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1 **Landscape features impact connectivity between soil populations: a comparative study**
2 **of gene flow in earthworms**

3

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14

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17

18 **Running Title** : Landscape genetics of earthworms

19

20

21 **Abstract**

22

23 Landscape features are known to alter the spatial genetic variation of above ground
24 organisms. Here, we tested the hypothesis that the genetic structure of below ground
25 organisms also responds to landscape structure. Microsatellite markers were used to carry out
26 a landscape genetic study of two endogeic earthworm species, *Allolobophora chlorotica* (N =
27 440, 8 microsatellites) and *Aporrectodea icterica* (N = 519, 7 microsatellites), in an
28 agricultural landscape in the North of France, where landscape features were characterised
29 with high accuracy. We found that habitat fragmentation impacted genetic variation of
30 earthworm populations at the local scale. A significant relationship was observed between
31 genetic diversity (H_e , A_r) and several landscape features in *A. icterica* populations but not in
32 the *A. chlorotica* ones. Moreover, a strong genetic differentiation between sites was observed
33 in both species, with a low degree of genetic admixture and high F_{st} values. The landscape
34 connectivity analysis (MLPE) at the regional scale, including Isolation By Distance (IBD),
35 Least Cost Path (LCP) and Cost Weighted Distance (CWD) approaches, showed that genetic
36 distances were linked to landscape connectivity in both species. This indicates that the
37 fragmentation of natural habitats has shaped their dispersal patterns and local effective
38 population sizes. Landscape connectivity analysis confirmed that *a priori* favourable habitats
39 such as grasslands may constitute dispersal corridors for these species.

40

41 **Introduction**

42

43 A number of studies showed that spatial genetic variations of aboveground organisms
44 respond to changes in their landscape, through mechanisms involving movements of
45 organisms (review in Storfer *et al.* 2010; Manel & Holderegger 2013; Hall & Beissinger
46 2014). It is now well-grounded that landscape features alter aboveground organisms' genetic
47 structure. Comparatively little is known about the impact of landscape-scale habitat
48 heterogeneity on belowground organisms, such as soil invertebrates, whose mobility is more
49 restricted (Vanbergen *et al.* 2007). Despite their importance in ecosystem functioning and in
50 the delivery of many ecosystem services (Lavelle *et al.* 1997; Jouquet *et al.* 2006; Lavelle *et*
51 *al.* 2006; Blouin *et al.* 2013), we still do not have a grasp of even basic information about
52 population genetic structure of soil organisms. For instance, Costa *et al.* (2013) found only
53 sixteen different species among the collembolans, earthworms and isopods groups of soil
54 invertebrates for which a population genetics study was carried out. Some of these papers
55 investigated the spatial genetic structure of soil organisms at a fine-scale (Sullivan *et al.* 2009;
56 Novo *et al.* 2010; Dupont *et al.* 2015), but none addressed the effect of landscape features on
57 genetic variation. However, terrestrial habitat heterogeneity is known to affect the diversity of
58 soil species' assemblages by producing variation in the diversity of plant and litter
59 (Vanbergen *et al.* 2007). It is therefore assumed that aboveground structure and diversity
60 could profoundly impact the population genetic structure of belowground organisms.

61

62 The methodology of landscape genetics allows one to test the influence of the landscape
63 and environmental characteristics on microevolutionary processes and metapopulation
64 dynamics, including gene flow and local adaptation (Manel *et al.* 2003; Storfer *et al.* 2007;

65 Holderegger & Wagner 2008). Landscape connectivity is a twofold parameter made up of
66 structural connectivity and functional connectivity. Structural connectivity refers to the
67 physical relationship between landscape elements while functional connectivity may be
68 defined as the ease with which a landscape can be crossed by an organism (Taylor et al. 2006).
69 Depending on the organisms, the permeability of the landscape will differ and some
70 constituent elements of the landscape can facilitate dispersal (i.e. “corridors”) while others
71 can impede or reduce the passage of dispersers (i.e. “barriers”) (Taylor et al. 1993).
72 Landscape structure can also have an important effect on passive dispersers by altering the
73 abiotic and biotic conditions that affect movement (Matthysen 2012). In order to understand
74 how landscape characteristics influence functional connectivity, resistance surfaces are
75 usually computed and translated into measures of inter-population connectivity principally
76 using two kinds of models. Least-cost path models (Adriaensen et al. 2003) assume that
77 movement or gene flow rates between each pair of sites is related to the total cumulative
78 resistance or ‘cost’ (sum of per-pixel resistance values) along a single optimal path, while
79 circuit-theory based models (McRae 2006) incorporate all possible pathways across
80 landscapes, and their parameters and predictions can be expressed in terms of random walk
81 probabilities (Cost Weighted Distance "CWD", or "resistance approach"). Spear *et al.* (2010)
82 highlighted that both models provide complementary indices of connectivity with Least-cost-
83 path distances being more informative at local scales and circuit theoretic models being
84 particularly useful for incorporating effects of gene flow over multiple generations.

85

86 Here, we were interested in landscape features impacting genetic variation and functional
87 connectivity in earthworms. Dispersal by passive mechanisms, such as zoochory, wind, water
88 and human activities is believed to be implicated in their long-distance movement (Eijsackers

89 2011; Costa et al. 2013; Dupont et al. 2015), whilst their active dispersal is dependent on
90 habitat quality, conspecific density, and habitat modification by conspecifics in endogeic (i.e.
91 species living in the upper organo-mineral soil layers and forming horizontal non-permanent
92 burrows, Bouché 1977) and anecic (i.e. species forming permanent or semi-permanent
93 vertical burrows in the soil which open at the surface where the earthworm emerges to feed,
94 Bouché 1977) species (Mathieu et al. 2010; Caro et al. 2012; Caro et al. 2013). The
95 distribution of these restricted dispersers is known to be controlled by soil parameters at the
96 field scale and by land use (forest, grassland and agricultural field), soil management, soil
97 type and climatic conditions at larger scales; studies at the landscape scale are thus
98 challenging since small-scale heterogeneity as well as gradients affecting large-scale patterns
99 have to be accounted for (Palm et al. 2013).

