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1 **Agricultural intensification alters marbled newt genetic**
2 **diversity and gene flow through density and dispersal**
3 **reduction**

4
5 Running title: Newts landscape genetic in farmlands
6

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28 **Abstract**

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

48

49 **Keywords :** Landscape genetics, demographic inferences, dispersal, genetic diversity,
50 microsatellites, *Triturus marmoratus*

51

52 **Introduction**

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

67

68 In European agricultural hedgerow landscapes, land conversion has caused loss of permanent
69 habitats and their connection in space (Benton, Vickery, & Wilson, 2003). While land
70 conversion threatens all taxa, amphibians constitute one of the most impacted ones (Ceballos,
71 Ehrlich, & Raven, 2020; Crawford, Peterman, Kuhns, & Eggert, 2016; Cushman, 2006; Hof,
72 Araújo, Jetz, & Rahbek, 2011; McCartney-Melstad & Shaffer, 2015). This sensitivity is
73 primarily due to i) their specific habitat requirements and complex life cycle involving
74 spatially distinct breeding and foraging habitats (Karraker & Gibbs, 2009; Sztatecsny et al.,
75 2004), and ii) their low dispersal capacity (Hillman, Drewes, Hedrick, & Hancock, 2014;
76 Smith & Green, 2005). Among amphibians, urodeles (newts and salamanders) have lower

77 mobility (Smith & Green, 2005), and are particularly vulnerable to temperature and water
78 constraints (Riddell, Roback, Wells, Zamudio, & Sears, 2019; Riddell & Sears, 2020). Most
79 European newt species live in water during their larval stage and for reproduction but they are
80 generally terrestrial for the rest of the year. Newts mainly reach breeding ponds following
81 corridors connecting ponds with woodlands, and avoid arable crops (Marty, Angélibert, Giani,
82 & Joly, 2005). Therefore, land conversion for intensive agriculture, including the loss of
83 hedgerows, shelters and corridors, could affect populations by decreasing effective population
84 size and connectivity, both resulting in loss of genetic diversity and gene flow.

85

86 Landscape genetics combines tools from population genetics, spatial statistics and landscape
87 ecology to relate landscape features directly to population structure, genetic diversity, and
88 gene flow (Manel & Holderegger, 2013; Manel et al., 2003; Storfer et al., 2007). Detection of
89 barriers and genetic clusters is a first step in landscape genetics but, because many
90 populations are continuously distributed, barriers may only exist at large biogeographic
91 scales. However, at smaller spatial scales, gene flow and functional connectivity might be
92 gradually modulated by landscape and environmental heterogeneity (isolation by resistance,
93 IBR, McRae, 2006). In addition, because species dispersal abilities are generally limited,
94 geographic distance is expected to play a significant role in the explanation of genetic
95 differentiation (isolation by distance, IBD, Rousset, 1997; Wright, 1943). IBD is generally
96 considered as the null model in landscape genetics and methods such as causal modelling are
97 employed to compare different models of landscape resistance and IBD (Cushman,
98 McKelvey, Hayden, & Schwartz, 2006). More recently, multiple regression methods are
99 becoming more popular as they allow to identify landscape variables influencing genetic
100 differentiation in a more robust statistical framework (Shirk, Landguth, & Cushman, 2017;
101 Wang, 2013), and rank them according to their importance in shaping genetic structure while

102 accounting for correlations among them (Prunier et al., 2015). While matrix-based analyses
103 (i.e. relating genetic differentiation to landscape structure) are the most popular in landscape
104 genetics, node-based analyses (i.e. relating local genetic diversity to surrounding landscape
105 structure) might provide complementary information on landscape genetics relationships
106 (Flavenot, Fellous, Abdelkrim, Baguette, & Coulon, 2015). However, both approaches are
107 mostly correlative and only allow identifying landscape genetics relationships without
108 assessing the demographic parameters (e.g. effective population size and dispersal) by which
109 landscape influences genetic patterns. This is particularly important regarding two related
110 issues. First, landscape genetics is mostly used in conservation studies where populations are
111 rarely at a demographic equilibrium (Segelbacher et al., 2010). Furthermore, genetic patterns
112 at a large spatial scale might be integrative of many generations that are likely to have
113 experienced numerous changes in population size and dispersal. It is thus challenging to
114 assess whether landscape genetic patterns result from variations in effective population size or
115 changes in dispersal (Richardson, Brady, Wang, & Spear, 2016). Landscape genetics studies
116 often interpret increases in measures of genetic differentiation in terms of reduced gene flow
117 and loss functional connectivity, neglecting the effect of local genetic drift on genetic
118 structure. Thus, demographic inferences of population parameters, i.e. effective population
119 size and dispersal rate and distance, in a spatially explicit framework are required to improve
120 the landscape genetics toolbox. Indeed, methods based on models specifying population size
121 and dispersal function might allow inferring whether genetic diversity (or genetic
122 differentiation) is affected by variations in population size, variations in dispersal or both. The
123 second issue is methodological. There is actually a large body of methods in population
124 genetics producing demographic inferences on N_e and dispersal (migration) rate (m) but they
125 are based on the simple, not spatially explicit, island model, while most natural populations
126 (and landscape genetics approaches) follow the IBD model. There is a real lack of use of

127 demographic inference methods based on the IBD model, including methods within the ABC
128 framework or likelihood based methods (Bertorelle, Benazzo, & Mona, 2010; Rousset &
129 Leblois, 2012). To investigate landscape genetics effects, comparative approaches involving
130 multiple landscapes or gradients of landscape composition are recommended (Goldberg &
131 Waits, 2010). These approaches should be particularly appropriate to infer and compare
132 demographic parameter estimates in contrasted landscapes, which can provide further
133 information for conservation efforts (Beebee & Griffiths, 2005).

