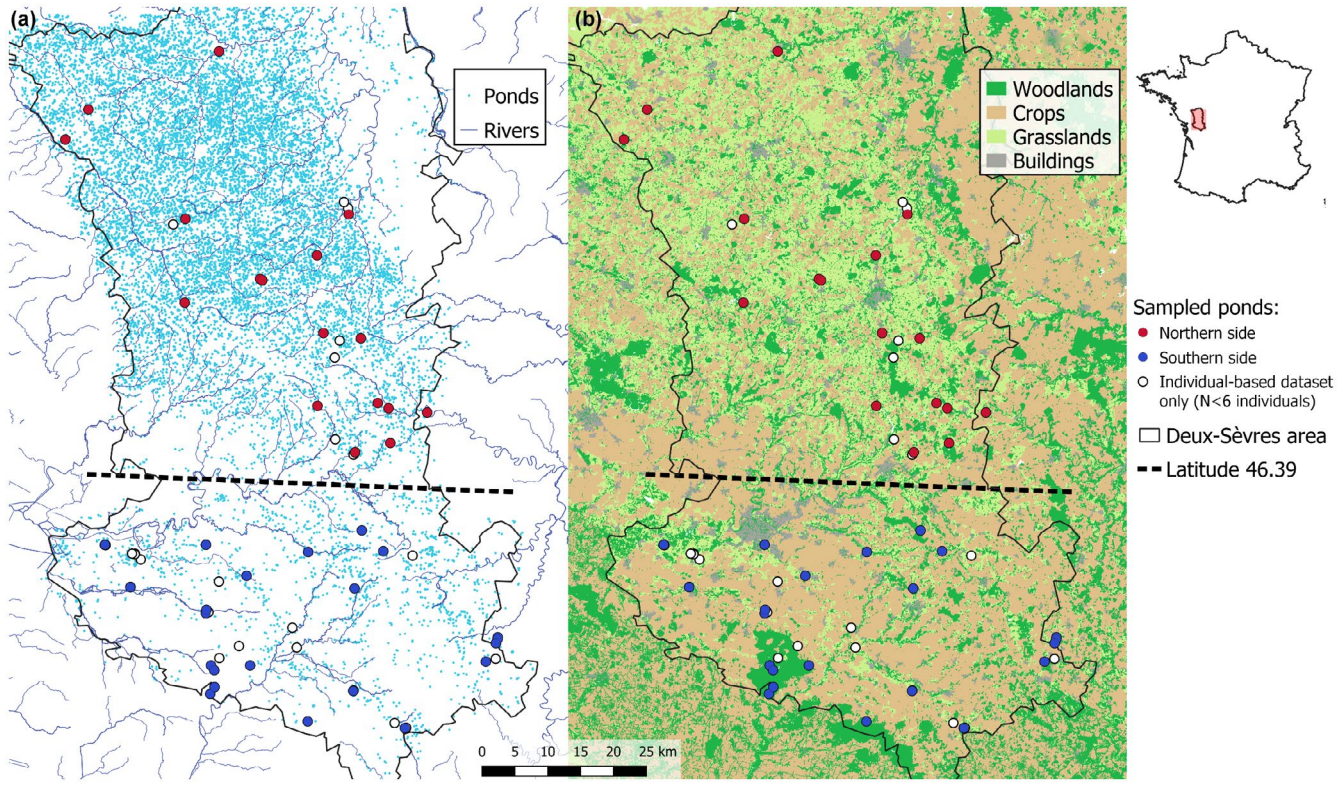

FIGURE 1 Map of sampling area in the Deux-Sèvres department with (a) rivers network and ponds and (b) main land use categories according to CORINE Land Cover 2012 and BDTOPO 2015. Dots indicate sampling locations with red and blue colours referring to the population data set and the northern and southern parts of the study area, respectively

2 | MATERIALS AND METHODS

2.1 | Study area and sample collection

The study area covers the entire Deux-Sèvres department (6,000 km²), in Western France. It is a farmland area with the northern part dominated by preserved hedged farmland with permanent grasslands and meadows dedicated to livestock farming while the southern part is more open and mostly dedicated to intensive cereal crops production (Figure 1). Sampling occurred during the newt breeding season from February to May, in 2014 and 2015. A total of 734 individuals from 67 ponds were sampled (15 ponds in 2014 and 52 in 2015). At night, individuals were localized using a flashlight and captured using a dip net. Newts were then put into a bucket half filled with water. DNA samples were obtained by rubbing the bottom of the mouth, cheeks and tongue with cotton swab to collect epithelial cells (Pidancier et al., 2003). Cotton swabs were stored in an alcohol solution (solution EDTA buffer: Ethylene Diamine Tetra-Acetic). All individuals were released once the pond sampling was completed.

2.2 | Genetic data collection

DNA extraction from cotton swabs was performed using Macherey-Nagel genomic DNA extraction kit. We used a set of 10 microsatellite markers (see Costanzi et al., 2018), including nine developed specifically for the marbled newt (Costanzi et al., 2015) and one (Tcri27) originally developed for the crested newt (Krupa et al., 2002). Details of microsatellites amplification are presented in Appendix S1 in the Supporting Information. Alleles were scored using GENEMAPPER v 4.0 (Applied Biosystems) and checked manually.

There was a relatively high amount of missing data (13.41% over the 734 samples) related to low yield of DNA extraction in some pond samples, probably due to low quality DNA conservation. A total of 612 individuals from 64 ponds were successfully genotyped at eight or more loci. From this global data set, ponds less than 150 m apart were pooled and ponds with less than seven individuals were removed. The final data set included 550 individuals from 39 ponds (Table S1.2). For sake of consistency, node-based and matrix-based landscape genetics analyses as well as demographic inferences were all performed on this population-based data set.

2.3 | Genetic diversity

We tested linkage disequilibrium between all pairs of loci on the global 612 individuals data set using GENEPOP4.2 (Rousset, 2008). Tests for deviations from Hardy–Weinberg equilibrium for each locus and linkage equilibria for each locus pair were performed using GENEPOP globally, and in each of the 39 ponds. Significance of tests was assessed following a false discovery rate correction for multiple tests (Benjamini & Hochberg, 1995) with a nominal significance level of 5%. Observed and expected heterozygosities (H_o and H_e) and Weir and Cockerham's estimate of F_{IS} (Weir & Cockerham, 1984) were calculated using GENEPOP globally and for each pond. Using the rarefaction procedure implemented in FSTAT 2.9.3.2 (El Mousadik & Petit, 1996; Goudet, 2012), we calculated allelic richness corrected for sample size (A_r) for each locus in all 39 ponds.

2.4 | Landscape features and changes overtime

We characterized the landscape over the study area using CORINE Land Cover 2012 (resolution 1/100,000) and BDTOPO 2015 (®IGN) in QGIS3.0.1 (Qgis Development Team, 2018). Eight landscape features were selected based on their functional role for *T. marmoratus*:

(i) The area of five classes of land cover were extracted, including woodlands, which represents the terrestrial habitat of *T. marmoratus*, hedgerows and grasslands, which might constitute alternative terrestrial habitats and favorable habitats for *T. marmoratus* movement, and arable crops and urbanized land covers, which are expected to constitute unfavourable features for *T. marmoratus*.

(ii) Rivers and roads (linear features) were extracted, as they can constitute barriers to movements of some amphibians.

(iii) Ponds, which represents the breeding habitat of *T. marmoratus* were exhaustively inventoried in the Deux-Sèvres department (N = 17,400) and manually georeferenced for the purpose of this study.

In order to evaluate habitat change since 1950 we also characterized past and present landscape structure based on hedgerows linear digitalization from aerial photos taken in 1950 and 2015, hereafter $Hedgerow_{1950}$ and $Hedgerow_{2015}$. This historical analysis was focused on the landscape surrounding the 39 ponds. We manually digitalized linear distance of hedgerows within 2 km buffers around the ponds. Our approach was restricted to hedgerows because some parameters (such as pond density) were not available on historical images. Yet, hedgerows are integrative of landscape quality and correlated with pond density and meadows. Indeed, Pearson's correlation coefficient calculated over the entire Deux-Sèvres department with a 5 km grid indicated strong correlation between hedgerow and pond density and between hedgerows and meadows (Pearson's r = 0.85, p-value < .001 and Pearson's r = 0.91, p < .001, respectively).

