Chapitre 1: "Multi-species gene flow in a fragmented environment"

Jonathan Remon[1,2*] Sylvain Moulherat[1] Jérémie H. Cornuau[1]
Lucie Gendron[1] Murielle Richard[2] Michel Baguette[2,3] Jérôme G. Prunier[2]

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*Corresponding author: jonathan.remon@wanadoo.fr

[1] TerrOïko, 14 Rue Ferlus, BP 26, 81540 Sorèze, France

[2] CNRS-Université Paul Sabatier, UMR 5321, Station d'Ecologie Théorique & Expérimentale, 2 Route du CNRS, 09200 Moulis, France

[3] Museum National d'Histoire Naturelle, UMR 7205, Institut de Systématique, Evolution, Biodiversité, Rue St Hillaire, 75005 Paris, France

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Abstract

Barrier effects of Large-scale Transportation Infrastructures (LTIs; roads, railways, *etc.*) are among the main factors contributing to habitat fragmentation. Dispersal reduction across LTIs can drive small, local populations to extinction. Barrier effects detection is now facilitated by the field of landscape genetics. However, a main limitation in genetic studies is the focus on a single species. Multi-species approaches are required when trying to understand how biodiversity is affected by landscape features in general and by LTIs in particular. Accordingly, we followed two vertebrates species (the grass snake *Natrix helvetica* and the midwife toad *Alytes obstetricans*) and two invertebrate species (the butterfly *Maniola jurtina* and the ground beetle *Abax parallelepipedus*) in a landscape fragmented by six types of infrastructures: a secondary road network, a country road, a motorway, a railway, a gas pipeline and a power line. Using multiple linear regressions and commonality analyses on two types of genetic distances (classical and hierarchical genetic distances), we showed that LTIs accounted for 47 % of the explained variance in *A. obsetricans* genetic distances, 100 % in *N. helvetica*, 0 % in *M. jurtina* and 49 % in *A. parallelepipedus*. More precisely, we found that roads (country road and secondary road network) were acting as major barriers to gene flow in *A. obsetricans* and *A. parallelepipedus* but the secondary road network was enhancing gene flow in

the snake *N. helvetica*. The motorway limited *N. helvetica* dispersal but promoted gene flow in *A. obstetricans*. The railway impeded gene flow in *A. obsetricans* but enhanced *N. helvetica* dispersal. The gas pipeline reduced gene flow in *A. parallelepipedus* and the power line had no effect on gene flow in any species. We also assessed how other landscape elements (various landscape features, isolation-by-distance and altitude) affect gene flow in these four species. Our results revealed that infrastructures were mostly acting as barriers to gene flow in terrestrial species (85 % of the averaged unique contributions across data sets) but that they could also somehow promote it, because of alternative favourable landscape features provided by right-of-ways. Considering these results, we argue that species-specific mitigation measures on infrastructures are required. We also confirm that roads are acting as a major threat to biodiversity. Specific efforts are required for current and planned roads in order to offset their negative effects on gene flow.

1 INTRODUCTION

The fragmentation of natural habitats is one of the main cause driving the global biodiversity collapse (Fahrig, 2003; Haddad et al., 2015). The most ubiquitous form of habitat fragmentation is 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. In urbanized areas, they are expending considerably, creating dense transportation networks with deep impacts on natural ecosystems (Dulac, 2013; Laurance et al., 2014).

The most visible detrimental effect of LTIs is direct vehicular collisions with wildlife (Trombulak and Frissell, 2000). Most animals are affected by collisions, from small invertebrates to large mammals (Forman and Alexander, 1998; Trombulak and Frissell, 2000; Balkenhol and Waits, 2009; Fahrig and Rytwinski, 2009; Borda-de Agua et al., 2017). Besides collision, LTIs also induce behavioral modifications; leading to infrastructure avoidance (Ascensao et al., 2016). They avoid LTIs because of several reasons such as traffic noise, modification of their natural habitat, perturbation of their reproductive success or perturbation of their physiological state (Trombulak and Frissell, 2000). For example, reproductive success of amphibians can be perturbed by main roads due to sound interferences covering up calling calls of males (Bee and Swanson, 2007). These disturbances lead to a limitation of crossing events through LTIs and limit effective dispersal (the movement of individuals that sustains gene flow within landscapes (Ronce, 2007)). Barrier effects due to LTIs may create geographical isolation of populations which are not more linked by dispersal (Fahrig and Rytwinski, 2009; Beyer et al., 2016). When populations are isolated and small, they exhibit higher rates of inbreeding and genetic drift, resulting in a decrease in heterozygosity and in an increase in the risk of population extinction (McCauley, 1991; Fagan and Holmes, 2006).

In practice, LTIs do not always impede organism's dispersal but their effects are context dependent.

Classical LTIs are roads, motorways, railways, power lines, pipelines and canals. Roads and motorways are the most studied infrastructures. They have strong barrier effects on a large range of animal species (Fahrig and Rytwinski, 2009). Railways are barriers for certain species (Whittington et al., 2004; Bartoszek and Greenwald, 2009; Breyne et al., 2014), are neutral to movement (Vandevelde et al., 2012), increase species richness and abundance near infrastructures (Li et al., 2010) or create corridors (Penone et al., 2012). Power lines create openings in woodlands environments. Sometimes, wildlife avoid power lines (e.g. prairie grouse (Pruett et al., 2009)); but few studies were able to detect a consistent effect of this infrastructure type on animal movements (Latch et al., 2011; Bartzke et al., 2015; Jahner et al., 2016). Power lines can even attract wildlife by providing perches for hunting activities of birds (Morelli et al., 2014). The other types of LTIs (gas pipelines, canals, *etc.*) have been seldom studied and require more investigations (but see Dyer et al., 2002; Coulon et al., 2006; Breyne et al., 2014; Kaya Özdemirel et al., 2016).

The construction of LTIs are usually restricted by landscape features such as valleys and coastlines. At such places, LTIs are often build parallel and close to each other because of technical and economical reasons. For a given species, some LTIs can be strong barriers to gene flow, while other LTIs can be neutral or provide corridors for dispersal (Bartzke et al., 2015). For example, Paquet and Callagan (1996) followed wolves in a Canadian landscape fragmented by a railway, a major motorway and power lines. They found that the motorway was as strong barrier impeding wolves to cross but the railway and the power lines redirected wolves movements and were acting as corridors (Paquet and Callagan, 1996). Similarly, Latch et al. (2011) found that desert tortoises gene flow was affected by roads but not by power lines.

In addition, species may respond differently to the same type of infrastructure depending on the landscape configuration. For example, Van Buskirk (2012) found that a motorway was limiting gene flow in the alpine newt *Ichthyosaura alpestris* in Switzerland but Prunier et al. (2014) found that a similar motorway did not affect gene flow in the same species in France. Therefore, when trying to understand how a species moves through the landscape, it is crucial to determine the effects of the different types of infrastructures present (Balkenhol et al., 2009).

In the past fifteen years, one of the most powerful tool to estimate landscape connectivity has been landscape genetics (Manel and Holderegger, 2013). This research field integrates population genetics, landscape ecology and spatial statistic tools (Manel et al., 2003; Holderegger and Wagner, 2008; Manel and Holderegger, 2013) in order to elucidate how the genetic variability (at neutral or adaptive markers) is influenced by landscape features. Genetic studies have been widely used in order to address connectivity questions (Storfer et al., 2010) and to estimate the barrier effects of LTIs (Holderegger and Di Giulio, 2010). Therefore, they have wide applications in species management and conservation (Segelbacher et al., 2010). However, one major limitation in genetic studies is the focus on a single species (Balkenhol

and Waits, 2009; Segelbacher et al., 2010; Keller et al., 2015). Balkenhol and Waits (2009) reviewed 33 studies that assessed road effects using molecular approaches. Only two of them focused on more than one species. Studies focusing on only one species can hardly be generalized to other species and management and conservation planning can only be applied to that particular species. This considerably limits the reach and relevance for conservation planning (Keller et al., 2015). Multi-species approaches that go beyond studying related species (e.g. Riley et al., 2006) are required in order to understand how biodiversity is affected by landscape features in general and by LTIs in particular. Those evaluations are particularly requested by local authorities to design mitigation measures (EEA, 2015).

Our main goal in this study was to identify what were the main landscape features affecting gene flow in several sympatric terrestrial species. More precisely, we aimed to understand whether species were affected mostly by natural landscape features or by anthropized elements including LTIs. Accordingly, we monitored four species with various life history traits (two vertebrates and two invertebrates) in a landscape fragmented by six types of LTIs in south-western France: a secondary road network, a country road, a motorway, a railway, a gas pipeline and a power line. We used recent molecular and statistical tools to estimate how landscape features influence gene flow in these four species. We predicted that roads would impede gene flow in most of the studied species but that the motorway, built in 2004, may be too recent to allow detecting effects on genetic metrics (Anderson et al., 2010). Finally we hypothesized that the railway, the power line and the gas pipeline would have no effect on gene flow due to low traffic density for the railway and low maintenance perturbations for the two others.

2 MATERIAL AND METHODS

2.1 Study area

The study was carried out in the 'Périgord' region in the south-western France between Brive-La-Gaillarde and Périgueux (45°07'31.8"N; 0°58'56.9"E; Fig. 1).

It is a $300km^2$ rural landscape composed of limestone plateaux with low human density. This landscape includes crops, mowed meadows, deciduous forests and small villages. The hydrology is limited to small sized rivers and ponds. Altitude ranges from 91 to 294 m above sea level. Six types of Large-scale Transportation Infrastructures (LTIs) cross this study area: the fenced motorway "A89", built in 2004; a low traffic single-track railway built in the 19th century; a high traffic country road historically present since the 18th century; a gas pipeline built in 1955, a power line constructed in 1962 and a network of 1370 km of secondary roads (Fig. 1).

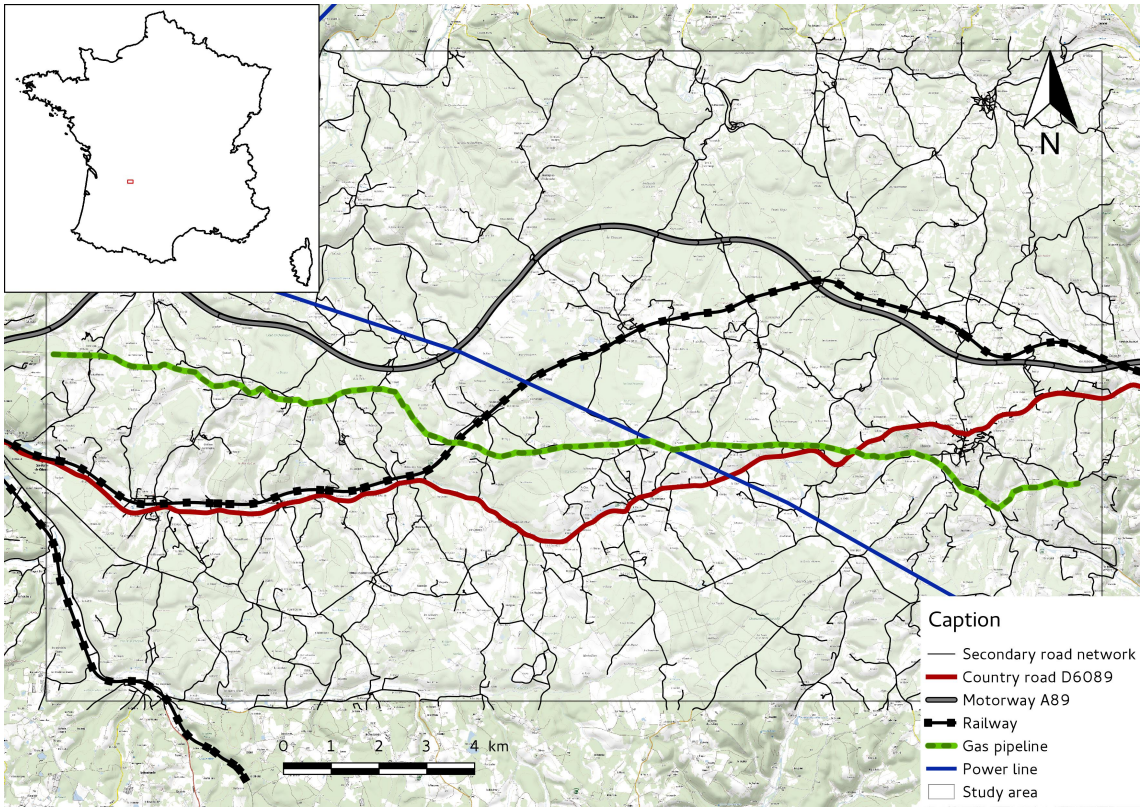

Figure 1: Study area in south-western France

2.2 Biological models

We estimated how this fragmented landscape influences gene flow in four species. The species were selected based on a compromise between abundances on the field (in order to collect large genetic data sets) and the availability of neutral genetic markers. We also chose species with various life history traits. Accordingly, we monitored two vertebrates (a reptile and an amphibian) and two invertebrates (a butterfly and a ground beetle).

The amphibian studied was the midwife toad *Alytes obstetricans*, a small toad widely distributed in western Europe. This species is characterized by an interesting reproductive strategy with a semi-terrestrial egg development stage. Just after reproduction, males carry the clutches on their back until hatching. This species is of particular conservation interest due to its sensitivity to the chytrid fungus *Batrachochytrium dendrobatidis* (Bosch et al., 2001). Fragmentation is an additional threat because local populations are known to function as relatively independent entities with strong genetic structure detected among populations (Tobler et al., 2013; Maia-Carvalho et al., 2014; Albert et al., 2015). Little is known on the dispersal ability of this species. Trochet et al. (2014) reviewed a maximal dispersal distance of 500 m.

The reptile studied was the grass snake (*Natrix natrix* sensu lato). Grass snakes are non-venomous and are the most common snake species in Europe with a wide geographical range. Wetlands are typical

habitats and their diet consists mostly of amphibians (Gregory and Isaac, 2004). Mean home-range size is about 40 ha (Wisler et al., 2008) and they are considered as good dispersers with individuals travelling more than 1 km distances in less than a month (Pettersson, 2014). The taxonomy of grass snakes as been revised recently and three new species have been proposed instead of the previous unique species (Kindler et al., 2013; Pokrant et al., 2016; Kindler, Chèvre, Ursenbacher, Böhme, Hille, Jablonski, Vamberger and Fritz, 2017; Kindler, de Pous, Carranza, Beddek, Geniez and Fritz, 2017). In this study, we focused on the species *Natrix helvetica* previously considered as the subspecies *Natrix natrix helvetica* (Kindler, Chèvre, Ursenbacher, Böhme, Hille, Jablonski, Vamberger and Fritz, 2017). A previous study showed that there was no genetic structure in this species in a intensively used agricultural landscape, suggesting good landscape connectivity in fragmented environments (Meister et al., 2010).