134

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

149

150 We studied *T. marmoratus* in a 6000 km² agricultural landscape in Western France. The study
151 area is heterogeneous with preserved hedgerow farmland landscape with permanent

152 grasslands and meadows (hereafter grasslands) in the northern part, while recently opened
153 landscape dedicated to arable crops (mostly cereals) dominate the southern part (see Figure
154 1). We sampled 67 ponds distributed in these two contexts. Previous studies showed that pond
155 disappearance was associated with a decrease in grasslands and an increase in arable crop
156 land covers (Curado, Hartel, & Arntzen, 2011). In the study area, ponds were initially created
157 to water the livestock and are thus associated to grasslands. When grasslands are converted to
158 arable crops ponds are usually drained to increase cultivated land (Arntzen, Abrahams,
159 Meilink, Iosif, & Zuiderwijk, 2017). We characterized landscape changes (hedgerow loss)
160 over the last 60 years in the study area using historical landscape data and analyzed genetic
161 data collected in order to address the following goals:

162 (i) Identify the effect of landscape composition at various spatial scales on within pond
163 genetic diversity. We expected genetic diversity to increase with pond density and forested
164 land cover (woodlands and hedgerows) in the local landscape and to decrease with arable crop
165 land cover;

166 (ii) Characterize population genetic structure and test whether it was homogeneous across the
167 study area. In particular, we expected the northern and southern parts of the study area to
168 show contrasted levels of genetic structuring with stronger IBD in the southern part;

169 (iii) Identify landscape features that affect genetic differentiation. We expected roads, arable
170 crops and urbanized land cover to negatively affect gene flow while the opposite was
171 expected for pond density and forested land cover.

172 (iv) Infer population demographic parameters, including effective population density
173 (equivalent to effective population size in the continuous population model), dispersal rate
174 and the shape of dispersal distribution between contrasted landscape contexts. Enhanced
175 dispersal and larger population effective densities were expected in more connected areas.

176

177

178 **Materials and methods**

179 **Study area and sample collection**

180 The study area covers the entire Deux-Sèvres department (6000 km²), in Western France. It is
181 a farmland area with the northern part dominated by preserved hedged farmland with
182 permanent grasslands and meadows dedicated to livestock farming while the southern part is
183 more open and mostly dedicated to intensive cereal crops production (Figure 1). Sampling
184 occurred during the newt breeding season from February to May, in 2014 and 2015. A total of
185 734 individuals from 67 ponds were sampled (15 ponds in 2014 and 52 in 2015). At night,
186 individuals were localized using a flashlight and captured using a dip net. Newts were then
187 put into a bucket half filled with water. DNA samples were obtained by rubbing the bottom of
188 the mouth, cheeks and tongue with cotton swab to collect epithelial cells (Pidancier, Miquel,
189 & Miaud, 2003). Cotton swabs were stored in an alcohol solution (solution EDTA buffer:
190 Ethylene Diamine Tetra-Acetic). All individuals were released once the pond sampling was
191 completed.

192

193 **Genetic data collection**

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

200 There was a relatively high amount of missing data (13.41 % over the 734 samples) related to
201 low yield of DNA extraction in some pond samples, likely due to low quality DNA

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

208

209 **Genetic diversity**

210 We tested linkage disequilibrium between all pairs of loci on the global 612 individuals
211 dataset using GENEPOP4.2 (Rousset, 2008). Tests for deviations from Hardy–Weinberg
212 equilibrium for each locus and linkage equilibria for each locus pair were performed using
213 GENEPOP globally, and in each of the 39 ponds. Significance of tests was assessed following a
214 false discovery rate correction for multiple tests (Benjamini & Hochberg, 1995) with a
215 nominal significance level of 5%. Observed and expected heterozygosities (H_o and H_e) and
216 Weir and Cockerham’s estimate of F_{IS} (Weir & Cockerham, 1984) were calculated using
217 GENEPOP globally and for each pond. Using the rarefaction procedure implemented in FSTAT
218 2.9.3.2 (El Mousadik & Petit, 1996; Goudet, 2012), we calculated allelic richness corrected
219 for sample size (A_r) for each locus in all 39 pond.

220

221 **Landscape features and changes overtime**

222 We characterized the landscape over the study area using CORINE Land Cover 2012
223 (resolution 1/100 000) and BDTPOPO® 2015 (® IGN) in QGIS3.0.1(QGISDevelopment Team,
224 2018). Eight landscape features were selected based on their functional role for *T.*
225 *marmoratus*:

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

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

233 (iii) Ponds, which represents the breeding habitat of *T. marmoratus* were exhaustively
234 inventoried in the Deux-Sèvres department (N = 17400) and manually georeferenced for the
235 purpose of this study.

236 In order to evaluate habitat change since 1950 we also characterized past and present
237 landscape structure based on hedgerows linear digitalization from aerial photos taken in 1950
238 and 2015, hereafter Hedgerow₁₉₅₀ and Hedgerow₂₀₁₅. This historical analysis was focused on
239 the landscape surrounding the 39 ponds. We manually digitalized linear distance of
240 hedgerows within 2 km buffers around the ponds. Our approach was restricted to hedgerows
241 because some parameters (such as pond density) were not available on historical images. Yet,
242 hedgerows are integrative of landscape quality and correlated with pond density and
243 meadows. Indeed, Pearson correlation coefficient calculated over the entire Deux-Sèvres
244 department with a 5 km grid indicated strong correlation between hedgerow and pond density
245 and between hedgerows and meadows (Pearson's $r = 0.85$, P-value < 0.001 and Pearson's $r =$
246 0.91 , P-value < 0.001 , respectively).

247

248 **Effects of local landscape composition on genetic diversity**

249 Local genetic diversity depends on both population size and landscape connectivity. We
250 investigated which landscape features affected the genetic diversity of *T. marmoratus*. Within

251 circular buffers centered on the 39 ponds, we calculated the area of four different classes of
252 land cover (woodlands, grasslands, arable crops and urbanized), the length of roads and rivers,
253 and the number of ponds. Considering hedgerows, we used the linear data digitalized in 2 km
254 buffers around the ponds (Hedgerow₂₀₁₅) as it was more precise compared to the land cover
255 database and allowed us to include historical data to derive an index of landscape
256 intensification (Intensification_{index}) as $1 - (\text{Hedgerow}_{2015} / \text{Hedgerow}_{1950})$. Intensification_{index}
257 was fixed to 0 for ponds located in large forest patches, where woodland represented > 75%
258 of the total buffers area, because there are no hedgerows in those patches. There was no
259 intensification_{index} < 0 (i.e. no increase in hedgerow from 1950 to 2015 in any buffers). To
260 explore the scale at which landscape features influence genetic diversity, we calculated
261 landscape composition in buffer of different radius (500, 1000 and 2000 m). The maximal
262 distance was set to 2000 m to avoid artificially large correlation due to overlapping of buffers
263 and because buffers tended to be out of the mapped area at larger scales. To address possible
264 confounding effects due to correlations between landscape features, we computed pairwise
265 Pearson correlations between landscape features for each buffer radius. Pairs of landscape
266 features with Pearson correlation above the 0.70 threshold were considered highly correlated
267 (Dormann et al., 2013). We thus excluded Hedgerow₂₀₁₅ from the set of landscape descriptors
268 for all models, grasslands from the set of landscape descriptors for 500 model, and
269 Intensification_{index} from the set of landscape descriptors for 1000 m model (see details on
270 correlations in Appendix S2, Supplementary Information). A_r and H_o , calculated for each
271 locus and each pond 39 ponds, were alternatively used as response variable. A_r is expected to
272 be more sensitive to recent reductions in population size since it is more impacted by loss of
273 rare allele than H_o (Schwartz, Luikart, & Waples, 2007).