2.5 | Effects of local landscape composition on genetic diversity

Local genetic diversity depends on both population size and landscape connectivity. We investigated which landscape features affected the genetic diversity of *T. marmoratus*. Within circular buffers centred on the 39 ponds, we calculated the area of four different classes of land cover (woodlands, grasslands, arable crops and urbanized), the length of roads and rivers, and the number of ponds. Considering hedgerows, we used the linear data digitalized

in 2 km buffers around the ponds ($Hedgerow_{2015}$) as it was more precise compared to the land cover database and allowed us to include historical data to derive an index of landscape intensification ($Intensification_{index}$) as 1-($Hedgerow_{2015}$ / $Hedgerow_{1950}$). $Intensification_{index}$ was fixed to 0 for ponds located in large forest patches, where woodland represented >75% of the total buffers area, because there are no hedgerows in those patches. There was no $intensification_{index}$ <0 (i.e., no increase in hedgerow from 1950 to 2015 in any buffers). To explore the scale at which landscape features influence genetic diversity, we calculated landscape composition in buffer of different radius (500, 1,000 and 2,000 m). The maximal distance was set to 2,000 m to avoid artificially large correlation due to overlapping of buffers and because buffers tended to be out of the mapped area at larger scales. To address possible confounding effects due to correlations between landscape features, we computed pairwise Pearson's correlations between landscape features for each buffer radius. Pairs of landscape features with Pearson's correlation above the 0.70 threshold were considered highly correlated (Dormann et al., 2013). We thus excluded $Hedgerow_{2015}$ from the set of landscape descriptors for all models, grasslands from the set of landscape descriptors for 500 model, and $Intensification_{index}$ from the set of landscape descriptors for 1,000 m model (see details on correlations in Appendix S2, Supporting Information). A_r and H_o, calculated for each locus and each pond 39 ponds, were alternatively used as response variable. A_r is expected to be more sensitive to recent reductions in population size since it is more impacted by loss of rare allele than H_o (Schwartz et al., 2007).

We used linear mixed effect models to quantify the contribution of landscape features on genetic diversity using the lmer function implemented in the LME4 package (Bates et al., 2015) in R 4.0.2 (R Development Core Team, 2014). We treated selected landscape features as fixed factors and they were *z*-transformed (i.e., standardized to zero mean and a standard deviation of one). Locus identity was included as a random intercept to account for differences in allelic diversity among loci. First, we compared R^2 of the full models (interactions were not considered) at 500, 1,000 and 2,000 m to assess the best spatial scale. Second, for the best spatial scale, we ran models with all possible combinations of explanatory variables using dredge function implemented in the MuMIn package (Barton, 2015). Models were fitted with maximum likelihood to compute reliable Akaike information criterion (AIC) scores, and ranked according to AICc values (the small-sample-size corrected version of AIC). To take into account uncertainty in model selection we then used model averaging on models with Delta AICc values <4 to get estimates of final parameters. Models were validated a posteriori by checking plots of residuals. We also checked for autocorrelation in the model residuals.

2.6 | Regional genetic structure

To assess the extent to which ponds might be isolated from each other and form separate populations, the population genetic structure was investigated by two complementary approaches. (i) We used the Bayesian clustering method implemented in STRUCTURE Version 2.2 (Pritchard et al., 2000), with the admixture model and correlated allele frequencies (Falush et al., 2003) to determine the number of genetic clusters (K). Ten replicate runs of 2×10^6 Markov chain Monte Carlo (MCMC) iterations, after an initial burnin period of 5×10^5 iterations, were performed for values of K ranging from 1 to 10. Results were summarized using the standard pipeline on the CLUMPAK web server (Kopelman et al., 2015). The most likely number of clusters (K) was explored using the estimated logarithm of likelihood (LnP[D]) and the Evanno et al. (2005) ΔK method that finds the point of greatest change in the distribution of LnP(D) with STRUCTURE HARVESTER Version 0.6.92 (Earl & vonHoldt, 2012). We used all 612 individuals for this individual-based analysis. (ii) The level of pairwise genetic differentiation between the 39 ponds was quantified by pairwise F_{ST} (Weir & Cockerham, 1984), and tested using the exact probability test for population differentiation implemented in GENEPOP. IBD was then analysed by regressing pairwise estimates of F_{ST} /(1−F_{ST}) against the logarithm of the geographical distances (Rousset, 1997), and tested using a Mantel test (10,000 permutations). To visualize IBD and whether it was homogeneous across our study area, we used LocalDiff 1.5 (Duforet-Frebourg & Blum, 2014), a method that infers local genetic differentiation based on Bayesian kriging. The pairwise F_{ST}/(1−F_{ST}) values between the 39 ponds was used as input measure of pairwise dissimilarity. The method requires fictive neighbouring populations to be introduced at the vicinity of sampling sites (i.e., short distance compared to the dimension of the region under study), as a means to provide measures of local genetic differentiation that are comparable between sampling sites. Hence, four fictive neighbouring populations were introduced at 2 km from the 39 ponds. The output of LocalDiff generates a minimum convex polygon that encompasses all sampling sites, where warmer colors represent areas with stronger local genetic differentiation. We used Pearson's correlations to test the relationship between estimates of genetic diversity (A_r and H_o) within ponds and estimates of local genetic differentiation computed with LocalDiff.

2.7 | Effects of landscape composition on genetic structure

To investigate the effect of landscape features on spatial genetic structure, we performed multiple regression of distance matrices (MRDM) (Legendre et al., 1994) and multiple matrix regression with randomization MMRR (Wang, 2013). Pairwise genetic distances were computed between the 39 ponds using F_{ST}/(1−F_{ST}) as genetic distance. To compute pairwise resistance distances a specific layer was created for each landscape feature. We overlaid a 250 m grid on these raster data, and calculated the percentage per grid cell of each categorical feature (Woodlands, Hedgerows, Arable crops, Urbanized, and Grasslands), attributed a value of one for each grid cell intersected by linear features (roads and rivers) and calculated the number of ponds in each cell of the grid. To allow comparison

among landscape features, these layers were finally rescaled to range from 1 to 100 and used in CIRCUITSCAPE 4.0.5 (Mcrae et al., 2013) to compute pairwise resistance distances between ponds.

MRDMs are similar to classical multiple ordinary least-square regressions, except that the significance of model fit and beta weights (β) is assessed through permutations (Legendre et al., 1994). Beta weights could be heavily impacted by multicollinearity among landscape variables (Ray-Mukherjee et al., 2014), so we used a commonality analysis (CA) (Prunier et al., 2015; Ray-Mukherjee et al., 2014) to dissect the complexity of landscape features relative contribution to the model fit. CA is a variance-partitioning procedure that disentangles the individual *vs*. shared contribution of each predictor to R^2. This procedure is particularly useful when the predictors are themselves correlated (Prunier et al., 2015). Pearson's correlations among pairwise resistance distances computed for each landscape feature ranged from 0.02 to 0.69, while variance inflation factor (VIF) ranged from 1.62 to 2.96 (Table S4 in Supporting Information). All pairwise resistance distances were kept in the model as we considered Pearson's correlations and VIF to be high above thresholds of 0.7 and 10, respectively (Dormann et al., 2013; Zuur et al., 2009). To determine the contribution of pairwise resistance distances relatively to IBD, pairwise geographic distance was also included in the model. CA-MRDM was run using the R packages ECODIST (Goslee & Urban, 2007) and YHAT (Nimon et al., 2013) following the CAonDM script provided by Prunier et al. (2015). Significant levels were assessed with 10,000 permutations after sequential Bonferroni correction (Holm, 1979). Commonalities and 95% confidence intervals were computed using a bootstrap procedure with 1,000 replicates based on a random selection of 10% of samples (out of 39 populations) without replacement (Prunier et al., 2015).

Alternatively, we ran MMRR according to Wang (2013). MMRR was implemented in R using the package PopGenReport (Adamack & Gruber, 2014). We used pairwise geographic and the eight pairwise resistance distances as the explanatory variables and 999 permutations to assess the additive effect of both independent factors (geographical distance and landscape features). All matrices were standardized using the "scale" function implemented in R before running the MMRR. Finally, we tested each resistance distances and geographic distance in eight separate models.

2.8 | Inferences on demographic parameters

Spatial variation in IBD was explored by the maximum likelihood method implemented in MIGRAINE that infers model parameters using importance sampling algorithms (De Iorio et al., 2005), extended to consider IBD as a model for population structure (Rousset & Leblois, 2007, 2012). Based on LOCALDIFF results, ponds from the northern and southern parts of the study area were analysed separately to infer area specific demographic parameters. Our specific aim here, was to assess whether differences in IBD between these two parts of the study area result from differences in effective population size and/or differences in dispersal. We excluded the three northermost ponds to get parameter estimates from a more homogeneous landscape and with a continuous distribution of sampled ponds. Based on a geometric distribution for dispersal and a K allele model for mutation, MIGRAINE provides point estimates, 95% coverage confidence intervals (CIs) and two-dimensional parameter likelihood profiles for several parameters: (i) The scaled local population size $\theta = 2 \times N_{genes} \times \mu$, where N_{genes} is the local population size expressed in number of genes and μ the mutation rate per locus per generation. (ii) The scaled emigration rate per generation: $\gamma = 2 \times N_{genes} \times m$, where m is the total emigration rate per generation for a local population. (iii) The parameter of the geometric distribution of dispersal (g). (iv) The neighbourhood size, $Nb = 4\pi \times N \times \sigma^2$, where N is the effective population size and σ^2 the mean squared parent–offspring dispersal distance. All MIGRAINE runs were performed under a two-dimensional model of IBD. The spatial binning of samples was performed using MIGRAINE-GUI and resulted in 16 × 10 and 20 × 11 bins for the northern and southern parts of the study area, respectively. Bin size, 3,275 × 3,275 m, was set constant over the two analyses to facilitate results interpretation. We used the following computing parameters: 2,000 trees, 100 points and three iterations. We translated the parameters inferred from MIGRAINE into effective population size using a mutation rate range commonly used for microsatellites: $1 \times 10^{-4}(5 \times 10^{-3} - 5 \times 10^{-5})$ (Sun et al., 2012).