The butterfly studied was the meadow brown *Maniola jurtina*, an univoltine butterfly which is very common in Europe with locally very high densities. The ideal habitat for this species consists of open grasslands. Median life span of adults is 6.55 days (Bubová et al., 2016). Flight period is about 67 days between June and September (Bubová et al., 2016). Caterpillars feed on a wide range of grass species with some preferences for *Poa spp.*, *Agrostis spp.* and *Lolium spp.* (Brakefield, 1982; Thomas and Lewington, 1991). *Maniola jurtina* has a medium dispersal capacity with mean dispersal distances ranging from about 50 to 300 m (Schneider et al., 2003; Ouin et al., 2008; Stevens et al., 2013).

The ground beetle studied was *Abax parallelepipedus*. This species is an opportunist carnivorous beetle (Loreau, 1983) that inhabits the upper layer of litter in forest environments (Loreau, 1987). Hedges in agricultural landscapes are also important refuges (Fournier and Loreau, 1999). This beetle has a low growth rate and a great longevity for a beetle ($>$ 2 years) (Chaabane et al., 1997). A study in a beechwood in Belgium calculated that density was about 2000 individuals per hectare (Loreau and Nolf, 1993) and Keller et al. (2004) estimated density between 632 and 1707 individuals per hectare in a fragmented mixed forest in Switzerland. The mean distance covered per day was estimated between 0.6 and 2.3 m (Brouwers and Newton, 2009) with a home range of approximately 660 m^2 (Loreau and Nolf, 1993). *Abax parallelepipedus* has typically a low dispersal capacity with high sensitivity to fragmentation due to roads (Keller et al., 2004).

2.3 Genetic data sets

For all species, tissues were collected between April and September in 2015 and 2016. For the two vertebrate species (*N. helvetica* and *A. obstetricans*), we followed an individual-based sampling design due to their low abundances in the field. Individual-based sampling design has been proved to be a good alternative method to population-based sampling design as less individuals are required per sampling location (1 to 4) and more geographical locations can be sampled over the landscape (Prunier et al., 2013, 2014). Accordingly, the entire study area was prospected at night to collect individuals of *A. obstetricans*

(with also opportunistic detection of *N. helvetica*), completed by day surveys to collect individuals of *N. helvetica*. We focused mainly on sampling sites with high suitability of presence such as wetlands, ponds, rivers, woodland edges and small villages. Sites were prospected on foot and headtorches were used during night to locate individuals of both species. Because snakes were hard to detect, 108 artificial shelters were laid across the study area to attract snakes and facilitate data collection. When an individual was detected, it was hand-captured and manipulated directly in the field. A GPS location (Garmin Etrex20, USA) was taken for each captured individual. See Fig. 2 and 3 for sampling locations. Each individual was sexed, measured, weighted, marked (to avoid sampling individual twice) and a genetic sample was taken. We used 7x1.35 mm FDX-B Passive Integrated Transponder (PIT) tags (Loligo Systems, Denmark) to mark *A. obstetricans* individuals. For each captured *A. obstetricans*, we collected a non-destructive genetic sample using buccal swab. The mouths of captured individuals were gently opened using a little metal spoon and were then swabbed for about 10 s. For snakes, we individually marked individuals by clipping ventral scales following Brown and Parker (1976) method. The clipped scales were then used for genotyping. We also collected genetic samples of encountered dead snakes and amphibians (road kill or predation) and snake shed skins.

Tissues from the two invertebrates species (*M. jurtina* and *A. parallelepipedus*) were collected using a classic population sampling design with 30 sites sampled. The locations of the sites were obtained by dividing the study area in a 5x6 grid with 30 subsections on QGIS (V. 2.8). In each subsection, the definitive sampling site was defined based on the presence of suitable habitats for both species (woodlands for *A. parallelepipedus* and grasslands for *M. jurtina*). In each site, 30 individuals were sampled, resulting in 900 genetic samples per species. See Fig. 2 and 3 for sampling locations. Butterflies were captured during day time with nets. *A. parallelepipedus* were trap collected using non-lethal pittfals. At each retained site, we set up 15 dry pitfall traps arranged in circles at regular intervals of 5 m. Traps were 20 cm in diameter and 15 cm in depth. They were emptied every day until 30 individuals were captured. For both invertebrate species, genetic samples were collected by removing a leg. To avoid sampling the same individual twice, we always removed the same leg from all individuals (middle right when seen from above).

All genetic samples from the four species were stored in 70 % EtoH until DNA extraction. Care was taken to minimise animal handling and stress. All material for marking animals and collecting genetic samples were washed and disinfected using absolute ethanol between each individual. Animals were rapidly released on the place of capture after manipulation.

2.4 Laboratory procedures

we amplified 13 (Pokrant et al., 2016), 14 (Tobler et al., 2013; Maia-Carvalho et al., 2014), 15 (Richard et al., 2015) and 14 (Marcus et al., 2013) polymorphic microsatellite loci, for the species *N. helvetica*,

A. obstetricans, *M. jurtina* and *A. parallelepipedus*, respectively. For a detailed procedure of DNA extraction, amplification and genotyping, see Appendix A.

We used Genepop 4.2 (Rousset, 2008) to test for linkage disequilibrium among pairs of loci and deviation from Hardy-Weinberg Equilibrium after sequential Bonferroni correction to account for multiple related tests (Rice, 1989). The presence of null alleles was tested using MICROCHECKER 2.2.3 (Van Oosterhout et al., 2004).

2.5 Final data sets

The presence of related individuals (siblings for example) in data sets is known to over-estimate the number of clusters when assessing population structure (Anderson and Dunham, 2008) and bias subsequent genetic analyses. Therefore, we used COLONY2 (Jones and Wang, 2010) to identify full-sib and parent-offspring groups among our individual-based data sets (*N. helvetica* and *A. obstetricans*). We used the full-likelihood approach based on the individual multilocus genotypes. For *A. obstetricans*, we assumed that males and females were polygamous. For *N. helvetica* we assumed that only males were polygamous. All individuals were considered as potential offspring and no a priori candidate parental genotypes was defined. Allele frequencies were determined directly from genetic datasets. We ran three independent long runs with various seed numbers to test for congruence among results. Only relationships with an associated inclusion probability higher than 95 % were considered as significant. In each group of related individuals, we randomly retained one genotype. Accordingly, 76 genotypes in the *A. obstetricans* data set were discarded. In the *N. helvetica* data set, two genotypes were identical. These two genotypes corresponded to an adult male and a shed skin collected on the same site, 100 m apart, in 2016. Therefore, we discarded the shed skin sample as it probably belonged to the same individual. In addition, because some sites were unevenly sampled for *N. helvetica* and *A. obstetricans*, we only retained a maximum number of three random genotypes per sampling location (Prunier et al., 2013). Some individuals could not be genotyped mainly due to insufficient DNA amount. Therefore, in the population data sets, we only retained populations for which more than 15 genotypes were available. Finally, genotypes with more than 2 loci presenting missing values were discarded to allow robust genetic analyses. The final data sets comprised 848 genotypes (30 populations) in *A. parallelepipedus*, 508 genotypes (21 populations) in *M. jurtina*, 115 genotypes in *N. helvetica* (68 sampling locations) and 132 genotypes in *A. obstetricans* (56 sampling locations).

2.6 Hierarchical genetic clustering

For each of the four final data sets (either individual or population based data sets), genetic clustering was investigated using the program STRUCTURE 2.3.4 (Pritchard et al., 2000) with the admixture and the correlated allele frequency models and prior population information when structure in the data was

weak. We followed a hierarchical genetic clustering procedure (Coulon et al., 2008). At each hierarchical level, we tested the number K of clusters from 1 to 10 and repeated analyses for each value 5 times. Runs were performed with a burn-in period of 50 000 and the 50 000 subsequent Markov chain Monte Carlo repetitions were retained. We also checked that the alpha value (looking at alpha plots created by STRUCTURE) had stabilized before the end of the burn-in period to ensure convergence. If convergence was not reached, we used a burn-in period of 100 000 and the 100 000 subsequent Markov chain Monte Carlo repetitions were retained. We used STRUCTURE HARVESTER (Earl and VonHoldt, 2012) to obtain Log-likelihood plots and deltaK statistics to infer the optimal K-value. We used the optimal K-value to performed 20 runs with a burn-in period of 200 000 and the 200 000 subsequent Markov chain Monte Carlo repetitions retained. We compiled the ten best runs using CLUMPP (Jakobsson and Rosenberg, 2007) to obtained the individual or population Q-values. Each Individual or population was assigned to the cluster for which its Q-value was higher than 0.6 (Prunier, Colyn, Legendre and Flamand, 2017). We then repeated the analysis for each inferred cluster separately until no more structure was found in the data. For each hierarchical level, we used Q-values to compute pairwise matrices (among individuals or populations depending on the design) of ancestry-based hierarchical genetic distance (HGD) (Balkenhol et al., 2014; Prunier, Colyn, Legendre and Flamand, 2017).

2.7 Dependent variables

In each of the four final data sets (one per species), we calculated two types of dependent variables. The first one is a standard genetic distance, calculated from the Bray-Curtis (bc) percentage dissimilarity metric (Legendre and Legendre, 1998) for the individual-based data sets (*N. helvetica* and *A. obstetricans*). For the two other species, *M. jurtina* and *A. parallelepipedus*, we calculated inter population genetic distances based on Fst. Classical genetic distances are powerful to detect regional and surface elements affecting gene flow but may be unwilling to detect isolation due to linear elements (Prunier, Colyn, Legendre and Flamand, 2017). Therefore, we used a second type of dependent variable described as hierarchical genetic distances (HGD), which is powerful to detect mainly linear elements affecting gene flow at a more local scale (Prunier, Colyn, Legendre and Flamand, 2017). HGD was calculated only for species where a genetic structure was detected using the STRUCTURE software. When more than one hierarchical level was detected, each hierarchical level (HGD1, HGD2...) was considered as a dependent variable.

2.8 Landscape predictors

In total, we considered 13 landscape features present in our study area as predictors likely to explain the variance in the two types of dependent variables across the four data sets. Six predictors described soil occupancy. They were defined by digitalizing the entire study area on QGIS (V. 2.8) using national

maps and aerial photographs (BD Ortho from National Geographic Institute, France). Every elements of the landscape was classified into 49 habitat types. Field botanic expertises were also performed in 2015 to confirm the affiliation of certain habitat types. We combined these features into six main predictors (Appendix B): Water (stagnant water bodies, streams and rivers), Crops (intensive and non intensive cultures), Woodlands (all types of forests), Grasslands (open lands that are not cultivated), Urban (villages, agricultural installations, industrial sites, stone quarries, *etc.*) and Roads (all roads excluding small trails). These six spatial classes were rasterized at a 1 m resolution using ARCGIS 10.2.2 and the SPATIAL ANALYST extension. Each spatial class was used to compute a resistance surface based on the spatial density of the corresponding element in the landscape. To do so, we overlaid a 20 m grid on each spatial class and calculated the percentage of the element in each grid (400 m^2) (Balkenhol et al., 2014; Prunier, Colyn, Legendre and Flamand, 2017). For each resistance surface, we rescaled pixel resistance values to range from 1 (null or extremely low densities) to 100 (the element covered the entire pixel). These six resistance surfaces were used in CIRCUITSCAPE 4.0 (McRae, 2006; McRae et al., 2013), implementing a method that determines all possible pathways between two points by analogy to electrical resistance. We obtained electric current values between each pair of locations for our six resistance surfaces. In addition to these six predictors, we included an isolation-by-distance predictor (IBD) and an Altitude predictor using euclidean distances and altitude difference between pairs of locations, respectively. Finally, we included five predictors likely to create isolation-by-barriers in our data sets: Motorway A89, Railway, D6089 country road, Gas pipeline and Power line (Fig. 1). For each of these five linear elements we computed a binary pairwise matrix between all pairs of locations where 0 indicated that pairs were on the same side of the element and 1 indicated that pairs were on either side of the element.

2.9 Spatial scale of analyses

The spatial scale retained in landscape genetic analyses can deeply influence the conclusions of studies (Keller et al., 2013). The local influence of landscape elements on genetic distances can remain unnoticed if spatial scale retained is wide in comparison to dispersal capacities of individuals (Anderson et al., 2010). Accordingly, we did not use all possible pairs of populations or individuals in our data sets. For each dataset, we retained a subset of pairwise data by defining a maximum euclidean distance between pairs. The maximum euclidean distance was selected as the neighboring distance maximizing the R^2 of our full model including all predictors in a classical multiple linear regression. This retained distance was higher that the minimum distance in a neighboring graph that ensures that no individual is excluded from the network (Jombart et al., 2008). It was estimated using Gabriel graphs with the "adegenet" package (Jombart, 2008) in R 3.3.2 (R Core Team, 2015). Subsequent analysis were only ran with pairwise data associated with Euclidean distances lower than the computed maximum neighboring distance.

2.10 Multiple linear regression and commonality analysis on genetic distances

For each of the four data subsets and the two types of dependent variables (standard genetic distances or HGD), a complete linear model including the 13 predictors was designed. All predictors were centered. We explored the relationship between the explanatory variable and the predictors using multiple linear regression on vectors (Smouse et al., 1986; Prunier et al., 2015). We used multiple linear regression on vectors instead of matrices because we did not considered complete matrices of pairwise distances but a subset based on the maximum neighboring distance. The contribution of predictors to the dependent variable was assessed using commonality analyses (CA). Commonality analyses is a procedure of variance partitioning that provides decisive support when trying to assess the reliability of model parameters (beta weights and confidence intervals) in face of multicollinearity (for more detailed informations on CA, see Prunier et al. (2015)). In commonality analyses, the effect of each predictor can be decomposed into a unique (U) and common (C; shared with other predictors) effect. For a given predictor, the sum of unique and common effects corresponds to the total contribution (T), equal to its squared zero-order correlation with the dependent variable ($U + C = T = r^2$). Therefore, CA represents a good opportunity to assess the reliability of predictors to explain the dependent variable in face of collinearity. The magnitude of suppression among predictors is indicated by negative commonalities. Negative commonalities represent the amount of predictive power that would be lost by other predictors if the suppressor variable was not included in the regression model. Accordingly, we can distinguish three specific types of suppressor (Conger, 1974). (i) A classical suppressor corresponds to a predictor whose unique contribution is totally counterbalanced by its negative common contribution ($U + C = 0$). (ii) A reciprocal suppressor also described as a partial suppressor is a predictor with a negative common effect but that does not counterbalance its unique contribution to the variance in the dependent variable ($T = U + C > 0$). Finally, (iii) cross-over suppressor is similar to a partial suppressor but with reversal sign. Cross-over suppressors are detected by a sign inversion between the structure coefficients and the beta weights (Prunier, Colyn, Legendre and Flamand, 2017). We performed multiple linear regressions and CA using packages ecodist (Goslee and Urban, 2007) and yhat (Nimon et al., 2008) in R 3.3.2 (R Core Team, 2015). To remove classical suppressors, we discarded predictors presenting low univariate squared correlation against the dependent variables (r^2 lower than 0.1). Low correlated predictors are likely to act as classical suppressors leading to the distortion of regression coefficients (Prunier et al., 2015; Prunier, Dubut, Chikhi and Blanchet, 2017). When we discarded those non-informative predictors, we ended up with simplified models containing a reduced number of predictors likely to explain the variance in the dependent variables. Predictors that were identified as cross-over and reciprocal suppressors were discarded from our model and subsequent models were ran without these suppressors until no more suppressors could reasonably be discarded from the model (that is, we kept reciprocal suppressors showing

a non-negligible unique contribution). We also removed predictors with synergistic (S) association with other predictors, which have a unique contribution to the dependent variable equal to zero but presenting synergistic association with other predictors ($C > 0$)(Appendix D).

