

Patterns of gene flow across multiple anthropogenic infrastructures: insights from a multi-species approach

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Abstract

Large-scale Transportation Infrastructures (LTIs; roads, railways, *etc.*) are among the main determinants of landscape fragmentation, with strong impacts on animal dispersal movements and functioning of metapopulations. Although the detection of the impacts of LTIs is now facilitated by landscape genetic tools, studies are often conducted on a single species, while it is acknowledged that different species might react differently to the same obstacle. Multi-specific approaches are thus required to get a better overview of the impacts of human-induced fragmentation, especially in landscapes crossed by multiple LTIs whose impacts can accumulate. We surveyed two vertebrates species (the grass snake *Natrix helvetica* and the midwife toad *Alytes obstetricans*) and two insect species (the butterfly *Maniola jurtina* and the ground-beetle *Abax parallelepipedus*) in a landscape fragmented by six LTIs: a motorway, a railway, a country road, a gas pipeline, a power line and a secondary road network. Using multiple linear regressions and commonality analyses on both classical and hierarchical genetic distances computed over reduced spatial scales, we showed that 38% of the overall explained genetic variability across all species was due to LTIs. While the butterfly was seemingly not impacted by any LTI, the genetic structure of the three ground-dwelling species was mostly influenced by roads, motorway and railway. LTIs, and especially roads, mostly acted as barriers to gene flow, barrier effects accounting for 85% of the overall variance in genetic distances explained by LTIs across species. Although the power line did not affect any studied species and the

gas pipeline only impacted gene flow in the ground-beetle through forest fragmentation, other LTIs systematically affected at least two species. Importantly, we showed that some LTIs could somehow promote gene flow, embankments probably providing favourable habitats for vertebrate species. Considering the high variability in species response to LTIs, we argue that drawing general conclusions on landscape connectivity from the study of a single species may lead to counterproductive mitigation measures and that multi-species approaches are to be more systematically considered.

Key-words: connectivity; fragmentation; dispersal; individual-based; hierarchical genetic distance; commonality analysis; spatial scale; conservation

INTRODUCTION

The human-induced fragmentation of natural habitats is one of the main determinants of the global biodiversity collapse (Fahrig, 2003; Haddad et al., 2015). The most ubiquitous form of habitat fragmentation is due to large-scale transportation infrastructures (LTIs) (Forman and Alexander, 1998; Trombulak and Frissell, 2000; Balkenhol and Waits, 2009). LTIs are linear infrastructures allowing the transportation of goods, vehicles or energy, such as roads, motorways, railways, power lines, pipelines and canals. They are expanding considerably, creating dense transportation networks with profound impacts on natural ecosystems (Dulac, 2013; Laurance et al., 2014). It notably deeply affects metapopulation dynamics through a reduction in population sizes in response to direct habitat degradation but also through a reduction in demographic and genetic exchanges between populations in response to a decrease in the permeability of the landscape matrix to dispersal (Balkenhol et al., 2009b). As populations become smaller and isolated, they might exhibit higher rates of inbreeding through genetic drift, resulting in an increased risk of population extinction (McCauley, 1991; Legendre et al., 1999; Fagan and Holmes, 2006). Understanding the influence of LTIs on wildlife dispersal patterns is thus of crude importance to fuel conservation policies.

The most obvious detrimental effects of LTIs on dispersal success are direct collisions with vehicles and physical crossing impediment when infrastructures are for instance fenced (Forman and Alexander, 1998; Trombulak and Frissell, 2000; Hels and Buchwald, 2001; Beebee, 2013; Barrientos et al., 2019). Most animals are affected, from small invertebrates to large mammals (Balkenhol and Waits, 2009; Fahrig and Rytwinski, 2009; Borda-de Agua et al., 2017). LTIs may also induce behavioral alterations that further affect nearby populations (Trombulak and Frissell, 2000; Ascensao et al., 2016). For example, both breeding migrations and reproductive success of anurans can be perturbed by main roads due to sound interference with males mating calls (Bee and Swanson, 2007; Troïanowski et al., 2017), in turn possibly impacting effective dispersal and thus gene flow (Ronce, 2007).

Over the past fifteen years, "molecular road ecology" has emerged as a fully-fledged discipline to thoroughly estimate landscape functional connectivity (Holderegger and Di Giulio, 2010). Building on

65 population genetics, landscape ecology and spatial statistic tools (Manel et al., 2003; Holderegger and
66 Wagner, 2008; Manel and Holderegger, 2013), its objective is to elucidate how the genetic variability is
67 influenced by LTIs and other anthropogenic obstacles, with numerous applications in species management
68 and conservation (Segelbacher et al., 2010). However, one major limitation of such studies is that they
69 generally focus on a single species (Balkenhol and Waits, 2009; Keller et al., 2015), while different species
70 may actually respond differently to the same type of infrastructure. Furthermore, they also often focus
71 on a single LTI, while multiple LTIs are commonly built next to each other because of technical and
72 economic constraints, notably within valleys or along coastlines: although the impacts of LTIs are then
73 expected to add up and result in a “cumulative” barrier effect, some LTIs might actually be neutral to
74 movement or even create corridors to dispersal (Penone et al., 2012; Vandeveldel et al., 2012; Bartzke
75 et al., 2015), these antagonistic effects making the whole picture even more complex. For example,
76 Paquet and Callagan (1996) showed that a motorway strongly impeded crossing events in wolves but
77 that a railway and power lines located within the same study area together redirected wolves movements
78 and thus rather acted as corridors. In the same vein, Latch et al. (2011) found that gene flow in the
79 desert tortoise *Gopherus agassizii* was affected by roads but not by power lines. In highly fragmented
80 landscapes, it is thus highly advisable to assess the concomitant influence of all existing LTIs using a
81 multi-species, and ideally a multi-taxa, approach to adopt efficient conservation policies (Balkenhol et al.,
82 2009a; Keller et al., 2015; Richardson et al., 2016; EEA, 2015).

83 In this study, We used recent molecular and statistical tools to assess the respective and cumulative
84 impacts of six LTIs in four parapatric terrestrial species with contrasted life history traits (two vertebrates
85 and two insects including a flying species, sampled in south-western France). We hypothesized that
86 flying species would be less affected by LTIs than ground-dwelling ones and that large infrastructures
87 carrying vehicles (roads, motorways, railways) would overall be more impactful than infrastructures
88 carrying energy (power lines, gas pipelines). We also hypothesized that the impacts of some LTIs might
89 accumulate to shape spatial patterns of gene flow in studied species.

90 MATERIAL AND METHODS

91 Study area and biological models

92 The study was carried out in the 'Périgord' region in South-Western France between Brive-La-Gaillarde
93 and Périgueux (45°07'31.8"N; 0°58'56.9"E; Fig. 1). It is a 300km² rural landscape composed of limestone
94 plateaus including crops, mowed meadows, deciduous forests and small villages. The hydrology is limited
95 to small streams and ponds. Altitude ranges from 91 to 294 m above sea level. Six types of LTIs are
96 present in this study area (from the widest to the narrowest): a fenced motorway (“A89”) commissioned
97 in 2004; a low traffic single-track railway built in the 19th century; a high traffic country road (“D6089”)

