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

Running title: Newts landscape genetic in farmlands

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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 ten 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 :** Landscape genetics, demographic inferences, dispersal, genetic diversity, microsatellites, *Triturus marmoratus*

## 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, Bérubé, & Allendorf, 2007; Scoble & Lowe, 2010; Sgrò, Lowe, & Hoffmann, 2011).

In European agricultural hedgerow landscapes, land conversion has caused loss of permanent habitats and their connection in space (Benton, Vickery, & Wilson, 2003). While land conversion threatens all taxa, amphibians constitute one of the most impacted ones (Ceballos, Ehrlich, & Raven, 2020; Crawford, Peterman, Kuhns, & Eggert, 2016; Cushman, 2006; Hof, Araújo, Jetz, & Rahbek, 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, Drewes, Hedrick, & Hancock, 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, Roback, Wells, Zamudio, & Sears, 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, Angélibert, Giani, & Joly, 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, McKelvey, Hayden, & Schwartz, 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, Landguth, & Cushman, 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, Fellous, Abdelkrim, Baguette, & Coulon, 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, Brady, Wang, & Spear, 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 *Ne* 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, Benazzo, & Mona, 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 6000 km² 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, Hartel, & Arntzen, 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, Abrahams, Meilink, Iosif, & Zuiderwijk, 2017). We characterized landscape changes (hedgerow loss) over the last 60 years in the study area using historical landscape data and analyzed genetic data collected in order to address the following goals:

(i) 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.

## Materials and methods

### Study area and sample collection

The study area covers the entire Deux-Sèvres department (6000 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, Miquel, & Miaud, 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.

### 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 Supplementary 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, likely due to low quality DNA

conservation. A total of 612 individuals from 64 ponds were successfully genotyped at 8 or more loci. From this global dataset, ponds less than 150 m apart were pooled and ponds with less than 7 individuals were removed. The final dataset included 550 individuals from 39 ponds (Table S1.2, Supplementary Information). For sake of consistency, node-based and matrix-based landscape genetics analyses as well as demographic inferences were all performed on this population-based dataset.

**Genetic diversity**

We tested linkage disequilibrium between all pairs of loci on the global 612 individuals dataset 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 pond.

**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(QGISDevelopment 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 unfavorable 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 = 17400) 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 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 $< 0.001$ and Pearson's $r = 0.91$, P-value $< 0.001$, respectively).

**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 centered 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, 1000 and 2000 m). The maximal distance was set to 2000 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 correlations between landscape features for each buffer radius. Pairs of landscape features with Pearson 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 1000 m model (see details on correlations in Appendix S2, Supplementary 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, Luikart, & Waples, 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,

Maechler, Bolker, & Walker, 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, 1000 and 2000 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.

**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, Stephens, & Donnelly, 2000), with the admixture model and correlated allele frequencies (Falush, Stephens, & Pritchard, 2003) to determine the number of genetic clusters (K). Ten replicate runs of $2 \times 10^6$ Markov chain Monte Carlo (MCMC) iterations, after an initial burn-in period of $5 \times 10^5$ iterations, were performed for values of K ranging from one to ten. Results were summarized using the standard pipeline on the CLUMPAK web server (Kopelman, Mayzel, Jakobsson, Rosenberg, & Mayrose, 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 analyzed 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 neighboring 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 neighboring 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 correlations to test the relationship between estimates of genetic diversity ($A_r$, $H_e$ and $H_o$) within ponds and estimates of local genetic differentiation computed with LOCALDIFF.

**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, Shah, & Mohapatra, 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 multi-collinearity among landscape variables (Ray-Mukherjee et al., 2014), so we used a commonality analysis (CA) (J. G. 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 Supplementary 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, Oswald, & Roberts, 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 1000 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.

**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, Griffiths, Leblois, & Rousset, 2005), extended to consider IBD as a model for population structure (Rousset & Leblois, 2012; Rousset & Leblois, 2007). Based on LOCALDIFF results, ponds from the northern and southern parts of the study area were analyzed 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: 1) 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 $\mu$ the mutation rate per locus per generation. 2) 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. 3) The parameter of the geometric distribution of dispersal ($g$). 4) The neighborhood size, $Nb = 4\pi\times N\times\sigma^2$, where N is the effective population size and $\sigma^2$ 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 x 10 and 20 x 11 bins for the northern and southern parts of the study area, respectively. Bin size, 3275 x 3275 m, was set constant over the two analyses to facilitate results interpretation. We used the following computing parameters: 2000 trees, 100 points and 3 iterations. We translated the parameters inferred from MIGRAINE into effective population size using a mutation rate range commonly used for microsatellites: $1\times10^{-4}[5\times10^{-3} – 5\times10^{-5}]$ (Sun et al., 2012).