3 | RESULTS

3.1 | Landscape characteristics and changes through time

Pond density was almost five times higher in the northern part with 3.79 ponds/km² compared to 0.8 ponds/km² in the southern part of the study area (Figure 1). Consistently, arable crops represented 41.16% and 63.88% and meadows 48.59% and 21.49% of land cover in the northern and southern parts of the study area, respectively. Based on the digitalized hedgerow in 2 km buffers around the 39 ponds, we found a significant difference in the proportion of hedgerow loss between the northern and southern parts of the study area (two sample t test, p- < .001). On average loss was 34% and 53% in the northern and southern parts of the study area, respectively.

3.2 | Genetic markers and diversity

The number of alleles per locus ranged from 4 to 16 in the global data set (Table S1.1), with an average value of 8.5. Expected heterozygosity for each locus ranged from 0.047 to 0.895, with an average value of 0.576. Observed heterozygosity ranged from 0.046 to 0.813, with an average value of 0.486. There was a marginally significant overall heterozygosity deficit in the global data set (p-value = .056, F_{IS} = 0.127) that could be related to the presence of spatial structure ("Wahlund effect"). Indeed, only one of the 39

ponds with at least seven individuals showed significant departure from HWE (Table S1.2). After FDR correction, there was no pair of loci showing significant LD in the global data set.

3.3 | Effect of landscape composition on ponds genetic diversity

Within pond allelic richness corrected for sample size (A_r) ranged from 2.684 to 3.773 (Table S1.2). R^2 comparison among linear mixed models based on landscape descriptors calculated in 500, 1,000 and 2,000 m buffers indicated 1,000 m was the best spatial scale to explain A_r (marginal R^2 = 0.005, 0.007 and 0.005 for 500, 1,000 and 2,000 m buffers, respectively). Several plausible best models (i.e., with delta AICc < 4) were identified at 1,000 m (Table S2.4). Model averaging indicated that pond density in the surrounding landscape had a significant and positive effect on within pond A_r while the negative effect of arable crop land cover was only marginal (Table 1 and Figure 2). Consistently, models at 500 and 2,000 m also reported a significant and positive effect of the amount of ponds in the surrounding landscape.

Regarding H_o, R^2 comparison among linear mixed mode indicated 500 m was the best spatial scale (marginal R^2 = 0.006, 0.001 and 0.004 for 500, 1,000 and 2,000 m buffers, respectively). However, no significant effect of landscape composition on H_o was found at any spatial scale (Table S2.5).

3.4 | Regional pattern of genetic structure

The most likely value of *K* from the STRUCTURE analysis based on the method of Evanno et al. (2005) was two (Table S3). Consistently, the estimated logarithm of likelihood for data was highest for K = 2. However, for most individuals, the estimated membership coefficients in each cluster Q was low (i.e., >60% individuals had Q < 0.7) and inspection of STRUCTURE barplot was more consistent with an IBD pattern than to the presence of two or more genetic clusters (Figure S1). Indeed, a pattern of IBD was supported by the positive relationship between $F_{ST}/(1-F_{ST})$ and the logarithm of geographic distance (Figure 3, Slope = 0.027, 95% CI: 0.018–0.037, Mantel *test* $p < .001$) over the 741 pairs of ponds. The average F_{ST} value between ponds was 0.066 and for 621 pairs (84%) genetic differentiation was significant. The analysis performed with LOCALDIFF indicated a heterogeneous pattern of IBD across the study area. The southern part was characterized by strongest values of local genetic differentiation, whereas smaller local genetic differentiation was found in the northern part of the study area (Figure 4). We measured a significant negative correlation between allelic richness (Ar) and estimates of local genetic differentiation computed with LOCALDIFF (cor = −0.59, $p < .01$), while the correlation was non-significant when H_o (cor = −0.19, $p = .24$) was used as a measure of genetic diversity (Figure 5).

TABLE 1 Results of model averaging on models of allelic richness with delta AIC <4 for the spatial scale with the highest R^2 (i.e., 1,000 m buffer size)

Landscape variable	Estimate	Std. Error	z-value	*p*-value
Arable crops	−0.065	0.037	1.750	.080
Ponds	**0.107**	**0.035**	**3.021**	**.003**
Woodlands	−0.026	0.060	0.441	.659
Rivers	0.021	0.034	0.604	.546
Buildings	0.020	0.036	0.554	.579
Grasslands	0.027	0.052	0.511	.610
Roads	−0.008	0.039	0.197	.844

Note: Estimates and *p*-values are presented for each landscape descriptor tested. Significant relations are in bold.

3.5 | Effect of landscape composition on genetic structure

The MRDM was significant and explained 35.7% of the variance in $F_{ST}/(1-F_{ST})$ (Table 2). After sequential Bonferroni corrections, only arable crop land cover had a significant positive effect on genetic differentiation (β_{crops} = 0.33, $p = .016$). $F_{ST}/(1-F_{ST})$ increased by 0.33 standard deviations with a one standard deviation change in arable crops resistance distance, all other predictors being held constant. Commonality coefficients showed that arable crop land cover uniquely contributed 8.2% of the total variance in $F_{ST}/(1-F_{ST})$ and to 22.8% of the 35.7% of the variance explained by the regression model. Unique contributions of other landscape features were negligible or counterbalanced by their common contribution with other predictors (indicative of classical suppressor effect according to Prunier et al. 2017) (Table 2).

The MMRR model including geographic distance and all resistance distances explained nearly 30% of the variability in genetic differentiation (R^2 = 0.291, $p < .001$). It revealed that arable crop land cover was the only landscape feature significantly influencing genetic differentiation (β_{crops} = 0.009, $p = .033$, Table 2 and Figure S2).

3.6 | Demographic inferences

Outputs from the MIGRAINE software confirmed a clear contrast in demographic parameters between the northern and southern parts of the study area (Table 3). N_b was found to be significantly higher in the northern part (557.5, 95% CI: 206.8–22134) compared to the southern part (68.77, 95% CI: 48.34–107.2) of the study area as was *g*, the parameter of the geometric distribution describing dispersal (Table 3). Using 10^{-4} as mutation rate for microsatellites it was possible to derive a theoretical area specific effective population density (D_e) per bin and migration rate among bins (*m*) (see Table 3 for figures) and draw a theoretical dispersal kernel (Figure 6). The latter

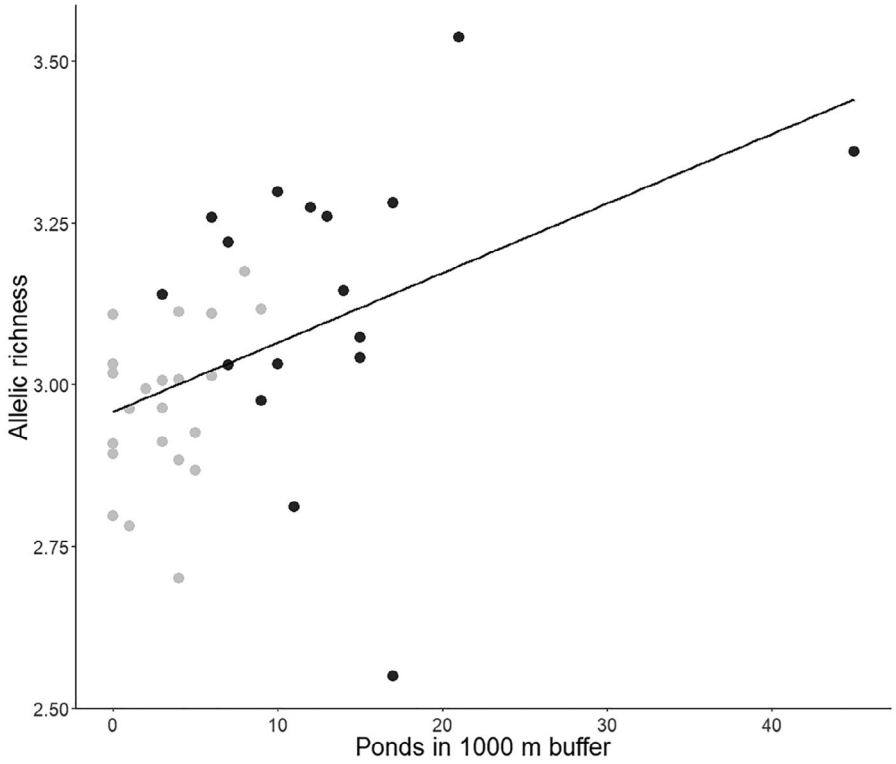

FIGURE 2 Relation between Allelic richness (A_r) and pond density in a 1,000 m buffer around the 39 ponds. Black dots and grey dots represent ponds from the northern and southern sides of the study area, respectively