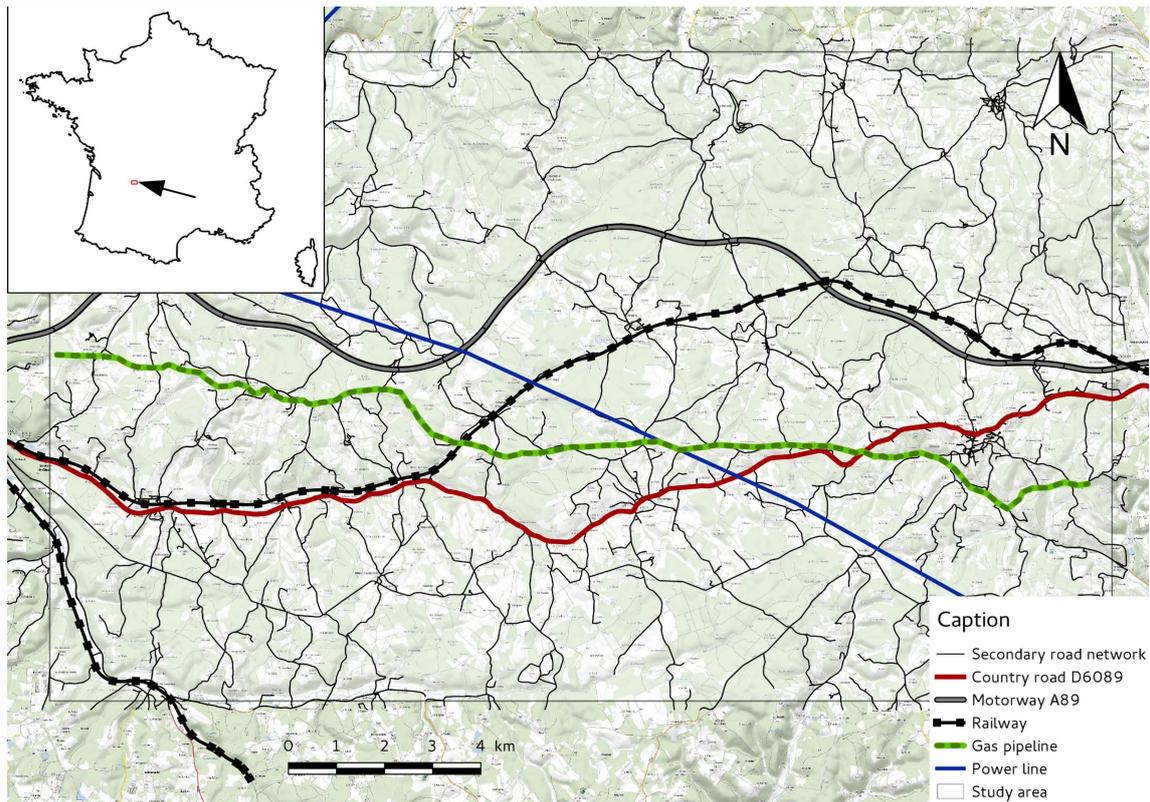


Figure 1: Study area in south-western France

98 present since the 18th century; a power line and a gas pipeline constructed in 1962 and 1955, respectively,
99 both associated with breaches in forest cover; a 1370 km dense network of low traffic secondary roads
100 (Fig. 1).

101 We considered four species with various life history traits in order to span a large amount of biological
102 variability: two vertebrates (the snake *Natrix helvetica* and the midwife toad *Alytes obstetricans*) and two
103 insects (the butterfly *Maniola jurtina* and the ground-beetle *Abax parallelepipedus*). *Alytes obstetricans* is
104 a small toad widely distributed in Western Europe. It is highly sensitive to fragmentation because local
105 populations are known to function as relatively independent entities with strong genetic structuring
106 (Tobler et al., 2013; Maia-Carvalho et al., 2014; Albert et al., 2015). *Natrix helvetica* is also widely
107 distributed in Western Europe (Kindler et al., 2017). This snake species is considered to exhibit good
108 dispersal abilities, with individuals travelling over more than 1 km in less than a month (Petterson,
109 2014). A previous study did not detect any genetic structure in this species in a intensively used
110 agricultural landscape, indeed suggesting good dispersal ability in fragmented environments (Meister
111 et al., 2010). *Maniola jurtina* is an univoltine butterfly which is very common in Europe with locally
112 very high densities. It shows medium dispersal capacity with mean dispersal distances ranging from
113 about 50 to 300 m (Schneider et al., 2003; Ouin et al., 2008; Stevens et al., 2013). Previous studies
114 revealed that both land cover (arable lands and forests) and LTIs (motorway and railway) could affect

115 its dispersal (Villemey et al., 2016; Remon et al., 2018). Finally, *Abax parallelepipedus* is an opportunistic
116 carnivorous ground-beetle (Loreau, 1983) that inhabits the upper layer of litter in forest environments
117 (Loreau, 1987). It typically shows limited dispersal capacity with an avoidance behaviour face to open
118 habitats (Charrier et al., 1997) and a high sensitivity to fragmentation due to roads (Keller et al., 2004).

119 Sampling and genotyping

120 For all species, tissues were collected between April and September in 2015 and 2016. For the two
121 vertebrate species (*N. helvetica* and *A. obstetricans*), we followed an individual-based sampling design due
122 to low abundances in the field. Individual-based sampling design has been proved to be a good alternative
123 method to population-based sampling design as less individuals are required per sampling location (1
124 to 4) and more geographical locations can be sampled over the landscape (Prunier et al., 2013, 2014).
125 Accordingly, the entire study area was prospected to collect toads and snakes, at night and at day time,
126 respectively. We mainly focused on sampling locations with high probability of presence such as wetlands,
127 ponds, rivers, woodland edges and small villages. To attract snakes and facilitate data collection, 108
128 artificial shelters were laid across the study area. When an individual was detected, it was hand-captured
129 and manipulated directly in the field. A GPS location (Garmin Etrex20, USA) was recorded for each
130 captured individual (see Fig. 2 and 3 for sampling locations). Each individual was sexed, measured,
131 weighted, marked (to avoid sampling individual twice) and a genetic sample was collected. Captured
132 toads were marked using 7x1.35 mm FDX-B Passive Integrated Transponder (PIT) tags (Loligo Systems,
133 Denmark) and a non-destructive genetic sample was collected by gently opening mouth with a little metal
134 spoon and swabbing mouth cavity for about 10 seconds. We used ventral scales clipping following Brown
135 and Parker (1976) to both mark snakes and collect DNA. We also opportunistically collected genetic
136 samples from snakes and amphibians found dead (road kill or predation) and from snake shed skins. The
137 two insect species (*M. jurtina* and *A. parallelepipedus*) were sampled within 30 sites using a classical
138 population-based sampling design. Site locations were obtained by dividing the study area into 30
139 sectors using a 5x6 regular grid in QGIS (V. 2.8). In each sector and each species, a single sampling
140 site was chosen according to the presence of suitable habitats (woodlands for beetles and grasslands for
141 butterflies). At each sampling location, 30 individuals were sampled, resulting in 900 genetic samples
142 per species (see Fig. 2 and 3 for sampling locations). Butterflies were captured during day time with
143 nets. Beetles were trapped using non-lethal dry pitfalls. Pitfalls were 20 cm in diameter and 15 cm in
144 depth and were arranged in circles at regular intervals of 5 m. They were emptied every day until 30
145 individuals were captured. For both insect species, we collected the middle right leg of each captured
146 individual, as both a source of DNA and a way to avoid sampling the same individual twice.

147 All genetic samples were stored in 70 % EtoH until DNA extraction. All material for marking animals
148 and collecting genetic samples was washed and disinfected using absolute ethanol between each individual