100 In order to investigate whether landscape features impact the genetic structure of
101 earthworm populations, we carried out a regional-scale comparative survey of genetic
102 variation in two species commonly found in European agricultural landscapes, the green
103 morph of *Allolobophora chlorotica* (Savigny, 1826) and *Aporrectodea icterica* (Savigny,
104 1826). Both species are endogeic but present several ecological differences. *A. chlorotica*
105 typically lives between soil surface and the upper 60 mm soil layer (Sims & Gerard 1999) and
106 is theoretically able to travel over 167 m.year⁻¹ in constant suitable conditions (Caro et al.
107 2013). However, in the field, dispersal distances ranging from 6.82 to 7.56m per year were
108 estimated in a recent population genetics study at fine spatial scales (Dupont et al. 2015). *A.*
109 *icterica* is found deeper in soils and is considered to be more mobile, being theoretically able
110 to travel up to 500 m.year⁻¹ under constant artificial conditions (Mathieu et al. 2010; Caro et
111 al. 2013). An extremely low signal of genetic structure was obtained for this species in a fine-
112 scale population genetics study at the within plot scale (100x80m). This result was explained

113 by the great dispersal capacity of the species (Dupont et al. 2015). Moreover, no pattern of
114 isolation by distance (IBD, i.e. decrease in the genetic similarity among populations as the
115 geographic distance between them increases) was observed among six *A. icterica* populations
116 separated by less than 13km (Torres-Leguizamon et al. 2014).

117 We analysed the relationship between landscape features and genetic variation in these
118 two common earthworm species, at fine and regional scales (Fig. 1), in an agricultural
119 landscape in North of France, where both species are common (e.g. Richard et al. 2012). First,
120 we tested the hypothesis that the mosaic of habitats created by anthropogenic drivers alters the
121 genetic diversity in earthworm populations using a buffer approach. It consists in assessing
122 the correlation between the genetic variation of the earthworm population and the local
123 landscape structure. Second, we tested the hypothesis that the different elements of the
124 landscape could act either as dispersal barriers or corridors for earthworms with a landscape
125 connectivity analysis at the regional scale (Zeller et al. 2012), encompassing Isolation by
126 Distance (IBD), Least Cost Path (LCP) and resistance (CWD) approaches. The role of three
127 elements, i.e. grasslands, crops and roads, was specifically tested. Grassland represents a
128 suitable habitat that could be easily crossed by endogeic species (Bouché 1972; Decaens et al.
129 2008) while soil tillage and the use of pesticides in cultivated soils are known to have a
130 detrimental effect on earthworms (Bertrand et al. 2015) and roads have been shown to
131 represent dispersal corridors for invasive earthworms (Cameron & Bayne 2009).

132

133 **Material and Methods**

134

135 *Study Area and Sampling*

136 Earthworms were collected in Normandy (northern France, Fig. 2) in 2009 and 2010.
137 The first sampling campaign was carried out in March and April 2009 in two pastures (P_A and
138 P_B) ~ 500m apart at the local agricultural school “Lycée Agricole d’Yvetot”. Details of these
139 sampling sites and methods are given in Dupont *et al.* (2015). The second sampling campaign
140 was carried out in April 2010, during which 39 other pastures were prospected. *A. chlorotica*
141 and *A. icterica* were found in 14 and 19 pastures, respectively (Fig. 2 and Table 1). We
142 selected pastures that had similar management histories, in order to reduce the effect of local
143 environmental variations. The location of the plots was chosen in order to maximise the
144 normality of the pairwise distance between plots. All pastures were at least 5 years old, and
145 the great majority was grazed by cattle. Within each plot of 10 x 10m, 30 individuals were
146 captured by sampling five monoliths of soil (25 x 25cm x 30 cm deep). If a species was
147 present in the samples of a plot, but less than 30 individuals were captured, we sampled other
148 monoliths - less than 10 m apart from the others- until the target of 30 individuals per
149 population was reached. Individuals were preserved in pure ethanol for DNA analysis.

150

151 *DNA extraction, microsatellite genotyping and basic genetic statistics*

152 Total genomic DNA of *A. icterica* was extracted using either the CTAB extraction
153 protocol, as described in Torres-Leguizamon *et al.* (2014) or the DNeasy 96 Blood & Tissue
154 Kit (Quiagen). The latter was also used for *A. chlorotica*.

155 *A. chlorotica* individuals were genotyped at the eight microsatellite loci described in
156 Dupont *et al.* (2011) while *A. icterica* individuals were genotyped at seven microsatellite loci

157 described in Torres-Leguizamon *et al.* (2012) and Dupont *et al.* (2015). Loci were amplified
158 by polymerase chain reaction (PCR) following protocols detailed in Dupont *et al.* (2011),
159 Torres-Leguizamon *et al.* (2012) and Dupont *et al.* (2015). The migration of PCR products
160 was carried out on a 3130xl Genetic Analyser using the LIZ500 size standard, alleles were
161 scored using GENESCAN V3.7 and GENOTYPER V3.7 software (Applied Biosystems,
162 Foster City, CA, USA).

163 Individuals missing 3 or more loci (e.g. failed PCR, poor-quality DNA extract) were
164 excluded from our dataset and mean genotyping error rates per locus and per allele
165 (Pompanon *et al.* 2005) were estimated from repeat genotyping of 5% of samples (24
166 individuals per species). The null hypothesis of independence between loci was tested from
167 statistical genotypic disequilibrium analysis using GENEPOP v. 4.4 (Rousset 2008). Evidence
168 of null alleles was examined using the software MICRO-CHECKER (Van Oosterhout *et al.*
169 2004) and from the frequency of null homozygote within populations. The statistical power to
170 detect genetic divergence was measured for all the samples and markers using POWSIM 4.0
171 to evaluate the hypothesis of genetic homogeneity under Fisher's exact tests (Ryman & Palm
172 2006). Microsatellite loci were tested for departure from Hardy–Weinberg equilibrium
173 (HWE) within each sampling population using exact tests implemented in GENEPOP v. 4.4.
174 To adjust for multiple comparisons, the FDR method (Benjamini & Hochberg 1995) as
175 implemented in the software SGoF (<http://webs.uvigo.es/acraaj/SGoF.htm>) was applied.

176

177 *Genetic variation of earthworm populations*

178 For each population, the genetic diversity was analysed by computing allelic richness
179 standardized for sample size (A_r ; $N = 26$ and $N = 9$ for *A. chlorotica* and *A. icterica*
180 respectively) using the program FSTAT v2.9.3.2 (Goudet 2000) and expected heterozygosity

181 (H_e) using Genetix v 4.05 (Belkhir et al. 2004). Weir and Cockerham's (1984) estimator of
182 the inbreeding coefficient F_{is} was calculated using GENEPOP v. 4.4 (Rousset 2008). The
183 distribution of the genetic diversity within populations can diverge from equilibrium models
184 due to demographic changes. We tested whether the populations recently experienced a
185 reduction of their effective size using the approach detailed in Cornuet & Luikart (1996) and
186 implemented in their software BOTTLENECK v. 1.2.02. Using a Wilcoxon test, the observed
187 heterozygosity was compared with the heterozygosity expected under equilibrium,
188 considering a two-phase mutation model (TPM) recommended for microsatellite data (Piry et
189 al. 1999) with 90% single-step mutations and 10% multiple-step mutations (and a variance
190 among multiple step of 12). Populations exhibiting a significant heterozygosity excess would
191 be considered as having experienced a recent genetic bottleneck whereas expanding
192 populations (e.g. recovering from a bottleneck) are characterized by loci exhibiting a
193 heterozygosity deficiency (Cornuet & Luikart 1996).