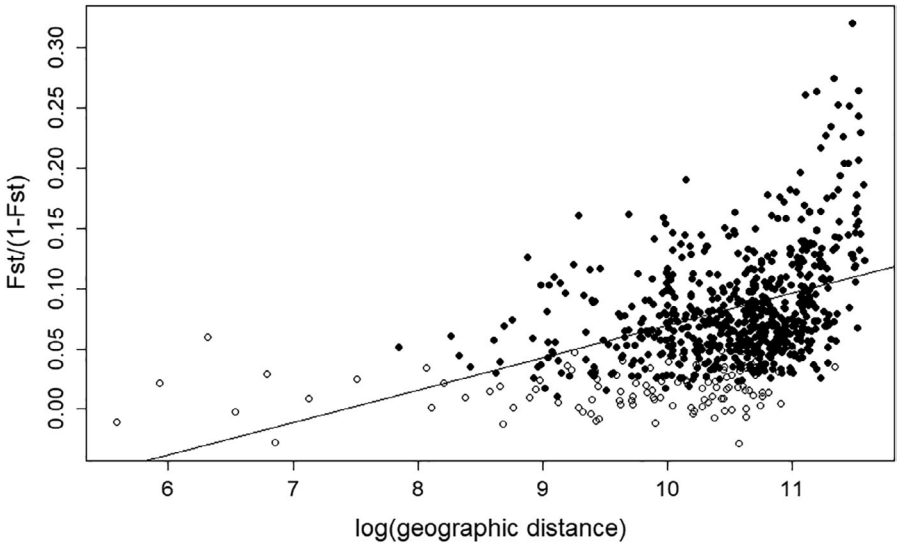

FIGURE 3 Correlation between pairwise genetic differentiation (F_{ST}/[1−F_{ST}]) and logarithm of the geographic distance between pairs of ponds. Filled dots indicate pairs of ponds that were significantly different (GENEPOP exact probability test for population differentiation)

indicated higher dispersal rate and distance in the northern part of the study area.

4 | DISCUSSION

In this study, we identified landscape effects on genetic structure, genetic diversity and gene flow in *T. marmoratus* an amphibian species with supposed limited mobility. Our results support the hypothesis that land conversion (i.e., loss of habitats and their connectivity) that occurred in the last 60 years impacted genetic diversity and functional connectivity. In addition, we were able to conduct demographic inferences to characterize effective population density and dispersal according to landscape context. Both demographic parameters varied with landscape composition.

4.1 | Population genetic structure

In Western France, at a local spatial scale (up to 6.5 km) previous genetic studies on *T. marmoratus* reported distinct genetic clusters with STRUCTURE, an average pairwise F_{ST} of 0.11 (range: 0.007–0.303) and no significant IBD (Jehle, Burke et al., 2005; Jehle, Wilson et al., 2005). In our study, at distance <6.5 km, the average pairwise F_{ST} was only 0.025 (range: −0.03 to 0.07) with nine out of 25 pairs of ponds significantly differentiated. A single population exhibiting IBD was

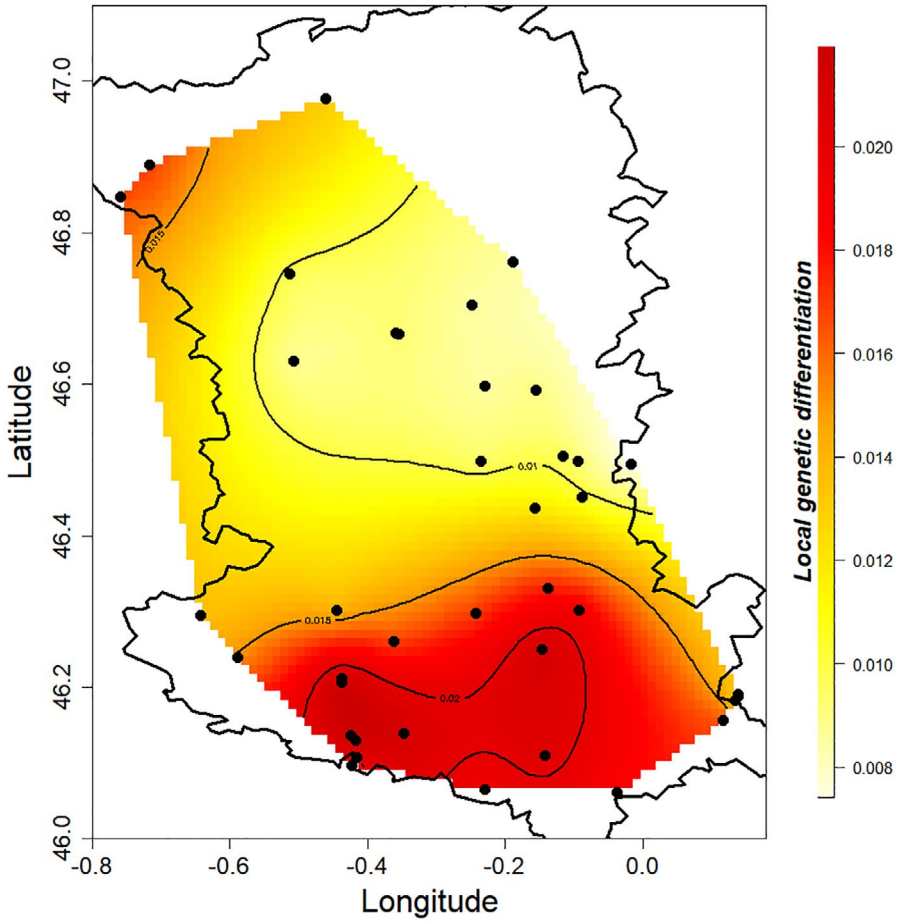

FIGURE 4 Map of local genetic differentiation inferred by the Bayesian kriging method implemented in LocalDiff. Shape represent the minimum convex polygon that encompasses the 39 ponds. Measures of local genetic differentiation correspond to $F_{ST}/[1-F_{ST}]$ calculated between the sampled ponds and four fictive neighbouring populations, not shown, located at 2 km. Warmer colours indicates higher local genetic differentiation, that is, higher genetic distances between sampled ponds and interpolated fictive neighbours

found, and genetic structure was not affected by rivers or motorways crossing the study area, similar to other newt studies (Costanzi et al., 2018; Luqman et al., 2018; Prunier et al., 2013). Based on 11 *T. marmoratus* populations sampled across Western France, Costanzi et al. (2018) identified strong genetic structure at a large scale (>100 km), including distinct genetic clusters in the area of our study. Their result is probably due to IBD which might create spurious genetic clusters when geographical sampling is clumped (Blair et al., 2012; Frantz et al., 2009). This supports that continuous distribution of samples with individual-based sampling is the best strategy to uncover unbiased spatial genetic structure. Our results also side with the conclusion of Smith and Green (2005) that although amphibians are predominantly philopatric with poor dispersal capacities, they could move distances much greater than anticipated. Another relevant finding of our study is the clear heterogeneous IBD pattern across the study area as evidences by the LocalDiff analysis. Results from our landscape genetics analyses strongly suggest this contrast might be related to differences in landscape composition between the northern and southern parts of the study area as previously suggested (Costanzi et al., 2018).

4.2 | Landscape influence on genetic diversity and genetic structure

Genetic diversity is affected by effective population size and connectivity (Flavenot et al., 2015). It is an important parameter in conservation genetics since reduced genetic diversity could translate to lower fitness and subsequently vortex of extinction (Fagan & Holmes, 2006). A review analysed 19 studies that directly quantified genetic diversity - fitness relationships in amphibians, among which 15 provided evidence that levels of genetic diversity affected important traits such as growth or survival (Allentoft & O'Brien, 2010). Pond density was the only landscape feature affecting local genetic diversity in our study with a positive influence on allelic richness. Interestingly, we did not find any effect of woodlands land cover, the main terrestrial habitat of the species. This result emphasizes the importance of the breeding habitat in newt life cycle and suggests larger effective population density with increased pond density. Higher pond density may also facilitate dispersal and resulting gene flow, as it is easier for a dispersing newt to encounter a new pond. This result might be related

