

1 Jérôme G. Prunier ^{a, b}, Bernard Kaufmann ^b, Jean-Paul Léna ^b, Serge Fenet ^c, François Pompanon ^d and
2 Pierre Joly ^b

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4 **A forty-year-old divided highway does not prevent gene flow**
5 **in the alpine newt *Ichthyosaura alpestris*.**

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7 ^a *Ecosphère, 3bis rue des Remises, 94100 Saint Maur des Fossés, France*

8 ^b *Université de Lyon; UMR5023 Ecologie des Hydrosystèmes Naturels et Anthropisés; Université Lyon*
9 *1; ENTPE; CNRS; Villeurbanne, F-69622, France.*

10 ^c *Université de Lyon, UMR 5205 Laboratoire d'Informatique en Image et Systèmes d'information, Bat.*
11 *Nautibus, Université Lyon 1; CNRS; Villeurbanne, F-69622, France.*

12 ^d *Université Joseph Fourier, Laboratoire d'Ecologie Alpine, CNRS, UMR 5553, 2233 Rue de la Piscine,*
13 *38041 Grenoble Cedex 9, France.*

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15 **Corresponding author:**

16 Jérôme G. Prunier

17 *Current address : Université Catholique de Louvain, 4-5 Croix du Sud, bte L7.07.14, Institute of Life*
18 *Sciences (ISV), Louvain-la-Neuve, B-1348, Belgium*

19 *Fax : (+32) 10 47 38 72*

20 *Email: jerome.prunier@gmail.com*

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23 **Abstract**

24 Roads are of major concern in conservation biology, as they are known to restrict animal movements
25 through landscape fragmentation, and may therefore impact genetic patterns in native terrestrial
26 organisms. We assessed the effect of two large-scale transportation infrastructures, a 40 year-old highway
27 and a 30 year-old high-speed railway, on the spatial genetic structure of the alpine newt *Ichthyosaura*
28 *alpestris*, a highly nomadic amphibian. Genetic data were gathered following a targeted individual-based
29 sampling scheme and analysed using both overlay and correlative methods. While simulations suggested
30 that the highway may be old enough for a significant barrier effect to be detected, large-scale
31 transportation infrastructures were never detected as barriers to gene flow: inferred genetic boundaries
32 rather coincided with transition zones between major landscape entities. Furthermore, spatial principal
33 component analysis, a method designed to reveal cryptic genetic spatial patterns in high gene flow
34 species, counter-intuitively suggested that the highway may act as a potential dispersal corridor in low-
35 quality habitats, thus challenging traditional hypotheses on road impacts in amphibians. Our study
36 showed that considering local interactions between species, infrastructures and landscape-specific
37 characteristics is essential for better understanding the potential impacts of roads on movement patterns in
38 terrestrial organisms.

39

40 **Keywords:** amphibian, Bayesian clustering methods, correlative analyses, landscape genetics, highway,
41 sPCA.