274 We used linear mixed effect models to quantify the contribution of landscape features on
275 genetic diversity using the ‘lmer’ function implemented in the LME4 package (Bates,

276 Maechler, Bolker, & Walker, 2015) in R 4.0.2 (R Development Core Team, 2014). We treated
277 selected landscape features as fixed factors and they were z -transformed (i.e. standardized to
278 zero mean and a standard deviation of one). Locus identity was included as a random
279 intercept to account for differences in allelic diversity among loci. First, we compared R^2 of
280 the full models (interactions were not considered) at 500, 1000 and 2000 m to assess the best
281 spatial scale. Second, for the best spatial scale, we ran models with all possible combinations
282 of explanatory variables using dredge function implemented in the MUMIN package (Barton,
283 2015). Models were fitted with maximum likelihood to compute reliable Akaike information
284 criterion (AIC) scores, and ranked according to AICc values (the small-sample-size corrected
285 version of AIC). To take into account uncertainty in model selection we then used model
286 averaging on models with Delta AICc values < 4 to get estimates of final parameters. Models
287 were validated a posteriori by checking plots of residuals. We also checked for
288 autocorrelation in the model residuals.

289

290 **Regional genetic structure**

291 To assess the extent to which ponds might be isolated from each other and form separate
292 populations, the population genetic structure was investigated by two complementary
293 approaches.

294 (i) We used the Bayesian clustering method implemented in STRUCTURE Version 2.2
295 (Pritchard, Stephens, & Donnelly, 2000), with the admixture model and correlated allele
296 frequencies (Falush, Stephens, & Pritchard, 2003) to determine the number of genetic clusters
297 (K). Ten replicate runs of 2×10^6 Markov chain Monte Carlo (MCMC) iterations, after an
298 initial burn-in period of 5×10^5 iterations, were performed for values of K ranging from one
299 to ten. Results were summarized using the standard pipeline on the CLUMPAK web server
300 (Kopelman, Mayzel, Jakobsson, Rosenberg, & Mayrose, 2015). The most likely number of

301 clusters (K) was explored using the estimated logarithm of likelihood (LnP(D)) and the
302 Evanno et al. (2005) ΔK method that finds the point of greatest change in the distribution of
303 LnP(D) with STRUCTURE HARVESTER Version 0.6.92 (Earl & vonHoldt, 2012). We used all
304 612 individuals for this individual-based analysis.

305 (ii) The level of pairwise genetic differentiation between the 39 ponds was quantified by
306 pairwise F_{ST} (Weir & Cockerham, 1984), and tested using the exact probability test for
307 population differentiation implemented in GENEPOP. IBD was then analyzed by regressing
308 pairwise estimates of $F_{ST} / (1 - F_{ST})$ against the logarithm of the geographical distances
309 (Rousset, 1997), and tested using a Mantel test (10,000 permutations). To visualize IBD and
310 whether it was homogeneous across our study area, we used LOCALDIFF 1.5 (Duforet-
311 Frebourg & Blum, 2014), a method that infers local genetic differentiation based on Bayesian
312 kriging. The pairwise $F_{ST} / (1 - F_{ST})$ values between the 39 ponds was used as input measure of
313 pairwise dissimilarity. The method requires fictive neighboring populations to be introduced
314 at the vicinity of sampling sites (*i.e.* short distance compared to the dimension of the region
315 under study), as a means to provide measures of local genetic differentiation that are
316 comparable between sampling sites. Hence, four fictive neighboring populations were
317 introduced at 2 km from the 39 ponds. The output of LOCALDIFF generates a minimum
318 convex polygon that encompasses all sampling sites, where warmer colors represent areas
319 with stronger local genetic differentiation. We used Pearson correlations to test the
320 relationship between estimates of genetic diversity (A_r , H_e and H_o) within ponds and estimates
321 of local genetic differentiation computed with LOCALDIFF.

322

323 **Effects of landscape composition on genetic structure**

324 To investigate the effect of landscape features on spatial genetic structure, we performed
325 multiple regression of distance matrices (MRDM) (Legendre et al., 1994) and multiple matrix

326 regression with randomization MMRR (Wang, 2013). Pairwise genetic distances were
327 computed between the 39 ponds using $F_{ST}/(1 - F_{ST})$ as genetic distance. To compute pairwise
328 resistance distances a specific layer was created for each landscape feature. We overlaid a 250
329 m grid on these raster data, and calculated the percentage per grid cell of each categorical
330 feature (Woodlands, Hedgerows, Arable crops, Urbanized, and Grasslands), attributed a value
331 of one for each grid cell intersected by linear features (roads and rivers) and calculated the
332 number of ponds in each cell of the grid. To allow comparison among landscape features,
333 these layers were finally rescaled to range from 1 to 100 and used in CIRCUITSCAPE 4.0.5
334 (Mcrae, Shah, & Mohapatra, 2013) to compute pairwise resistance distances between ponds.

335 MRDMs are similar to classical multiple ordinary least-square regressions, except that the
336 significance of model fit and beta weights (β) is assessed through permutations (Legendre et
337 al., 1994). Beta weights could be heavily impacted by multi-collinearity among landscape
338 variables (Ray-Mukherjee et al., 2014), so we used a commonality analysis (CA) (J. G.
339 Prunier et al., 2015; Ray-Mukherjee et al., 2014) to dissect the complexity of landscape
340 features relative contribution to the model fit. CA is a variance-partitioning procedure that
341 disentangles the individual vs. shared contribution of each predictor to R^2 . This procedure is
342 particularly useful when the predictors are themselves correlated (Prunier et al., 2015).
343 Pearson's correlations among pairwise resistance distances computed for each landscape
344 feature ranged from 0.02 to 0.69, while variance inflation factor (VIF) ranged from 1.62 to
345 2.96 (Table S4 in Supplementary Information). All pairwise resistance distances were kept in
346 the model as we considered Pearson's correlations and VIF to be high above thresholds of 0.7
347 and 10, respectively (Dormann et al., 2013; Zuur et al., 2009). To determine the contribution
348 of pairwise resistance distances relatively to IBD, pairwise geographic distance was also
349 included in the model. CA-MRDM was run using the R packages ECODIST (Goslee & Urban,
350 2007) and YHAT (Nimon, Oswald, & Roberts, 2013) following the CAonDM script provided