**Results**

**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 samples t-test, P-value < 0.001). On average loss was 34 % and 53 % in the northern and southern parts of the study area, respectively.

**2) Genetic markers and diversity**

The number of alleles per locus ranged from four to 16 in the global dataset (Table S1.1, Supplementary Information), 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 dataset (P-value = 0.056, $F_{IS}$ = 0.127) that could be related to the presence of spatial structure ("Wahlund effect"). Indeed, only one of the 39 ponds with at least 7 individuals showed significant departure from HWE (Table S1.2, Supplementary Information). After FDR correction, there was no pair of loci showing significant LD in the global dataset.

**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, Supplementary Information). $R^2$ comparison among linear mixed models based on landscape descriptors calculated in 500, 1000 and 2000 m buffers indicated 1000 m was the best spatial scale to explain $A_r$ (marginal $R^2$ = 0.005, 0.007 and 0.005 for 500, 1000 and 2000 m buffers, respectively). Several plausible best models (i.e. with delta AICc < 4) were identified at 1000 m (Table S2.4, Supplementary Information). 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 m and 2000 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, 1000 and 2000 m buffers, respectively).

However, no significant effect of landscape composition on $H_o$ was found at any spatial scale (Table S2.5, Supplementary Information).

**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, Supplementary Information). 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-value < 0.001) 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-value < 0.01), while the correlation was non-significant when $H_o$ (cor = -0.19, P-value = 0.24) was used as a measure of genetic diversity (Figure 5).

**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 ($\beta_{crops}$ = 0.33, P-value = 0.016). $F_{ST}/(1-F_{ST})$ 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 < 0.001). It revealed that arable crop land cover was the only landscape feature significantly influencing genetic differentiation ($\beta_{crops}$ = 0.009, P = 0.033, Table 2 and Figure S2, Supplementary Information).

**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). *Nb* 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 (*De*) per bin and migration rate among bins (*m*) (see Table 3 for figures) and draw a theoretical dispersal kernel (Figure 6). The latter indicated higher dispersal rate and distance in the northern part of the study area.

## 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.

**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, & Arntzen, 2005; Jehle, Wilson, Arntzen, & Burke, 2005). In our study, at distance < 6.5 km, the average pairwise $F_{ST}$ was only 0.025 (range: -0.03 – 0.07) with 9 out of 25 pairs of ponds significantly differentiated. A single population exhibiting IBD was 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 likely due to IBD which might create spurious genetic clusters when geographical sampling is clumped (Blair et al., 2012; Frantz, Cellina, Krier, Schley, & Burke, 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 et al. (2005) that although amphibians are predominantly philopatric with poor dispersal capacities, they could move distances much greater than anticipated. Another

500 relevant finding of our study is the clear heterogeneous IBD pattern across the study area as
501 evidences by the LOCALDIFF analysis. Results from our landscape genetics analyses strongly
502 suggest this contrast might be related to differences in landscape composition between the
503 northern and southern parts of the study area as previously suggested (Costanzi et al., 2018).
504
505 **Landscape influence on genetic diversity and genetic structure**
506 Genetic diversity is affected by effective population size and connectivity (Flavenot et al.,
507 2015). It is an important parameter in conservation genetics since reduced genetic diversity
508 could translate to lower fitness and subsequently vortex of extinction (Fagan & Holmes,
509 2006). A review analyzed 19 studies that directly quantified genetic diversity - fitness
510 relationships in amphibians, among which 15 provided evidence that levels of genetic
511 diversity affected important traits such as growth or survival (Allentoft & O'Brien, 2010).
512 Pond density was the only landscape feature affecting local genetic diversity in our study with
513 a positive influence on allelic richness. Interestingly, we did not find any effect of woodlands
514 land cover, the main terrestrial habitat of the species. This result emphasizes the importance
515 of the breeding habitat in newt life cycle and suggests larger effective population density with
516 increased pond density. Higher pond density may also facilitate dispersal and resulting gene
517 flow, as it is easier for a dispersing newt to encounter a new pond. This result might be related
518 to reduction of pond density associated with intensification that affected both parts of the
519 study area, though to a higher degree in the south. Both MRDM and MMRR identified arable
520 crop land cover as the only landscape variable affecting significantly gene flow, likely
521 through reduced functional connectivity. It is also supported by the LOCALDIFF analysis that
522 showed stronger IBD in the southern part of the study area. It is interesting to note that node-
523 and matrix-based landscape genetics analyses provided complementary results. In the node-
524 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 micro-evolutionary process driving genetic differentiation (Coleman, Weeks, & Hoffmann, 2013). Altogether, our study exemplifies the difficulty to disentangle the influence of effective population density 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.