In the final simplified model, we assessed the linear relationship among our predictors to test for multicollinearity by using Pearson's correlation coefficients r and Variance Inflation Factors VIF (Dormann et al., 2013). Because data are not independent, the p-values were necessary biased and, therefore, were not calculated (Legendre and Legendre, 1998). Yet, we computed 95 % confidence intervals around regression coefficients using a bootstrap procedure, with 1000 replicates based on a random removal of 10 % of individuals without replacement (Prunier et al., 2015). These confidence intervals were used to assess the significance of the predictor's contributions to the variance in the dependent variable. We considered that when the confidence intervals did not include 0, the predictor was a robust contributor to the variance in the response.

This framework was repeated for each of the two types of dependent variables and for each data subset. It can be summarized as follow:

1. Define spatial scale between pairs of locations maximizing the R^2
2. Discard predictors with low squared correlations with the dependent variable, likely to act as classical suppressors
3. Run commonality analyses, discard cross-over and reciprocal suppressors
4. Assess collinearity among final predictors
5. From the final model, get regression coefficients, unique contributions and confidence intervals for the retained predictors

A given predictor with a positive β value was associated with an increase of genetic distances. It was interpreted as a predictor that impeded gene flow and created barrier to dispersal. A predictor with a negative β was associated with a reduction of genetic distances. It was interpreted as a predictor promoting gene flow and enhancing dispersal (Jacquot et al., 2017).

2.11 Output summary

In order to summarize all the results, we built three 100 % stacked barplots showing averaged unique contributions of all retained predictors across the two types of dependent variables. In the first plot, averaged unique contributions were presented per species across predictors merged into three main classes: natural predictors (IBD, Altitude, Woodlands, Grasslands and Water), anthropized predictors (Crops and Urban) and infrastructures (the six types of LTIs). In the second plot, we presented averaged unique contributions per species across infrastructures with their two types of effects (increase or reduction of

genetic distances). Finally, in the last plot, we presented averaged unique contribution per type of infrastructure effect (increase or reduction of genetic distances) across all species. Predictors that were absent in the final models were given a unique contribution of 0.

3 RESULTS

3.1 Genetic data

In the *A. obstetricans* data set, there was no evidence of linkage disequilibrium among loci. We found evidence of null alleles for locus Aly7. Accordingly, we retained 13 loci for subsequent analysis (Aly28, Aly3, Aly4, Aly17, Aly19, Aly20, Aly23, Aly24, Aly25, Aobst14, Aobst15, Aobst16 and Aobst17).

In the *N. helvetica* data set, two loci could not be amplified (Nsμ3 and 3TS) either in multiplex or in standalone PCR. There was no evidence of null alleles, but we found evidence of linkage disequilibrium between loci Natnat05 and μNt8new and between loci Natnat05 and TbuA09. Therefore, we only retained 10 loci for subsequent analysis (Natnat09, μNt8new, μNt3, μNt7, Natnat06, Natnat11, Eobμ 1, Eobμ13, TbuA09 and 30).

In the *M. jurtina* data set, the locus Mj2410 was discarded as it showed sex linkage (Richard et al., 2015; Villemey et al., 2016). Similar to Villemey et al. (2016), we found evidence of frequent null alleles for loci: Mj5522, Mj5287, Mj5647, Mj3956, Mj5563, Mj0272, Mj0283 and Mj3637. Thus, we only retained six loci for subsequent analysis (Mj0008, Mj7132, Mj0247, Mj7232, Mj4870 and Mj5331).

In the *A. parallelepipedus* data set, there was no evidence of linkage disequilibrium among loci. We found evidence of null alleles for loci: apar14, apar44, apar46 and apar50. Then, we retained 10 loci for subsequent analysis (apar20, apar50, apar27, apar34, apar32, apar12, apar23, apar25, apar02, apar46, apar05, apar44, apar14, apar06). Appendix A provides tables summarizing markers characteristics in each species.

3.2 Genetic structure

STRUCTURE revealed that all individuals from the *N. helvetica* and *M. jurtina* data sets belonged to a single cluster. The logarithm estimates of the probability of the data [ln Pr(X—K)] were maximal for K = 1. Implementing sampling locations as locprior did not help STRUCTURE to find more than one cluster in the two data sets.

In the *A. obstetricans* individual data set, we identified two hierarchical levels (Fig. 3). At the first level, one cluster (A) surrounded a second cluster (B) with no clear geographical boundaries explaining this pattern (Fig. 3). Ten individuals could not be assigned to any of these two clusters (cross-assigned) suggesting some exchanges between these two clusters. At the second hierarchical level, only cluster A was further divided into three clusters: A1, A2 and A3. These three clusters were not separated by

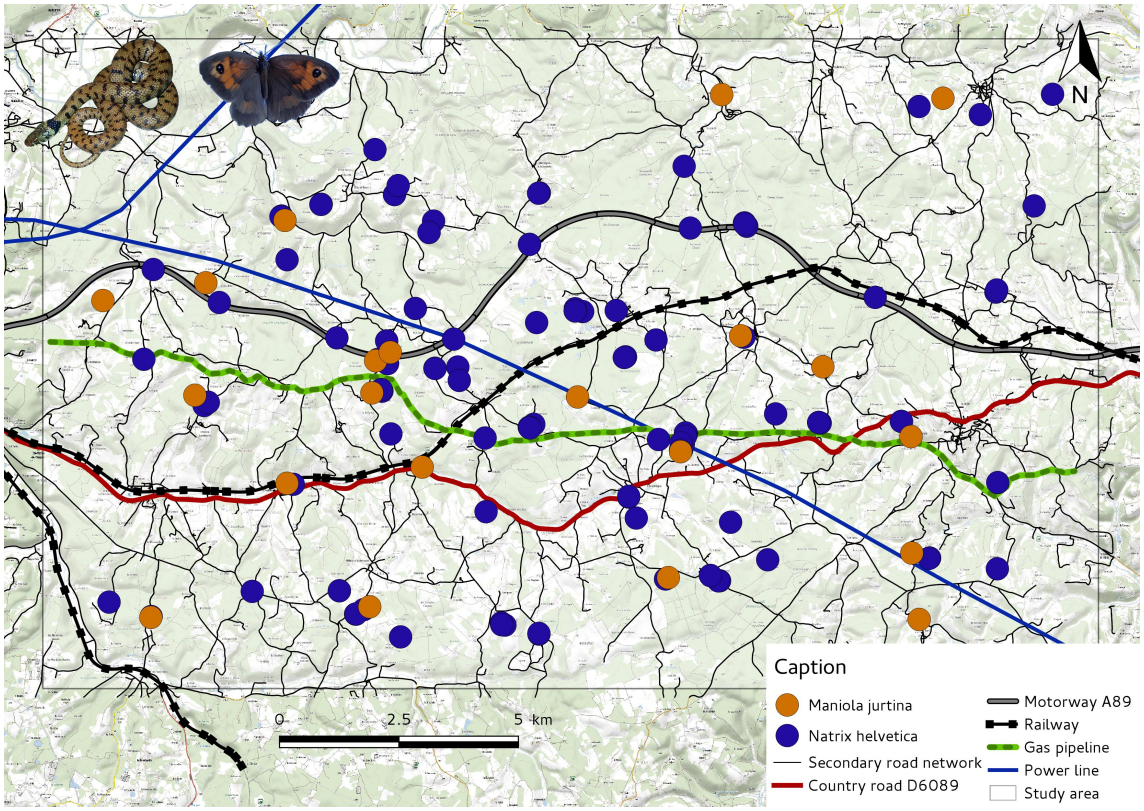

Figure 2: Sampling locations of the species *Natrix helvetica* and *Maniola jurtina* in the study area. Samples were collected in 2015 and 2016. Each *N. helvetica* location represents an individual. Each *M. jurtina* location represents a sampled population (about 30 individuals per population). For these two species, there was no genetic structure identified with the STRUCTURE software (see text).

clear geographical patterns. At the second hierarchical level, a high number of individuals (21) could no be assigned to any of these three sub-clusters suggesting frequent exchanges among them. In total, we identified four final clusters (Fig. 3).

In the *A. parallelepipedus* population data set, we identified two hierarchical levels (Fig. 3). At the first level, 19 populations were assigned to cluster A and ten were assigned to cluster B. Cluster A included populations sampled mostly in the western part of the study area and overall north of the road "D6089" (Fig. 3). One population at the extreme south-west could not be assigned to any of these two clusters (cross-assigned). Cluster B, was further divided into two sub-clusters at the second hierarchical level. Cluster B1 comprised five populations north of the "D6089" and the gas pipeline and cluster B2 comprised four populations south of the "D6089" and the gas pipeline. At the second hierarchical level, only one population could not be assigned to any of these two clusters (cross-assigned). This population was located between the road "D6089" and the gas pipeline exactly in-between clusters B1 and B2 suggesting some exchanges between these two clusters. In total, we identified three final clusters (Fig. 3).

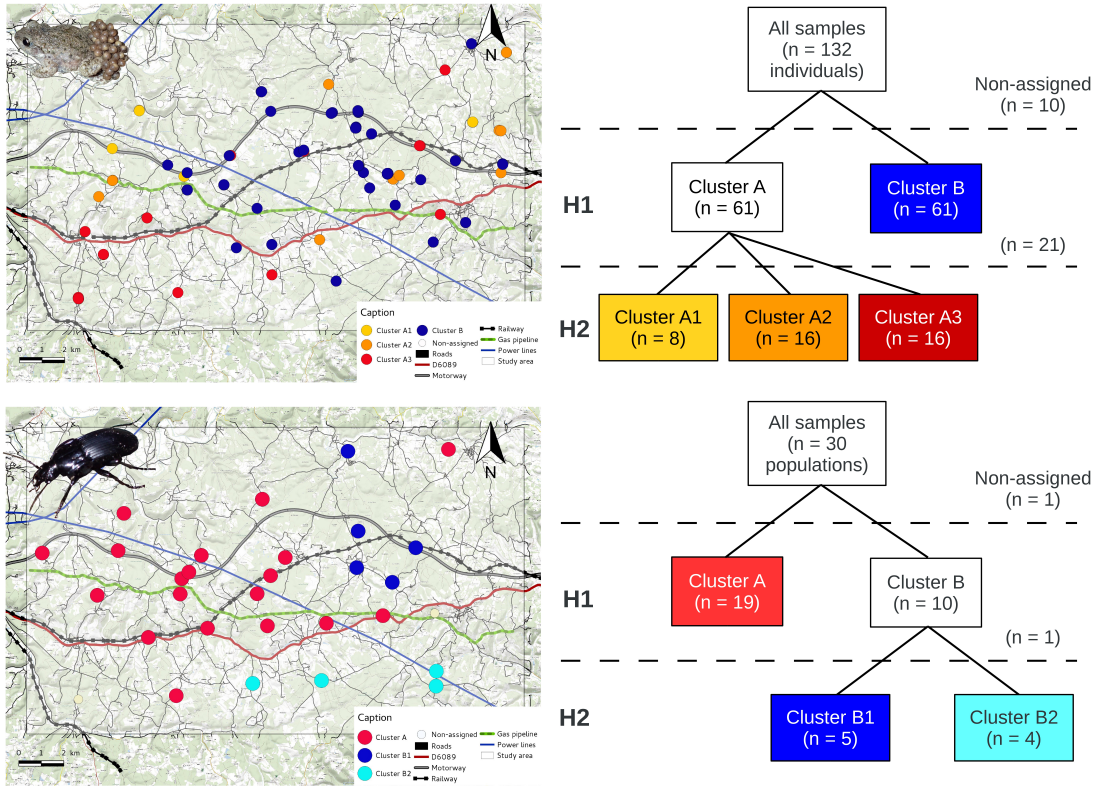

Figure 3: STRUCTURE outputs for the species *A. obstetricans* (132 individuals in 56 sampling locations) and *A. parallelepipedus* (30 populations of about 30 individuals) plotted over the study area. Right panels represent the hierarchical splits of clusters inferred with STRUCTURE from the first to the second hierarchical level. n is the number of samples (individuals for *A. obstetricans* and populations for *A. parallelepipedus*) assigned to each cluster. On the right-hand side of panels, we present the number of non-assigned samples at each hierarchical level (Q-values < 0.6).

3.3 Spatial scale of analysis

In the four data sets, the minimum neighboring distances detected with the Gabriel graphs were 2400 m, 2700 m, 5100 m and 4500 m for the species *A. obstetricans*, *N. helvetica*, *M.jurtina* and *A. parallelepipedus*, respectively (Appendix C). In the *A. obstetricans* data set, the spatial scales maximizing the R^2 between pairs were 3000 m, 2400 m and 3500 m for the Bray-Curtis genetic distance, HGD1 and HGD2, respectively. In the *N. helvetica* data set, the spatial scale maximizing the R^2 was 2800 m. In the *M. jurtina* data set, the spatial scale maximizing the R^2 was 5500 m. In the *A. parallelepipedus* data set, the spatial scales maximizing the R^2 were 6500 m, 18500 m and 4500 m for the Fst genetic distance, HGD1 and HGD2, respectively (Appendix C).

3.4 Correlation among final predictors

Across all data sets and all types of dependent variables, values of Pearson's correlation coefficients among predictors that were retained ranged from -0.303 to 0.489 and Variance Inflation Factors (VIF) ranged from 1.00 to 1.70 (Appendix E). These results suggested little collinearity among predictors (Dormann

et al., 2013), and thus little distortion in regression outputs (Prunier et al., 2015).

3.5 Multiple linear regression and commonality analyses for *A. obstetricans*

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 type: classical genetic distances (GD) calculated either with the Bray-Curtis dissimilarity metric (bc) or with Fst and hierarchical genetic distances (HGD1 and HGD2 for first an second level of hierarchy, respectively). For each model, the model fit (Multiple R^2) was estimated with the spatial scale retained between pairs of locations (Distance). For each retained predictor per model, we estimated the structure coefficient (rs), beta weight (β), unique (U), common (C) and total (T) contributions. Significance of the predictor's contribution to the DV was estimated using confidence intervals (CI-inf and CI-sup). A CI that included 0 was considered as a non-representative predictor (indicated in bold). Gray color indicates predictors with negative relationship to the dependent variable (negative β). They correspond to predictors that are associated with a decrease in genetic distances and may thus be considered as promoting gene flow.