351 by Prunier et al. (2015). Significant levels were assessed with 10,000 permutations after
352 sequential Bonferroni correction (Holm, 1979). Commonalities and 95% confidence intervals
353 were computed using a bootstrap procedure with 1000 replicates based on a random selection
354 of 10% of samples (out of 39 populations) without replacement (Prunier et al., 2015).
355 Alternatively, we ran MMRR according to Wang (2013). MMRR was implemented in R
356 using the package PopGenReport (Adamack & Gruber, 2014). We used pairwise geographic
357 and the eight pairwise resistance distances as the explanatory variables and 999 permutations
358 to assess the additive effect of both independent factors (geographical distance and landscape
359 features). All matrices were standardized using the 'scale' function implemented in R before
360 running the MMRR. Finally, we tested each resistance distances and geographic distance in
361 eight separate models.

362

363 **Inferences on demographic parameters**

364 Spatial variation in IBD was explored by the maximum likelihood method implemented in
365 MIGRAINE that infers model parameters using importance sampling algorithms (De Iorio,
366 Griffiths, Leblois, & Rousset, 2005), extended to consider IBD as a model for population
367 structure (Rousset & Leblois, 2012; Rousset & Leblois, 2007). Based on LOCALDIFF results,
368 ponds from the northern and southern parts of the study area were analyzed separately to infer
369 area specific demographic parameters. Our specific aim here, was to assess whether
370 differences in IBD between these two parts of the study area result from differences in
371 effective population size and / or differences in dispersal. We excluded the three northernmost
372 ponds to get parameter estimates from a more homogeneous landscape and with a continuous
373 distribution of sampled ponds. Based on a geometric distribution for dispersal and a K allele
374 model for mutation, MIGRAINE provides point estimates, 95% coverage confidence intervals
375 (CIs) and two-dimensional parameter likelihood profiles for several parameters: 1) The scaled

376 local population size $\theta=2\times N_{\text{genes}}\times\mu$, where N_{genes} is the local population size expressed in
377 number of genes and μ the mutation rate per locus per generation. 2) The scaled emigration
378 rate per generation: $\gamma = 2\times N_{\text{genes}}\times m$, where m is the total emigration rate per generation for a
379 local population. 3) The parameter of the geometric distribution of dispersal (g). 4) The
380 neighborhood size, $Nb = 4\pi\times N\times\sigma^2$, where N is the effective population size and σ^2 the mean
381 squared parent–offspring dispersal distance. All MIGRAINE runs were performed under a two-
382 dimensional model of IBD. The spatial binning of samples was performed using MIGRAINE-
383 GUI and resulted in 16 x 10 and 20 x 11 bins for the northern and southern parts of the study
384 area, respectively. Bin size, 3275 x 3275 m, was set constant over the two analyses to
385 facilitate results interpretation. We used the following computing parameters: 2000 trees, 100
386 points and 3 iterations. We translated the parameters inferred from MIGRAINE into effective
387 population size using a mutation rate range commonly used for microsatellites: 1×10^{-4} [5×10^{-3}
388 $- 5\times 10^{-5}$] (Sun et al., 2012).

389

390 **Results**

391 **1) Landscape characteristics and changes through time**

392 Pond density was almost five times higher in the northern part with 3.79 ponds / km²
393 compared to 0.8 ponds / km² in the southern part of the study area (Figure 1). Consistently,
394 arable crops represented 41.16 % and 63.88 % and meadows 48.59 % and 21.49 % of land
395 cover in the northern and southern parts of the study area, respectively. Based on the
396 digitalized hedgerow in 2 km buffers around the 39 ponds, we found a significant difference
397 in the proportion of hedgerow loss between the northern and southern parts of the study area
398 (two samples t-test, P-value < 0.001). On average loss was 34 % and 53 % in the northern and
399 southern parts of the study area, respectively.

400

401 **2) Genetic markers and diversity**

402 The number of alleles per locus ranged from four to 16 in the global dataset (Table S1.1,
403 Supplementary Information), with an average value of 8.5. Expected heterozygosity for each
404 locus ranged from 0.047 to 0.895, with an average value of 0.576. Observed heterozygosity
405 ranged from 0.046 to 0.813, with an average value of 0.486. There was a marginally
406 significant overall heterozygosity deficit in the global dataset (P -value = 0.056, F_{IS} = 0.127)
407 that could be related to the presence of spatial structure (“Wahlund effect”). Indeed, only one
408 of the 39 ponds with at least 7 individuals showed significant departure from HWE (Table
409 S1.2, Supplementary Information). After FDR correction, there was no pair of loci showing
410 significant LD in the global dataset.

411

412 **3) Effect of landscape composition on ponds genetic diversity**

413 Within pond allelic richness corrected for sample size (A_r) ranged from 2.684 to 3.773 (Table
414 S1.2, Supplementary Information). R^2 comparison among linear mixed models based on
415 landscape descriptors calculated in 500, 1000 and 2000 m buffers indicated 1000 m was the
416 best spatial scale to explain A_r (marginal R^2 = 0.005, 0.007 and 0.005 for 500, 1000 and 2000
417 m buffers, respectively). Several plausible best models (i.e. with delta AICc < 4) were
418 identified at 1000 m (Table S2.4, Supplementary Information). Model averaging indicated
419 that pond density in the surrounding landscape had a significant and positive effect on within
420 pond A_r while the negative effect of arable crop land cover was only marginal (Table 1 and
421 Figure 2). Consistently, models at 500 m and 2000 m also reported a significant and positive
422 effect of the amount of ponds in the surrounding landscape.

423 Regarding H_o , R^2 comparison among linear mixed mode indicated 500 m was the best spatial
424 scale (marginal R^2 = 0.006, 0.001 and 0.004 for 500, 1000 and 2000 m buffers, respectively).