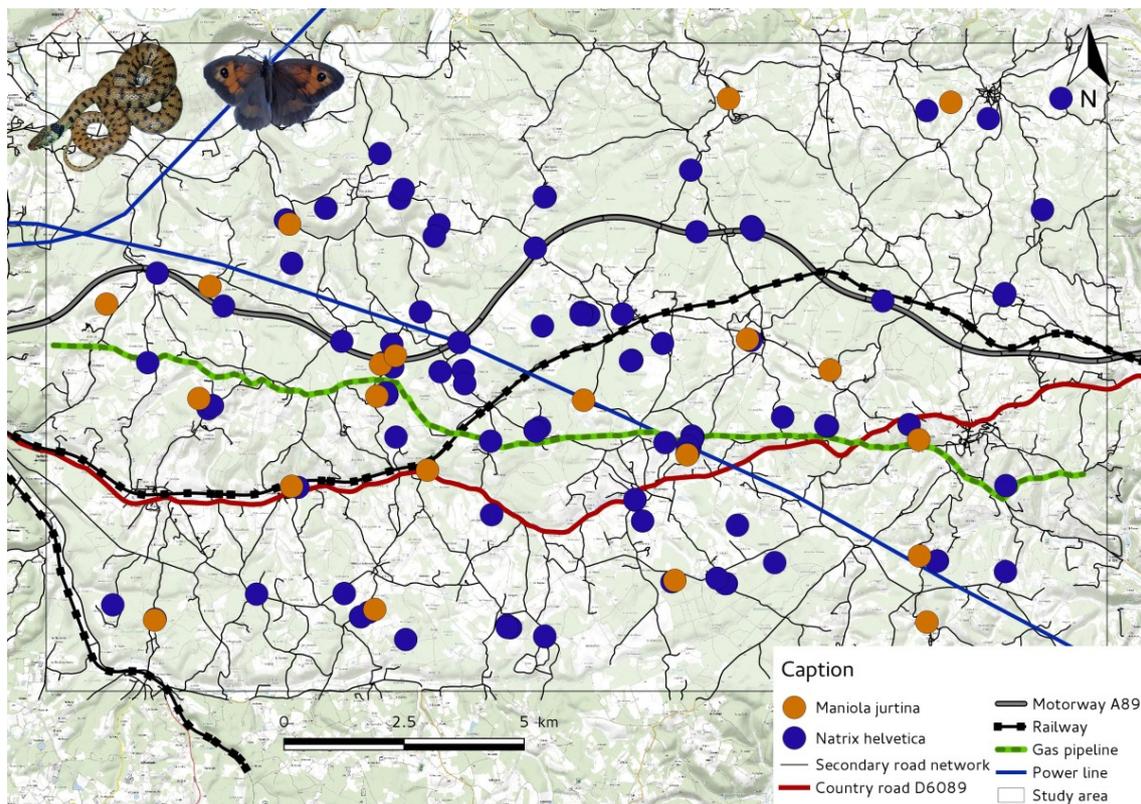


Figure 2: Sampling locations of the snake *Natrix helvetica* and the butterfly *Maniola jurtina* within the study area. Each snake location represents an individual and each butterfly location represents a sampled population (about 30 individuals per population). For these two species, no genetic structure was identified (see result section).

149 sampling. Care was taken to minimise animal handling and stress and all individuals were rapidly released
150 at the place of capture after manipulation. We amplified 13 (Pokrant et al., 2016), 14 (Tobler et al.,
151 2013; Maia-Carvalho et al., 2014), 15 (Richard et al., 2015) and 14 (Marcus et al., 2013) polymorphic
152 microsatellite loci in *N. helvetica*, *A. obstetricans*, *M. jurtina* and *A. parallelepipedus*, respectively. For a
153 detailed procedure of DNA extraction, amplification and genotyping, see Appendix 1. Some individuals
154 could not be correctly genotyped because of insufficient amounts of DNA: genotypes with more than
155 2 loci presenting missing values were discarded to allow robust subsequent genetic analyses. We used
156 Genepop 4.2 (Rousset, 2008) to test for linkage disequilibrium among pairs of loci and deviation from
157 Hardy-Weinberg Equilibrium after sequential Bonferroni correction to account for multiple related tests
158 (Rice, 1989). The presence of null alleles was tested using MICROCHECKER 2.2.3 (Van Oosterhout
159 et al., 2004). Loci with null alleles and/or in linkage disequilibrium were discarded, resulting in the
160 final selection of 13, 10, 6 and 10 microsatellite loci in toads, snakes, butterflies and beetles, respectively
161 (Appendix 1).

162 **Classical and hierarchical genetic distances**

163 The presence of related individuals in data sets may lead to an over-estimate of the number of clusters
164 when assessing population structure (Anderson and Dunham, 2008) and thus bias subsequent genetic
165 analyses. We therefore used COLONY2 (Jones and Wang, 2010) to identify and discard siblings within
166 our individual-based data sets (*N. helvetica* and *A. obstetricans*, Appendix 2). In addition, because sites
167 were unevenly sampled for snakes and toads, we only retained a maximum of three randomly picked
168 genotypes per sampling location (Prunier et al., 2013). In the population data sets, we only retained
169 populations for which at least 15 genotypes were available. The final data sets comprised 848 genotypes
170 (30 populations) in *A. parallelepipedus*, 508 genotypes (21 populations) in *M. jurtina*, 115 genotypes in
171 *N. helvetica* (68 sampling locations) and 132 genotypes in *A. obstetricans* (56 sampling locations).

172 For each of the four final data sets (either individual- or population-based data sets), genetic structure
173 was investigated using STRUCTURE 2.3.4 (Pritchard et al., 2000) with the admixture and the correlated
174 allele frequency models and prior sampling location information when structure in the data was too weak.
175 We followed a hierarchical genetic clustering procedure (Coulon et al., 2008). At each hierarchical level,
176 we tested the number K of clusters from 1 to 10 and repeated analyses for each value 5 times. Runs were
177 performed with a burn-in period of 50 000 and 50 000 subsequent Markov chain Monte Carlo (MCMC)
178 repetitions. We also checked that the alpha value had stabilised before the end of the burn-in period to
179 ensure algorithm convergence. If convergence was not reached, we used a burn-in period of 100 000 and
180 100 000 MCMC repetitions. We then used STRUCTURE HARVESTER (Earl and VonHoldt, 2012) to
181 obtain deltaK statistics to infer the optimal K-value. We used this optimal K-value to perform 20 runs
182 with a burn-in period of 200 000 and 200 000 MCMC repetitions. We finally compiled the ten best runs

183 using CLUMPP ([Jakobsson and Rosenberg, 2007](#)) to obtain individual or population Q-values. Each
184 individual or population was assigned to the cluster for which the Q-value was higher than 0.6 ([Prunier
185 et al., 2017](#)). We then repeated the analysis for each inferred cluster separately until no more structure
186 was found in the data. For each hierarchical level, we used Q-values to compute pairwise matrices
187 (among individuals or populations depending on the sampling design) of ancestry-based hierarchical
188 genetic distances (HGD) ([Balkenhol et al., 2014](#); [Prunier et al., 2017](#)). HGD were only calculated for
189 species displaying a significant genetic structure. When more than one hierarchical level was detected,
190 each hierarchical level (HGD1, HGD2...) was considered separately. We also computed classical genetic
191 distances (GD), using the Bray-Curtis (bc) percentage dissimilarity index ([Legendre and Legendre, 1998](#))
192 for the individual-based data sets (*N. helvetica* and *A. obstetricans*) and Fst for the population-based
193 data sets (*M. jurtina* and *A. parallelepipedus*). While these classical genetic distances are well suited to
194 detect surface elements affecting gene flow at a regional scale, HGD have been shown to allow a better
195 detection of sharp genetic variations caused by linear elements such as LTIs ([Prunier et al., 2017](#)).

196 **Multiple linear regressions and commonality analyses**

197 Both classical and hierarchical genetic distances were tested against the six types of LTIs present in our
198 study area, along with a number of covariates likely to affect patterns of genetic differentiation (isolation-
199 by-distance IBD, difference in altitude and the following landcover features: water, crops, woodlands,
200 grasslands and urban areas), although assessing the respective influence of these non-LTIs features was
201 not the main scope of this study. Five LTIs (motorway, railway, D6089 country road, gas pipeline and
202 power line) were coded into binary pairwise matrices, with 0 indicated that individuals/populations of
203 each pair were on the same side of the LTI and 1 indicated that they were on either side of the LTI.
204 Because of the density of the secondary road network in the study area, this sixth LTI was treated as other
205 landcover features. Landcover features were defined by digitalizing the entire study area in QGIS (V. 2.8)
206 using national maps and aerial photographs (National Geographic Institute, France). Every elements of
207 the landscape was classified into 49 habitat types of the EUNIS Habitat Classification System ([Davies
208 and Moss, 1999](#)). Botanic field surveys were also performed in 2015 to confirm the affiliation of certain
209 habitat types. We combined these 49 elements into six main landcover predictors (Appendix 3): Water
210 (stagnant water bodies, streams and rivers), Crops (intensive and non intensive cultures), Woodlands
211 (all types of forests), Grasslands (uncultivated open lands), Urban (villages, industrial sites, *etc.*) and
212 Secondary road network (all roads excluding small trails, motorway and D6089 country road). These
213 six landcover classes were each rasterised at a 1 m resolution using ARCGIS 10.2.2 and its SPATIAL
214 ANALYST extension. Each raster was then used to create a resistance surface based on the spatial
215 density of the corresponding element in the landscape. To do so, we overlaid a 20 m grid on each spatial
216 class and calculated the percentage of the element in each grid ([Balkenhol et al., 2014](#); [Prunier et al.,](#)

217 2017). For each resistance surface, we rescaled pixel resistance values to range from 1 (null or extremely
218 low densities) to 100 (the element covers the entire pixel) and the final rescaled resistance surface was
219 used in CIRCUITSCAPE 4.0 (McRae, 2006; McRae et al., 2013) to compute pairwise effective distances
220 between sampling locations (individuals or populations). The IBD pairwise matrix was similarly obtained
221 by running CIRCUITSCAPE on a uniform resistance surface only composed of pixels of value 1. Finally,
222 altitude pairwise matrices were computed as the absolute values of pairwise differences in altitude between
223 sampling locations.