**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

550 in the southern part of the study area, where land conversion for intensive arable crops
551 production induced substantial *T. marmoratus* aquatic and terrestrial habitat loss (we
552 estimated a 53 % decrease in hedgerows since 1950). Demographic inferences conducted with
553 MIGRAINE were consistent with this hypothesis and indicated contrasted values between the
554 two parts of the study area, in line with previous results. First, *Nb*, the neighborhood size, was
555 significantly lower in the south, with *Nb* = 68.77 and 557.5 in the southern and northern parts
556 of the study area, respectively. While *Nb* depends on both effective population size and
557 dispersal (Neel et al., 2013), MIGRAINE analysis also allowed us to infer area-specific effective
558 population density and dispersal separately. Although not significantly different, MIGRAINE
559 estimate of area-specific effective population density was almost 35% lower in the south, with
560 2.56 and 3.86 individuals per km² in the southern and northern parts of the study area,
561 respectively. This contrast is consistent with the positive effect of local pond density on
562 genetic diversity, since pond density was five times lower in the southern part of the study
563 area. Overall, our effective population density estimates might seem low. However, in
564 amphibians, the effective population size (*Ne*) is usually much lower than the census
565 population size (*N*) (see Table 4 in Schmeller & Merila 2007). Indeed, *T. marmoratus Ne*/*N*
566 ratio was estimated to range from 0.05 to 0.65 in five ponds of Western France (Jehle,
567 Wilson, et al., 2005). In the same study, the effective population size was estimated in the
568 order of 100–200 individuals in a 26.25 km² study site (~ 3.8 to 7.6 individuals per km²), thus
569 consistent with our effective population density estimate in the northern part of the study area.
570 Interestingly, landscape structure in the northern part of the study area and in the Jehle et al
571 (2005) study area were similar. Finally, area-specific MIGRAINE estimates of dispersal rate *m*
572 and of the parameter of the dispersal function *g* also showed contrasted situation between the
573 two parts of the study area. Dispersal rate was 46 % lower in the south, and the significant
574 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 > 1km 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 et al. (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.

**Conclusion and recommendation for conservation**

Our study contributes to a growing body of literature suggesting that agricultural intensification is harmful for pond breeding amphibians (Crawford, Peterman, Kuhns, & Eggert, 2016; Curado et al., 2011; Joly et al., 2001; Marty et al., 2005, Boissinot et al. 2019). 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 likely 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, Besnard, & Lourdais, 2019; Semlitsch, 2008).

**Acknowledgements**

This work was financially supported by the Conseil Régional du Poitou-Charentes (Projet "Bocage et Biodiversité"), the Fundation Lisea (Projet "Bocage") and the CNRS. This publication has been written with the support of the AgreenSkills+ fellowship programme, which has received funding from the EU's Seventh Frame- work Programme under grant agreement N° FP7-609398 (AgreenSkills+ contract). We address special thanks to people who accompanied us on the field. More specifically, we wish to thank Leïla Migault, Jérémie Souchet, Fabrice Connort, Jérôme Lallemand, Florian Doré, Alexandre Baduel, Nathan Braconnier, Jean-Pierre Lassaire, Stéphane Bonnet, Antoine Pasquier, Sabrina Lefebvre, Frédéric Migault, Morgan Pourrageau, Yannick Besson, Alain Texier, Clément Gouraud, Sébastien Billaud, Sabrina Maiano, Cédric Auburtin, Justine Vidal, Virginie Lassaire, Loïc Nau, Anthony Leguen and Eddy Leguen. We are also grateful to the local farmers in the study area for allowing us to study the ponds on their land. The study was carried out in compliance with all applicable animal care guidelines, and all required French permits were obtained (from the Préfecture des Deux-Sèvres).

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## Data Accessibility

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

## Author Contributions

A.B. O.L. initiated and designed the study. A.B. O.L. P.G. collected the data, and C.R. performed molecular analyses. A.B. and V.Q. conducted current and historical hedgerow data digitalization. B.G. and V.Q. conducted connectivity, landscape genetics and statistical analyses. B.G. and R.L. performed demographic inferences. B.G. wrote the manuscript and prepared the figures. O.L A.B. R.L. S.M. D.P. edited the article and all authors approved the current version.