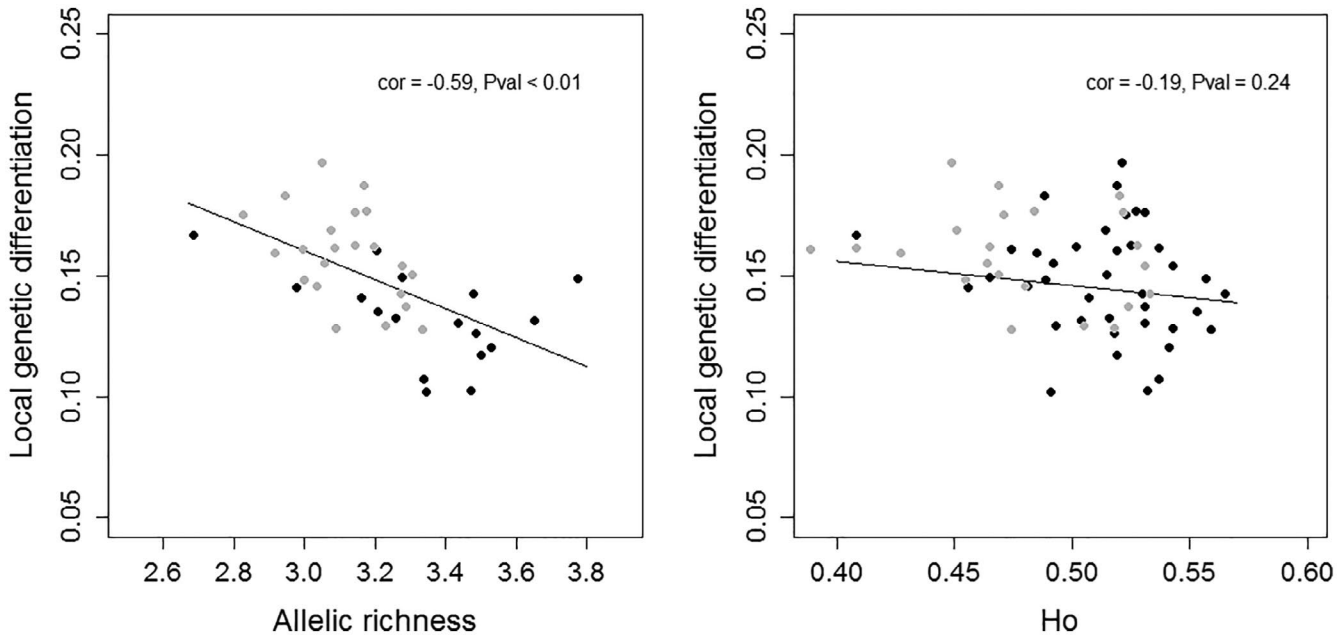

FIGURE 5 Relationship between genetic diversity and estimates of local genetic differentiation from LocalDiff for the 39 ponds (a) with allelic richness and (b) with observed heterozygosity as measures of genetic diversity. Black and grey colours indicate the northern and southern parts of the study area, respectively

TABLE 2 MRDM and MMRR results and additional parameters derived from commonality analysis: model fit index (multivariate R^2; ***p-value <.001), beta weights β and p-values and unique, common and total contributions of landscape variables to the variance in $F_{ST}/(1-F_{ST})$

	MRDM						MMRR		
Landscape feature	**multiple R^2**	**B**	***p*-value**	**Unique**	**Common**	**Total**	**R^2**	**β**	***p*-value**
Geographic distance	0.38**	**0.398**	**.043**	**0.024**	**0.252**	**0.275**	0.29***	0.012	.081
Rivers		−0.034	.791	0.001	0.004	0.005		0.000	.940
Grasslands		0.205	.213	0.015	−0.015	0		0.010	.162
Arable crops		**0.327**	**.016**	**0.054**	**0.142**	**0.196**		**0.012**	**.024**
Woodlands		−0.055	.696	0.002	−0.001	0.001		0.000	.943
Roads		0.114	.49	0.005	0.153	0.158		0.008	.409
Hedgerows		−0.289	.106	0.027	−0.022	0.006		−0.010	.205
Ponds		−0.069	.727	0.001	0.152	0.153		−0.001	.899
Urbanized		−0.028	.853	0	0.116	0.116		−0.005	.575

Note: Significant contributions are in bold.

to reduction of pond density associated with intensification that affected both parts of the study area, though to a higher degree in the south. Both MRDM and MMRR identified arable crop land cover as the only landscape variable affecting significantly gene flow, probably through reduced functional connectivity. It is also supported by the LocalDiff analysis that showed stronger IBD in the southern part of the study area. It is interesting to note that node- and matrix-based landscape genetics analyses provided complementary results. In the node-based analysis, we only considered the local landscape (i.e., up to 2 km² buffers around sampled ponds). The landscape feature selected in the model, pond density, was more representative of local landscape quality in terms of habitat availability. For the matrix-based analyses, we considered the full landscape matrix and the landscape feature selected in MRDM and MMRR models, arable crop land cover, was more indicative of reduced landscape permeability between habitat patches. However, since gene flow depends on both effective population density and dispersal, population decline in the southern side of the study area could also explain the negative effect of arable crop land cover on genetic differentiation found with MRDM and MMRR. The negative relationship between genetic diversity and estimates of local genetic differentiation from LocalDiff supports the hypothesis that genetic drift is a predominant microevolutionary process driving genetic differentiation (Coleman et al., 2013). Altogether, our study exemplifies the difficulty to disentangle the influence of effective population density

TABLE 3 Inferences on demographic parameters by the software MIGRAINE. Point estimate values with data range outputted in brackets are shown. Scaled local population size (2Nμ), scaled emigration rate (2Nm), parameter of the geometric distribution of dispersal (g) and neighbourhood size (Nb) are indicated

Parameters	South	North
2Nμ	0.011 (0.008–0.014)	0.016 (0.012–0.022)
2Nm	25.4 (18.68–38.78)	70.85 (47.12–141.5)
g	0.15 (0.003–0.31)	0.41 (0.031–1)
Nb	68.77 (48.34–107.2)	557.5 (206.8–22134)
De	27 (5.4–54)	40.8 (8.15–81.5)
m	0.235 (0.118–1)	0.435 (0.217–1)

Note: De, the effective density per 3,250 × 3,250 m bin and m, the migration rate among bins were estimated using 10^{-4} (5×10^{-4} – 5×10^{-5}) as value and range for the mutation rate.

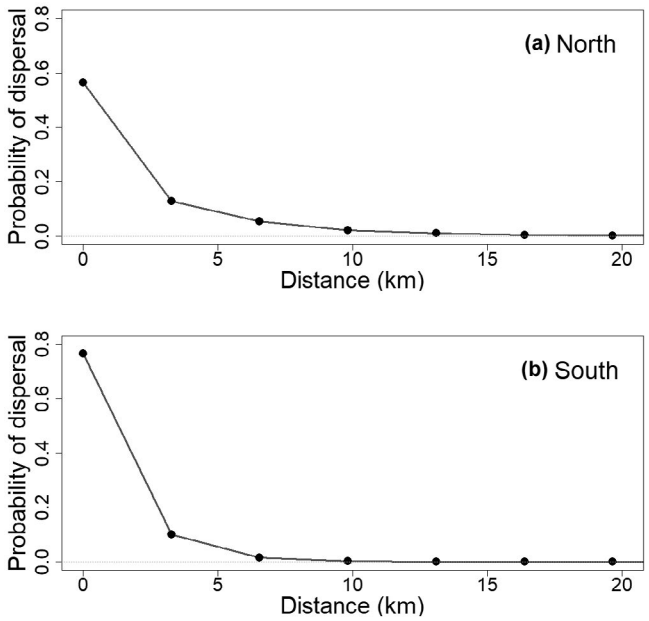

FIGURE 6 Estimates of dispersal kernel based on MIGRAINE in the northern (a) and southern (b) parts of the study area. Migration rate was estimated using a mutation rate of 10^{-4} for microsatellite markers

and dispersal on gene flow from patterns of genetic diversity and genetic structure. More specifically, it is challenging to assess whether landscape structure and land conversion affect natural populations through reduction in effective population size or dispersal.