224 The local influence of landscape features may go unnoticed if all pairs of genetic distances are retained,
225 as isolation-by-distance might take over the influence of isolation-by-barriers or isolation-by-resistance
226 (Anderson et al., 2010), with strong implications in terms of biological interpretation of results (Keller
227 et al., 2013; Schregel et al., 2018). We thus considered subsets of pairwise data by defining a maximum
228 euclidean distance threshold between sampling locations. Following Cayuela et al. (2019), this distance
229 threshold was selected for each species and each metric of genetic distances (GD or HGD) as the neigh-
230 bouring distance maximizing the model fit of a classical multiple linear model including all predictors (see
231 Appendix 4 for details). For each species, we then explored the relationship between subsets of each type
232 of genetic distances (GD or HGD) and the corresponding 13 predictors using standard multiple linear
233 regressions. The contributions of predictors to the dependent variables were assessed using commonality
234 analyses (CA). Commonality analysis is a variance partitioning procedure allowing the detection and the
235 withdrawal of statistical suppressors that are responsible for a distortion of model estimates (beta weights
236 and confidence intervals), thus providing decisive support when trying to assess the reliability of model
237 parameters in face of multicollinearity (for more details about CA, see Appendix 5 and Ray-mukherjee
238 et al. (2014); Prunier et al. (2015, 2017)). We performed model simplification by discarding predictors
239 identified as statistical suppressors in an iterative way, following Prunier et al. (2017) and Cayuela et al.
240 (2019) (see Appendix 6 for details).

241 In each final simplified model, we assessed final levels of collinearity among predictors using Vari-
242 ance Inflation Factors VIF (Dormann et al., 2013). Because pairwise data are not independent, the
243 p-values inferred from simplified models could not be interpreted (Legendre and Legendre, 1998): we
244 thus computed 95 % confidence intervals around regression estimates using a jackknife procedure, with
245 1000 replicates based on a random removal of 10 % of individuals without replacement (Peterman et al.,
246 2014; Prunier et al., 2015). These confidence intervals were used to assess the significance of the predic-
247 tors contributions to the variance in the corresponding genetic distances. We considered that a predictor
248 was a robust contributor to the variance in the response variable as soon as the confidence interval about
249 the corresponding β value did not include 0. A predictor with a positive β value was associated with
250 an increase in the genetic distances and was interpreted as impeding gene flow . On the contrary, a
251 predictor with a negative β was associated to a reduction in genetic distances and was thus interpreted

252 as promoting gene flow (Jacquot et al., 2017).

253 In order to summarise model results across species and predictors, we built three 100 % stacked
254 barplots. In the first plot, averaged unique contributions of predictors to the variance in genetic variables
255 were presented per species and across all species with contributions combined into two main classes:
256 those associated with LTIs and those associated with non-LTI predictors (IBD, Altitude, Woodlands,
257 Grasslands, Water, Crops and Urban). In the two last plots, averaged unique contributions of LTIs were
258 either presented per species (and across all species) or per LTI (and across LTIs), with contributions
259 combined into two main classes: those associated with an increase and those associated with a decrease
260 in genetic distances. Predictors that were absent from final simplified models were given a unique
261 contribution of 0.

262 RESULTS

263 Genetic structures

264 Structure outputs indicated a single genetic cluster in both the grass snake *N. helvetica* and the butterfly
265 *M. jurtina*, suggesting high gene flow across the study area in these species. On the contrary, we found
266 strong hierarchical genetic clustering in the toad *A. obstetricans* and in the beetle *A. parallelepipedus*
267 (Fig. 3). In toads, we identified two hierarchical genetic clusters. At the first level, one cluster (B)
268 was surrounded by a second cluster (A) with no clear geographical boundaries explaining this pattern
269 (Fig. 3). Ten individuals could not be assigned to any of these two clusters (cross-assigned individuals),
270 suggesting some exchanges between these two clusters. At the second hierarchical level, only cluster A was
271 further divided into three sub-clusters: A1, A2 and A3. These three sub-clusters were not separated by
272 clear geographical patterns and a high number of individuals (21) were cross-assigned, again suggesting
273 frequent exchanges among them. We similarly identified two hierarchical clustering levels in beetles
274 (Fig. 3). At the first level, 19 populations were assigned to cluster A and ten were assigned to cluster
275 B. Cluster A included populations sampled mostly in the western part of the study area and north of
276 the road D6089 (Fig. 3). One population at the extreme south-west could not be assigned to any of
277 these two clusters (cross-assigned). Cluster B, was further divided into two sub-clusters at the second
278 hierarchical level. Clusters B1 and B2 were separated by the D6089 and the gas pipeline, with B1 in
279 the north comprising five populations and B2 in the south comprising four populations. At the second
280 hierarchical level, only one population could not be assigned to any of these two clusters (cross-assigned).
281 This population was located between the road “D6089” and the gas pipeline, exactly in-between clusters
282 B1 and B2.

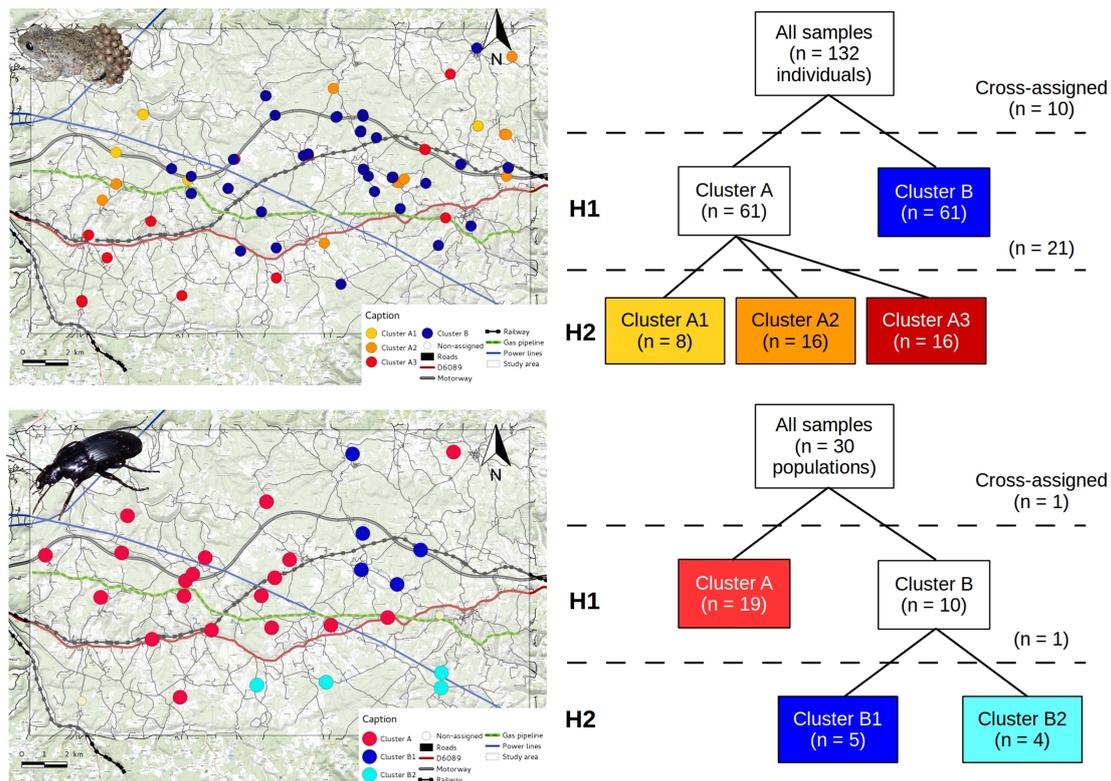


Figure 3: Left panels: STRUSTRUCTURE outputs for *A. obstetricans* (132 individuals in 56 sampling locations) and *A. parallelepipedus* (30 populations of about 30 individuals each) plotted over the study area. Right panels: hierarchical splits of inferred clusters from the first to the second hierarchical level. Each box represents a cluster, with n the number of samples (individuals for *A. obstetricans* and populations for *A. parallelepipedus*) assigned to it. The number of cross-assigned samples at each hierarchical level (Q-values < 0.6) is also indicated.