## Tables and figures

**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.* 1000 m buffer size). Estimates and P-values are presented for each landscape descriptor tested. Significant relations are in bold.

| Landscape variable | Estimate | Std. Error | z-value | P-value |
|---|---|---|---|---|
| Arable crops | -0.065 | 0.037 | 1.750 | 0.080 |
| **Ponds** | **0.107** | **0.035** | **3.021** | **0.003** |
| Woodlands | -0.026 | 0.060 | 0.441 | 0.659 |
| Rivers | 0.021 | 0.034 | 0.604 | 0.546 |
| Buildings | 0.020 | 0.036 | 0.554 | 0.579 |
| Grasslands | 0.027 | 0.052 | 0.511 | 0.610 |
| Roads | -0.008 | 0.039 | 0.197 | 0.844 |

**Table 2:** MRDM and MMRR results and additional parameters derived from Commonality Analysis: model fit index (multivariate $R^2$; ***: P-value <0.001), beta weights β and P-values and unique, common and total contributions of landscape variables to the variance in $F_{ST}/(1-F_{ST})$. Significant contributions are in bold.

| Landscape feature | MRDM | | | | | | MMRR | | |
|---|---|---|---|---|---|---|---|---|---|
| | multiple $R^2$ | β | P-value | Unique | Common | Total | $R^2$ | β | P-value |
| Geographic distance | 0.38** | **0.398** | **0.043** | **0.024** | **0.252** | **0.275** | 0.29*** | 0.012 | 0.081 |
| Rivers | | -0.034 | 0.791 | 0.001 | 0.004 | 0.005 | | 0.000 | 0.940 |
| Grasslands | | 0.205 | 0.213 | 0.015 | -0.015 | 0 | | 0.010 | 0.162 |
| **Arable crops** | | **0.327** | **0.016** | **0.054** | **0.142** | **0.196** | | **0.012** | **0.024** |
| Woodlands | | -0.055 | 0.696 | 0.002 | -0.001 | 0.001 | | 0.000 | 0.943 |
| Roads | | 0.114 | 0.49 | 0.005 | 0.153 | 0.158 | | 0.008 | 0.409 |
| Hedgerows | | -0.289 | 0.106 | 0.027 | -0.022 | 0.006 | | -0.010 | 0.205 |
| ponds | | -0.069 | 0.727 | 0.001 | 0.152 | 0.153 | | -0.001 | 0.899 |
| Urbanized | | -0.028 | 0.853 | 0 | 0.116 | 0.116 | | -0.005 | 0.575 |

**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 ($2N\mu$), scaled emigration rate (2Nm), parameter of the geometric distribution of dispersal (g) and neighborhood size (Nb) are indicated. *De*, the effective density per 3250 x 3250 m bin and *m*, the migration rate among bins were estimated using $10^{-4}$ [$5 \times 10^{-4} - 5 \times 10^{-5}$] as value and range for the mutation rate.

| Parameters | South | North |
|---|---|---|
| **$2N\mu$** | 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 - NA] |
| **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] |

**Figures 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 colors referring to the population dataset and the northern and southern parts of the study area, respectively.