194 We estimated genetic differentiation between populations by calculating Weir and
195 Cockerham's (1984) estimator of pairwise F_{st} values and carrying out exact tests of allelic
196 differentiation between populations using GENEPOP v.4.0. To adjust for multiple
197 comparisons, the FDR correction was used. Due to the frequent presence of null alleles, we
198 used the program FREENA to calculate pairwise F_{st} estimates corrected for null alleles
199 (F_{st_COR}) using the so-called ENA method (Chapuis & Estoup 2007). This software was also
200 used to estimate the Cavalli-Sforza and Edwards (1967) genetic distance for each pair of
201 populations (D_c) and this distance was also estimated using the INA correction described in
202 Chapuis and Estoup (D_{c_COR} , 2007). Matrices of pairwise genetic distances were compared
203 with Mantel tests (Mantel 1967) using the R program (R Development Core Team 2012).

204 We used the program BAPS v.6 (Corander & Marttinen 2006; Corander *et al.* 2008) to
205 detect clusters of genetically similar populations and to estimate individual coefficients of
206 ancestry with regard to the detected clusters. When testing for population clusters, we ran 5
207 replicates for $k = 5$, $k = 10$, $k = 15$, $k = 20$, $k = 25$ and $k = 30$, where k is the maximum number of
208 genetically divergent groups (populations). When estimating individual ancestry coefficients
209 *via* admixture analysis we used recommended values of (i) the number of iterations used to
210 estimate the admixture coefficients for the individuals (100), (ii) the number of reference
211 individuals from each population (200) and (iii) the number of iterations used to estimate the
212 admixture coefficients for the reference individuals (20).

213

214 *Landscape genetics*

215 Landscape elements were mapped at high resolution (precision ~2m) over the whole
216 area. Land use cover and linear elements such as roads and rivers were obtained by merging
217 different sources of data. As background data, we used databases from the French National
218 Geographic Institute (IGN), encompassing shapefiles (BD TOPO, accuracy ~1m), and raster
219 (BD Ortho, resolution = 0.5m) of the year 2010. We crossed this information with field work
220 with a differential GPS with 10 cm real time accuracy, in order to check the boundaries of
221 plots and their management. We also compared our data with Corine Land cover 2006 to
222 identify any inconsistencies. Historical and management information was gained with google
223 maps, from interviews with the farmers, and checked with the different version of Corine
224 Land Cover. The data base and the different geographical layers were built up in ArcGis 10.1
225 (ESRI) in the projection Lambert 93 (EPSG: 2154). Data were stored in a vector format and
226 rasterized at 10m resolution in order to perform the landscape analysis. Polygons and linear
227 elements were rasterized separately and merged in raster format. Linear elements were

228 buffered before rasterization in order to avoid artefact gaps. Landscape structure variables
229 were computed in Fragstats (McGarigal et al. 2012) and were computed at patch scale or at
230 the buffer scale (500m of radius) depending on the metric. Landscape descriptors were then
231 normalized (centred and reduced) and selected for the statistical modelling process based on
232 their Variance Inflation Factor value (VIF), in order to avoid collinearity. There is no
233 theoretical base to choose the threshold of the VIF value to exclude variables, and it is usually
234 recommended to use a predictors with a VIF below ten (Montgomery & Peck 1992; Zuur *et*
235 *al.* 2010; Dormann *et al.* 2013). We used a threshold of six in this study. Landscape structure
236 descriptors were correlated to genetic diversity indices (A_r , H_e) with a forward multiple
237 regression with AIC criterion. This approach produces r^2 goodness of fit and avoid over-
238 fitting, thanks to the AIC criteria. It assesses the significance of the separate effects, but the
239 corresponding coefficient needs to be interpreted with caution. In particular, because to
240 inherent correlation between landscape descriptors, the sign of the parameters cannot be
241 interpreted straightforwardly: a positive parameter does not imply a positive net effect of the
242 variable on the genetic variation.

243 Landscape connectivity was performed by defining different scenarios of cost of
244 movements within landscape elements, based on species ecology. Elements were classified in
245 three categories: Barrier, Neutral or Corridors, which corresponded to decreasing movement
246 cost (50, 20, 1 respectively). The result is called a resistance surface map. In all scenarios,
247 urban areas were considered to be barriers; forested areas, hedges and permanent water bodies
248 were considered neutral and temporary water bodies were considered to be corridors. The
249 other elements – grasslands, crops, roads - were considered differently according to the
250 scenario (for details see Table 2). Combining all these possibilities yielded 27 scenarios of
251 resistance surface. In order to test the robustness of our results we also run the analyses by

252 multiplying the costs by 100 in each scenario (giving costs of 5000, 2000, 100). The results
253 were well congruent with initial costs. Connectivity was assessed in three ways. First, simple
254 geographical distance along a straight line between all localities (Euclidian distance) was used
255 to estimate the distance between localities. This scenario makes the assumption that landscape
256 elements do not play a role in dispersal, and is usually referred to as Isolation by Distance
257 (IBD). Second we calculated the least cost path between each pair of site for each of the 27
258 scenarios. This approach makes the assumption that individuals disperse optimally regarding
259 landscape structure, and is usually referred to as Least Cost Path (LCP). Last, we calculated
260 for all the 27 scenarios all paths between each pair of site, weighted by their cumulative cost,
261 to produce 27 corresponding cost weighted distance matrices (CWD), which are usually
262 referred to as resistance distances in circuit theory (McRae & Nürnberger 2006). All these
263 spatial analyses were performed in R with the package {gdistance}. Once all pairwise
264 distances were computed, we looked for the ones that best matched to the (logit transformed)
265 genetic differentiation between populations ($F_{st}/1-F_{st}$ and $F_{st_COR}/1-F_{st_COR}$). This was done
266 using Maximum Likelihood Population Effect (MLPE, Clark et al. 2010; Van Strien et al.
267 2012), a type of linear mixed model that takes into account the non-independence of values
268 within pairwise distance matrices. For this we adapted an R script supplied by Marteen J. Van
269 Strien.