DV	Species	Multiple R²	Distance	Predictor	rs	B	CI-inf	CI-sup	U	C	T
GD(bc)	*A. obstetricans*	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	A. obstetricans	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	A. obstetricans	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)	*N. helvetica*	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)	*M. jurtina*	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)	*A. parallelepipedus*	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	*A. parallelepipedus*	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
HGD2	*A. parallelepipedus*	26.76%	4500 m	Altitude	0.445	0.223	0.056	0.397	0.049	0.004	0.053
				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

When using the genetic distance based on the Bray-Curtis dissimilarity index (bc), the multiple linear regression explained 11.8 % of the variance (Table 1). Five final predictors explained the dependent variable: IBD, Altitude, Woodlands, Roads and the road D6089. All β values were positive, indicating that these predictors were associated with an increase of genetic distance in *A. obsetricans*. Natural predictors (IBD, Altitude and Woodlands) explained most of the variance in the dependent variable (67 % of the averaged unique contributions). Woodlands was the landscape element with the highest unique contribution to the genetic distances (U = 0.018). Two infrastructures were associated with an increase of genetic distances in this model: the secondary road network and the country road "D6089". Both explained about 33 % of the averaged unique contribution and had similar unique contributions to the dependent variable (U = 0.009 and 0.008, respectively).

When using the first level of hierarchical genetic distance (HGD1), the linear regression explained 10.76 % of the variance. In the final model, four predictors explained HGD1 and all were associated with an increase of genetic distances (positive β values). Crops was the predictor with the highest contribution

to the dependent variable (U = 0.032) followed by Roads (U = 0.024). In this model, Woodlands was also associated with an increase of genetic distances but was the predictor with the lowest unique contribution (U = 0.010). Railway was associated with an increase of genetic distances with a unique contribution of 0.011 to the dependent variable.

With the second level of hierarchical genetic distance (HGD2), a higher portion of the variance in the dependent variable was explained by our model: 20 %. The final model comprised five predictors: Woodlands, Urban, Roads, D6089 and Motorway. Woodlands, Roads and the road D6089 were associated with an increase of genetic distances in *A. obsetricans* (positive β values) but urbanization and the motorway had negative β values indicating that these two predictors were associated with a reduction of genetic distances in *A. obstetricans*. The Motorway predictor was the one explaining the lowest part of variance in the dependent variable (U = 0.014). Urbanization was the landscape element affecting the highest part of the variance in the dependent variable (U = 0.047). Woodlands, Roads and the road D6089 were all associated with an increase of genetic distances in this model with unique contribution of 0.031, 0.033 and 0.037, respectively.

When the unique contribution from the three dependent variables were merged, gene flow of *A. obstetricans* was mostly explained by infrastructures (47 % of the variability, Fig. 4). Natural and anthropized predictors explained each about 26 % of the variability. Infrastructures were mostly associated with an increase of genetic distances in *A. obsetricans* with 90 % of the variability in unique contributions explained by barrier effects of infrastructures (Fig. 5). The secondary road network and the main road "D6089" were driving most of this pattern (82 % of the unique contributions) and the railway to a smaller extent (8 % of the unique contributions). The 10 % left were associated with a reduction of genetic distances detected across the motorway when using the second level of hierarchical genetic distance (HGD2)(Fig. 5).

3.6 Multiple linear regression and commonality analyses for *N. helvetica*

With the dependent variable (bc), the multiple linear regression explained a small proportion (4.15 %) of the variance (Table 1). The motorway was associated with an increase of genetic distances in *N. helvetica* (positive β value) and explained most of the variance in the dependent variable (U = 0.021). The two other types of infrastructures (the secondary road network and the railway) had unique contribution of 0.015 and 0.008, respectively. Both had negative β values, indicating that they were associated with a reduction of genetic distances in the snake.

The entire variability detected in *N. helvetica* was due to infrastructures (Fig. 4). When unique contribution of predictors were merged, 50 % of the variability was associated with an increase of genetic distances supported by the motorway and the 50 % left was associated with a reduction of genetic distances (Roads = 34 % and Railway = 17.7 %; Fig. 5).

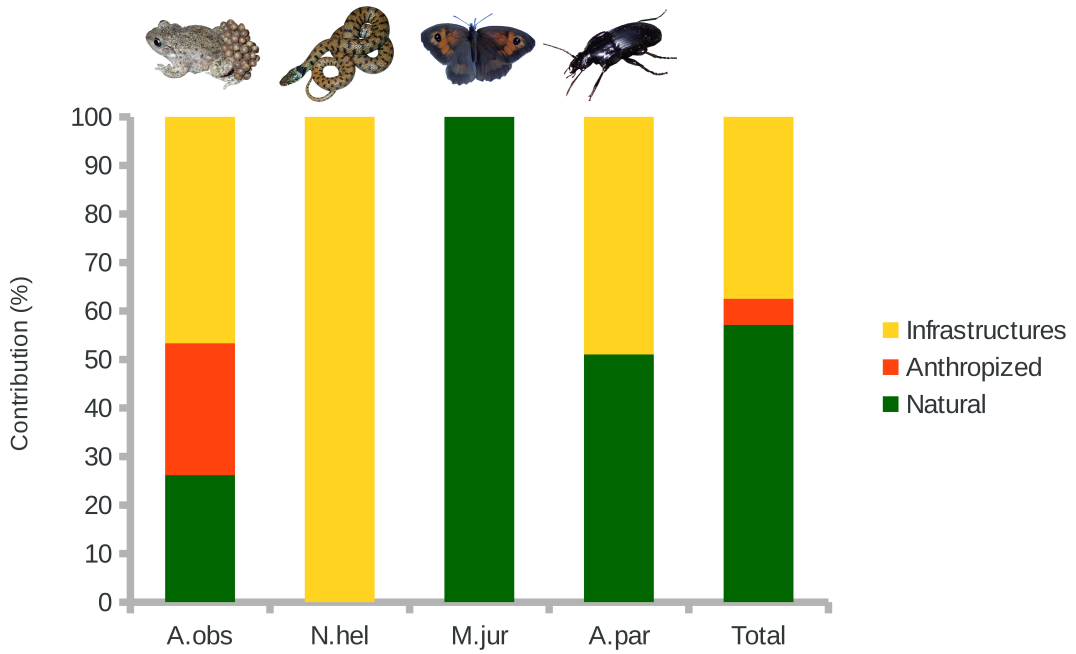

Figure 4: Averaged unique contributions of natural predictors (IBD, Altitude, Woodlands, Water, Grasslands), anthropized predictors (Crops and Urban) and infrastructures (all linear infrastructures) to each species and combined results (Total) across all datasets.

3.7 Multiple linear regression and commonality analyses for *M. jurtina*

The butterfly genetic distances were calculated using Fst. The multiple linear regression explained 20 % of the variance in the dependent variable (Table 1). After non-informative predictors and suppressors were discarded, only IBD, Woodlands and the Power line remained in the final model. The 95 % confidence interval of the Power line effect included 0, indicating that this predictor did not significantly contributes to the variance in the dependent variable. Woodlands were associated with an increase of genetic distances (positive β values) in *M. jurtina* and explained most of the variance (U = 0.089). The rest of the explained variance was due to isolation by distance (IBD, U = 0.066). Therefore, the entire variability detected in *M. jurtina* genetic distances was explained by natural predictors (Fig. 4).

3.8 Multiple linear regression and commonality analyses for *A. parallelepipedus*

When using the genetic distance based on Fst, the multiple linear regression explained 26 % of the variance in the dependent variable (Table 1). Two final predictors explained the dependent variable: Altitude and Grasslands. Altitude did not significantly explain genetic distances (95 % confidence intervals included 0). Therefore the variance explained by our model was only due to Grasslands associated with an increase of genetic distances in *A. parallelepipedus* (U = 0.248).

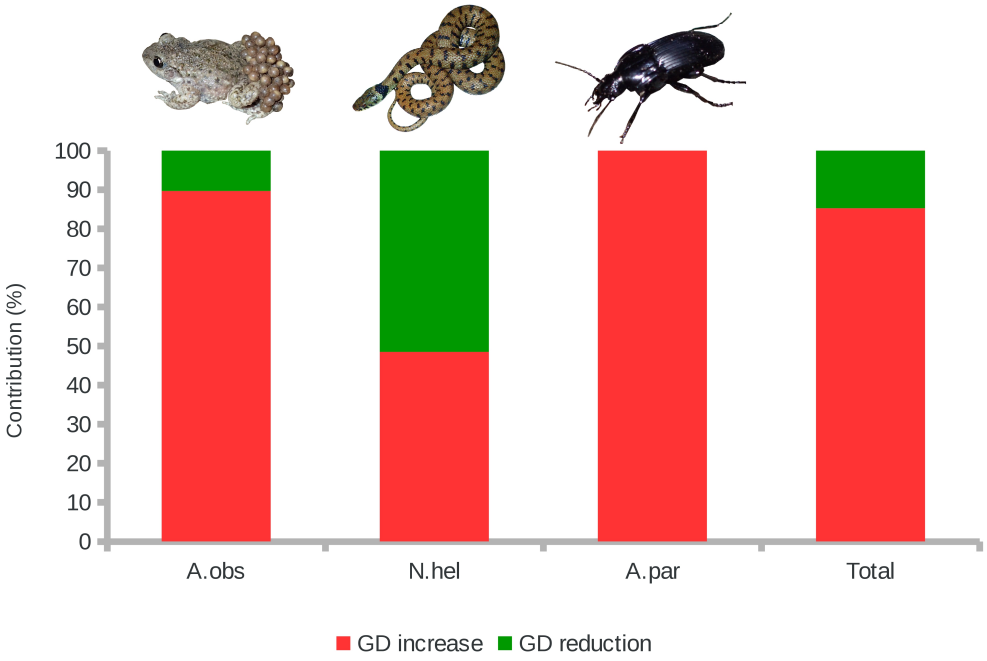

Figure 5: Averaged unique contributions of genetic distances (GD) increase or reduction of the six types of linear infrastructures (Roads, D6089, Motoraway A89, Railway, Gas pipeline and Power line) to each species and combined results (Total) across all datasets. A reduction in GD is associated with a gene flow enhancement and an increase in GD is associated with a barrier effect impeding gene flow.

When using the first level of hierarchical genetic distance (HGD1), the linear regression explained 17 % of the variance in the dependent variable. HGD1 was explained entirely by predictors associated with an increase of genetic distances in the beetle (positive β values): the secondary road network (U = 0.063) and the country road D6089 (U = 0.059).

When using the second level of hierarchical genetic distance (HGD2), the linear regression explained 27 % of the variance in the dependent variable. Four predictors remained in the final model: Altitude, the road D6089, the motorway and the gas pipeline. The 95 % confidence interval around the β value of the motorway included 0 indicating that the motorway was not significantly explaining HGD2. The three remaining predictors were all associated with an increase of genetic distances (positive β values). The road D6089 was explaining the highest part of the variability (U = 0.114) suggesting a strong barrier effect of this infrastructure on gene flow. The gas pipeline and Altitude had both a unique contribution to the dependent variable of 0.049.

When the unique contribution from the three dependent variables were merged, gene flow of *A. parallelepipedus* was explained by infrastructures (49 %) and natural predictors (51 %) (Fig. 4). In this species, infrastructures were all associated with an increase of genetic distances (Fig. 5).

3.9 Summary of infrastructure effects

In total, 38 % of the genetic variability across all species was due to infrastructures (Fig. 4). The secondary road network (12 %) and the country road D6089 (15 %) were the LTIs most affecting genetic distances in the four studies species. The motorway (5 %), the railway (2.5 %) and the gas pipeline (3.5 %) had moderate effects on genetic distances and the power line had no effect on gene flow in any species.

When unique contributions were presented per type of infrastructure and averaged across species, five of the six tested infrastructures were associated with an increase of genetic distances in at least one of the studied species (Fig. 6).

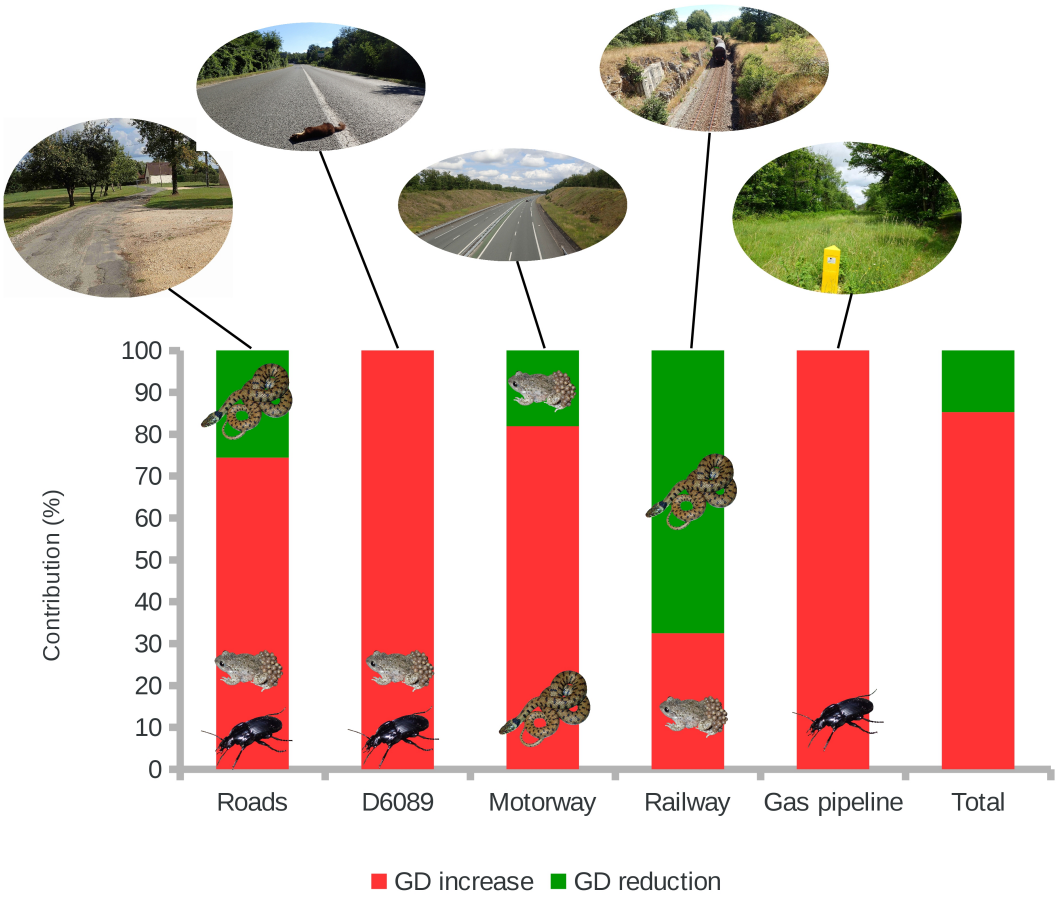

Figure 6: Proportions of the averaged unique contributions of genetic distances (GD) increase or reduction of five linear infrastructure types (Roads, D6089, Motoraway A89, Railway and Gas pipeline) across species. The power line is not represented as no species were affected by this infrastructure (see results). Total represents the combined results across datasets. A reduction in GD is associated with a gene flow enhancement and an increase in GD is associated with a barrier effect impeding gene flow.