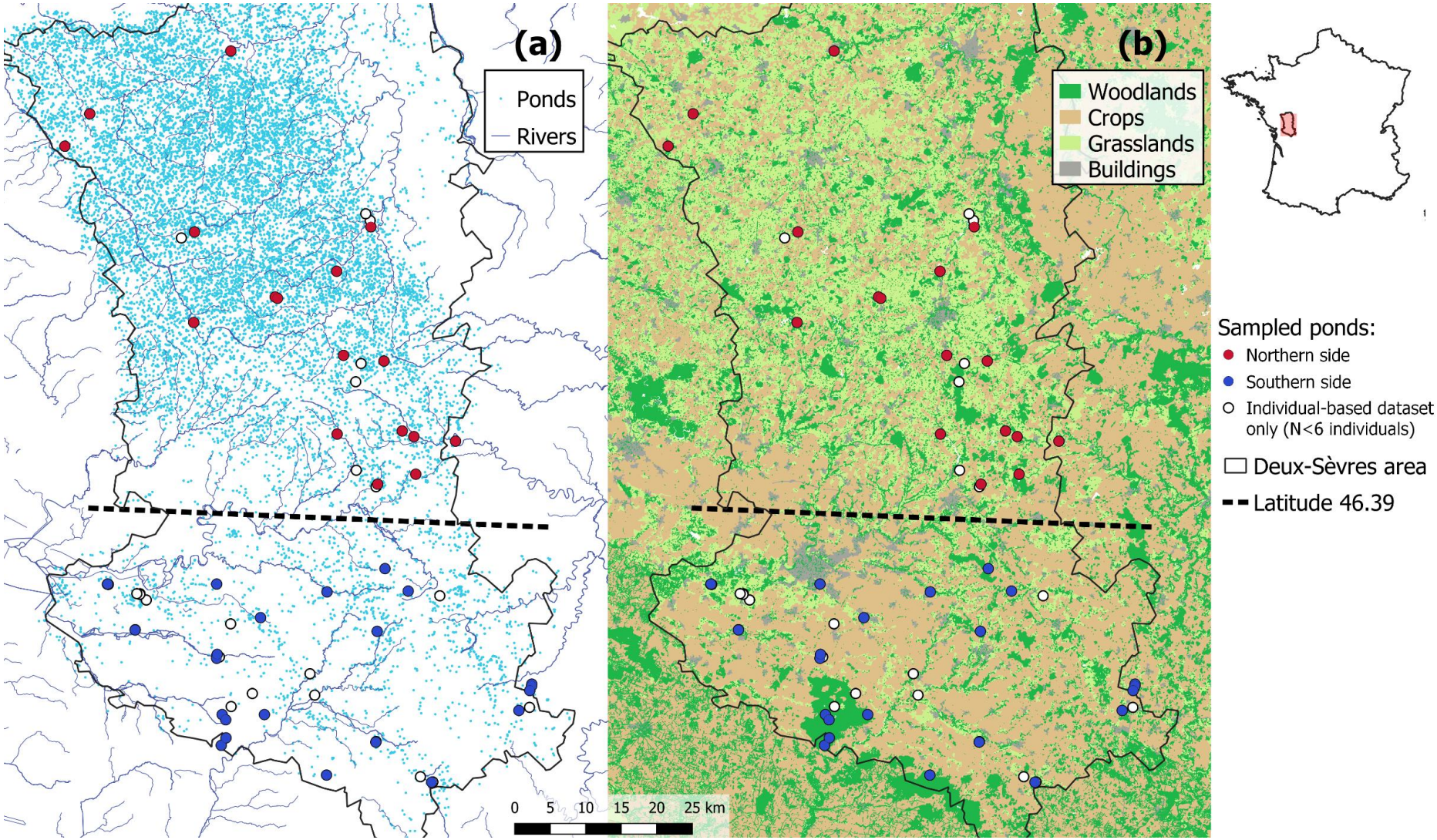

**Figure 2.** Relation between Allelic richness ($A_r$) and pond density in a 1000 m buffer around the 39 ponds. Black dots and gray dots represent ponds from the northern and southern sides of the study area, respectively.