270

271

272 **Results**

273

274 *Microsatellite data*

275 All microsatellite markers were polymorphic across all populations, with 4–19 and 4 -
276 21 alleles per locus for *A. chlorotica* and *A. icterica*, respectively (Supplementary data Tables
277 S1 and S2 respectively). We did not find any evidence of genotypic linkage disequilibrium at
278 any pair of loci in any species. The mean genotyping error rate per locus was 3.12 % and 4.65
279 % in *A. chlorotica* and *A. icterica*, respectively (ranging from 0% to 8.33% and from 0% to
280 14.29%, respectively), mainly due to allelic dropouts. The mean genotyping error rate per
281 allele was 1.56 % and 3.11 % in *A. chlorotica* and *A. icterica*, respectively (ranging from 0%
282 to 4.17% and from 0% to 9.52%, respectively). Significant departures from HWE were
283 observed in 39 of 112 and in 33 of 118 single-locus exact tests after FDR correction in *A.*
284 *chlorotica* and *A. icterica*, respectively. Across all populations, the presence of null alleles
285 was suggested by MICRO-CHECKER for all *A. chlorotica* loci except Ac 476, with
286 frequencies ranging from 0.08 to 0.34 (Supplementary data Table S1) and for PB10, 2PE70
287 and C4 *A. icterica* loci, with frequencies ranging from 0.13 to 0.41 (Supplementary data
288 Table S2). However, no locus showed null alleles in all populations. A few failures of
289 amplification could be interpreted as null homozygotes that would confirm the presence of
290 null alleles at some loci (Supplementary data Tables S1 and S2). However, amplification
291 failures observed at loci that did not present heterozygote deficit, highlighted that the lack of
292 amplification may be due to causes other than null alleles such as degraded DNA.

293

294

295

296 *Genetic variation within populations*

297 Higher values of genetic diversity were obtained for *A. chlorotica* than for *A. icterica*
298 (Table 1). For example, standardized allelic richness (A_r) ranged from 6.30 to 9.28 and from
299 1.73 to 3.69 in *A. chlorotica* and *A. icterica*, respectively. A significant heterozygosity excess
300 (Wilcoxon test, $P < 0.05$) was detected in 1 *A. chlorotica* and 5 *A. icterica* populations (Table
301 1). Significant F_{is} was observed in almost all populations except in P_B and I36 for *A.*
302 *chlorotica* and in I03, I10 and I31 for *A. icterica* (Table 1).

303

304 *Relationship between genetic diversity and local landscape structure*

305 Landscape features in buffers were correlated to genetic diversity in *A. icterica* but not
306 in *A. chlorotica* (Table 3). In *A. icterica*, the r^2 between A_r and H_e , and landscape features
307 were respectively 0.41 and 0.56 and both were significant ($p < 0.05$). In this species, patch
308 diversity was significantly linked to A_r and H_e , and patch richness was linked to A_r .

309

310 *Genetic structure at regional scale*

311 The statistical power for both sets of microsatellite loci to detect various levels of true
312 population differentiation (F_{st}) between populations is presented Table 4. Both sets of markers
313 will detect a true F_{st} of 0.005 or larger with a probability of 96% or more. The alpha error
314 (corresponding to the probability of obtaining false significances when true $F_{st} = 0$) was close
315 to 5% in all cases.

316 All genetic distances matrices ($F_{st}/1-F_{st}$, $F_{st_COR}/1-F_{st_COR}$, D_c and D_{c_COR}) were
317 significantly correlated with Mantel r value ranging from 0.77 ($p = 0.001$) to 0.99 ($p = 0.001$)
318 in *A. icterica* and from 0.88 ($p = 0.001$) to 0.99 ($p = 0.001$) in *A. chlorotica* (Supplementary
319 data Tables S3, S4 and S5).

320 F_{st} analysis showed significant genetic structure at the level of the whole study for
321 both species ($F_{st} = 0.059$, $F_{st_COR} = 0.055$, $P < 0.001$ and $F_{st} = 0.152$, $F_{st_COR} = 0.138$, $P < 0.001$
322 for *A. chlorotica* and *A. icterica*, respectively). Pairwise F_{st} estimates ranged from 0.008 to
323 0.116 (F_{st_COR} ranged from 0.009 to 0.105) and 0.005 to 0.430 (F_{st_COR} ranged from 0.004 to
324 0.412) for *A. chlorotica* and *A. icterica*, respectively (Supplementary data Tables S3 and S4
325 respectively). All exact tests of allelic differentiation were significant ($P \leq 0.005$). Analyses
326 using BAPS identified 8 and 12 genetic clusters in *A. chlorotica* and *A. icterica*, respectively
327 ($P = 0.99$ and $P = 1$ respectively). For *A. chlorotica*, one cluster was composed of 4
328 populations that were close geographically to one another (P_A , P_B , I32 and I33), another
329 cluster was composed of the I07, I10, I15 and I18 populations and all other populations
330 corresponded to a different cluster. For *A. icterica*, 5 clusters were composed of two
331 geographically close populations (P_A and P_B , I02 and I03, I04 and I32, I07 and I08, I11 and
332 I25) while all other clusters were composed of only one population. Low levels of admixture
333 were observed among the clusters (Supplementary data Fig S1).

334

335 *Relationship between genetic differentiation and landscape connectivity*

336 Genetic variations were linked to landscape connectivity in both species (Table 5, Fig
337 3). In both species resistance distance (CWD) had the most explanatory power, followed by
338 least cost path (LCP) and finally by isolation by distance (IBD). In *A. chlorotica*, the best
339 scenarios were those in which grasslands were considered to be corridors, whereas crops and
340 roads were considered to be barriers (Table 5). In *A. icterica*, no significant isolation by
341 distance was found except with the non-corrected Dc distance and the only common point
342 between the several likely scenarios was that crops were considered as barriers (Table 5). The
343 best congruent models between the two species, taking into account the results from the

344 different genetic indices, were scenarios 9, 10 and 11. The most frequent role of the different
345 landscape element in these scenarios was corridor for grasslands and barrier for crops and
346 roads.

347

348 **Discussion**

349

350 *Microsatellite markers in earthworms*

351 Microsatellites markers have been developed for only a few earthworm species (i.e. 7
352 species so far, review in Torres-Leguizamon et al. 2012; Souleman et al. 2016) and these
353 markers have rarely been used for population genetics studies (but see Velavan et al. 2009;
354 Novo et al. 2010; Dupont et al. 2015). Two different research groups have tried to developed
355 reliable markers for one of the most emblematic European earthworm species, *Lumbricus*
356 *terrestris*. Of the ten markers obtained in this species by Velavan *et al.* (2007), only three
357 were used in a subsequent study (Velavan et al. 2009) suggesting difficulties in genotyping
358 the samples with the other ones. Souleman et al. (2016) couldn't obtain reliable results with
359 these markers. Thus, they developed eight new markers for which they obtained a low
360 amplification success and a significant heterozygote deficit, suggesting null alleles. In our
361 study, null alleles were suspected at seven out of eight loci in *Allobophora chlorotica* and at
362 four out of eight loci in *Aporrectodea icterica*. It is already known that the development of
363 microsatellite molecular markers can be problematic in some taxa (e.g. in molluscs,
364 McInerney et al. 2011). It was proposed that such methodological difficulties may have been
365 caused by genomic complexities contained within microsatellite flanking regions. In
366 particular, unstable flanking regions may arise when indels or mutations occur at PCR primer
367 binding sites, thereby causing null alleles (McInerney et al. 2011). We therefore believe that
368 microsatellite flanking regions are particularly variable in earthworm species. This could be
369 verified by gathering more genomics data on these taxa. Nevertheless, we showed that the
370 estimation of genetic divergence was not significantly altered by the presence of null alleles in

371 the dataset. Indeed, similar results were obtained with all indices of genetic divergence (F_{st} ,
372 F_{st_COR} , D_c , D_{c_COR}), and correction for the presence of null alleles did not change the results.