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

427

428 **4) Regional pattern of genetic structure**

429 The most likely value of K from the STRUCTURE analysis based on the method of Evanno *et al.*
430 (2005) was two (Table S3, Supplementary Information). Consistently, the estimated logarithm
431 of likelihood for data was highest for $K = 2$. However, for most individuals, the estimated
432 membership coefficients in each cluster Q was low (i.e. > 60 % individuals had $Q < 0.7$) and
433 inspection of STRUCTURE barplot was more consistent with an IBD pattern than to the
434 presence of two or more genetic clusters (Figure S1). Indeed, a pattern of IBD was supported
435 by the positive relationship between $F_{ST}/(1-F_{ST})$ and the logarithm of geographic distance
436 (Figure 3, Slope = 0.027, 95% CI: (0.018 – 0.037), Mantel test P-value < 0.001) over the 741
437 pairs of ponds. The average F_{ST} value between ponds was 0.066 and for 621 pairs (84%)
438 genetic differentiation was significant. The analysis performed with LOCALDIFF indicated a
439 heterogeneous pattern of IBD across the study area. The southern part was characterized by
440 strongest values of local genetic differentiation, whereas smaller local genetic differentiation
441 was found in the northern part of the study area (Figure 4). We measured a significant
442 negative correlation between allelic richness (Ar) and estimates of local genetic differentiation
443 computed with LOCALDIFF (cor = -0.59, P-value < 0.01), while the correlation was non-
444 significant when H_o (cor = -0.19, P-value = 0.24) was used as a measure of genetic diversity
445 (Figure 5).

446

447 **5) Effect of landscape composition on genetic structure**

448 The MRDM was significant and explained 35.7% of the variance in $F_{ST}/(1-F_{ST})$ (Table 2).
449 After sequential Bonferroni corrections, only arable crop land cover had a significant positive

450 effect on genetic differentiation ($\beta_{\text{crops}} = 0.33$, P-value = 0.016). $F_{\text{ST}}/(1-F_{\text{ST}})$ increased by 0.33
451 standard deviations with a one standard deviation change in arable crops resistance distance,
452 all other predictors being held constant. Commonality coefficients showed that arable crop
453 land cover uniquely contributed 8.2% of the total variance in $F_{\text{ST}}/(1-F_{\text{ST}})$ and to 22.8% of the
454 35.7% of the variance explained by the regression model. Unique contributions of other
455 landscape features were negligible or counterbalanced by their common contribution with
456 other predictors (indicative of classical suppressor effect according to Prunier et al. 2017)
457 (Table 2).

458 The MMRR model including geographic distance and all resistance distances explained
459 nearly 30 % of the variability in genetic differentiation ($R^2 = 0.291$, $P < 0.001$). It revealed
460 that arable crop land cover was the only landscape feature significantly influencing genetic
461 differentiation ($\beta_{\text{crops}} = 0.009$, $P = 0.033$, Table 2 and Figure S2, Supplementary Information).

462

463 **6) Demographic inferences**

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

473

474 **Discussion**

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

482

483 **Population genetic structure**

484 In Western France, at a local spatial scale (up to 6.5 km) previous genetic studies on *T.*
485 *marmoratus* reported distinct genetic clusters with STRUCTURE, an average pairwise F_{ST} of
486 0.11 (range: 0.007 - 0.303) and no significant IBD (Jehle, Burke, & Arntzen, 2005; Jehle,
487 Wilson, Arntzen, & Burke, 2005). In our study, at distance < 6.5 km, the average pairwise F_{ST}
488 was only 0.025 (range: -0.03 – 0.07) with 9 out of 25 pairs of ponds significantly
489 differentiated. A single population exhibiting IBD was found, and genetic structure was not
490 affected by rivers or motorways crossing the study area, similar to other newt studies
491 (Costanzi et al., 2018; Luqman et al., 2018; Prunier et al., 2013). Based on 11 *T. marmoratus*
492 populations sampled across Western France, Costanzi et al. (2018) identified strong genetic
493 structure at a large scale (> 100 km), including distinct genetic clusters in the area of our
494 study. Their result is likely due to IBD which might create spurious genetic clusters when
495 geographical sampling is clumped (Blair et al., 2012; Frantz, Cellina, Krier, Schley, & Burke,
496 2009). This supports that continuous distribution of samples with individual-based sampling
497 is the best strategy to uncover unbiased spatial genetic structure. Our results also side with the
498 conclusion of Smith et al. (2005) that although amphibians are predominantly philopatric with
499 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

525 sampled ponds). The landscape feature selected in the model, pond density, was more
526 representative of local landscape quality in terms of habitat availability. For the matrix-based
527 analyses, we considered the full landscape matrix and the landscape feature selected in
528 MRDM and MMRR models, arable crop land cover, was more indicative of reduced
529 landscape permeability between habitat patches. However, since gene flow depends on both
530 effective population density and dispersal, population decline in the southern side of the study
531 area could also explain the negative effect of arable crop land cover on genetic differentiation
532 found with MRDM and MMRR. The negative relationship between genetic diversity and
533 estimates of local genetic differentiation from LOCALDIFF supports the hypothesis that genetic
534 drift is a predominant micro-evolutionary process driving genetic differentiation (Coleman,
535 Weeks, & Hoffmann, 2013). Altogether, our study exemplifies the difficulty to disentangle
536 the influence of effective population density and dispersal on gene flow from patterns of
537 genetic diversity and genetic structure. More specifically, it is challenging to assess whether
538 landscape structure and land conversion affect natural populations through reduction in
539 effective population size or dispersal.

540

541 **Demographic inferences on dispersal and population effective density**

542 Several lines of evidence support a recent demographic decline in the southern part of the
543 study area. Landscape influence on local genetic diversity was only found for allelic richness,
544 while it was not significant for heterozygosity that is less affected by recent demographic
545 changes (Schwartz et al., 2007). Consistently, correlation between estimates of local genetic
546 differentiation from LOCALDIFF and genetic diversity were significant for allelic richness and
547 not for heterozygosity, suggesting *T. marmoratus* populations are declining to a greater
548 degree in the southern side of the study area. This supports the hypothesis that landscape
549 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, N_b , the neighborhood size, was
555 significantly lower in the south, with $N_b = 68.77$ and 557.5 in the southern and northern parts
556 of the study area, respectively. While N_b 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 (N_e) is usually much lower than the census
565 population size (N) (see Table 4 in Schmeller & Merila 2007). Indeed, *T. marmoratus* N_e/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

575 part of the study area, consistently with a previous radiotracking study (Trochet et al., 2017).
576 Our landscape scale estimates of dispersal rates and distances might appear much higher than
577 was previously thought (Jehle et al. 2005). These authors concluded that dispersers could not
578 travel distances > 1km but their estimates of pond specific immigration rate were only based
579 on three closely located ponds as potential sources. Our analysis based on the IBD model and
580 the sampling of more than 39 ponds over a large area appear more robust to infer landscape-
581 scale dispersal distances and rates. In their review paper on amphibians dispersal, Smith et al.
582 (2005) concluded that for salamanders ponds may receive migrating individuals from
583 distances up to 8-9 km. Although *T. marmoratus* was not included in this review, our results
584 suggest some individuals might occasionally travel similar distances.