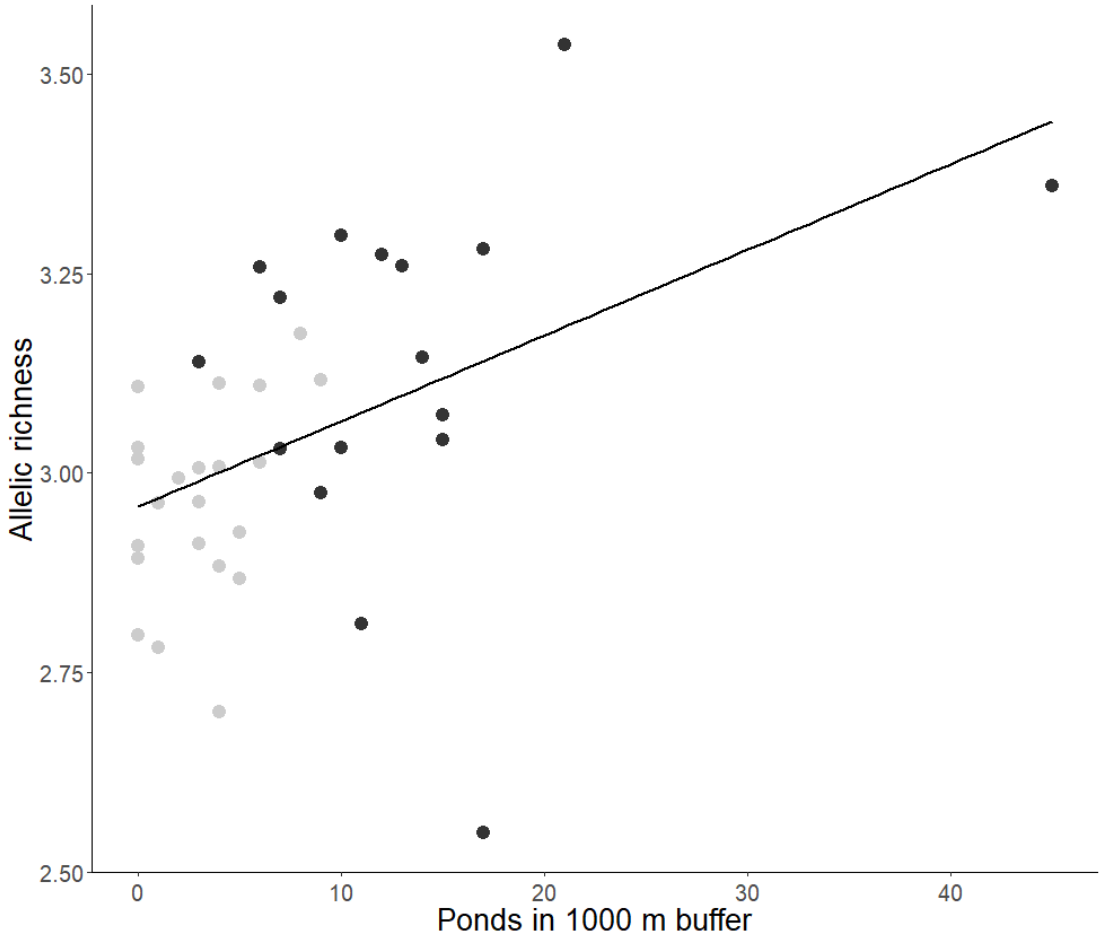

**Figure 3.** Correlation between pairwise genetic differentiation ($F_{ST}$/(1 -$F_{ST}$)) and logarithm of the geographic distance between pairs of ponds. Filled dots indicate pairs of ponds that were significantly different (GENEPOP exact probability test for population differentiation).