373

374 *Landscape structure and population genetic diversity*

375 Agriculture and urbanization result in habitat loss and fragmentation that variously
376 impact many animal groups. Anthropogenic landscape fragmentation results in reduced size
377 and increased isolation of habitat patches. Fragmented populations are thus expected to
378 experience increased genetic drift and reduced gene flow, which result in the erosion of
379 genetic diversity and the increase of genetic differentiation among local populations
380 (Keyghobadi 2007). Moreover, small populations isolated by surrounding inhospitable
381 landscapes are more vulnerable to demographic variability, environmental stochasticity and
382 genetic processes including inbreeding depression, the random fixation of deleterious alleles
383 and the loss of adaptive potential (Frankham 1995).

384 In this study, we tested how landscape structure in a man-made environment impacted
385 genetic diversity of earthworm populations by characterizing landscape at the buffer scale. A
386 significant relationship was observed between genetic diversity indices (H_e and A_r) and two
387 landscape features (i.e. patch diversity and patch richness, Table 3) in *A. icterica* while no
388 correlation was detected for *A. chlorotica*. We thus confirmed that geographic isolation of *A.*
389 *icterica* populations due to natural and artificial barriers to gene flow probably accentuate the
390 loss of genetic variability through genetic drift, such as already suggested in a previous
391 population genetic study of this species (Torres-Leguizamon et al. 2014). Interestingly, one
392 quarter of the *A. icterica* populations seemed recently founded, such as revealed by the
393 heterozygosity excess in these populations. Overall, these results suggest that demographic

394 changes occur more frequently in *A. icterica* than in *A. chlorotica* and that these demographic
395 changes can be explained by the local landscape structure.

396

397 *Genetic differentiation between populations*

398 A local decline of effective population size may be explained by the disruption of
399 historical patterns of gene flow in a fragmented habitat (Keyghobadi 2007). Analyses of
400 spatial patterns of genetic structure showed the presence of a strong genetic differentiation in
401 both species, with a low degree of genetic admixture and high F_{st} values. F_{st} values were
402 higher for *A. icterica*, highlighting that these populations are more genetically isolated than
403 the ones of *A. chlorotica*. This was not expected because *A. icterica* has a higher potential for
404 active dispersal. Caro *et al.* (2013) indeed demonstrated in a mesocosm study that *A. icterica*
405 had a higher dispersal rate than two other endogeic species, namely *A. chlorotica* and
406 *Aporrectodea caliginosa*. Moreover, in a recent population genetic study at very fine scale,
407 Dupont *et al.* (2015) showed a low signal of genetic structure within two *A. icterica*
408 populations sampled in two plots of less than 1 ha separated by ~ 500m while *A. chlorotica*
409 populations showed spatial neighbourhood structure in the same sites. This difference was
410 interpreted as a higher dispersal capacity of *A. icterica*. In the light of the results at very fine
411 scale (Dupont *et al.* 2015) and at landscape scale (this study), we can assume that *A.*
412 *chlorotica* essentially disperse through passive mechanisms over larger distance while passive
413 dispersal might be more restricted for *A. icterica*. *A. chlorotica* is a small bodied species and
414 lives near the soil surface in the upper 60 mm soil layer (Sims & Gerard 1999), two features
415 probably facilitating dispersal *via* various vectors (e.g. zoochory, wind, water and soil transfer
416 *via* human activities) while *A. icterica* is found deeper in the soil and is bigger.

417

418 *Landscape connectivity at regional scale*

419 The landscape connectivity analysis revealed that genetic structure was linked to
420 landscape connectivity in both species, with resistance distance (cwd) having the most
421 explanatory power. Thus, landscape features better explain genetic structure than Euclidian
422 distances. We specifically tested the hypothesis that linear features such as roads may
423 function as dispersal corridors (see for instance Tyser & Worley 1992; Cameron & Bayne
424 2009) for these species. It has indeed been shown that European earthworms that are invasive
425 in Canada and the northern USA were introduced and spread along road networks (review in
426 Cameron & Bayne 2009). It was however not clear whether the spread of earthworms along
427 roads is more likely to occur *via* initial transport of earthworms or their cocoons in soil or
428 gravel during road construction or *via* transport by vehicles after the road has been built
429 (Cameron & Bayne 2009). In addition, roads and sidewalks could also function as dispersal
430 corridors when earthworms crawl out of the soil and disperse at night after heavy rain, as is
431 often observed in some species (e.g. Chuang & Chen 2008). Our results rather suggested that
432 roads constitute obstacles for earthworm dispersal. Using MLPE, we indeed showed that the
433 majority of the most likely landscape connectivity scenarios considered roads as barriers
434 (Table 5).

435 The second hypothesis tested was that grasslands represent a suitable habitat that
436 could be easily crossed by endogeic species and thus represent dispersal corridors, while soil
437 under crops has a detrimental effect on earthworms. These expectations were confirmed by
438 the MLPE analysis, the most likely landscape connectivity scenarios generally considered
439 grasslands as corridors and crops as barriers.

440

441

442 **Conclusion**

443 Simultaneously investigating two ecologically similar species highlighted several
444 common features in the response of each species to the landscape. We showed that functional
445 connectivity was impacted by landscape features and that a favourable habitat could act as a
446 corridor for the dispersal of earthworms. We thus confirmed that the aboveground landscape
447 has a fundamental role in dispersal and gene flow of below-ground organisms. However, we
448 also observed some differences between species which could be linked to the dispersal and
449 life history attributes of each species. Indeed, population genetic diversity was significantly
450 influenced by the local landscape structure in *A. icterica* but not in *A. chlorotica*. This result
451 highlights that the exact effect of each habitat type on genetic variation over space and time
452 and of agricultural practices on earthworm dispersal should be studied using specific
453 sampling strategies.