The only infrastructure that was not affecting genetic distances across all species was the power line. The secondary road network affected the genetic distances in *N. helvetica*, *A. obsetricans* and *A. paral-*

lelepipedus. 74 % of unique contributions of secondary roads were associated with an increase of genetic distances in the species *A. obsetricans* and *A. parallelepipedus* (Fig. 6). 26 % of unique contributions of secondary roads were associated with a reduction of genetic distances in *N. helvetica*. The country road D6089 was influencing genetic distances in two species (*A. obsetricans* and *A. parallelepipedus*) and 100 % of unique contributions were associated with an increase of genetic distances (Fig. 6). The motorway affected genetic distances of the two vertebrate species (*A. obsetricans* and *N. helvetica*). 82 % of unique contributions of the motorway were associated with an increase of genetic distances in *N. helvetica*. The 18 % left corresponded to a reduction of genetic distances in *A. obstetricans*. Similarly, the railway influenced only the two vertebrate species. 32 % of the unique contributions of the railway were associated with an increase of genetic distances in *A. obstetricans* and 68 % of the unique contributions were associated with a reduction of genetic distances in the snake. The gas pipeline was only affecting genetic distances in the beetle *A. parallelepipedus* and was associated with an increase of genetic distances.

4 DISCUSSION

In this study we assessed landscape connectivity in four species in a fragmented environment in south-western France. We were particularly interested in the convergent effects of six types of large-scale transportation infrastructures. We used individual and population based analyses, restricted spatial scale and regression commonality analyses to evaluate the relative contribution of various landscape predictors to the variance in both, classical and hierarchical genetic distances.

4.1 Analytical framework

Individual-based sampling scheme is a recent promising tool in landscape genetics. Because less individuals are needed per sampling location (3-4 individuals), more sampling locations can be covered. It allows to capture a wide amount of genetic variation an provide an optimal representation of the landscape heterogeneity (Prunier et al., 2013). In our study, we used individual-based analyses for the snake *N. helvetica* and the toad *A. obstetricans*, as a population-based sampling scheme would requires between 20 to 30 individuals per population (Prunier et al., 2013). Considering the ecology of these two species, an individual-based sampling scheme is optimal. The grass snake as a random distribution in the landscape with low detectability, which makes the use of a population-based sampling scheme almost impossible. The midwife toad as a clumped distribution in the landscape but population sizes are small. Sampling between 20 to 30 individuals would require both a huge time investment in the field and an optimal landscape configuration with large populations. By using individual-based analyses with the Bray-Curtis dissimilarity index, we were able to explain 4 and 12 % of the genetic variability in *N. helvetica* and *A. obstetricans*, respectively. These amounts were lower than the variance explained for the two other

species studied using population-based analyses (20 % in *M. jurtina* and 26 % in *A. parallelepipedus*). Prunier et al. (2013) argue that individual-based methods should outperform population methods based on allelic frequencies but a direct comparison between individual and population based-methods on the same biological model is still required to test this hypothesis (Luximon et al., 2014).

By using restricted spatial scales in our analyses, we were able to optimize the detection of landscape features likely to explain the variability in genetic distances (Keller et al., 2013). Some local influences of landscape elements on genetic distances can remain unnoticed if all pairs of genetic distances are retained. This is especially true for pairs separated by important distances where isolation by distance is likely to cover up the variability explain by isolation by barriers or isolation by resistance (Anderson et al., 2010). For example, if all pairs were retained in the *A. obstetricans* data set with classical (bc) genetic distances, the variability explained would be reduced to 5 %, which corresponds to a diminution of more than 50 % compared to the variability explained by the restricted spatial scale (Appendix C).

The use of hierarchical genetic distances (HGD) in addition to classical distances is a great improvement in landscape genetic analyses (Balkenhol et al., 2014; Prunier, Colyn, Legendre and Flamand, 2017). HGD allow the detection of sharp genetic variations caused by linear elements, whereas classical genetic distances considered the sampled area as a single continuous genetic unit and inform on the regional landscape permeability. The use of both metrics give a deep understanding of the landscape features affecting gene flow at different geographical scales (Prunier, Colyn, Legendre and Flamand, 2017). In our study, this was particularly true for *A. parallelepipedus*. When using the classical genetic distances we found that only the feature Grasslands was identify as affecting gene flow in this beetle. However, linear elements affecting gene flow in this species were detected when using HGD. In the first level (HGD1), the secondary road network and the country road D6089 explained the genetic variability, indicating that these two features were impeding dispersal at the regional scale (Prunier, Colyn, Legendre and Flamand, 2017). In the second level (HGD2), the country road and the gas pipeline were explaining most of the genetic distances variability, indicating that these features limited dispersal at the local scale (Prunier, Colyn, Legendre and Flamand, 2017). Among the four studied species, we could calculate HGD only for *A. obstetricans* and *A. parallelepipedus*. STRUCTURE was not able to find clusters for the two other species. In *A. obstetricans*, the informations provided by HGD (HGD1 and HGD2) were recurrent with the informations from the classical genetic distances. For example, Woodlands and roads were elements affecting gene flow when using the bray-curtis dissimilarity index, HGD1 and HGD2. However, the use of HGD, revealed that the railway and the motorway were two linear elements affecting HGD1 (regional) and HGD2 (local), respectively.

The use of commonality analyses has been used in previous landscape genetic studies (e.g. Gouskov et al., 2016; Prunier, Colyn, Legendre and Flamand, 2017; Renner et al., 2016; Seeholzer and Brumfield, 2017; Prunier et al., 2018) and is a powerful framework to identify synergistic association among predic-

tors and suppressors likely to bias the interpretation of genetic results (Prunier et al., 2015). In our study, the use of commonality analyses was a great tool to end up with a reduced number of predictors with little collinearity among them (Dormann et al., 2013), and thus little distortion in regression outputs (Prunier et al., 2015).

The use of CA, give us strong support of the effect (β) of the retained predictors. A predictor with a negative β value was associated with a reduction of genetic distances and interpreted as promoting gene flow across this feature. On the opposite, a landscape feature that is associated with an increase of genetic distances (positive β value) was interpreted as impeding gene flow across this feature and creating barrier to dispersal.

4.2 Fragmentation due to the secondary road network and the country road D6089

The secondary road network and the country road D6089 were affecting gene flow in three of the four studied species (all but the butterfly). They were mostly acting as barriers to gene flow (Fig. 6) and corresponded to the LTIs with the strongest effects on gene flow across species. Together, the secondary road network and the country roads were responsible of about 27 % of the total explained variability in genetic distances across species.

Among vertebrates, amphibians are one of the groups mainly affected by LTIs (Fahrig and Rytwinski, 2009). This statement was confirmed in this study. Across the four studied species, *A. obsetricans* was the most impacted by LTIs, with four of the six studied LTIs impeding gene flow (Table 1). The secondary road network and the country road D6089 were the main threats to dispersal in *A. obsetricans* as they were affecting both, the classical genetic distance (bc) and the second hierarchical level (HGD2). In addition, the secondary road network impeded gene flow in the first hierarchical level (HGD1). Our results are similar to Garcia-Gonzalez et al. (2012) who found that all roads, including small secondary roads, are obstacles for gene flow in *A. obsetricans* in northern Spain. Roads are creating barriers to gene flow mostly because of road kills (Forman and Alexander, 1998; Hels and Buchwald, 2001; Beebee, 2013), which obviously limit gene exchanges across roads. Amphibians are particularly vulnerable to road kills because of their seasonal migration between breeding water bodies and shelters. In addition, they have slow moving capacities (Trochet et al., 2014) with limiting ability to escape an approaching vehicle. This study is an additional clue revealing that roads have a tremendous negative effect on amphibian dispersal and that mitigation measures are crucial in order to limit road kill (Beebee, 2013).

Roads are also responsible of a tremendous number of killing in snakes (Rosen and Lowe, 1994). Snakes are known to bask on road surfaces to absorb radiant heat; this behavior increases the likelihood of collisions (Rosen and Lowe, 1994) and results in a reduction of gene flow across roads (Clark et al., 2010). However, our results suggest the exact reverse pattern. We found that the secondary road network

present in our study area enhanced gene flow in *N. helvetica*. This conflicting result could be explained by an attractive effect of roads that provides basking surfaces coupled with a low risk of roadkill. Low roadkill probability can be explained by the small width of secondary roads and the weak traffic volume. In addition, this result could be linked to the particular life-history traits of this species. Grass snakes' distribution is strongly dependent on wetlands because of their diet. Secondary roads are often alongside water-filled ditches providing interesting alternative habitats full of amphibian preys (Matos et al., 2012). This could result in a local increase of abundance of grass snakes along roads, favoring road crossings and gene flow. A similar explanation was proposed by Johansson et al. (2005) who found a positive effect of gravel roads (with ditches surrounding them) on genetic distances in the common frog (*Rana arvalis*).

Gene flow in *A. parallelepipedus* was impeded by these two types of LTIs. The country road D6089 and the secondary road network explained the whole variance at the first hierarchical level (HGD1) resulting in clusters A and B (Fig. 3). At the second hierarchical level (HGD2) the country road D6089 (but also the gas pipeline) was a barrier to gene flow and explained the separation of cluster B in two sub-clusters (Fig. 3). Our results are congruent with Keller et al. (2004) who found that roads are barriers to dispersal in *A. parallelepipedus* but also in other ground beetle species (e.g. Keller and Largiader, 2003). Roads may act as barrier to gene flow because of road kills but also because ground beetles may be reluctant to cross roads due to behavior changes (Holderegger and Di Giulio, 2010).

4.3 Fragmentation due to the motorway A89

The motorway A89 was affecting gene flow in the two vertebrate species (positively for the toad and negatively for the snake). 5 % of the total explained variability in genetic distances across species was due to the motorway.

Motorways are usually known to impede gene flow in amphibians. For example, Van Buskirk (2012) found that a Switzerland motorway reduced gene flow in the alpine newt (*Ichthyosaura alpestris*) and the frog *Rana temporaria*. Yet, in our study, *A. obsetricans* gene flow was promoted by the motorway at the second hierarchical level (Table 1). This counter-intuitive genetic pattern could be explained by the alternative open habitats provided by right-of-ways. For instance, adults and tadpoles of *A. obstetricans* were detected in eight out of the ten retention basins present along the studied motorway (data not shown). These retention basins may provide interesting breeding water bodies free of predatory fish and with sand or gravel in close vicinity (ideal substrates to build their burrows). Besides interesting alternative habitats, the motorway is crossed by underneath culverts and tracks which are good dispersal ways for amphibians, especially when their are filled with water (Veenbaas and Brandjes, 1999). This is not the first study showing a potential positive effect of a motorway on amphibian gene flow. Prunier et al. (2014) revealed that a 40-years old motorway was not a barrier for the alpine newt (*Ichthyosaura alpestris*) and could even serve as a longitudinal dispersal corridor due to recent landscape changes.

Interestingly, they even found negative beta values indicating that gene flow across the motorway was enhanced. But because they analyzed the data using one-tailed Mantel test, their method was not designed to reveal such effect (Prunier et al., 2014). Even if 10-years old LTIs can affect gene flow (Yu et al., 2017), our results must be interpreted with caution due to the recent age of the motorway (< 15 years old). This genetic pattern could be explained by ancestral landscape configurations before the building of the motorway such as high proportion of wetlands and optimal habitats for this species. Direct approaches such as Mark-Release-Recapture surveys will be necessary to confirm this pattern.

Genetic studies estimating gene flow of reptiles across LTIs are dramatically lacking (Holderegger and Di Giulio, 2010) (but see Clark et al., 2010). Here, we revealed that the motorway A89 impeded gene flow in *N. helvetica* and accounted for half of the explained variance. Because the motorway is fenced with fine mesh, snakes can only reach the other side by using crossing structures (bridges, underpasses, culverts, roads). These crossing structures may be seldom use by grass snakes due to inadequate placement, architectural design and behavior of snakes (Woltz et al., 2008). Thermoregulatory behavior of reptiles is probably the main reason why individuals would not use underpasses (Rodriguez et al., 1996), as a 50 m-length underpass under the motorway would provide inadequate thermal conditions due to the absence of sunlight. In addition, Baxter-Gilbert et al. (2015) evaluated the effectiveness of different mitigation measures implemented to reduce reptile road mortality (including underneath culverts). They found that these structures were seldom used by reptiles (Baxter-Gilbert et al., 2015).

4.4 Fragmentation due to the railway

The railway was explaining a low proportion (2.5 %) of the total explained variability in genetic distances across species. However, the railway was significantly affecting gene flow of the two vertebrate species (negatively for the toad and positively for the snake).

In the first hierarchical level, *A. obsetricans* gene flow was impeded by the railway (Table 1) although cluster A and cluster B were not clearly separated by this LTI (Fig. 3), suggesting a modest effect of the railway on gene flow. Railways are known to restrict gene flow in some amphibian species such as frogs or salamanders (e.g. Reh et al., 1990; Bartoszek and Greenwald, 2009) and many studies on train collision with wildlife reported a high abundance of amphibian killed (Borda-de Agua et al., 2017) representing up to 47 % of all vertebrate records (Heske, 2015). However, the railway in our study area has a low traffic density with about 10 trains/day. It seems not plausible that train collisions alone drive the gene flow limitation in *A. obstetricans*. The physical features of the railway are likely to explain this pattern. Amphibians have a high probability to be trapped between the rails, depending on their agility to overcome the rails and be more vulnerable to railway mortality than other vertebrates (Budzik and Budzik, 2014). The age of the railway is also an important driver of the detected effect. A recent study on the alpine newt (*Ichthyosaura alpestris*) revealed that a high-speed railway was not a barrier for gene

flow in this species (Prunier et al., 2014). However, the authors argue that the railway was too recent (29 years old) to detect any genetic isolation. In our study area, the railway was older than 150 years, which seems a reasonable time length to detect a barrier effect (Cushman and Landguth, 2010; Epps and Keyghobadi, 2015). We revealed that even a low traffic secondary railway may be an important driver of genetic isolation in amphibians.

Finally, we found that the railway promoted dispersal in the snake species. Reptiles are among the vertebrates species with the lowest probability to be impacted by railways (Borda-de Agua et al., 2017). Railways embankments provide important alternative habitats for reptiles with optimal thermal conditions for basking (Graitson, 2006; Stoll, 2013). Even active lines with optimal sunny areas have particularly high richness of reptiles (Graitson, 2006). The absence of human presence along the rails provides a peaceful environment with many shelters (Borda-de Agua et al., 2017). Railways may even contribute to gene flow by creating dispersal corridors (Graitson, 2006). Snakes may avoid collision with trains thanks to their developed perceptions. When trains are approaching, the vibration transmitted through the rails and the ballast can be felt by snakes. This warning message might help snakes to reach a shelter before collision. Similar to the secondary road network, the railway in the study area probably has an attractive effect on snakes and explain the detected gene flow enhancement across the railway.