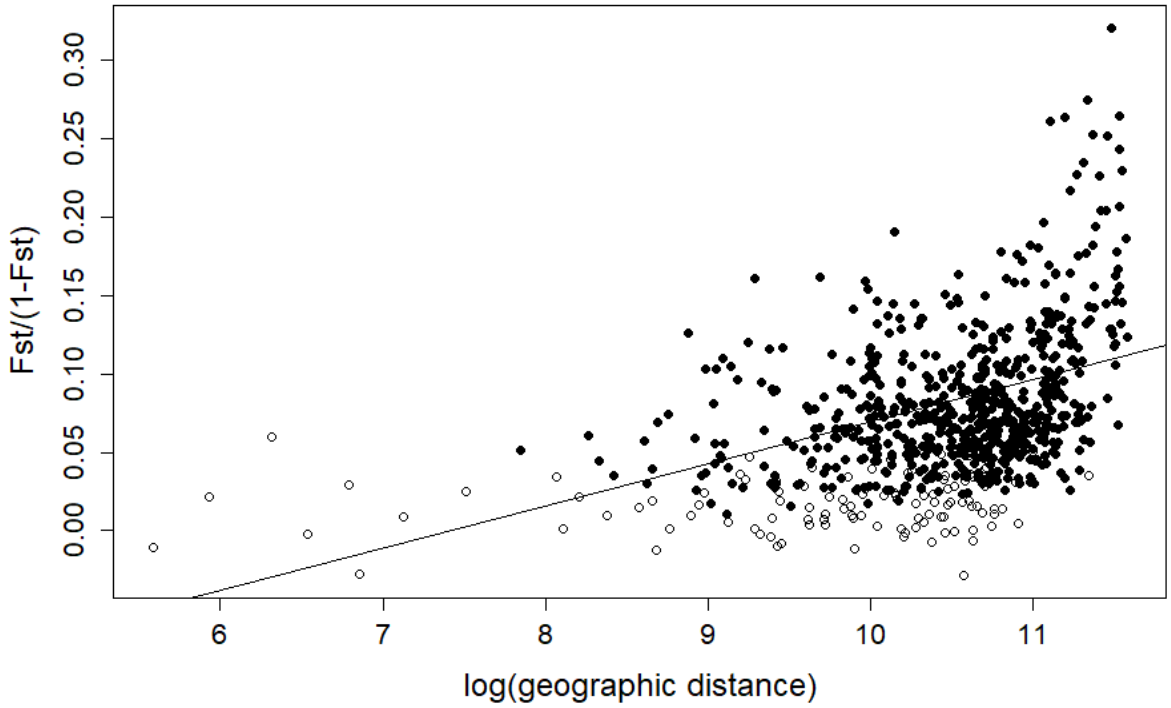

**Figure 4.** Map of local genetic differentiation inferred by the Bayesian kriging method implemented in LOCALDIFF . Shape represent the minimum convex polygon that encompasses the 39 ponds. Measures of local genetic differentiation correspond to $F_{ST}/(1-F_{ST})$ calculated between the sampled ponds and 4 fictive neighboring populations, not shown, located at 2 km. Warmer colors indicates higher local genetic differentiation, i.e. higher genetic distances between sampled ponds and interpolated fictive neighbors.

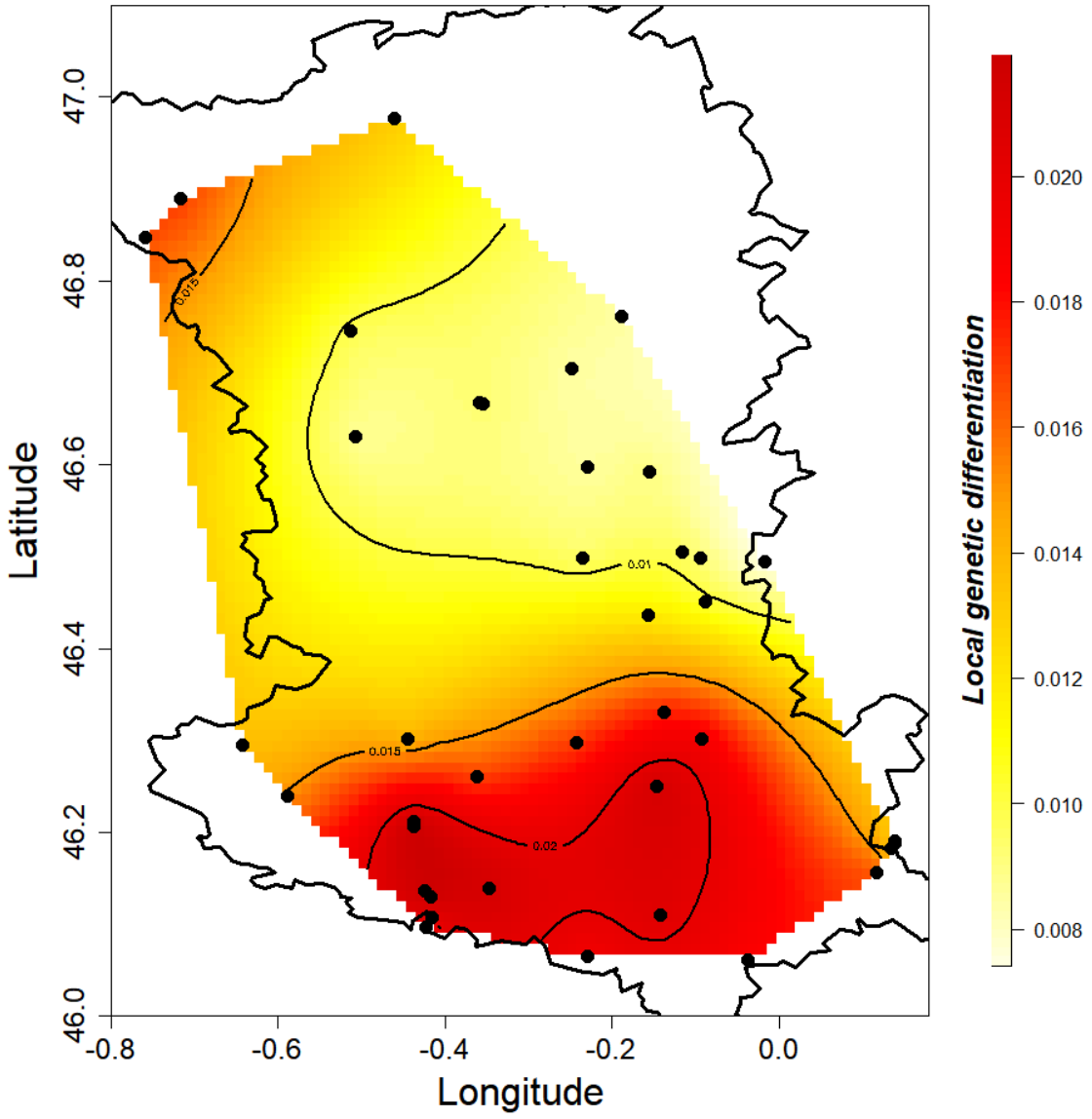

**Figure 5.** Relationship between genetic diversity and estimates of local genetic differentiation from LOCALDIFF for the 39 ponds (a) with allelic richness and (b) with observed heterozygosity as measures of genetic diversity. Black and grey colors indicate the northern and southern parts of the study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