454

455

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462

463

464

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624 **Data Accessibility**

625 The data sets with the microsatellite genotypes of *A. chlorotica* and *A. icterica* are available
626 from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.sc6bb>

627

628

629 **Author Contributions**

630 J.M initiated the project. L.D. and J.M. designed the study. L.D. drafted the manuscript. L.D.,
631 M.T.L., P. R.-C. and J.M. collected the data. L.D and P. R.-C. performed molecular analyses.
632 J.M. built the GIS database and conducted the landscape genetic analyses (Buffer and
633 connectivity). L.D. and J.M. performed statistical analyses and wrote the manuscript.

634

635

636 **Figure legends**

637

638 **Figure 1**

639 Illustration of the different landscape genetic approaches used in this work. a) Local genetic
640 structure is compared to local landscape features within a buffer around each population; b)
641 genetic difference is linked to geographical distance between populations (IBD); c) genetic
642 difference is linked to the functional distance between populations, calculated as the least cost
643 path between populations (LCP), based on a resistance map; d) genetic difference is linked to
644 the functional distance between populations, calculated as the sum of the cost weighted paths
645 between populations (cwd), based on a resistance map.

646

647 **Figure 2**

648 Geographical distribution of earthworm sampling sites near Yvetot in Normandy (France).
649 “No species” means that neither *A. icterica* nor *A. chlorotica* were found.

650

651 **Figure 3**

652 Best correlation found between genetic distance (D_c distance) and resistance distance scenario
653 (cwd) for *A. chlorotica* (A) and *A. icterica* (B).

654

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657

Table 1. Geographical location^{*}, sample size (N), genetic diversity (A_r : standardized allelic richness; H_e : expected heterozygosity), fixation index F_{is} (significant values are in bold) and Wilcoxon test P value for heterozygote excess compared to expectations at mutation-drift equilibrium (p_{wil}) for each study plot and each species. ^{*}Latitude/longitude range in Lambert II étendu.

Plot	Latitude	Longitude	<i>Allolobophora chlorotica</i>					<i>Aporrectodea icterica</i>				
			N	A_r	H_e	F_{is}	p_{wil}	N	A_r	H_e	F_{is}	p_{wil}
P _A	484578.509	2513691.360	42	8.61	0.777	0.128	0.727	22	2.73	0.523	0.352	0.055
P _B	484100.668	2513845.836	30	8.44	0.794	0.091	0.527	30	2.51	0.481	0.094	0.016
I02	484907.071	2511340.636	-	-	-	-	-	12	2.27	0.397	0.274	0.281
I03	484824.797	2511382.199	-	-	-	-	-	28	2.32	0.395	0.115	0.015
I04	483636.894	2511737.970	-	-	-	-	-	12	2.55	0.438	0.103	0.344
I07	483713.045	2511076.294	28	8.60	0.775	0.185	0.371	24	2.95	0.494	0.380	0.281
I08	483823.122	2511110.760	-	-	-	-	-	31	2.71	0.484	0.209	0.406
I10	483411.237	2511337.635	32	7.75	0.781	0.233	0.422	14	2.61	0.495	0.167	0.008
I11	485746.167	2507374.639	27	7.45	0.765	0.195	0.527	29	2.81	0.449	0.172	0.406
I15	481854.533	2514843.110	27	9.28	0.777	0.141	0.727	30	2.54	0.461	0.065	0.008
I18	479823.687	2512611.239	27	8.08	0.742	0.205	0.973	30	3.69	0.575	0.277	0.344
I19	492929.229	2508850.328	-	-	-	-	-	43	3.00	0.493	0.335	0.711
I20	477908.835	2515839.136	29	7.06	0.725	0.293	0.808	33	2.15	0.373	0.184	0.312
I25	480555.744	2508635.999	29	6.30	0.720	0.229	0.527	31	2.84	0.506	0.263	0.078

I27	489902.819	2513148.683	30	8.81	0.788	0.156	0.770	34	2.62	0.485	0.317	0.023
I31	482053.828	2513328.538	-	-	-	-	-	27	1.73	0.226	0.115	0.594
I32	483297.736	2513724.785	29	8.16	0.786	0.179	0.273	30	2.70	0.422	0.305	0.656
I33	481742.786	2511459.498	32	8.61	0.761	0.182	0.902	29	2.52	0.454	0.179	0.148
I34	482430.931	2510886.051	32	7.95	0.747	0.237	0.875	30	2.33	0.366	0.403	0.078
I36	487833.362	2509076.597	46	6.67	0.777	0.067	0.010	-	-	-	-	-

Table 2 Role and cost of landscape elements in the different landscape scenarios tested in this study. The cost of grasslands, crops and roads changed among scenarios. Costs of permanent water bodies (P.W.B.), temporary water bodies (T.W.B.), deciduous forest (D. Forest), coniferous forest (C. Forest), hedges and urban area (Urban A.) were fixed in all scenarios.

Scenario	Role of elements			Cost of elements								
	Grasslands	Crops	Roads	Grasslands	Crops	Roads	P. W. B.	T. W. B.	D. Forest	C. Forest	Hedges	Urban A.
1	Barrier	Barrier	Barrier	50	50	50	20	1	20	20	20	50
2	Barrier	Barrier	Neutral	50	50	20	20	1	20	20	20	50
3	Barrier	Barrier	Corridor	50	50	1	20	1	20	20	20	50
4	Barrier	Corridor	Barrier	50	1	50	20	1	20	20	20	50
5	Barrier	Corridor	Neutral	50	1	20	20	1	20	20	20	50
6	Barrier	Corridor	Corridor	50	1	1	20	1	20	20	20	50
7	Barrier	Neutral	Barrier	50	20	50	20	1	20	20	20	50
8	Barrier	Neutral	Neutral	50	20	20	20	1	20	20	20	50
9	Barrier	Neutral	Corridor	50	20	1	20	1	20	20	20	50
10	Corridor	Barrier	Barrier	1	50	50	20	1	20	20	20	50
11	Corridor	Barrier	Neutral	1	50	20	20	1	20	20	20	50
12	Corridor	Barrier	Corridor	1	50	1	20	1	20	20	20	50
13	Corridor	Corridor	Barrier	1	1	50	20	1	20	20	20	50
14	Corridor	Corridor	Neutral	1	1	20	20	1	20	20	20	50

15	Corridor	Corridor	Corridor	1	1	1	20	1	20	20	20	50
16	Corridor	Neutral	Barrier	1	20	50	20	1	20	20	20	50
17	Corridor	Neutral	Neutral	1	20	20	20	1	20	20	20	50
18	Corridor	Neutral	Corridor	1	20	1	20	1	20	20	20	50
19	Neutral	Barrier	Barrier	20	50	50	20	1	20	20	20	50
20	Neutral	Barrier	Neutral	20	50	20	20	1	20	20	20	50
21	Neutral	Barrier	Corridor	20	50	1	20	1	20	20	20	50
22	Neutral	Corridor	Barrier	20	1	50	20	1	20	20	20	50
23	Neutral	Corridor	Neutral	20	1	20	20	1	20	20	20	50
24	Neutral	Corridor	Corridor	20	1	1	20	1	20	20	20	50
25	Neutral	Neutral	Barrier	20	20	50	20	1	20	20	20	50
26	Neutral	Neutral	Neutral	20	20	20	20	1	20	20	20	50
27	Neutral	Neutral	Corridor	20	20	1	20	1	20	20	20	50