4.5 Fragmentation due to the gas pipeline and the power line

The gas pipeline was affecting negatively gene flow only in the beetle *A. parallelepipedus*. It accounted for about 3.5 % of the total explained variability in genetic distances across species. The limitation of dispersal across the gas pipeline might be due to an inability to move through dense vegetation cover on the litter layer.

The power line was not affecting any of the four studied species. It confirms previous studies showing the limited impact of power lines on wildlife dispersal (Latch et al., 2011; Bartzke et al., 2015; Jahner et al., 2016).

4.6 Non-linear elements affecting gene flow

Infrastructures accounted for about 38 % of the total explained variability in genetic distances across species. The 62 % left were explained by natural (57 %) and anthropized (5 %) features (Fig. 4).

The non-linear features influencing gene flow in *A. obsetricans* were isolation by distance (IBD), altitude differences, crops, woodlands and urban areas (Table 1). Despite classical knowledge on amphibians (Van Buskirk, 2012), we revealed that woodland is a strong driver and is a main barrier to gene flow because it affected the classical genetic distances (bc), the first and second hierarchical level (HGD1 and HGD2). Several hypothesis can be suggested to explain this observation. Individuals may be reluctant to move through woodlands because of inadequate soil characteristics, higher predation level, mitigation of

their calling calls due to dense vegetation or absence of optimal breeding water bodies. We were able to detect IBD in this study area that was not detected in the same species in Spain (Garcia-Gonzalez et al., 2012) probably because they used mitochondrial DNA instead of microsatellites which are less variable at narrow geographical scale. Individuals separated by high altitude differences were more genetically distant than individuals sampled at similar altitude level. This result could be linked to a hydrology gradient with individuals sampled in the same water catchment more prone to be close genetically. Crops impeded gene flow at the first hierarchical level (HGD1). A similar result was found for the frog *Rana temporaria* in Germany (Lenhardt et al., 2017). Individuals may be unwilling to cross this landscape feature or be killed while crossing crops because of pesticide exposures (Brühl et al., 2013) or dehydration risk. Finally, urban areas are landscape elements promoted gene flow in *A. obstetricans*. Urban areas are usually considered as inappropriate habitats, limiting gene flow in amphibians (Goldberg and Waits, 2010; Van Buskirk, 2012). Our result could be explained due to the habitat requirements of this species. Old farmhouses are ideal habitats because they combine permanent water bodies (watering trough, cattle ponds, wells, *etc.*), open areas and shelters (stone walls, rubble piles, sand piles, tarps, *etc.*). In the rural landscape studied, old farmhouses are the main urbanized features with only a few small villages. It is likely that in more intensive landscapes with large towns, this genetic pattern would differ.

In our study area, the genetic structure of *N. helvetica* was weak. The software STRUCTURE detected only one cluster (interpreted as one main population) indicating that gene flow through this landscape was important. This result may explain the low proportion of the genetic variance explained by landscape features (4 % of the variance). In a comparable landscape in Switzerland, Meister et al. (2010) also found that grass snakes belong to one main population. In this study, we found that *N. helvetica* gene flow was affecting only by infrastructures (roads, motorway A89 and the railway). In seems that, at the local scale, grass snake dispersal is not affected by intensively used landscape features such as crops or urban areas (Wisler et al., 2008; Meister et al., 2010, 2012). Isolation by distance explains the genetic variance at the regional level (Meister et al., 2012) and genetic structuring can be detected only at the biogeographical level (Kindler et al., 2013; Pokrant et al., 2016; Kindler, Chèvre, Ursenbacher, Böhme, Hille, Jablonski, Vamberger and Fritz, 2017; Kindler, de Pous, Carranza, Beddek, Geniez and Fritz, 2017).

Compared to a previous individual-based study that explained less than 5 % of the genetic variance in three sites across France in the butterfly *M. jurtina* (Villemey et al., 2016), we were able to explain about 20 % of the variance when using a population-based method and a restricted spatial scale (maximum neighboring distance = 5500 m). STRUCTURE was not able to find any genetic structure in the data, probably because of high abundance, low specialization and great dispersal capacity in this butterfly (Villemey et al., 2016). Interestingly, we were able to detect an isolation-by-distance effect. This IBD effect was not detected in Villemey et al. (2016) with pairwise distances up to 60 km apart. We found

that woodlands were impeding gene flow in *M. jurtina*, a result similar to Villemey et al. (2016). The absence of sunlight and the dense vegetation may limit the movements through woodlands. None of the six LTI types was influencing gene flow in this species despite evidence of previous studies showing that roads (Polic et al., 2014) and motorways (Remon et al. submitted) can hinder crossing events of butterfly. Remon et al. (submitted) were using direct Mark-Release-Recapture surveys in the same landscape on *M. jurtina* and found that crossing events through the motorway were fivefold reduced compare to adjacent habitats. In this study, we used indirect method based on genetic, which is subject to population sizes bias (Prunier, Dubut, Chikhi and Blanchet, 2017) and time lag bias due to the recent construction of the motorway (Anderson et al., 2010). However, even with very wide infrastructures such as motorways, some butterflies are able to reach the other side (Remon et al. submitted) and may sustain gene flow at landscape level (Munguira and Thomas, 1992). This confirms that genetic tools should not be used alone (Safner et al., 2011). A combination of Mark-Release-Recapture studies coupled with landscape genetic can inform precisely how animals move through landscapes.

Unlike Marcus et al. (2015), we found a strong genetic structure in the ground beetle *A. parallelepipedus* within the studied landscape. The explained proportion of the classical Fst genetic distance was due to grasslands acting as barrier to gene flow. Individuals may be reluctant to cross grasslands to reach other woodland patches because of herbaceous vegetation cover on the litter layer that hinders their movements. This encourages the maintenance of hedges in agricultural environments to favor landscape connectivity among woodland patches (Fournier and Loreau, 1999). Altitude affected gene flow at the second hierarchical level (HGD2), but its effect was modest (Table 1). In any case, the fragmentation of woodlands due to land conversion, roads or other kind of LTIs could lead to strong isolation of ground beetles populations. Population abundance are high in this species (Loreau and Nolf, 1993; Keller et al., 2004) but its dispersal capacity is very limited (Brouwers and Newton, 2009). Therefore, populations which are not linked by dispersal may suffer from geographical isolation (Fahrig and Rytwinski, 2009; Beyer et al., 2016).

5 Conclusion

In fragmented landscape such as the one we studied, the accumulation of many LTIs is likely to isolate more strongly populations than single LTIs. For instance, we found that the combination of roads and the railway reduced *A. obstetricans* dispersal. Similarly, roads and the gas pipeline constrained dispersal of the ground beetle *A. parallelepipedus*. According to our expectations, roads (the secondary road network and the country road D6089) were the most detrimental studied LTIs in this study which confirms current knowledge on their negative impacts on a wide range of species (Holderegger and Di Giulio, 2010). The construction of passages for amphibians under rural roads (Woltz et al., 2008) or

traffic calming (Jaarsma and Willems, 2002) could contribute to road kill limitation and favor landscape connectivity. However, proper evaluations are required in order to build effective mitigation measures (Beebee, 2013). The motorway A89 and the railway had conflicting results concerning the snake *N. helvetica* and the amphibian *A. obstetricans*. The railway affected positively snake dispersal but it affected negatively amphibian dispersal. Conversely, the motorway affected negatively snake dispersal but it affected positively amphibian dispersal. Although, the positive effect of the motorway on the amphibian need to be confirmed with direct surveys, we highlight the fact that species-specific mitigation measures are required (Glista et al., 2009). Amphibians require moist conditions, therefore designing crossing structures to allow water to moist the passage is essential (Veenbaas and Brandjes, 1999). On the other hand, snakes are very sensitive to thermal conditions; long and moist underneath crossing structures are likely to be inefficient for snakes (Baxter-Gilbert et al., 2015). For this group, over-passages would probably be more efficient in addition to benefit to other organisms such as mammals. We highlight the fact that focusing only on one type of crossing structure is not an appropriate strategy. Instead, the combination of multiple mitigation measures an crossing structure types would benefit to the widest range of species.

6 Authors' contributions

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

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Appendices

A Laboratory procedures and microsatellite markers

For all species, we used a Qiagen Type-it Microsatellite kit. We extracted total DNA from invertebrate legs, scales and swabs using the DNeasy Blood and Tissue kit (Qiagen, Valencia, CA). Before enzymatic digestion, each invertebrate leg and scale was cut in 4-6 pieces to facilitate DNA extraction. Buccal swabs were used as is. For *N. helvetica* and *A. obstetricans*, we amplified 13 (Pokrant et al., 2016) and 14 (Tobler et al., 2013; Maia-Carvalho et al., 2014) polymorphic microsatellite loci, respectively. For both species, loci were amplified in 10 μl reaction volumes containing 2 μl multiplex PCR Master Mix,

1.2 to 1.6 μl of primer mix (between 0.13 and 0.25 μM of each primer), 5.4 to 5.8 μl of purified water and 1 μl of template DNA (10-20 ng μl^{-1}).

For *Maniola jurtina*, we amplified 15 polymorphic microsatellite loci (Richard et al., 2015) in three Multiplexes, in 10 μl reaction volumes containing 2 μl multiplex PCR Master Mix, 0.7 μl of primer mix (between 0.03 and 0.08 μM of each primer), 4.3 μl of purified water and 3 μl of template DNA (1-10 ng μl^{-1}). For *Abax parallelepipedus*, we amplified 14 polymorphic microsatellite loci (Marcus et al., 2013) in three Multiplexes, in 5 μl reaction volumes containing 1 μl multiplex PCR Master Mix, 0.7 μl of primer mix (between 0.04 and 0.11 μM of each primer), 2.3 μl of purified water and 1 μl of template DNA (approx. 10 ng μl^{-1}).

Polymerase Chain Reaction (PCR) conditions were set on an Applied Biosystems thermal cycler. For the two vertebrate species, conditions were set as follows: initial denaturation 10 min at 95°C; 30 cycles of 30 s at 95°C, 90 s at 51 to 60°C (depending on the multiplex) and 30 s at 72°C; final elongation of 5 min at 72°C. For the two invertebrate species, conditions were set as follows: initial denaturation 10 min at 94°C; 40 cycles of 30 s at 94°C, 90 s (for the 10 first) or 30 s (for the 30 following) at 61°C (*A. parallelepipedus*) or 56°C (*M. jurtina*) and 30 s at 72°C; final elongation of 5 min at 72°C.

All PCR products were ten times diluted and were run on an ABI 3730 DNA Analyser (Applied Biosystems) with the GeneScan-600 LIZ size standard. Genotyping was performed with GENEMAPPER 5.0 (Applied Biosystems) and all peaks were manually confirmed.

The following tables describe the specificity of the microsatellite markers tested for the four species followed in this study. Gray colours represent markers that were not used in the landscape genetic analyses either because they could not be amplified, showed sex-linkage, presence of null alleles or linkage disequilibrium (see results section).

Locus	Primer sequences (5'- 3')	Repeat motif	Allele size range (bp)	Number of alleles	Multiplex set	Annealing temperature	Fluorescent label	Original reference
Alyobs3	f – CCAACATGTTCACTTTATAGAGCAG r – GGAACCTTGAATCTCGAAAGC	(TATC)28	168-276	27	1	52	FAM	Tobler *et al.* (2013)
Alyobs4	f – TTTTCCCTTGCTAAATCCTCAG r – AAAGTGTTGATGCACATTTTCC	(CTGT)11	117-161	9	1	52	NED	Tobler *et al.* (2013)
Alyobs7	f – AAGGACGTGCTTCTATCTGC r – AGTTCGCACACATTACATTGC	(TATC)16(TG)3(TA)3(TC)(TA)4			1	52	PET	Tobler *et al.* (2013)
Alyobs28	f – CCAGTGCTGTGGTTTTCTCA r – AAATATCAAGAGCCTTAGCTAACATTT	(GT)13(GA)3(GTGA)3	96-106	4	1	52	VIC	Tobler *et al.* (2013)
Alyobs17	f – TTCTCTTCAGCTGGGCAATC r – TGGAACTGAAGAGCGAGGAC	(GT)13	146-160	8	2	56	VIC	Tobler *et al.* (2013)
Alyobs19	f – TGAATGTGCCGGTGAAGAC r – AAACACATATGAACAGGTGAAAAGAG	(GT)12	72-112	13	2	56	NED	Tobler *et al.* (2013)
Alyobs20	f – GATGCAGCACATTTCTGAGC r – GGTGCATCTGCCATAGTGTG	(GT)12	105-115	5	2	56	PET	Tobler *et al.* (2013)
Alyobs23	f – TGCAGAGCTCAGCCACTTAG r – TGACCAATCCAATCATCCAG	(GT)13	208-238	6	2	56	PET	Tobler *et al.* (2013)
Alyobs24	f – TCCTCAAAATCTTGTGATGTGC r – ATGGCCAGATGTCCCAATAC	(CA)28	75-139	23	2	56	FAM	Tobler *et al.* (2013)
Alyobs25	f – CCTTCTGTCTACCTTGTCATATTTCC r – AAAGCGACTAATACAGAACACTGC	(GT)16	141-163	9	2	56	NED	Tobler *et al.* (2013)
Aobst14	f – TGTGGAACCTTTACATCATAA r – CCCTCCTCTAAGCCGTCA	(ACT)n	102-158	11	3	52	VIC	Maia-Carvalho *et al.* (2013)
Aobst15	f – TTGGATGGTGGGTACAATCA r – TGAGGACAAATGCCTGACAA	(AGAT)n	251-401	21	3	52	NED	Maia-Carvalho *et al.* (2013)
Aobst16	f – TCAGAATAAACAAGAGCTGCAAA r – GGAGATCCACGCTCAGGATA	(AGAT)n	445-497	8	3	52	FAM	Maia-Carvalho *et al.* (2013)
Aobst17	f – CGGTGTCCCCATCTTATCAA r – CCCAGTGCTCAAACCTCAAT	(ACC)n	244-268	6	3	52	PET	Maia-Carvalho *et al.* (2013)