Table 1: Outputs of multiple linear regressions and additional parameters from commonality analyses (CA) for each species and for each type of data set. DV represents the dependent variable: classical genetic distances (GD) calculated either with the Bray-Curtis dissimilarity index (bc) or with Fst and hierarchical genetic distances (HGD1 and HGD2 for first and second level of hierarchy, respectively). For each model, the model fit (Multiple R^2) was estimated from reduced scale analyses, with a maximum distance threshold between pairs of individuals or populations (Distance) ranging from 2800 to 18500m. In each model and for each retained predictor, we estimated the structure coefficient (rs), the beta weight (β), as well as unique (U), common (C) and total (T) contributions. Significance of a predictor's contribution to the dependent variable was estimated using confidence intervals (CI-inf and CI-sup). A CI that included 0 was considered as a non-informative predictor (indicated in bold). Grey colour indicates predictors with negative relationship with the dependent variable (negative β) that may thus be considered as promoting gene flow.

| DV | Species | Multiple R^2 | Distance | Predictor | rs | B | CI-inf | CI-sup | U | C | T |
|---------|--------------------------|----------------|----------|--------------|--------|--------|--------|--------|-------|--------|-------|
| GD(bc) | <i>A. obstetricans</i> | 11.82% | 3000 m | IBD | 0.823 | 0.126 | 0.066 | 0.198 | 0.009 | 0.071 | 0.080 |
| | | | | Altitude | 0.618 | 0.098 | 0.052 | 0.140 | 0.008 | 0.037 | 0.045 |
| | | | | Woodlands | 0.554 | 0.145 | 0.091 | 0.191 | 0.018 | 0.018 | 0.036 |
| | | | | Roads | 0.623 | 0.113 | 0.062 | 0.153 | 0.009 | 0.037 | 0.046 |
| | | | | D6089 | 0.320 | 0.091 | 0.043 | 0.142 | 0.008 | 0.004 | 0.012 |
| HGD1 | <i>A. obstetricans</i> | 10.76% | 2400 m | Woodlands | 0.461 | 0.100 | 0.037 | 0.172 | 0.010 | 0.013 | 0.023 |
| | | | | Crops | 0.687 | 0.185 | 0.099 | 0.254 | 0.032 | 0.018 | 0.051 |
| | | | | Roads | 0.675 | 0.159 | 0.100 | 0.203 | 0.024 | 0.025 | 0.049 |
| | | | | Railway | 0.442 | 0.108 | 0.048 | 0.178 | 0.011 | 0.010 | 0.021 |
| HGD2 | <i>A. obstetricans</i> | 19.92% | 2500 m | Woodlands | 0.538 | 0.188 | 0.134 | 0.240 | 0.031 | 0.026 | 0.058 |
| | | | | Urban | -0.465 | -0.241 | -0.276 | -0.203 | 0.047 | -0.004 | 0.043 |
| | | | | Roads | 0.448 | 0.184 | 0.134 | 0.238 | 0.033 | 0.006 | 0.039 |
| | | | | D6089 | 0.440 | 0.196 | 0.145 | 0.250 | 0.037 | 0.001 | 0.039 |
| | | | | Motorway | -0.278 | -0.120 | -0.159 | -0.076 | 0.014 | 0.002 | 0.016 |
| GD(bc) | <i>N. helvetica</i> | 4.15% | 2800 m | Roads | -0.533 | -0.125 | -0.193 | -0.062 | 0.015 | -0.003 | 0.012 |
| | | | | Motorway | 0.616 | 0.148 | 0.078 | 0.221 | 0.021 | -0.005 | 0.016 |
| | | | | Railway | -0.520 | -0.088 | -0.155 | -0.022 | 0.008 | 0.004 | 0.011 |
| GD(Fst) | <i>M. jurtina</i> | 19.91% | 5500 m | IBD | 0.468 | 0.264 | 0.001 | 0.490 | 0.066 | -0.023 | 0.044 |
| | | | | Woodlands | 0.685 | 0.315 | 0.077 | 0.519 | 0.089 | 0.004 | 0.093 |
| | | | | Power line | -0.595 | -0.180 | -0.388 | 0.046 | 0.030 | 0.040 | 0.071 |
| GD(Fst) | <i>A. parollepipedus</i> | 25.87% | 6500 m | Altitude | 0.203 | 0.121 | -0.023 | 0.251 | 0.015 | -0.004 | 0.011 |
| | | | | Grasslands | 0.971 | 0.498 | 0.372 | 0.610 | 0.248 | -0.004 | 0.244 |
| HGD1 | <i>A. parollepipedus</i> | 17.22% | 18500 m | Roads | 0.812 | 0.262 | 0.170 | 0.350 | 0.063 | 0.051 | 0.114 |
| | | | | D6089 | 0.797 | 0.254 | 0.159 | 0.338 | 0.059 | 0.051 | 0.110 |
| | | | | Altitude | 0.445 | 0.223 | 0.056 | 0.397 | 0.049 | 0.004 | 0.053 |
| HGD2 | <i>A. parollepipedus</i> | 26.76% | 4500 m | D6089 | 0.759 | 0.350 | 0.184 | 0.500 | 0.114 | 0.040 | 0.154 |
| | | | | Motorway | -0.316 | -0.114 | -0.273 | 0.041 | 0.012 | 0.015 | 0.027 |
| | | | | Gas pipeline | 0.518 | 0.225 | 0.070 | 0.368 | 0.049 | 0.022 | 0.071 |



283 Multiple linear regression and commonality analyses

284 The maximum euclidean distances between sampling locations that optimized the amount of variance
 285 in classical and hierarchical genetic distances (variance explained by full regression models) ranged from
 286 2800 to 3500m in individual-based data sets (vertebrate species) and from 4500 to 18500m in population-
 287 based data sets (insect species; Table 1; Appendix 4). After simplification (Appendix 6) and whatever
 288 the model, Variance Inflation Factors (VIF) ranged from 1.00 to 1.70 (Appendix 7), suggesting little
 289 collinearity among retained variables (Dormann et al., 2013; Prunier et al., 2015).

290 When considering classical genetic distances in toads, the multiple linear regression explained 11.8
 291 % of variance (Table 1). Two infrastructures (the road D6089 and the secondary road network) were
 292 associated with an increase in genetic distances in this model, thus suggesting barrier effects. Both
 293 explained about 33 % of the averaged unique contribution and similarly contributed to the dependent
 294 variable ($U = 0.009$ and 0.008 , respectively). When considering the first level of hierarchical genetic
 295 distance (HGD1), the linear regression explained 10.76 % of the variance. In the final model, the
 296 secondary road network ($U = 0.024$) and the railway ($U = 0.011$) were both associated with an increase

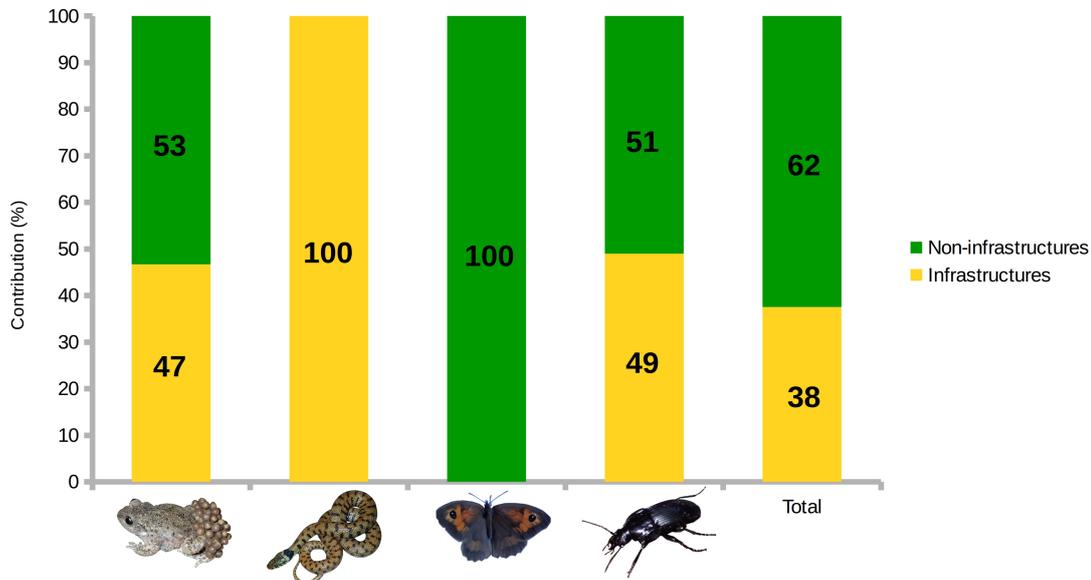


Figure 4: For each each species and for all species combined (Total), 100 % stacked barplots indicating the averaged unique contributions of non-LTI predictors (IBD, Altitude, Woodlands, Water, Grasslands, Crops and Urban) and LTIs (all infrastructures combined) to the overall variance explained by simplified models.