4.3 | Demographic inferences on dispersal and population effective density

Several lines of evidence support a recent demographic decline in the southern part of the study area. Landscape influence on local genetic diversity was only found for allelic richness, while it was not significant for heterozygosity that is less affected by recent demographic changes (Schwartz et al., 2007). Consistently, correlation between estimates of local genetic differentiation from LOCALDIFF and genetic diversity were significant for allelic richness and not for heterozygosity, suggesting *T. marmoratus* populations are declining to a greater degree in the southern side of the study area. This supports the hypothesis that landscape genetic effects found in the present study reflect recent demographic changes, predominantly in the southern part of the study area, where land conversion for intensive arable crops production induced substantial *T. marmoratus* aquatic and terrestrial habitat loss (we estimated a 53% decrease in hedgerows since 1950). Demographic inferences conducted with MIGRAINE were consistent with this hypothesis and indicated contrasted values between the two parts of the study area, in line with previous results. First, N_b, the neighbourhood size, was significantly lower in the south, with N_b = 68.77 and 557.5 in the southern and northern parts of the study area, respectively. While N_b depends on both effective population size and dispersal (Neel et al., 2013), MIGRAINE analysis also allowed us to infer area-specific effective population density and dispersal separately. Although not significantly different, MIGRAINE estimate of area-specific effective population density was almost 35% lower in the south, with 2.56 and 3.86 individuals per km² in the southern and northern parts of the study area, respectively. This contrast is consistent with the positive effect of local pond density on genetic diversity, since pond density was five times lower in the southern part of the study area. Overall, our effective population density estimates might seem low. However, in amphibians, the effective population size (N_e) is usually much lower than the census population size (N) (see Table 4 in Schmeller & Merila, 2007). Indeed, *T. marmoratus Ne/N* ratio was estimated to range from 0.05 to 0.65 in five ponds of Western France (Jehle, Wilson, et al., 2005). In the same study, the effective population size was estimated in the order of 100–200 individuals in a 26.25 km² study site (~3.8 to 7.6 individuals per km²), thus consistent with our effective population density estimate in the northern part of the study area. Interestingly, landscape structure in the northern part of the study area and in the Jehle, Wilson, et al., 2005) study area were similar. Finally, area-specific MIGRAINE estimates of dispersal rate m and of the parameter of the dispersal function g also showed contrasted situation between the two parts of the study area. Dispersal rate was 46% lower in the south, and the significant difference of area-specific g estimates indicated shorter dispersal range in this less connected part of the study area, consistently with a previous radiotracking study (Trochet et al., 2017). Our landscape scale estimates of dispersal rates and distances might appear much higher than was previously thought (Jehle et al., 2005). These authors concluded that dispersers could not travel distances >1 km but their estimates of pond specific immigration rate were only based on three closely located ponds as potential sources. Our analysis based on the IBD model and the sampling of more than 39 ponds over a large area appear more robust to infer landscape-scale dispersal distances and rates. In their review paper on amphibians dispersal, Smith & Green (2005) concluded that for salamanders ponds may receive migrating individuals from

distances up to 8–9 km. Although *T. marmoratus* was not included in this review, our results suggest some individuals might occasionally travel similar distances.

4.4 | Conclusion and recommendation for conservation

Our study contributes to a growing body of literature suggesting that agricultural intensification is harmful for pond breeding amphibians (Boissinot et al., 2019; Crawford et al., 2016; Curado et al., 2011; Joly et al., 2001; Marty et al., 2005). Combining demographic, movement, and genetic data is needed to fully understand spatial population dynamics for conservation (Cayuela et al., 2018; Wood et al., 2020). We demonstrated the necessity to move from site-specific to landscape level analyses to understand the population dynamics of *T. marmoratus*. Our results underline the need to base conservation planning at the landscape level (Cushman, 2006). In particular, increasing connectivity among populations appears to be a major issue for *T. marmoratus* and probably other amphibians in agricultural landscapes. The ultimate goal of conservation for amphibians should be long-term regional persistence by addressing issues at both local (notably quality of breeding-site) and landscape scale (Boissinot et al., 2019; Semlitsch, 2008).

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AUTHOR CONTRIBUTIONS

Alexandre Boissinot and Olivier Lourdais initiated and designed the study. Alexandre Boissinot, Olivier Lourdais and Pierre Grillet collected the data, and Cécile Ribout performed molecular analyses. Alexandre Boissinot and Vincent Quiquempois conducted current and historical hedgerow data digitalization. Bertrand Gauffre and Vincent Quiquempois conducted connectivity, landscape genetics and statistical analyses. Bertrand Gauffre and Raphael Leblois performed demographic inferences. Bertrand Gauffre wrote the manuscript and prepared the figures. Oliver Lourdais, Alexandre Boissinot, Raphael Leblois, Sophie Morin and Damien Picard edited the manuscript and all authors approved the current version.

DATA AVAILABILITY STATEMENT

The data sets with *T. marmoratus* sampling locations and microsatellite genotypes can be found on Dryad - https://doi.org/10.5061/dryad.mkkwh710s.

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REFERENCES

Adamack, A. T., & Gruber, B. (2014). PopGenReport: Simplifying basic population genetic analyses in R. *Methods in Ecology and Evolution*, *5*(4), 384–387. https://doi.org/10.1111/2041-210X.12158

Allentoft, M. E., & O'Brien, J. (2010). Global amphibian declines, loss of genetic diversity and fitness: A review. *Diversity*, *2*(1), 47–71. https://doi.org/10.3390/d2010047

Arntzen, J. W., Abrahams, C., Meilink, W. R. M., Iosif, R., & Zuiderwijk, A. (2017). Amphibian decline, pond loss and reduced population connectivity under agricultural intensification over a 38 year period. *Biodiversity and Conservation*, *26*(6), 1411–1430. https://doi.org/10.1007/s10531-017-1307-y

Barton, K. (2015). MuMIn: Multi-model inference. R package: Retrieved from. http://r-forge.r-project.org/projects/mumin/

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01

Beebee, T. J. C., & Griffiths, R. A. (2005). The amphibian decline crisis: A watershed for conservation biology? *Biological Conservation*, *125*(3), 271–285. https://doi.org/10.1016/j.biocon.2005.04.009

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B-Methodological*, *57*(1), 289–300.

Benton, T. G., Vickery, J. A., & Wilson, J. D. (2003). Farmland biodiversity: Is habitat heterogeneity the key? *Trends in Ecology and Evolution*, *18*(4), 182–188. https://doi.org/10.1016/S0169-5347(03)00011-9

Bertorelle, G., Benazzo, A., & Mona, S. (2010). ABC as a flexible framework to estimate demography over space and time: Some cons, many pros. *Molecular Ecology*, *19*(13), 2609–2625. https://doi.org/10.1111/j.1365-294X.2010.04690.x

Blair, C., Weigel, D. E., Balazik, M., Keeley, A. T. H., Walker, F. M., Landguth, E., Cushman, S., Murphy, M., Waits, L., & Balkenhol, N. (2012). A simulation-based evaluation of methods for inferring linear barriers to gene flow. *Molecular Ecology Resources*, *12*(5), 822–833. https://doi.org/10.1111/j.1755-0998.2012.03151.x

Boissinot, A., Besnard, A., & Lourdais, O. (2019). Amphibian diversity in farmlands: Combined influences of breeding-site and landscape attributes in western France. *Agriculture Ecosystems & Environment*, *269*, 51–61. https://doi.org/10.1016/j.agee.2018.09.016

Cayuela, H., Rougemont, Q., Prunier, J. G., Moore, J., Clobert, J., & Bernatchez, L. (2018). Demographic and genetic approaches to study dispersal in wild animal populations: A methodological review. *Molecular Ecology*, *27*(20), 3976–4010. https://doi.org/10.1111/mec.14848

Ceballos, G., Ehrlich, P. R., & Raven, P. H. (2020). Vertebrates on the brink as indicators of biological annihilation and the sixth mass extinction. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(24), 13596–13602. https://doi.org/10.1073/pnas.1922686117

Coleman, R. A., Weeks, A. R., & Hoffmann, A. A. (2013). Balancing genetic uniqueness and genetic variation in determining conservation and translocation strategies: A comprehensive case study of threatened dwarf galaxias, Galaxiella pusilla (Mack) (Pisces: Galaxiidae). *Molecular Ecology*, *22*(7), 1820–1835. https://doi.org/10.1111/mec.12227

Costanzi, J.-M., Mège, P., Boissinot, A., Isselin-Nondedeu, F., Guérin, S., Lourdais, O., Trochet, A., Le Petitcorps, Q., Legrand, A., Varenne, F., Grillet, P., Morin-Pinaud, S., & Picard, D. (2018). Agricultural landscapes and the Loire River influence the genetic structure of the marbled newt in Western France. *Scientific Reports*, *8*(1), 1–12. https://doi.org/10.1038/s41598-018-32514-y

Costanzi, J. M., Mège, P., Guérin, S., Petitcorps, Q. L., Carbonell, A., Kouassi, A. B., & Picard, D. (2015). Characterization of nine new microsatellite loci for the marbled newt, Triturus marmoratus. *Journal of Genetics*, *94*, 63–64. https://doi.org/10.1007/s12041-015-0586-x

Crawford, J. A., Peterman, W. E., Kuhns, A. R., & Eggert, L. S. (2016). Altered functional connectivity and genetic diversity of a threatened salamander in an agroecosystem. *Landscape Ecology*, *31*(10), 2231–2244. https://doi.org/10.1007/s10980-016-0394-6

Curado, N., Hartel, T., & Arntzen, J. W. (2011). Amphibian pond loss as a function of landscape change – A case study over three decades in an agricultural area of northern France. *Biological Conservation*, *144*(5), 1610–1618. https://doi.org/10.1016/j.biocon.2011.02.011

Cushman, S. A. (2006). Effects of habitat loss and fragmentation on amphibians: A review and prospectus. *Biological Conservation*, *128*(2), 231–240. https://doi.org/10.1016/j.biocon.2005.09.031

Cushman, S. A., McKelvey, K. S., Hayden, J., & Schwartz, M. K. (2006). Gene flow in complex landscapes: testing multiple hypotheses with causal modeling. *The American Naturalist*, *168*(4), 486–499. https://doi.org/10.1086/506976