Locus	Primer sequences (5'- 3')	Repeat motif	Allele size range (bp)	Number of alleles	Multiplex set	Annealing temperature	Fluorescent label	Original reference
Natnat09	f – TGTAAATAACACTGTACCATTTTGG r - TGACTGGGCAACAGAAAAGC	$(AC)_{22}$	96-132	15	1	55	FAM	Meister *et al.* (2009)
Natnat05	f – TCTGCACTGGGGATAGGAAG r - GTCCCTTTTTCAGTGCTGTTG	$(GT)_{16}$			1	55	VIC	Meister *et al.* (2009)
µNt8new	f – GTATCGTCCTTCCAGACAAG r - GCAAATCAAATAAATCTCACTGG	$(AC)_{15}$	81-123	14	1	55	NED	Meister *et al.* (2009)
Nsµ3	f – CTGACTCACTTCTGACCCTAAT r - AATATTTGCTTGGCTCAAAC	$(ATCT)_{14}$ $ATC(CA)_{20}$			1	55	PET	Prosser *et al.* (1999)
µNt3	f – GGCAGGCTATTGGAGAAATG r – GGCAAAACTCCAGGTGCTAC	$(AC)_{16}$	127-145	5	2	60	FAM	Gautschi, Widmer & Koella (2000)
µNt7	f – TTTGAAAGGAGAATGAATCGTG r – CGCGAGGAATCAGAATGAAC	$(AC)_{17}$	177-185	3	2	60	VIC	Gautschi *et al.* (2000)
Natnat11	f – GGCTGTTTTCCCAGTGAAGC r - GGTCTGGGGAAAAAGAAAGG	$(GA)_{13}$	106-118	4	3	55	FAM	Meister *et al.* (2009)
Natnat06	f – AATGGCATTCTCTCCAGCTC r - ACCCATATCCGTATCCATATCC	$(GT)_{21}$	159-187	13	3	55	VIC	Meister *et al.* (2009)
3TS	f – GGTCACTTAAATACAACGAAATTGGTTAGCT r - CGGACAGCTCTGGCTCCCTTG	$(GATA)_{19}$			3	55	PET	Garner *et al.* (2002)
30	f – CCCACTGGCTCATTTCAAGT r – CCACATTTGCATCGGAGTG	$(CA)_{14}$	250-274	13	—	60	NED	Burns & Houlden (1999)
Tbu A09	f – CATCTCAACCAAAGTCGCTTC r – GGATGTTGTGGGGTGTTTTC	$(AC)_{7}$	110-140	14	—	55	NED	Sloss *et al.* (2012)
Eobµ1	f – ATCAGTAGGAGTGAGAGCAACT r – CTGCATACTCTTCCAGAACC	$(TG)_{21}$	128-134	3	—	51	NED	Blouin-Demers & Gibbs (2003)
Eobµ13	f – TGATCTGAGTCTCTTTCTGG r - CAATTCAAATCCATTGGTTT	$(AC)_{20}$	138-162	9	—	51	PET	Blouin-Demers & Gibbs (2003)

Locus	Primer sequences (5'- 3')	Repeat motif	Allele size range (bp)	Number of alleles	Multiplex set	Annealing temperature	Fluorescent label	Original reference
Mj0008	f – CGTGTCGCCTAAACCACATC r – TGGCAACCCTAAACCCTACG	(ACAT)7	91-149	5	1	56	PET	Richard *et al.* 2015
Mj5287	f – GCTAGCTCGTGGGTACTCTG r – CTCCAAGCAATAAGACCGCC	(GATA)11			1	56	FAM	Richard *et al.* 2015
Mj7132	f – ATCTGCGGATTTGCAGTTGG r – CACTATTGAGCACGTGTGTCC	(TATG)13	165-213	19	1	56	NED	Richard *et al.* 2015
Mj5647	f – GCGTTCTGATTACCACCCTG r – GCGACAGTCCCCTAAGATCG	(TATG)13			1	56	PET	Richard *et al.* 2015
Mj5563	f – CGGTTTTGCCGATAGCGTAG r – CGCAAGGCAATAGACCACTC	(ATCT)7			1	56	VIC	Richard *et al.* 2015
Mj3956	f – CAACATCGGGAGTCGAAACG r – CTCAGCCAGGATACCCACTC	(GATA)7			2	56	PET	Richard *et al.* 2015
Mj7232	f – AAGTTACAAGAGCGTTGGCG r – GCGGGAACTCTTGGGTTTTC	(CTGT)7	144-214	19	2	56	FAM	Richard *et al.* 2015
Mj5522	f – TGATCTTTGCCAGCAGGAAC r – AGTGTAAGCTGGCCCTAAAC	(GATA)8			2	56	NED	Richard *et al.* 2015
Mj0247	f – ATTCCACAAACGAGCCAACG r – ACTCCGATGGTAAGAGGTGC	(GATG)8	182-328	53	2	56	PET	Richard *et al.* 2015
Mj0272	f – GTTGCATTGGCACACTCCTC r – CAGCTGCACACTACGACAAG	(AGAT)7			2	56	VIC	Richard *et al.* 2015
Mj5331	f – TTAGACCGTGATCCCACTGC r – ATTTCGATAGGCAACGAGGC	(TATC)10	100-204	25	3	56	PET	Richard *et al.* 2015
Mj4870	f – ATGATCCATAGCTGCGTTGC r – CTCCTTAGCGCTTACACGTC	(ATGT)7	156-184	13	3	56	FAM	Richard *et al.* 2015
Mj3637	f – CTTCCGCAAAATAACGTCTGC r – AGATACTCCATTGACCCGGC	(TCTA)7			3	56	NED	Richard *et al.* 2015
Mj2410	f – TAATTAGAGTTTGCGCGGGG r – CGCACACCGCAGTATAAGTG	(TGTA)7			3	56	PET	Richard *et al.* 2015
Mj0283	f – CCCTTAGAATAAGAACTCGGCTC r – TGTTCGCACATGCTTAGTCC	(AGAT)9			3	56	VIC	Richard *et al.* 2015

Locus	Primer sequences (5'- 3')	Repeat motif	Allele size range (bp)	Number of alleles	Multiplex set	Annealing temperature	Fluorescent label	Original reference
apar_20	f – ACACTCCACTCAAAGTTGCG r – AAACGGTCAACTTTCCACCC	(AC)	185-189	3	1	61	PET	Marcus *et al.* 2013
apar_50	f – GCTGGACTATTACAGAAGTCTTTTGC r – ATGTGGAGGAAGCACGTGTT	(CATA)			1	61	FAM	Marcus *et al.* 2013
apar_27	f – CCTCCTTACCAAGTAACGGG r – GTTTGGAAGCGACAGTCAACGTG	(AC)	251-255	2	1	61	NED	Marcus *et al.* 2013
apar_34	f – GTTTGCCATACTAGGTGCTCTGG r – ATCTCCCGTGAAATCAACGC	(AC)	103-111	4	1	61	PET	Marcus *et al.* 2013
apar_32	f – TTTACCAACACACGCAGGC r – GTTTGGACCACAACACGTTAGCAC	(AG)	92-94	2	2	61	NED	Marcus *et al.* 2013
apar_12	f – GACCGTCGAGTGTAATGACG r – CAATCTGCTCCTCAAGTTCAAG	(AG)	123-133	4	2	61	VIC	Marcus *et al.* 2013
apar_23	f – GTGCCTATCGTTCTTTGTCAC r – GTTTGCGATATTGTCTCTTGGCGG	(AC)	156-162	4	2	61	NED	Marcus *et al.* 2013
apar_25	f – GTTTCGTAGCGAAACAGTGCCTTG r – ATACTCCGGCGCTACTTTGG	(AC)	198-204	5	2	61	NED	Marcus *et al.* 2013
apar_02	f – GCCGCACGATATTAGCGAC r – TTGGGAGTAAGTCTGTCCGG	(AC)	165-169	3	2	61	PET	Marcus *et al.* 2013
apar_46	f – CAGTTCAGTTCATCACGGGC r – GTTTGGAACCCAACGCAGAAAGTC	(AAC)			2	61	PET	Marcus *et al.* 2013
apar_05	f – CAACAACATTACCGGCGGAG r – GCCGAGTCACTTGTTACGTG	(AG)	150-156	4	3	61	FAM	Marcus *et al.* 2013
apar_44	f – GTTTCTTAATGTTCCATGCCGCG r – TCTTCTTCGGCAAGCGTTAC	(AG)			3	61	VIC	Marcus *et al.* 2013
apar_14	f – GACATCTCGACTGCACCTAC r – CCCTGTCTTTCCAACATCGC	(AG)			3	61	NED	Marcus *et al.* 2013
apar_06	f – AAACATTCTGCGGTGACACC r – CTGCTGCCCTCTTGTAAACG	(AG)	284-308	5	3	61	PET	Marcus *et al.* 2013

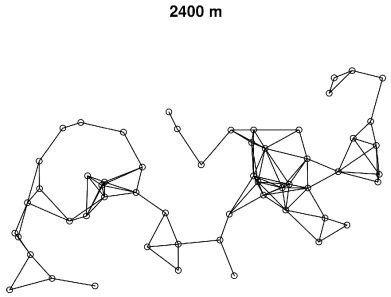
B Habitat predictors

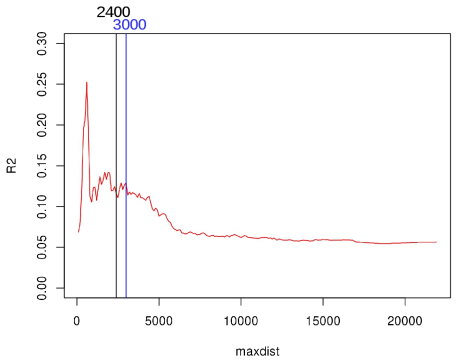
Habitat elements defining 6 of the retained landscape predictors used in genetic analysis.

Water	Crops	Woodlands	Grasslands	Urban	Roads
stagnant water	intensive monocultures	recent logged forests	grass stripes	agricultural buildings	gravelled roads
streams	gardens	coniferous forests	forest clearings	residential buildings	paved roads
ditches	orchards	deciduous forests	openings	waste disposals	
rivers	vineyards	riparian forests	grazed pastures	electrecity pylons	
	vegetable gardens or horticultures	mixed woodlands	dry grasslands	residential habitats	
		heathland	hayed meadows	water tanks	
		hedgerows	meadows	artificial gardens	
		tree plantations	trails ans paths	domestic gardens	
		bushlands	rocky lands	cemeteries	
			abandoned arable lands	sport equipements such as football fields	
				surroundings of agricultural buildings	
				campings	
				car parks	
				greenhouses	
				open cast mines	
				stone quarry	
				industrial sites	
				urban paved areas	
				windmills	

C Spatial scale of analysis

Identification of the maximum neighboring distance retained among pairs of individuals or populations in subsequent analyses. Gabriel graphs are presented for the four studied species and for two types of dependent variables: classic genetic distances (GD) based on the Bray-Curtis dissimilarity index (bc) or Fst and hierarchical genetic distances (HGD1 and HGD2). Left panels show the relation between the R^2 of the full model including all predictors in a classical multiple linear regression and euclidean distances among pairs of individuals or populations. Black lines correspond to the minimum distance insuring that all pairs are connected to at least one neighbor (top black Gabriel graph). Blue lines represent the retained spatial scale for subsequent analysis. Right panels represent the Gabriel graphs corresponding to the retained spatial scale.


2400 m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3000
R2
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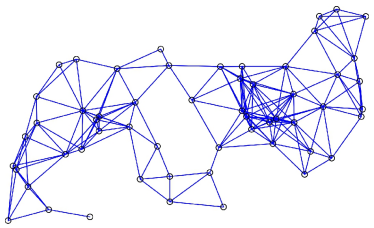
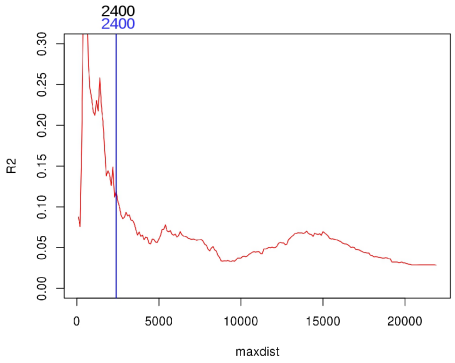
0.05
0.10
0.15
0.20
0.25
0.30
0
5000
10000
15000
20000
maxdist


GD(bc) 3000 m


2400
2400
R2
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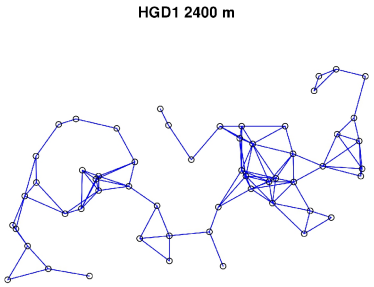
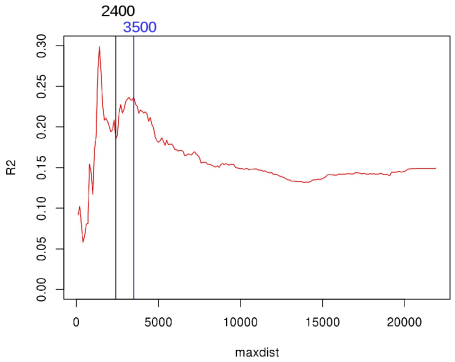
maxdist


HGD1 2400 m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R2
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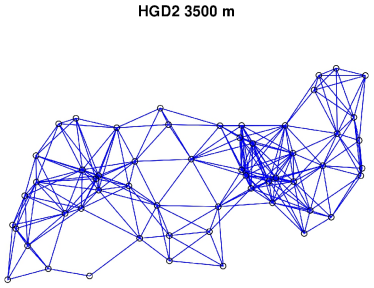
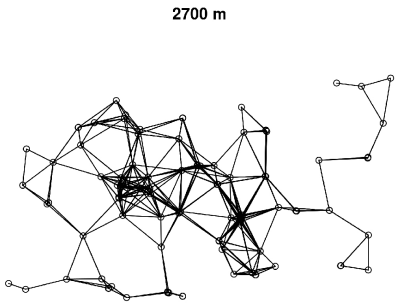
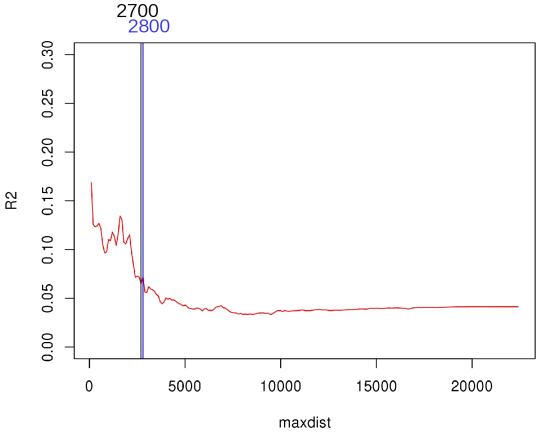
maxdist


HGD2 3500 m


2700 m


2700
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R2
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15000
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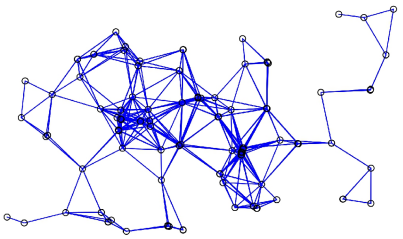
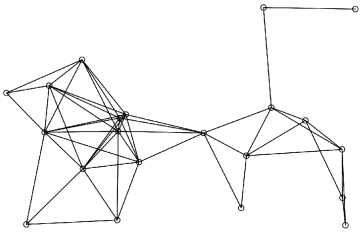
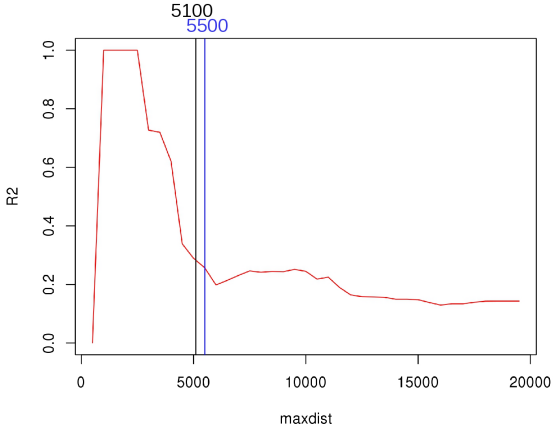
maxdist