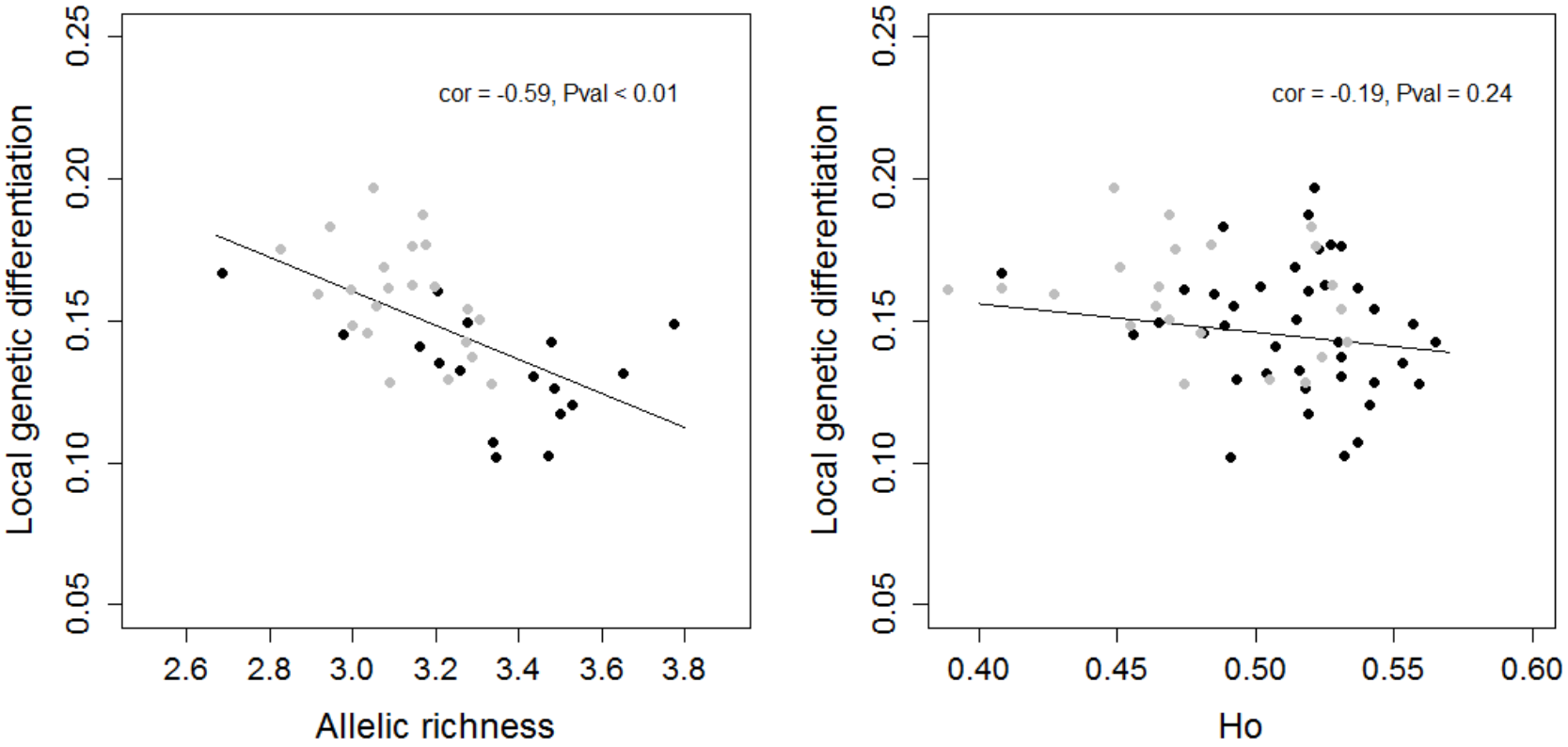

**Figure 6.** Estimates of dispersal kernel based on MIGRAINE in the northern (a) and southern (b) parts of the study area. Migration rate was estimated using a mutation rate of $10^{-4}$ for microsatellite markers.