Table 3 Summary of the forward multiple regression between landscape features (predictors) and genetic diversity (A_r : Allele Richness, H_e : expected heterozygosity) of the populations of the two earthworm species *A. icterica* and *A. chlorotica* in the region of Yvetot, Normandy, France. A patch represents an element in the landscape. Predictors are: Edge Density (length of patch edge/surface of the buffer), Patch Richness (number of different types of patches in the buffer), Patch Diversity (Shannon Diversity of the different Land Use types), Crop surface, Grassland surface and Total length of roads.

	<i>Aporrectodea icterica</i>		<i>Allolobophora chlorotica</i>	
	A_r	H_e	A_r	H_e
Anova table				
F	3.10	4.85	1.62	1.41
p - value	<0.05	<0.01	ns	ns
adjusted r2	0.41	0.56	0.22	0.16
Predictors				
Edge Density	ns	ns	ns	ns
Patch Richness	ns	-0.10	ns	ns
Patch Diversity	ns	0.74	ns	ns
Crop surface	ns	ns	ns	ns
Grassland surface	ns	ns	ns	ns
Total length of roads	ns	ns	ns	ns

Table 4 Statistical power for detecting various true levels of population differentiation (F_{st}) by means of Fisher's exact test when using both sets of microsatellite loci, allele frequencies, and sample sizes. The power is expressed as the proportion of simulations that provide statistical significance at the 0.05 level.

True F_{st}	<i>A. chlorotica</i>	<i>A. icterica</i>
0.000	0.064	0.070
0.001	0.417	0.196
0.002	0.852	0.432
0.005	1.000	0.964
0.010	1.000	1.000

Table 5 Summary of the network regression analyses of landscape connectivity comparing **A.** Euclidian distance and genetic differentiation (pairwise F_{st} , pairwise F_{st} estimates corrected for null alleles, D_c genetic distance and D_c genetic distance corrected for null alleles) between populations (Isolation by distance, IBD).and **B.** cost weighted distances (cwd) and genetic differentiation between populations. The most likely landscape connectivity scenarios are indicated. The roles of the landscape elements in the most likely scenarios are specified (b = barrier, c = corridor and n = neutral); when applicable the most frequent role is in bold. In **C.** the best congruent scenarios are presented. NA = Not Applicable

A. IBD								
	Genetic distance	Geographic distance	scenario	Grasslands	Crops	Roads	r2	pvalue
<i>A. chlorotica</i>	F_{st}	euclidian	NA	-	-	-	0.29	<10 ⁻⁵
	F_{st-COR}	euclidian	NA	-	-	-	0.28	<10 ⁻⁵
	D_c	euclidian	NA	-	-	-	0.4	<10 ⁻⁵
	D_{c-COR}	euclidian	NA	-	-	-	0.41	<10 ⁻⁵
<i>A. icterica</i>	F_{st}	euclidian	NA	-	-	-	0.01	0.12
	F_{st-COR}	euclidian	NA	-	-	-	0.02	0.09
	D_c	euclidian	NA	-	-	-	0.03	<10 ⁻⁵
	D_{c-COR}	euclidian	NA	-	-	-	0.02	0.06
B. Best species specific scenario								
	Genetic distance	Geographic distance	scenario	Grasslands	Crops	Roads	r2	pvalue
<i>A. chlorotica</i>	F_{st}	CWD	10	c	b	b	0.44	<10 ⁻⁵
	F_{st-COR}	CWD	10	c	b	b	0.43	<10 ⁻⁵

	D_c	CWD	8, 9, 10	b, c	b, n	b, n, c	0.54	$<10^{-5}$
	D_{c-COR}	CWD	8, 9, 14	b, c	n, c	n, c	0.54	$<10^{-5}$
<i>A. icterica</i>	F_{st}	CWD	1, 2, 3, 11, 12, 21	b, n, c	b, c	b, n, c	0.04	$<10^{-4}$
	F_{st-COR}	CWD	1, 2, 3, 11, 12, 21	b, n, c	b, c	b, n, c	0.05	$<10^{-4}$
	D_c	CWD	1, 2, 3, 11, 12, 21	b, n, c	b, c	b, n, c	0.08	$<10^{-5}$
	D_{c-COR}	CWD	1, 11, 12, 21, 25, 26, 27	b, n, c	b, n	b, n, c	0.06	$<10^{-5}$

C. Best congruent scenario

	Genetic distance	Geographic distance	scenario	Grasslands	Crops	Roads	r2	pvalue
<i>A.chlorotica</i>	F_{st}	CWD	10	c	b	b	0.44	$<10^{-5}$
	F_{st-COR}	CWD	10	c	b	b	0.43	$<10^{-5}$
	D_c	CWD	9, 10	b, c	n, b	b, c	0.54	$<10^{-5}$
	D_{c-COR}	CWD	9, 10, 11	b, c	n, b	b, n, c	0.54	$<10^{-5}$
<i>A.icterica</i>	F_{st}	CWD	10	c	b	b	0.04	$<10^{-4}$
	F_{st-COR}	CWD	10	c	b	b	0.04	$<10^{-4}$
	D_c	CWD	9, 10	b, c	n, b	b, c	0.07	$<10^{-5}$
	D_{c-COR}	CWD	9, 10, 11	b, c	n, b	b, n, c	0.06	$<10^{-4}$