De Iorio, M., Griffiths, R. C., Leblois, R., & Rousset, F. (2005). Stepwise mutation likelihood computation by sequential importance sampling in subdivided population models. *Theoretical Population Biology*, *68*(1), 41–53. https://doi.org/10.1016/j.tpb.2005.02.001

Dormann, C. F., Elith, J., Bacher, S., Buchmann, C., Carl, G., Carré, G., Marquéz, J. R. G., Gruber, B., Lafourcade, B., Leitão, P. J., Münkemüller, T., McClean, C., Osborne, P. E., Reineking, B., Schröder, B., Skidmore, A. K., Zurell, D., & Lautenbach, S. (2013). Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. *Ecography*, *36*(1), 27–46. https://doi.org/10.1111/j.1600-0587.2012.07348.x

Duforet-Frebourg, N., & Blum, M. G. B. (2014). Nonstationary patterns of isolation-by-distance: Inferring measures of local genetic differentiation with bayesian kriging. *Evolution*, *68*(4), 1110–1123. https://doi.org/10.1111/evo.12342

Earl, D. A., & vonHoldt, B. M. (2012). STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, *4*(2), 359–361. https://doi.org/10.1007/s12686-011-9548-7

El Mousadik, A., & Petit, R. J. (1996). High level of genetic differentiation for allelic richness among populations of the argan tree [Argania spinosa (L.) Skeels] endemic to Morocco. *Theoretical and Applied Genetics*, *92*(7), 832–839. https://doi.org/10.1007/BF00221895

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software structure: a simulation study. *Molecular Ecology*, *14*(8), 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Fagan, W. F., & Holmes, E. E. (2006). Quantifying the extinction vortex. *Ecology Letters*, *9*(1), 51–60. https://doi.org/10.1111/j.1461-0248.2005.00845.x

Falush, D., Stephens, M., & Pritchard, J. K. (2003). Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. *Genetics*, *164*(4), 1567–1587. https://doi.org/10.1111/j.1471-8286.2007.01758.x

Fisher, J., & Lindenmayer, D. B. (2007). Landscape modification and habitat fragmentation: a synthesis. *Global Ecology and Biogeography*, *16*, 265–280. https://doi.org/10.1111/j.1466-8238.2006.00287.x

Flavenot, T., Fellous, S., Abdelkrim, J., Baguette, M., & Coulon, A. (2015). Impact of quarrying on genetic diversity: an approach across landscapes and over time. *Conservation Genetics*, *16*(1), 181–194. https://doi.org/10.1007/s10592-014-0650-8

Foley, J. A., DeFries, R., Asner, G. P., Barford, C., Bonan, G., Carpenter, S. R., Chapin, F. S., Coe, M. T., Daily, G. C., Gibbs, H. K., Helkowski, J. H., Holloway, T., Howard, E. A., Kucharik, C. J., Monfreda, C., Patz, J. A., Prentice, I. C., Ramankutty, N., & Snyder, P. K. (2005). Global consequences of land use. *Science*, *309*(July), 570–574. https://doi.org/10.1126/science.1111772

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

Frankham, R., Ballou, J. D., Ralls, K., Eldridge, M. D. B., Dudash, M. R., Fenster, C. B., Lacy, R. C., & Sunnucks, P. (2017). *Genetic management of fragmented animal and plant populations*. Oxford University.

Frantz, A. C., Cellina, S., Krier, A., Schley, L., & Burke, T. (2009). Using spatial Bayesian methods to determine the genetic structure of a continuously distributed population: clusters or isolation by distance? *Journal of Applied Ecology*, *46*(2), 493–505. https://doi.org/10.1111/j.1365-2664.2008.01606.x

Goldberg, C. S., & Waits, L. P. (2010). Comparative landscape genetics of two pond-breeding amphibian species in a highly modified agricultural landscape. *Molecular Ecology*, *19*(17), 3650–3663. https://doi.org/10.1111/j.1365-294X.2010.04673.x

Goslee, S. C., & Urban, D. L. (2007). The ecodist package for dissimilarity-based analysis of ecological data. *Journal of Statistical Software*, *22*(7), 1–19. https://doi.org/10.18637/jss.v022.i07

Goudet, J. (2012). FSTAT (Version 1.2): A computer program to calculate F-statistics. *Journal of Heredity*, *86*, 485–486.

Hillman, S. S., Drewes, R. C., Hedrick, M. S., & Hancock, T. V. (2014). Physiological vagility and its relationship to dispersal and neutral genetic heterogeneity in vertebrates. *The Journal of Experimental Biology*, *217*, 3356–3364. https://doi.org/10.1242/jeb.105908

Hof, C., Araújo, M. B., Jetz, W., & Rahbek, C. (2011). Additive threats from pathogens, climate and land-use change for global amphibian diversity. *Nature*, *480*(7378), 516–519. https://doi.org/10.1038/nature10650

Holm, S. (1979). A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics*, *6*, 65–70.

Hutchison, D. W., & Templeton, A. R. (1999). Correlation of pairwise genetic and geographic distance measures : Inferring the relative influences of gene flow and drift on the distribution of genetic variability. *Evolution*, *53*(6), 1898–1914.

Jehle, R. (2000). The terrestrial summer habitat of radio-traked great crested newts (Triturus cristatus) and marbled newts(T. marmoratus). *Herpertological Journal*, *10*(April), 137–142.

Jehle, R., & Arntzen, J. W. (2000). Post-breeding migrations of newts (Triturus cristatus and T. marmoratus) with contrasting ecological requirements. *Journal of Zoology*, *251*(3), 297–306. https://doi.org/10.1017/S0952836900007032

Jehle, R., Burke, T., & Arntzen, J. W. (2005). Delineating fine-scale genetic units in amphibians: Probing the primacy of ponds. *Conservation Genetics*, *6*(2), 227–234. https://doi.org/10.1007/s10592-004-7832-8

Jehle, R., Wilson, G. A., Arntzen, J. W., & Burke, T. (2005). Contemporary gene flow and the spatio-temporal genetic structure of subdivided newt populations (Triturus cristatus, T. marmoratus). *Journal of Evolutionary Biology*, *18*(3), 619–628. https://doi.org/10.1111/j.1420-9101.2004.00864.x

Joly, P., Miaud, C., Lehmann, A., & Grolet, O. (2001). Habitat matrix effects on pond occupancy in newts. *Conservation Biology*, *15*(1), 239–248. https://doi.org/10.1111/j.1523-1739.2001.99200.x

Karraker, N. E., & Gibbs, J. P. (2009). Amphibian production in forested landscapes in relation to wetland hydroperiod: A case study of vernal pools and beaver ponds. *Biological Conservation*, *142*(10), 2293–2302. https://doi.org/10.1016/j.biocon.2009.05.002

Kopelman, N. M., Mayzel, J., Jakobsson, M., Rosenberg, N. A., & Mayrose, I. (2015). CLUMPAK : a program for identifying clustering modes and packaging population structure inferences across K. *Molecular Ecology Resources*, *15*(5), 1179–1191. https://doi.org/10.1038/nn.3945.Dopaminergic

Krupa, A. P., Jehle, R., Dawson, D. A., Gentle, L. K., Gibbs, M., Arntzen, J. W., & Burke, T. (2002). Microsatellite loci in the crested newt (Triturus cristatus) and their utility in other ttax. *Conservation Genetics*, *3*, 87–89.

Legendre, P., Lapointe, F., & Casgrain, P. (1994). Modeling brain evolution from behavior: A permutational regression approach. *Evolution*, *48*(5), 1487–1499. https://doi.org/10.1111/j.1558-5646.1994.tb02191.x

Luqman, H., Muller, R., Vaupel, A., Brodbeck, S., Bolliger, J., & Gugerli, F. (2018). No distinct barrier effects of highways and a wide river on the genetic structure of the Alpine newt (Ichthyosaura alpestris) in densely settled landscapes. *Conservation Genetics*, *19*, 673–685. https://doi.org/10.1007/s10592-018-1046-y

Manel, S., & Holderegger, R. (2013). Ten years of landscape genetics. *Trends in Ecology and Evolution*, *28*(10), 614–621. https://doi.org/10.1016/j.tree.2013.05.012

Manel, S., Schwartz, M. K., Luikart, G., & Taberlet, P. (2003). Landscape genetics: combining landscape ecology and population genetics. *Trends in Ecology & Evolution*, *18*(4), 189–197. https://doi.org/10.1016/S0169-5347(03)00008-9

Marty, P., Angélibert, S., Giani, N., & Joly, P. (2005). Directionality of pre- and post-breeding migrations of a marbled newt population (Triturus marmoratus): Implications for buffer zone management. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *15*(3), 215–225. https://doi.org/10.1002/aqc.672

McCartney-Melstad, E., & Shaffer, B. (2015). Amphibian molecular ecology and how it has informed conservation. *Molecular Ecology*, *24*, 5084–5109. https://doi.org/10.1111/mec.13391

McRae, B. H. (2006). Isolation by resistance. *Evolution: International Journal of Organic Evolution*, *60*(8), 1551–1561. https://doi.org/10.1554/05-321.1

Mcrae, B. H., Shah, V. B., & Mohapatra, T. K. (2013). Circuitscape 4 user guide. The Nature Conservancy, http://www.circuitscape.org

Neel, M. C., McKelvey, K., Ryman, N., Lloyd, M. W., Short Bull, R., Allendorf, F. W., Schwartz, M. K., & Waples, R. S. (2013). Estimation of effective population size in continuously distributed populations: there goes the neighborhood. *Heredity*, *111*(3), 189–199. https://doi.org/10.1038/hdy.2013.37

Nimon, K., Oswald, F. L., & Roberts, J. K. (2013). Interpreting regression effects. R package version, 2.0.