297 in genetic distances (positive β values), here again indicating barrier effects. At the second level of
 298 the hierarchy, our model explained 20 % of variance in HGD2. The secondary road network and the
 299 road D6089 were, again, associated with an increase in genetic distances (positive β values) but the
 300 motorway was also detected has having a weak but significant positive effect on toads effective dispersal
 301 ($U = 0.047$). Other features such as IBD, altitude, crops and woodlands were found to increase genetic
 302 distances whereas urbanisation was detected has having a positive effect on dispersal (see Appendix 8
 303 for more details). When unique contributions were averaged across genetic distances, LTIs accounted for
 304 47 % of overall explained variance (Fig. 4). Infrastructures were mostly associated with an increase in
 305 genetic distances, with 90 % of overall explained variance accounted for barrier effects of the road D6089
 306 and of the secondary road network (82%) and, to a smaller extent, of railway (8%; Fig. 5). The 10 %
 307 left were explained by a reduction in genetic distances across the motorway at the second level of the
 308 hierarchy (HGD2; Fig. 5).

309 In snakes, the simplified model explained a small amount (4.15%) of variance in the dependent
 310 variable (Table 1) but only comprised LTIs predictors (Fig. 4). The motorway was associated with an
 311 increase in genetic distances (positive β value) and accounted for 49% of explained variance ($U = 0.021$;
 312 Fig. 5). The two other types of infrastructures (the secondary road network and the railway) had unique

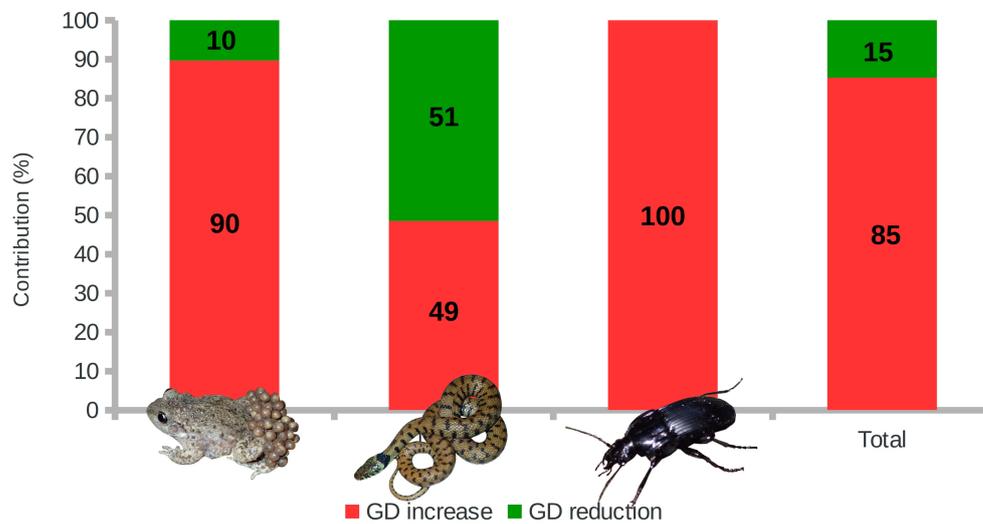


Figure 5: For each each species (except for *M. jurtina*, see main text for details) and for all species combined (Total), 100 % stacked barplots indicating the averaged unique contributions of LTIs associated with an increase (barrier effect) or with a decrease (corridor effect) in genetic distances, expressed as a % of the overall variance explained by simplified models.

313 contribution of 0.015 and 0.008, respectively, and both had negative β values, indicating that they were
314 associated with a reduction in genetic distances in this species, together accounting for 51% of explained
315 variance (Roads = 34% and Railway = 17.7%; Fig. 5).

316 In butterflies, the simplified model explained 20 % of variance in F_{st} values (Table 1). The only LTI
317 that remained in the final model was the power line but it did not significantly contribute to the model
318 predictive power (95% confidence interval including 0). The entire genetic variability in this species was
319 thus explained by isolation by distance and woodlands, both impeding gene flow (Fig. 4 and Appendix
320 8).

321 In the ground-beetle, the simplified model explained 26% of the variance in F_{st} values (Table 1).
322 The entire genetic variability was yet here explained by non-LTIs elements, open areas notably strongly
323 affecting gene flow in this species (see Appendix 8 for more details). When considering the first and
324 the second level of the inferred hierarchical genetic structure, simplified models explained 17% and 27%
325 of the variance in HGD1 and HGD2, respectively. In both cases, the road D6089 was associated with
326 an increase in genetic distances, indicating a consistent barrier effect ($U = 0.059$ in HGD1 and 0.114
327 in HGD2). In addition, the secondary road network (HGD1) and the gas pipeline (HGD2) were also
328 detected as having negative effects on gene flow ($U = 0.063$ and 0.049, respectively). In HGD2, the
329 motorway did not significantly contribute to the model predictive power. Overall, explained variance in
330 genetic distances was accounted for by both LTIs (49 %) and non-LTIs elements (51 %) (Fig. 4), with
331 all LTIs being associated with an increase in genetic distances (Fig. 5).

332 **Assessment of infrastructure effects**

333 Overall, 38 % of the explained variance in genetic distances across all species was due to LTIs (Fig. 4),
334 of which 85% was associated with an increase in genetic distances, that is, with a barrier effect (Fig. 5).
335 The only LTI that did not contribute to genetic distances in any species was the power line. On the
336 contrary, the road D6089 and the gas pipeline were both systematically associated with barrier effects,
337 in toads and beetles for the former and in beetles only for the latter. Other LTIs however showed more
338 nuanced impacts, with corridor effects detected in some species (15% of explained variance by LTIs).
339 While 82 % of the overall genetic variability explained by the motorway across genetic distances and
340 species corresponded to a barrier effect in snakes, the remaining 18 % corresponded to a reduction in
341 genetic distances in toads (Fig. 6). It was the opposite in the case of the railway, with 33 % corresponding
342 to a barrier effect in toads but 67 % to a reduction in genetic distances in snakes. Finally, the secondary
343 road network acted as a barrier to gene flow in both toads and beetles (74 %) but as a corridor in snakes
344 (26%; Fig. 6).