585

586 **Conclusion and recommendation for conservation**

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

599

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888 **Data Accessibility**

889 The data sets with *T. marmoratus* sampling locations and microsatellite genotypes can be
890 found on Dryad - <https://doi.org/10.5061/dryad.mkkwh710s>.
891

892 **Author Contributions**

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

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900 **Tables and figures**
901

902 **Table 1:** Results of model averaging on models of allelic richness with delta AIC < 4 for the spatial
 903 scale with the highest R² (*i. e.* 1000 m buffer size). Estimates and P-values are presented for each
 904 landscape descriptor tested. Significant relations are in bold.
 905

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

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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			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.

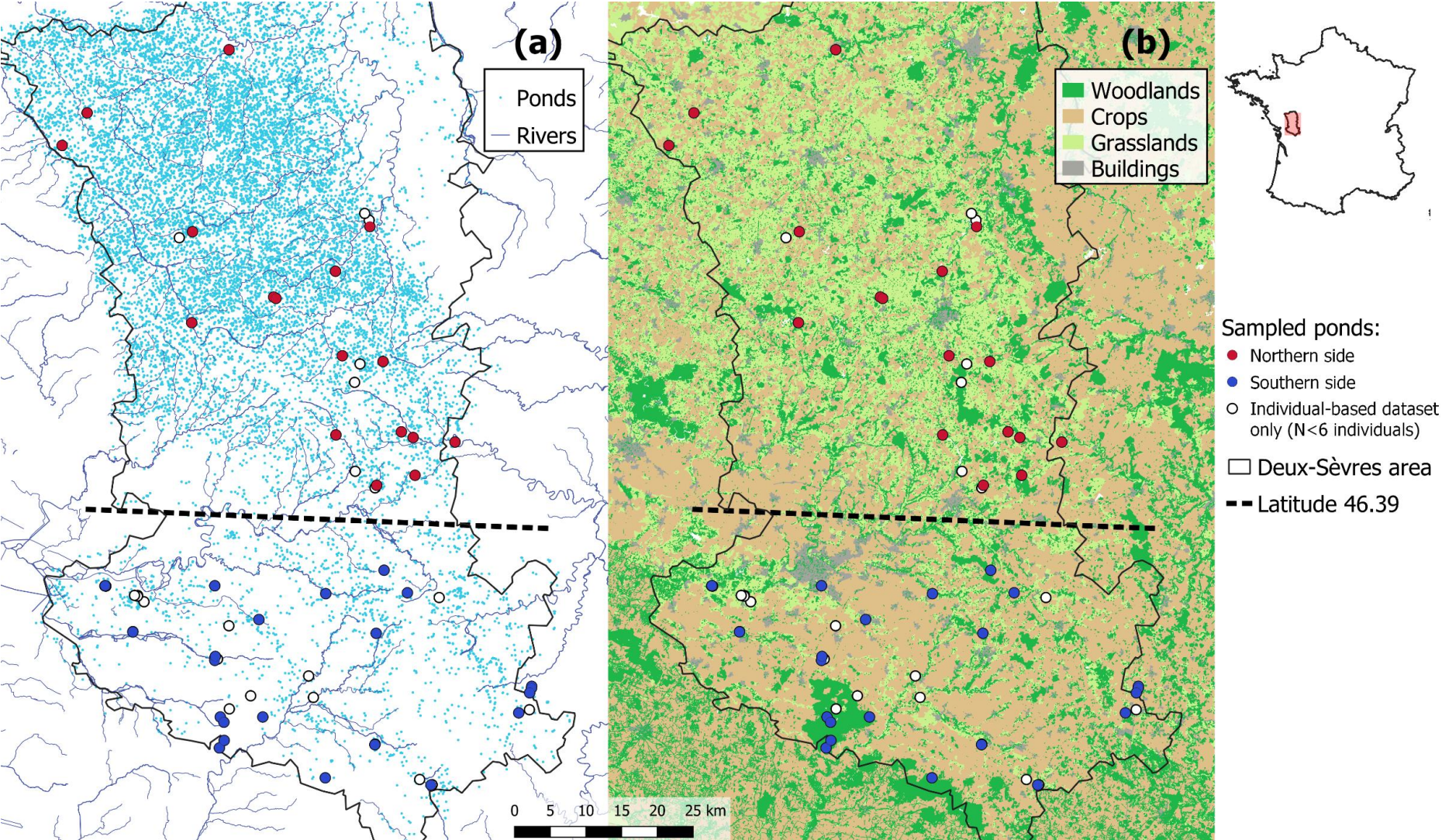


Figure 2. Relation between Allelic richness (A_r) and pond density in a 1000 m buffer around the 39 ponds. Black dots and gray dots represent ponds from the northern and southern sides of the study area, respectively.