GD(bc) 2800 m


5100 m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R2
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0.6
0.8
1.0
0
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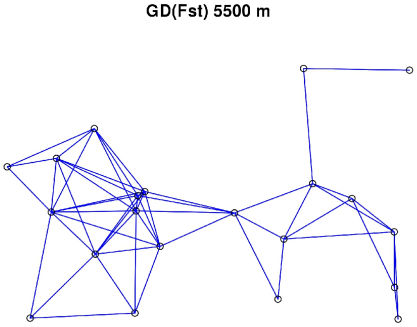
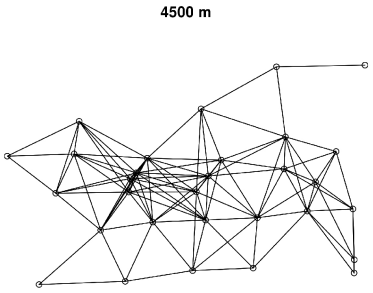
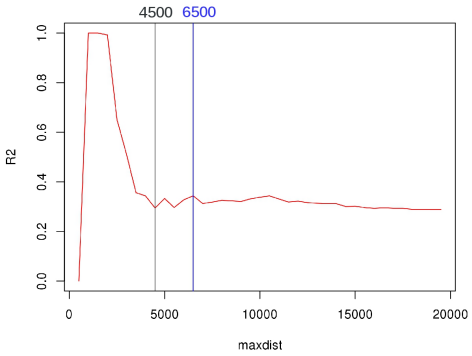
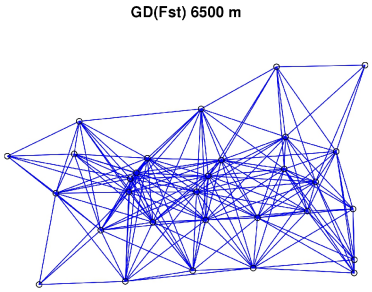
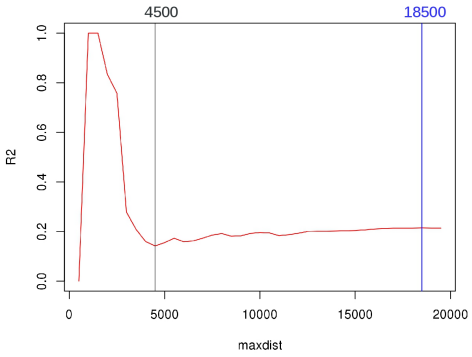
10000
15000
20000
maxdist

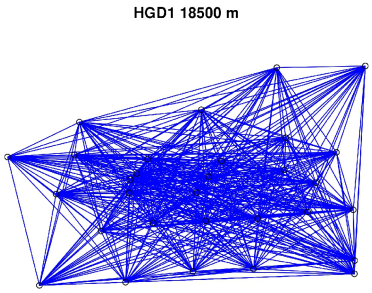

GD(Fst) 5500 m

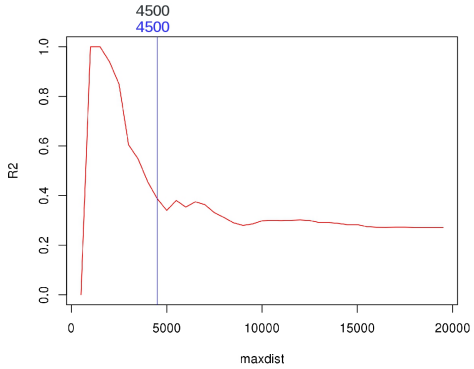


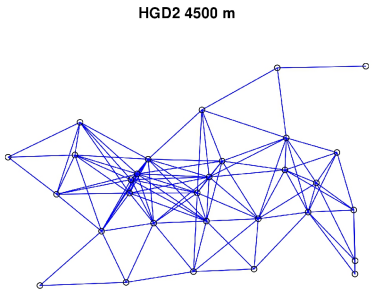












D Intermediate steps of commonality analyses on vectors

Runs of identification of unnecessary predictors for each species and dependent variable DV (GD: genetic distance either calculated with the Bray-Curtis (bc) dissimilarity index for individual-based method or

Fst for population-based method; HGD1 and HGD2 for hierarchical genetic distance based on first and second level of STRUCTURE outputs, respectively). Distance stands for the spatial scale retained in our analysis (Appendix C. Results of the different runs of multiple linear regressions (predictors, structure coefficient rs and standardized coefficient B), in addition to parameters derived from CA: unique (U), common (C) and total (T) contributions of predictors to the variance in the dependent variable. The rationale for withdrawal of predictors (Ra) is the following: CO: cross-over suppression; S: synergistic association with other predictors; PS: partial suppression (or reciprocal suppression). All predictors (IBD: isolation by distance; D6089: a large country road; Urban: urban areas; see Appendix B for additional informations on predictors) were coded as resistance. In bold: parameters allowing the identification of unnecessary predictors and suppressors. Note that situations of classical suppression were avoided by discarding any predictor with a squared zero-order correlation < 0.1.

DV	Species	Distance	Run	Pred	rs	B	U	C	T	Ra
GD(bc)	*A. obstetricans*	3000 m	1	IBD	0.809	0.172	0.012	0.068	0.080	
				Altitude	0.608	0.098	0.008	0.037	0.045	
				Woodlands	0.545	0.149	0.019	0.017	0.036	
				Water	**0.335**	**-0.078**	0.004	0.010	0.014	CO
				Roads	0.612	0.117	0.010	0.036	0.046	
				D6089	0.314	0.096	0.009	0.003	0.012	
				Railway	**0.346**	**-0.016**	0.000	0.014	0.015	CO
HGD1	*A. obstetricans*	2400 m	1	IBD	0.767	0.102	**0.004**	**0.062**	0.066	S
				Woodlands	0.451	0.081	0.005	0.018	0.023	
				Water	**0.381**	**-0.068**	0.003	0.013	0.016	CO
				Crops	0.672	0.182	0.030	0.021	0.051	
				Roads	0.660	0.135	0.011	0.038	0.049	
				Railway	0.433	0.081	0.005	0.016	0.021	
HGD2	*A. obstetricans*	3500 m	1	IBD	0.355	-0.006	**0.000**	**0.025**	0.025	S
				Woodlands	0.538	0.190	0.028	0.029	0.058	
				Urban	-0.465	-0.240	0.046	-0.003	0.043	
				Roads	0.448	0.268	0.051	-0.011	0.040	
				D6089	0.440	0.185	0.030	0.008	0.039	
				Motorway	-0.279	-0.118	0.013	0.003	0.016	
HGD2	*A. obstetricans*	3500 m	2	Woodlands	0.614	0.261	0.046	0.012	0.058	
				Urban	0.317	0.002	**0.000**	**0.015**	0.015	S
				Roads	0.512	0.187	0.035	0.006	0.040	
				D6089	0.502	0.196	0.036	0.002	0.039	
				Motorway	0.318	0.110	0.010	0.005	0.016	

DV	Species	Distance	Run	Pred	rs	B	U	C	T	Ra
GD(bc)	*N. hevetica*	2800 m	1	Roads	-0.533	-0.125	0.015	-0.003	0.012	
				Motorway	0.616	0.148	0.021	-0.005	0.016	
				Railway	-0.520	-0.088	0.008	0.004	0.011	

DV	Species	Distance	Run	Pred	rs	B	U	C	T	Ra
GD(Fst)	*M. jurtina*	5500 m	1	IBD	0.434	0.257	0.062	-0.019	0.044	
				Woodlands	0.636	0.349	0.072	0.022	0.093	
				Meadow	**-0.386**	**0.060**	0.002	0.032	0.035	CO
				D6089	-0.255	-0.178	0.030	-0.015	0.015	
				Power line	-0.553	-0.226	0.045	0.026	0.071	
GD(Fst)	*M. jurtina*	5500 m	2	IBD	0.436	0.254	0.061	-0.018	0.044	
				Woodlands	0.639	0.313	0.088	0.005	0.093	
				D6089	-0.257	-0.177	**0.030**	**-0.015**	0.015	PS
				Power line	-0.555	-0.220	0.043	0.028	0.071	

DV	Species	Distance	Run	Pred	rs	B	U	C	T	Ra
GD(Fst)	*A. parallelepipedus*	6500 m	1	Altitude	0.192	0.144	0.019	-0.008	0.011	
				Grasslands	0.918	0.683	0.213	0.031	0.244	
				Water	**0.360**	**-0.156**	0.010	0.028	0.038	CO
				Urban	**0.443**	**-0.023**	0.000	0.057	0.057	CO
				Roads	**0.445**	**-0.089**	0.001	0.056	0.057	CO
				Motorway	**0.226**	**-0.049**	0.002	0.013	0.015	CO
HGD1	*A. parallelepipedus*	18500 m	1	Grasslands	**0.283**	**-0.140**	0.010	0.006	0.016	CO
				Water	0.595	0.189	0.015	0.055	0.070	
				Crops	**0.546**	**-0.043**	0.001	0.058	0.059	CO
				Urban	**0.628**	**-0.172**	0.005	0.073	0.078	CO
				Roads	0.759	0.401	0.031	0.082	0.114	
				D6089	0.745	0.265	0.059	0.051	0.110	
HGD1	*A. parallelepipedus*	18500 m	2	Water	0.628	0.094	**0.005**	**0.065**	0.070	S
				Roads	0.801	0.194	0.018	0.096	0.114	
				D6089	0.787	0.261	0.062	0.048	0.110	
HGD2	*A. parallelepipedus*	4500 m	1	Altitude	0.440	0.212	0.044	0.009	0.053	
				Roads	0.404	0.085	**0.007**	**0.038**	0.045	S
				D6089	0.750	0.324	0.089	0.065	0.154	
				Motorway	-0.312	-0.119	0.013	0.014	0.027	
				Gas pipeline	0.511	0.226	0.050	0.022	0.072	

To explain the dependent variable based on the Bray-Curtis genetic distance in *A. obstetricans*, the predictors with a squared correlation (r^2) with the dependent variable higher than 0.1 were IBD, Altitude, Woodlands, Water, Roads, D6089 and Railway. Among these predictors, Water and Railway were cross-over suppressors and were discarded from subsequent analysis. To explain the first level of hierarchical genetic distance (HGD1) in *A. obstetricans*, the predictors with a r^2 higher than 0.1 were IBD, Woodlands, Water, Crops, Roads and Railway. IBD was a suppressor with synergistic association with other predictors. Water was a cross-over suppressor. These two predictors were discarded and the final model comprised four predictors: Woodlands, Crops, Roads and Railway. To explain the second

level of hierarchical genetic distance (HGD2) in *A. obstetricans*, the predictors with a r^2 higher than 0.1 were IBD, Woodlands, Urban, Roads, D6089 and Motorway. IBD and Urban were cross-over suppressors and were discarded from subsequent analysis.

In the *N. helvetica* data set, only three predictors had a r^2 higher than 0.1: Roads, Motorway and Railway. There was no suppressors among these three predictors and all were used in the final model.

For the species *M. jurtina*, five predictors had a r^2 higher than 0.1: IBD, Woodlands, Grasslands, D6089 and Power line. Grasslands was a cross-over suppressor and the roads D6089 was a partial suppressor. These two predictors were discarded from subsequent analysis resulting in a final model with three predictors: IBD, Woodlands and Power line.

To explain the Fst genetic distances in *A. parallelepipedus*, six predictors had a r^2 higher than 0.1: Altitude, Grasslands, Water, Urban, Roads and Motorway. Water, Urban, Roads and Motorway were cross-over suppressors. All were discarded from subsequent analysis. Only two predictors remained in the final model: Altitude and Grasslands.

To explain the first level of hierarchical genetic distance (HGD1) in *A. parallelepipedus*, we retained the predictors: Grasslands, Water, Crops, Urban, Roads and D6089 ($r^2 > 0.1$). Grasslands, Crops and Urban were cross-over suppressors and Water was a suppressor with synergistic association with other predictors. Therefore, we retained only Roads and D6089 to explain the dependent variable in the final data set.

To explain the second level of hierarchical genetic distance (HGD2) in *A. parallelepipedus*, we retained the predictors: Altitude, Roads, D6089, Motorway and Gas pipeline ($r^2 > 0.1$). The predictor Roads was a suppressor with synergistic association with other predictors and was discarded from subsequent analysis.

E Correlation among final predictors

Matrices of Pearson's correlation coefficients among final predictors depending on the dependent variables. The dependent variables are genetic distances (GD) based on the Bray-Curtis dissimilarity index (bc), Fst or hierarchical genetic distances based on first and second level of STRUCTURE outputs (HGD1 and HGD2). The variance inflation factors (VIF) are presented for each predictor.

Species	DV		Pearson's correlation coefficient				VIF
Alytes obstetricans		**Predictor**	IBD	Altitude	Woodlands	Roads	
	GD(bc)	IBD					1.704
		Altitude	0.427				1.244
		Woodlands	0.304	0.141			1.164
		Roads	0.489	0.308	0.029		1.368
		D6089	0.174	0.062	-0.111	0.062	1.067
		Predictor	Woodlands	Crops	Roads		
	HGD1	Woodlands					1.063
		Crops	0.045				1.053
		Roads	0.119	0.224			1.071
		Railway	0.220	0.006	0.086		1.055
		Predictor	Woodlands	Urban	Roads	D6089	
	HGD2	Woodlands					1.132
		Urban	-0.303				1.246
		Roads	0.003	0.318			1.142
		D6089	-0.112	0.015	0.084		1.041
		Motorway	0.008	-0.023	0.042	-0.125	1.021
Natrix natrix		**Predictor**	Roads	Motorway			
	GD(bc)	Roads					1.056
		Motorway	0.188				1.038
		Railway	0.131	-0.008			1.019
Maniola jurtina		**Predictor**	IBD	Woodlands			
	GD(Fst)	IBD					1.049
		Woodlands	-0.197				1.111
		Power line	-0.039	-0.239			1.069
Abax parallelepipedus		**Predictor**	Altitude				
	GD(Fst)	Altitude					1.001
		Grasslands	-0.034				1.001
		Predictor	Roads				
	HGD1	Roads					1.095
		D6089	0.295				1.095
		Predictor	Altitude	D6089	Motorway		
	HGD2	Altitude					1.010
		D6089	-0.019				1.071
		Motorway	0.046	-0.229			1.071
		Gas pipeline	0.087	0.095	0.090		1.030