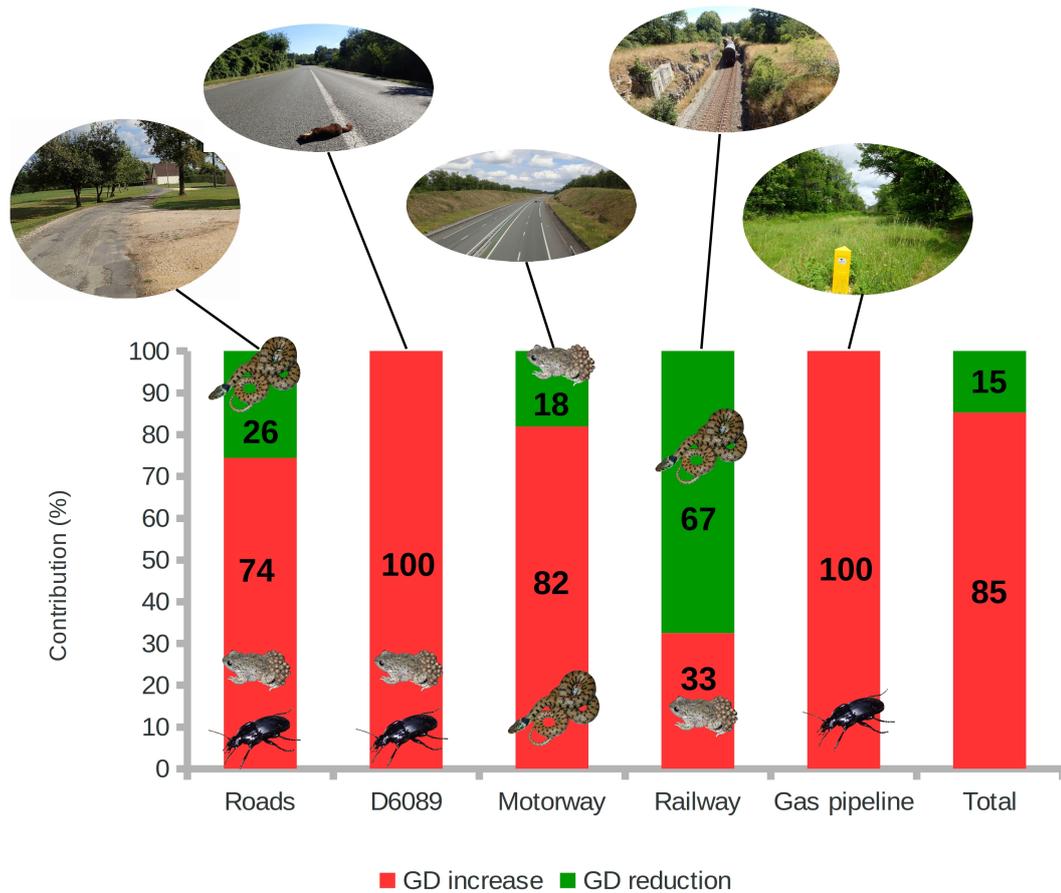


Figure 6: For each LTI and for all LTIs combined (Total), 100 % stacked barplots indicating the averaged unique contributions of LTIs associated with an increase (barrier effect) or in a decrease (corridor effect) in genetic distances, expressed as a % of the overall variance explained by simplified models across all datasets. The power line is not represented as no species was affected by this infrastructure (see results).

345 DISCUSSION

346 The goal of this study was to assess landscape functional connectivity in four parapatric species occupying
347 a landscape fragmented by multiple large-scale transportation infrastructures. We were particularly
348 interested in the potential cumulative or on the contrary the antagonistic effects of six LTIs. We used
349 individual- and population-based regression analyses along with commonality analyses over restricted
350 spatial scales to thoroughly evaluate the relative contribution of various landscape predictors to the
351 variance in both classical and hierarchical genetic distances. We notably showed that LTIs were overall
352 responsible for a significant amount of genetic variability across species but that the response of organisms
353 to these LTIs was highly species-dependant. Most importantly, we found that LTIs did not only act as
354 barriers to gene flow but might on the contrary promote gene flow, with some antagonistic effects across
355 species.

356 Overall, LTIs were found to have a strong influence (either positive or negative) on gene flow, ac-
357 counting for about 38% of the total explained genetic variability across species and genetic distances, as
358 against 62% explained by other non-LTI features (Fig. 5). All ground-dwelling species were affected by
359 LTIs, with contributions to the variance by LTIs ranging from about 50% in toads and beetles to 100%
360 in snakes, contrary to the flying species *M. jurtina* whose genetic variability was only negatively affected
361 by distance and woodlands, as expected from a previous study (Villemey et al., 2016). Although butter-
362 flies have a lower probability to be impacted by vehicles than ground-dwelling species, previous studies
363 showed that roads (Polic et al., 2014) and motorways (Remon et al., 2018) could hinder crossing events
364 in this species. A direct Mark-Release-Recapture survey conducted in the same study area notably found
365 that the motorway was responsible for a six-fold decrease in crossing events when compared to adjacent
366 habitats (Remon et al., 2018). It is possible that large population sizes in *M. jurtina* are responsible for
367 a temporal inertia in the setting-up of genetic differentiation since the creation, in 2004, of the motorway
368 (Gauffre et al., 2008; Landguth et al., 2010), but Remon et al. (2018) also showed that some butterflies
369 were able to cross it, thus possibly ensuring sufficient gene exchange across the landscape (Munguira
370 and Thomas, 1992). Although we could not ascertain the negative aftermaths of human-induced frag-
371 mentation in *M. jurtina* from our genetic data, our study highlights the potential benefits of combining
372 landscape genetics and Mark-Release-Recapture surveys (Safner et al., 2011; Cayuela et al., 2018).

373 As expected, LTIs were mainly associated with a reduction in gene flow, barrier effects accounting for
374 85% of the variance explained by LTIs across ground-dwelling species (Fig. 4). LTIs carrying vehicles
375 (roads, motorway and railway) were more impacting than infrastructures carrying energy (gas pipeline
376 and power line). Roads in particular (secondary road network and road D6089) were responsible for most
377 of inferred barrier effects in this landscape, with negative effects on gene flow in all ground-dwelling species
378 (Fig. 6). The motorway and the railway also accounted for non-negligible amounts of explained genetic
379 variability but to a lesser extent than roads, only negatively affecting snakes and toads, respectively.

380 In contrast, the contributions of LTIs carrying energy were less important. The gas pipeline negatively
381 affecting gene flow in the ground-beetle only, probably in response to associated breaches in forest cover
382 (Charrier et al., 1997), and the power line did not affect any studied species. These results indicate
383 that conservation measures should primarily focus on infrastructures carrying vehicles rather than on
384 infrastructures carrying energy (Shepard et al., 2008; Bartzke et al., 2015), although we acknowledge
385 that some taxa were not considered in this study, for instance birds, might be negatively affected by
386 LTIs such as power lines (Loss et al., 2014, 2015).

387 Despite these general negative impacts of LTIs on gene flow, we found that species showed very
388 different responses to the same LTI, which perfectly highlights the importance of considering functional
389 rather than just structural landscape connectivity in empirical studies (Taylor et al., 2006). Three of the
390 six studied LTIs were associated with an increase in genetic distances in toads (Table 1), these barrier
391 effects together accounting for 90% of genetic variance explained by LTIs (Fig. 4). The secondary road
392 network and the country road D6089 were the main barriers to dispersal in *A. obstetricans*, affecting
393 both classical (GD) and second-order hierarchical genetic distances (HGD2). In addition, the secondary
394 road network also impeded gene flow at the first level of the hierarchy (HGD1). Garcia-Gonzalez et al.
395 (2012) similarly found that all roads, including small secondary roads, acted as barriers to gene flow
396 in *A. obstetricans* in northern Spain. Amphibians are particularly vulnerable to road kills (Fahrig
397 and Rytwinski, 2009) because of their numerous movements during dispersal but also during seasonal
398 migrations between breeding water bodies and shelters. Although these results advocate for effective
399 mitigation measures to limit road kills of amphibians (Beebee, 2013) such as tunnels (Ottburg and Van
400 Der Grift, 2019), it is important to keep in mind that other road features such as traffic noise may also
401 affect amphibians population dynamics (Bee and Swanson, 2007; Troianowski et al., 2017).

402 In addition to toads, we found that roads also deeply impacted the ground-beetle *A. parallelepipedus*.
403 The country road D6089 and the secondary road network explained the whole genetic variance at the first
404 hierarchical level (HGD1) resulting in clusters A and B (Fig. 3). At the second hierarchical level (HGD2),
405 the country road D6089 (but also the gas pipeline) further impacted gene flow and was responsible for
406 the split of cluster B into two sub-clusters (Fig. 3). Our results are congruent with Keller et al. (2004)
407 who found that roads are barriers to dispersal in *A. parallelepipedus* but also in other ground-beetle
408 species (e.g. Keller and Largiader, 2003). Roads may act as barrier to gene flow because of road kills but
409 also because ground-beetles may be reluctant to cross roads due to behavioural changes (Holderegger
410 and Di Giulio, 2010).