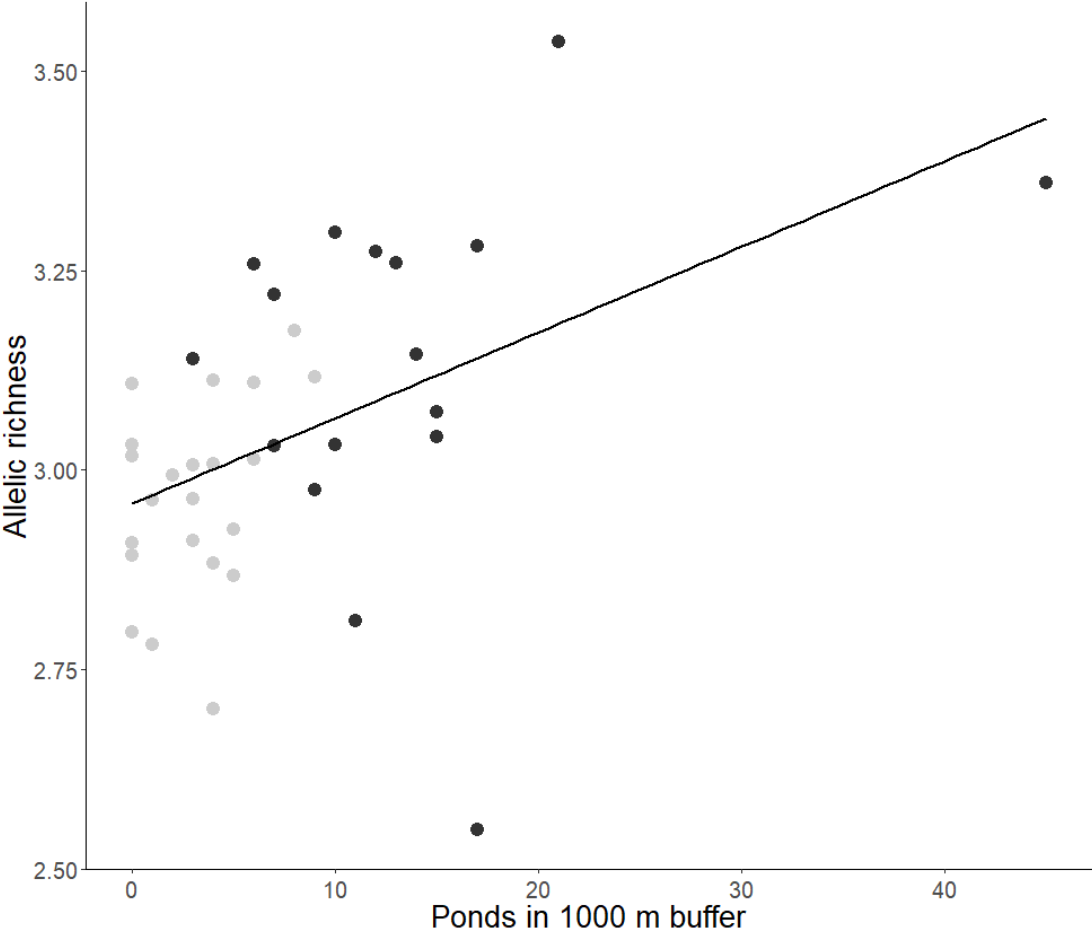


Figure 3. Correlation between pairwise genetic differentiation ($F_{ST}/(1 - F_{ST})$) and logarithm of the geographic distance between pairs of ponds. Filled dots indicate pairs of ponds that were significantly different (GENEPOP exact probability test for population differentiation).

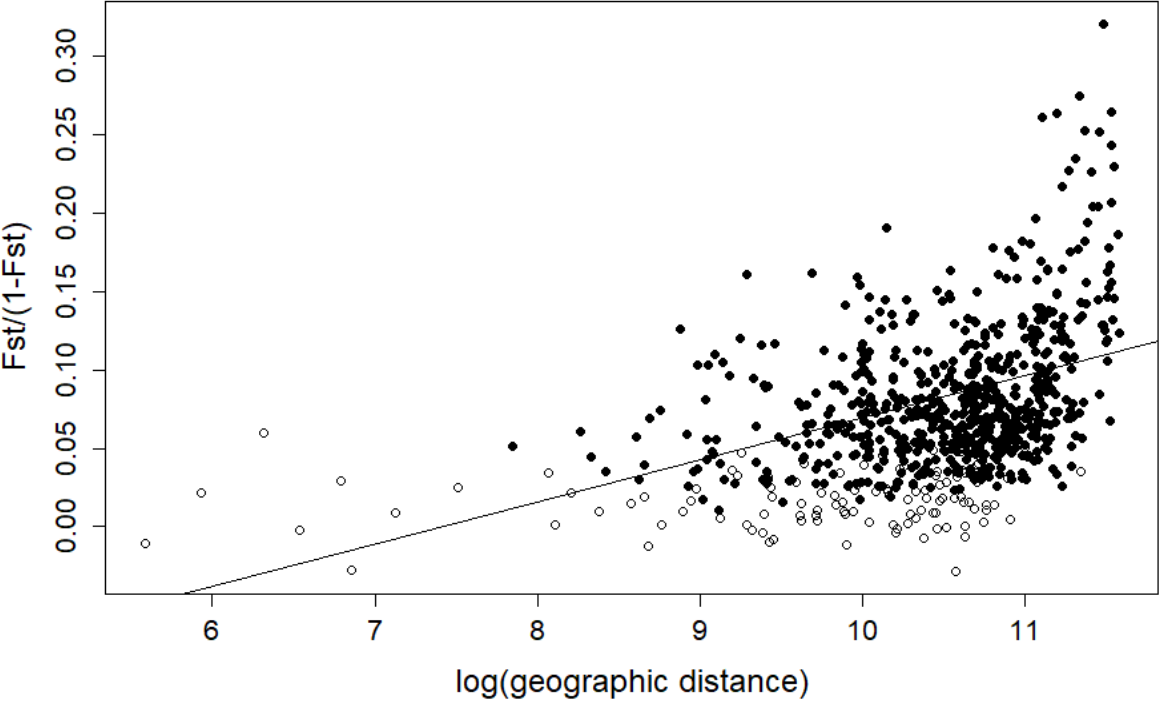


Figure 4. Map of local genetic differentiation inferred by the Bayesian kriging method implemented in LOCALDIFF . Shape represent the minimum convex polygon that encompasses the 39 ponds. Measures of local genetic differentiation correspond to $F_{ST}/(1-F_{ST})$ calculated between the sampled ponds 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.