Figure 1

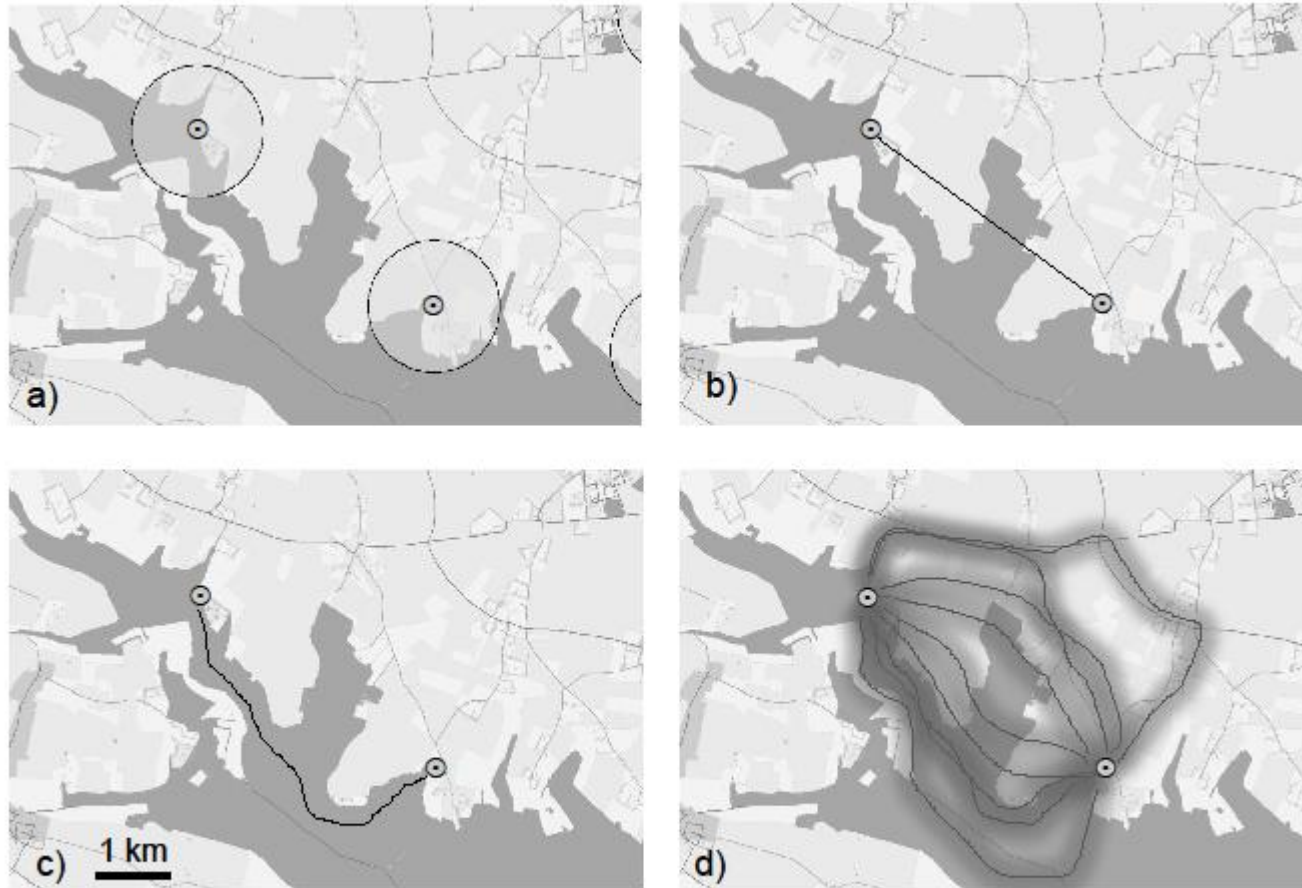


Figure 2

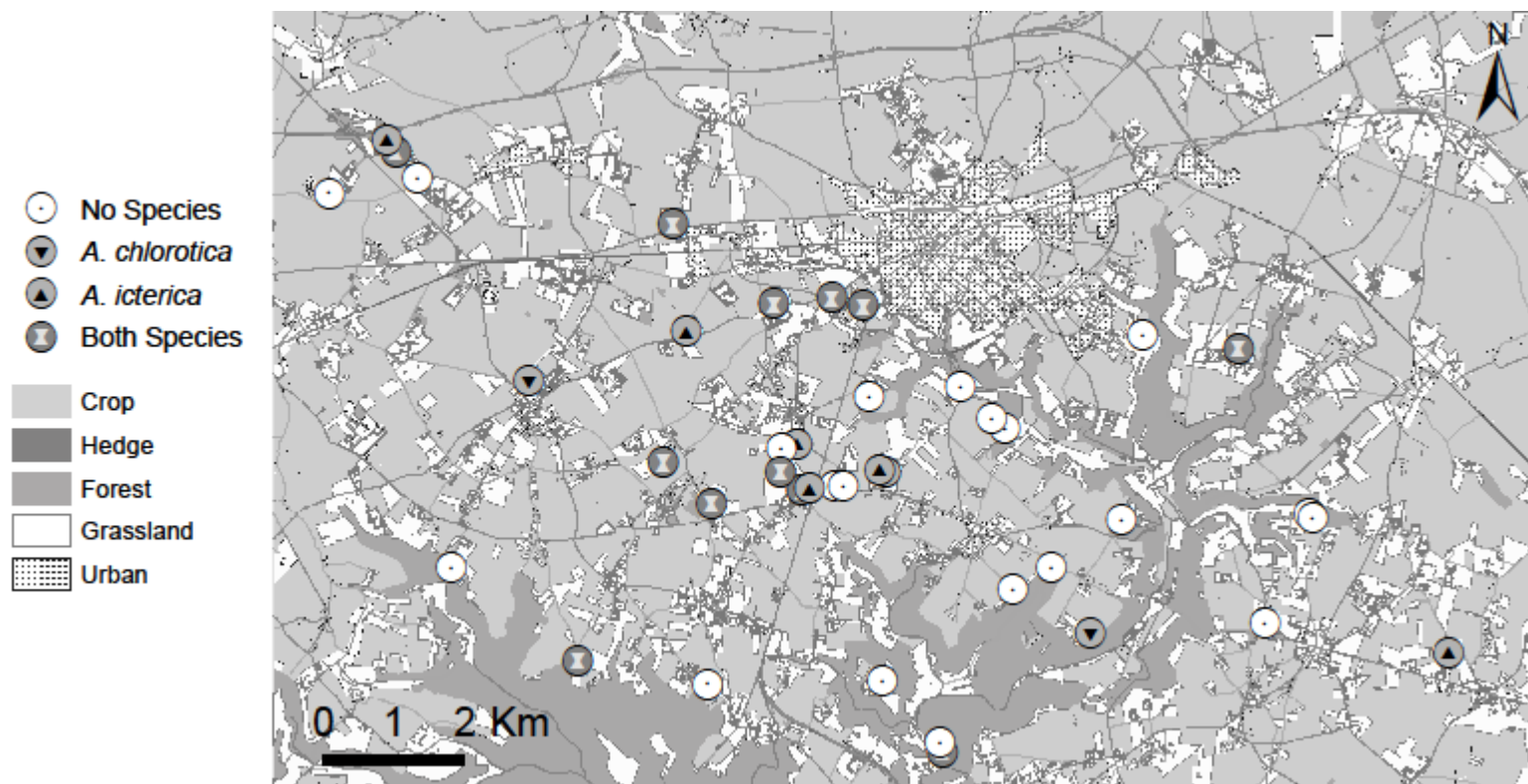


Figure 3