411 Contrary to roads, we found that the motorway and the railway showed limited barrier effects. The
412 only species that was negatively affected by the motorway was the snake *N. helvetica*. Genetic studies
413 estimating gene flow of reptiles across LTIs are lacking (Holderegger and Di Giulio, 2010) (but see Clark
414 et al., 2010) and we here revealed that half of the explained genetic variability in snakes resulted from the

415 negative impacts of the motorway. Because it is fenced with fine mesh, snakes can only reach the other
416 side by using crossing structures (bridges, underpasses, culverts, etc.). These crossing structures may
417 yet be seldom used by snakes due to inadequate placement, architectural design and snakes' behaviour
418 (Woltz et al., 2008). Thermoregulatory behaviour of reptiles is probably the main reason why individuals
419 would not use underpasses (Rodriguez et al., 1996), as a 50 m-length underpass would provide inadequate
420 thermal conditions due to the absence of sunlight. In addition, Baxter-Gilbert et al. (2015) evaluated
421 the effectiveness of different mitigation measures implemented to reduce reptile road mortality (including
422 underneath culverts) and found that these structures were seldom used by reptiles. Underpasses may yet
423 be used by other taxa such as amphibians (Prunier et al., 2014) and insects (Georgii et al., 2011), which
424 may explain why the motorway was only found as acting as a barrier in a single species. Similarly, only
425 one species was negatively affected by the railway. At the first level of the hierarchy (HGD1), we found
426 that a positive relationship between the presence of the railway and genetic distances in toads (Table 1),
427 although clusters A and B were not clearly separated by this LTI (Fig. 3), suggesting a modest barrier
428 effect. Railways are known to restrict gene flow in some amphibian species such as frogs or salamanders
429 (e.g. Reh et al., 1990; Bartoszek and Greenwald, 2009) and many studies on train collision with wildlife
430 reported a high abundance of amphibian kills (Borda-de Agua et al., 2017), representing up to 47 % of all
431 vertebrate records (Heske, 2015). However, the railway in our study area had a low traffic density with
432 approximately 10 trains/day, and train collisions may not be the only driver of the observed reduction in
433 gene flow in *A. obstetricans*. The physical features of the railway are more likely to explain this pattern
434 (Barrientos et al., 2019). Amphibians indeed have a high probability to be trapped between or along rail
435 tracks, making them more vulnerable to both collisions and desiccation than other vertebrates (Budzik
436 and Budzik, 2014). The studied railway was more than 150 years-old, which seems to be of sufficient
437 duration for the detection of a barrier effect from genetic data (Cushman and Landguth, 2010; Prunier
438 et al., 2014; Epps and Keyghobadi, 2015) and suggests that this LTI was actually permeable to the
439 movement of other species.

440 Our most striking finding is that, instead of acting as barriers, some LTIs might somehow promote
441 dispersal. This “corridor effect” accounted for 15% of the overall genetic variance explained by LTIs
442 across species (Fig. 4) and concerned both vertebrates. We first found that, at the second level of the
443 hierarchy (that is, at a more local scale), gene flow in toads was promoted by the motorway (Table
444 1). This counter-intuitive genetic pattern (Van Buskirk, 2012) could stem from the availability of new
445 habitats provided by the LTI. Adults and tadpoles of *A. obstetricans* were indeed detected in eight out
446 of the ten storm-water retention ponds present along the studied motorway (data not shown). These
447 ponds may provide favorable breeding habitats, free of predatory fish and surrounded by sand or gravel,
448 the ideal substrates to build their burrows. furthermore, the motorway is crossed by underneath culverts
449 and tracks which are good dispersal corridors for amphibians (Georgii et al., 2011), especially when

450 their are filled with water (Veenbaas and Brandjes, 1999; Lesbarrères et al., 2004). This is not the first
451 study showing a potential positive effect of a motorway on amphibian gene flow. Prunier et al. (2014)
452 revealed that a 40-years old motorway was not a barrier for the alpine newt (*Ichthyosaura alpestris*)
453 and could even serve as a longitudinal dispersal corridor when the surrounding landscape matrix is
454 highly unfavourable. Interestingly, they even found negative relationships between genetic distances and
455 presence of the motorway, indicating that, as in our study, gene flow across the motorway was probably
456 enhanced; but because they analysed their data using one-tailed Mantel tests, they did not discuss
457 this possibility (Prunier et al., 2014). These results might yet be interpreted with caution due to the
458 recent age of the motorway (< 15 years old): this genetic pattern could stem from ancestral landscape
459 configurations and direct monitoring surveys are now necessary to confirm that the motorway is indeed
460 not an obstacle for toads.

461 Despite limited explained variance in snakes, we also identified two LTIs acting as corridors in this
462 species, together accounting for 51% of genetic variance explained by LTIs (Fig. 4): the secondary road
463 network and the railway. Roads are known to be responsible for a high mortality in snakes (Rosen
464 and Lowe, 1994): they bask on road surfaces to absorb radiant heat but this behaviour increases the
465 probability of collisions (Rosen and Lowe, 1994) and can result in a reduction in gene flow across roads
466 (Clark et al., 2010). However, we found the exact reverse pattern, with the secondary road network
467 associated with a reduction in genetic distances in *N. helvetica*. This conflicting result can be explained
468 by an attractive effect of roads and road verges that provide basking surfaces, reinforced by a limited
469 traffic volume in our study area. In addition, the distribution of grass snakes being strongly dependent
470 on wetlands for foraging, water-filled ditches often found alongside secondary roads may provide rich
471 feeding areas (Matos et al., 2012), resulting in a local increase in snake abundance that favours road
472 crossings and gene flow. A similar explanation was proposed by Johansson et al. (2005) who found a
473 positive effect of gravel roads and associated ditches in the common frog (*Rana arvalis*). The railway
474 was probably as attractive as the secondary road network for snakes, which may similarly explain gene
475 flow enhancement observed in snakes. Railway embankments provide important alternative habitats
476 for reptiles with optimal thermal conditions for basking (Graitson, 2006; Stoll, 2013; Borda-de Agua
477 et al., 2017). Even active lines can harbour particularly high diversity in reptiles species (Graitson,
478 2006), notably because human presence is scarce and because reptiles can perceive vibrations transmitted
479 through the rail tracks and the ballast when a train approaches, allowing them to reach a shelter before
480 collision (Borda-de Agua et al., 2017).

481 Conclusion

482 The accumulation of LTIs within landscapes is emerging as an important concern and local conserva-
483 tion policies are to be fueled by a thorough assessment of landscape functional connectivity. Although
484 focusing on a single species may help corridor planning ([Baguette et al., 2013](#)), we here illustrated how
485 important it is to assess landscape connectivity from a multi-species perspective. Overall, we did not find
486 consistent evidence of a cumulative barrier effect of the six LTIs across species: indeed, butterflies were
487 not influenced by any LTI and snakes were only negatively impacted by the motorway. The case of toads
488 and beetles was yet much more compelling. These two species were the most heavily impacted, with
489 patterns of gene flow affected by various LTIs at different spatial scales. Roads were critical determinants
490 of gene flow across all hierarchical levels in both species, but the railway and the gas pipeline respectively
491 reinforced these impacts in *A. obstetricans* at the first hierarchical level and in *A. parallelepipedus* at
492 the second one. In these two species, the impact of the accumulation of LTIs was thus more a question
493 of a hierarchical than of a cumulative effect of barriers ([Bélisle and St. Clair, 2001](#); [Connelly, 2011](#)).
494 Importantly, we also showed that some LTIs, acting as barrier for some species, could somehow promote
495 gene flow in some others, leading to antagonistic LTIs effects: the motorway affected snakes but pro-
496 vided favourable habitats for toads, while the railway affected toads but provided favourable habitats
497 for snakes. Considering the high variability in species response to LTIs, we argue that considering a
498 single species may lead to counterproductive mitigation measures and that integrative approaches based
499 on multiple species are to be more systematically considered. As it obviously seems impossible to assess
500 functional connectivity in all existing species in a given landscape, it is also necessary to determine the
501 extent to which species-specific mitigation measures ([Jaarsma and Willems, 2002](#); [Woltz et al., 2008](#);
502 [Glista et al., 2009](#)) can benefit the largest number of species, and, more generally, to investigate which
503 life-history traits are driving organisms response to the presence of LTIs at a taxonomic level ([Blanchet
504 et al., 2017](#)).

505 Authors' contributions

506 JR, JGP, MB and SM contributed to the conception and design of the study. JR, SM and JHC collected
507 the data. LG digitalized the soil occupancy of the study area. JR and MR performed laboratory
508 analyses and genotyping. JR and JGP performed data analyses and wrote the manuscript. All authors
509 participated in critical revisions of the manuscript.

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