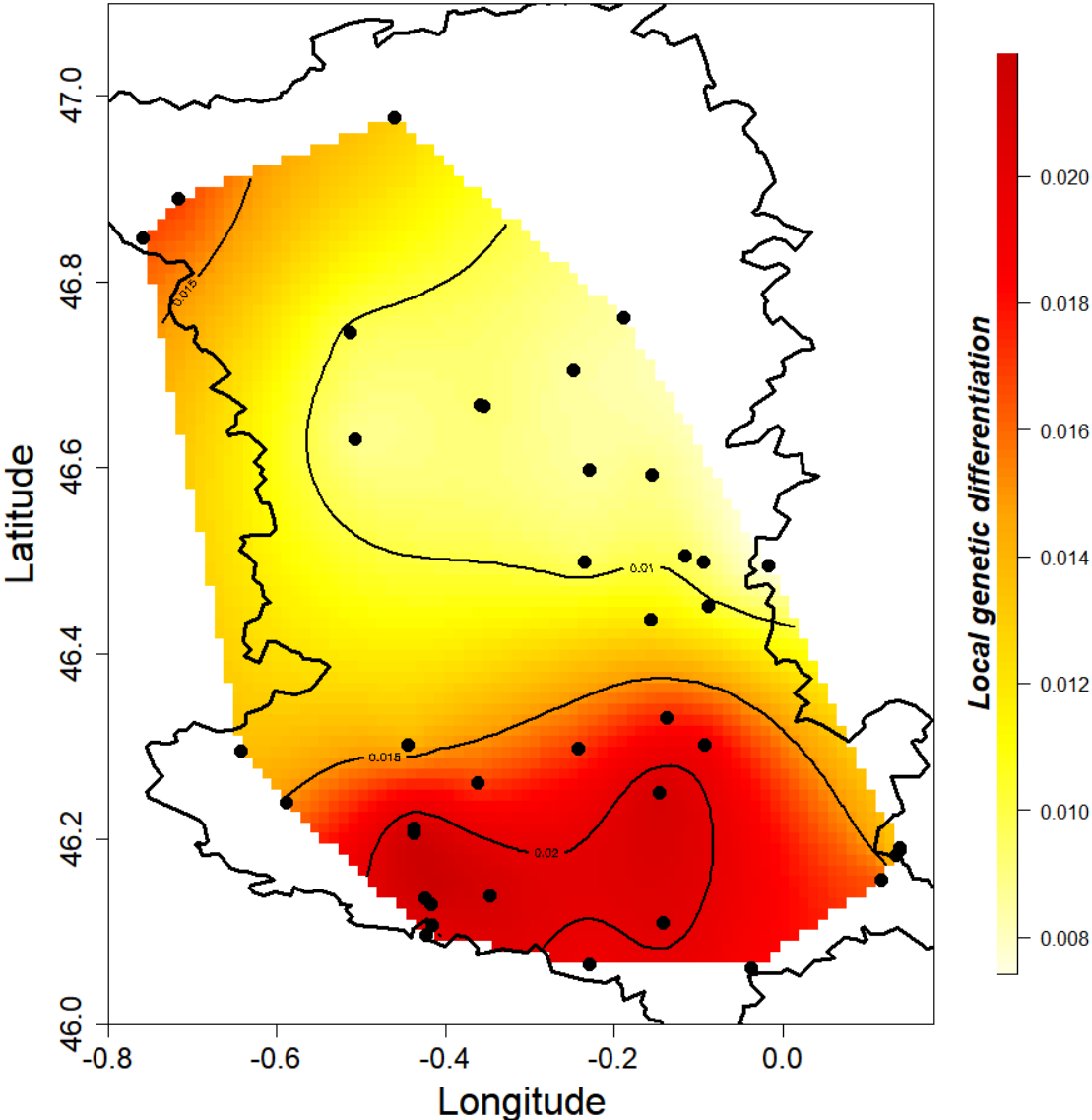


Figure 5. Relationship between genetic diversity and estimates of local genetic differentiation from LOCALDIFF for the 39 ponds (a) with allelic richness and (b) with observed heterozygosity as measures of genetic diversity. Black and grey colors indicate the northern and southern parts of the study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

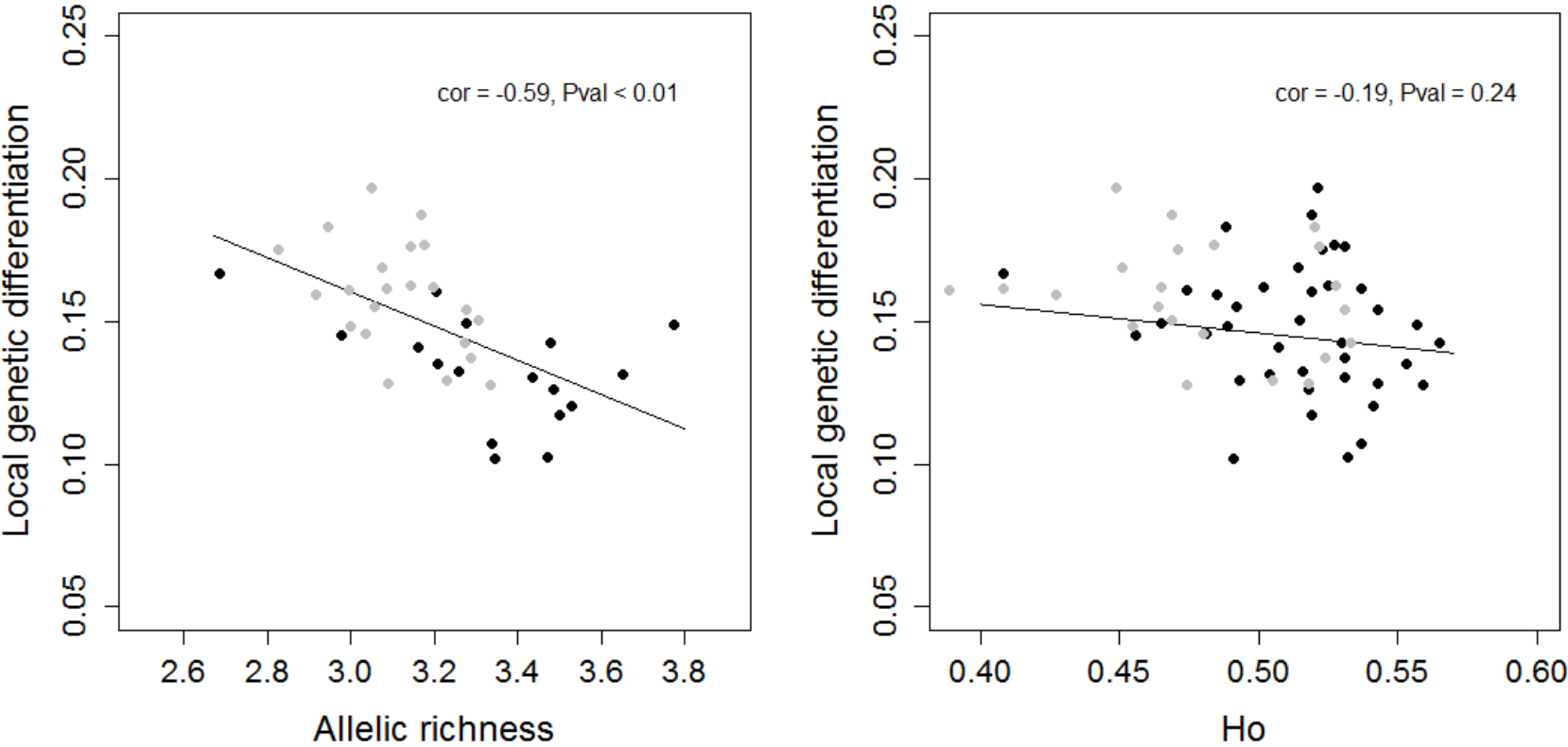


Figure 6. Estimates of dispersal kernel based on MIGRAINE in the northern (a) and southern (b) parts of the study area. Migration rate was estimated using a mutation rate of 10^{-4} for microsatellite markers.