Palsboll, P., Bérubé, M., & Allendorf, F. (2007). Identification of management units using population genetic data. *Trends in Ecology & Evolution*, *22*(1), 11–16. https://doi.org/10.1016/j.tree.2006.09.003

Pidancier, N., Miquel, C., & Miaud, C. (2003). Buccal swabs as a non-destructive tissue sampling method for DNA analysis in amphibians. *Herpetological Journal*, *13*(4), 175–178.

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*, 945–959. https://doi.org/10.1111/j.1471-8286.2007.01758.x

Prunier, J. G., Colyn, M., Legendre, X., & Flamand, M. C. (2017). Regression commonality analyses on hierarchical genetic distances. *Ecography*, *40*(12), 1412–1425. https://doi.org/10.1111/ecog.02108

Prunier, J. G., Colyn, M., Legendre, X., Nimon, K. F., & Flamand, M. C. (2015). Multicollinearity in spatial genetics: separating the wheat from the chaff using commonality analyses. *Molecular Ecology*, *24*(2), 263–283. https://doi.org/10.1111/mec.13029

Prunier, J., Kaufmann, B., Léna, J. P., Fenet, S., Pompanon, F., & Joly, P. (2013). A 40-year-old divided highway does not prevent gene flow in the alpine newt Ichthyosaura alpestris. *Conservation Genetics*, *15*, 453–468. https://doi.org/10.1007/s10592-013-0553-0

QGIS Development Team. (2018). QGIS geographic information system. Open source geospatial foundation project. http://qgis.osgeo.org

R Development Core Team (2014). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.

Ray-Mukherjee, J., Nimon, K., Mukherjee, S., Morris, D. W., Slotow, R., & Hamer, M. (2014). Using commonality analysis in multiple regressions: A tool to decompose regression effects in the face of multicollinearity. *Methods in Ecology and Evolution*, *5*(4), 320–328. https://doi.org/10.1111/2041-210X.12166

Richardson, J. L., Brady, S. P., Wang, I. J., & Spear, S. F. (2016). Navigating the pitfalls and promise of landscape genetics. *Molecular Ecology*, *25*(4), 849–863. https://doi.org/10.1111/mec.13527

Riddell, E. A., Roback, E. Y., Wells, C. E., Zamudio, K. R., & Sears, M. W. (2019). Thermal cues drive plasticity of desiccation resistance in montane salamanders with implications for climate change. *Nature Communications*, *10*, https://doi.org/10.1038/s41467-019-11990-4

Riddell, E., & Sears, M. W. (2020). Terrestrial salamanders maintain habitat suitability under climate change despite trade-offs between water loss and gas exchange. *Physiological and Biochemical Zoology*, *93*(4), 310–319. https://doi.org/10.1086/709558

Rousset, F. (1997). Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics*, *145*, 1219–1228.

Rousset, F. (2008). GENEPOP'007: A complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources*, *8*(1), 103–106. https://doi.org/10.1111/j.1471-8286.2007.01931.x

Rousset, F., & Leblois, R. (2007). Likelihood and approximate likelihood analyses of genetic structure in a linear habitat: Performance and robustness to model mis-specification. *Molecular Biology and Evolution*, *24*(12), 2730–2745. https://doi.org/10.1093/molbev/msm206

Rousset, F., & Leblois, R. (2012). Likelihood-based inferences under isolation by distance: Two-dimensional habitats and confidence intervals. *Molecular Biology and Evolution*, *29*(3), 957–973. https://doi.org/10.1093/molbev/msr262

Schmeller, D., & Merila, J. (2007). Demographic and genetic estimates of effective population and breeding size in the Amphibian *Rana temporaria*. *Conservation Biology*, *21*(1), 142–151. https://doi.org/10.1111/j.1523-1739.2006.00554.x

Schwartz, M., Luikart, G., & Waples, R. S. (2007). Genetic monitoring as a promising tool for conservation and management. *Trends in Ecology & Evolution*, *22*(1), 25–33. https://doi.org/10.1016/j.tree.2006.08.009

Scoble, J., & Lowe, A. J. (2010). A case for incorporating phylogeography and landscape genetics into species distribution modelling approaches to improve climate adaptation and conservation planning. *Diversity and Distributions*, *16*(3), 343–353. https://doi.org/10.1111/j.1472-4642.2010.00658.x

Segelbacher, G., Cushman, S. A., Epperson, B. K., Fortin, M.-J., Francois, O., Hardy, O. J., Holderegger, R., Taberlet, P., Waits, L. P., & Manel, S. (2010). Applications of landscape genetics in conservation biology: Concepts and challenges. *Conservation Genetics*, *11*(2), 375–385. https://doi.org/10.1007/s10592-009-0044-5

Semlitsch, R. D. (2008). Differentiating migration and dispersal processes for pond-breeding amphibians. *Journal of Wildlife Management*, *72*(1), 260–267. https://doi.org/10.2193/2007-082

Sgrò, C. M., Lowe, A. J., & Hoffmann, A. A. (2011). Building evolutionary resilience for conserving biodiversity under climate change. *Evolutionary Applications*, *4*(2), 326–337. https://doi.org/10.1111/j.1752-4571.2010.00157.x

Shirk, A. J., Landguth, E. L., & Cushman, S. A. (2017). A comparison of regression methods for model selection in individual-based landscape genetic analysis. *Molecular Ecology Resources*, *18*(1), 55–67. https://doi.org/10.1111/1755-0998.12709

Smith, M. A., & Green, D. M. (2005). Dispersal and the metapopulation in amphibian and paradigm ecology are all amphibian conservation: populations metapopulations? *Ecography*, *28*(1), 110–128. https://doi.org/10.1111/j.0906-7590.2005.04042.x

Storfer, A., Murphy, M. A., Evans, J. S., Goldberg, C. S., Robinson, S., Spear, S. F., Dezzani, R., Delmelle, E., Vierling, L., & Waits, L. P. (2007). Putting the 'landscape' in landscape genetics. *Heredity*, *98*(3), 128–142. https://doi.org/10.1038/sj.hdy.6800917

Sun, J. X., Helgason, A., Masson, G., Ebenesersdóttir, S. S., Li, H., Mallick, S., Gnerre, S., Patterson, N., Kong, A., Reich, D., & Stefansson, K. (2012). A direct characterization of human mutation based on microsatellites. *Nature Genetics*, *44*(10), 1161–1165. https://doi.org/10.1038/ng.2398

Sztatecsny, M., Jehle, R., Schmidt, B. R., & Arntzen, J. W. (2004). The abundance of premetamorphic newts (Triturus cristatus, T. marmoratus) as a function of habitat determinants: An a priori model selection approach. *Herpetological Journal*, *14*(2), 89–97. https://doi.org/10.5167/uzh-336

Trochet, A., Le Chevalier, H., Calvez, O., Barthe, L., Isselin-Nondedeu, F., Picard, D., Debelgarric, M., Pégourié, N., Rocher, R., & Ribéron, A. (2017). Postbreeding movements in Marbled newts (Caudata, Salamandridae): A comparative radiotracking study in two habitat types. *Herpetologica*, *73*(1), 1–9. https://doi.org/10.1655/Herpetologica-D-15-00072

Wang, I. J. (2013). Examining the full effects of landscape heterogeneity on spatial genetic variation: A multiple matrix regression approach for quantifying geographic and ecological isolation. *Evolution*, *67*(12), 3403–3411. https://doi.org/10.1111/evo.12134

Weir, B. S., & Cockerham, C. C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution*, *38*(6), 1358–1370.

Wood, D. A., Rose, J. P., Halstead, B. J., Stoelting, R. E., Swaim, K. E., & Vandergast, A. G. (2020). Combining genetic and demographic monitoring better informs conservation of an endangered urban snake. *PLoS One*, *15*(5), 1–27. https://doi.org/10.1371/journal.pone.0231744

Wright, S. (1943). Isolation by distance. *Genetics*, *28*, 114–138. https://doi.org/10.1093/genetics/28.2.114

Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A., & Smith, G. M., & Ebooks Corporation (2009). Mixed effects models and extensions in ecology with R. A. F. Zuur, E. N. Ieno, N. Walker, A. A. Saveliev & G. M. Smith (eds), *Statistics for biology and health*. (pp. 579). New York: Springer.

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