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45 **Introduction**

46 Roads constitute one of the most widespread forms of land use in contemporary landscapes and have been
47 the subject of numerous studies aiming to assess their impacts on natural populations (Trombulak and
48 Frissell 2000; Balkenhol and Waits 2009). Roads are known to act as barrier to dispersal and gene flow
49 (isolation-by-barrier, *IBB*), decreasing functional connectivity and increasing the genetic differentiation
50 among individuals on each side of the infrastructure (Balkenhol and Waits 2009; Holderegger and Di
51 Giulio 2010). The larger the size of a road (in terms of traffic volume and spatial dimensions), the higher
52 the expected impact on both landscape configuration (Trombulak and Frissell 2000) and dispersal patterns
53 (Holderegger and Di Giulio 2010). Large-scale transportation infrastructures (LTIs) such as highways and
54 high-speed railways are thus of major concern in conservation biology. Barrier effects may be explained
55 by mortality due to collisions, which obviously decreases gene exchanges across roads (Holderegger and
56 Di Giulio 2010), but also by the modification of effective dispersal patterns (e.g. Riley et al. 2006). The
57 presence of ‘Jersey barriers’ (Lesbarreres and Fahrig 2012) or fences for livestock exclusion (Dodd et al.
58 2004; Kuehn et al. 2007; Holderegger and Di Giulio 2010; Hepenstrick et al. 2012), as well as road
59 avoidance due to physical or chemical alteration of the local environment may prevent animals from
60 crossing LTIs and possibly lead them to increase their movement along road verges (Trombulak and
61 Frissell 2000; McGregor et al. 2008; Balkenhol and Waits 2009). However, barrier effects are often
62 alleviated by bridges, underpasses or specific road crossing structures, often coupled with guide fences
63 (McGregor et al. 2008; Woltz et al. 2008) that can be used by dispersing animals. Small ground-dwelling
64 animals such as amphibians are nevertheless suspected to be unable to encounter these crossing structures
65 because of poor locomotive performances and specific orientation capabilities (Joly and Miaud 1993;
66 Beebee 2013). As mortality due to collisions and road avoidance both depend on traffic volume and
67 infrastructure width, divided highways might be considered stronger barriers to dispersal than high-speed
68 railways. However, rails may also constitute a physical barrier for small ground-dwelling animals
69 (Bartoszek and Greenwald 2009), because tracks are in close contact to the ballast, leaving no space for
70 animals to move underneath, while the prominent head of tracks (when considering the cross sectional
71 shape of rail) may prevent animals from climbing over them.

72 On a larger scale, modifications of the surrounding landscape configuration due to LTI could also
73 impact wildlife dispersal patterns. For instance, LTIs are responsible for the alteration of surface-water

74 habitats through flow re-routing and wetlands destruction (Trombulak and Frissell 2000), impacting both
75 aquatic and semi-aquatic species. In the particular case of highways, road connections to urban areas
76 improve rural-urban access and increase farmland value (Drescher et al. 2001). This often leads to the
77 regrouping of cultivated farmlands, to the detriment of suitable landscape features such as wooded
78 patches, extensively pastured meadows and aquatic or hedgerows networks. Conversely, LTIs may also
79 contribute to the creation of large-scale, non-fragmented linear wetlands such as roadside ditches and
80 grasslands corridors such as roadside verges, favoring movement of native but also exotic species in areas
81 of low-quality or highly fragmented habitats (Tikka et al. 2001; Brisson et al. 2010; Holderegger and Di
82 Giulio 2010). All these potential effects depend on complex interactions between species, infrastructures
83 and landscape-specific characteristics, as well as the interactions with public policies and economic
84 context (Woltz et al. 2008; Balkenhol and Waits 2009).

85 Direct and indirect effects of roads on wildlife have been described for years (Trombulak and
86 Frissell 2000), leading to the emergence of road ecology as a specific scientific discipline (Forman et al.
87 2003). Among available tools, molecular genetic techniques recently emerged as particularly useful to
88 detect road impacts on population structures (Balkenhol and Waits 2009). In close collaboration with
89 local road planners, as recommended in Lesbarreres and Fahrig (2012), we carried out a broad-scale
90 analysis of the potential impacts of two LTIs, a divided highway and a high-speed railway, on spatial
91 genetic patterns in the alpine newt *Ichthyosaura alpestris*. Many landscape genetic studies assessing the
92 impact of roads on amphibians have detected a decrease in genetic diversity and an increase in genetic
93 differentiation in the vicinity of LTIs (Lesbarreres et al. 2006; Holderegger and Di Giulio 2010; Emel and
94 Storfer 2012), probably resulting in population fragmentation. The alpine newt is no exception, as shown
95 in a recent study in Switzerland (Van Buskirk 2012). Furthermore, both LTIs are located along the Paris-
96 Lyon axis, a sector already suspected to prevent genetic exchange in another species, the European
97 wildcat *Felis silvestris silvestris* (Say et al. 2012). We thus expected the two LTIs under study to
98 significantly alter gene flow in the alpine newt: the A6 highway and the LGV-PSE high-speed railway
99 should be associated with a significant increase in genetic distances between individuals located on either
100 side or should coincide with sharp genetic discontinuity between genetic clusters. To test this hypothesis,
101 we used both simulated and empirical genetic data, gathered following a regular individual-based
102 sampling scheme in order to optimize the number of sampled sites across the study area. This kind of

103 sampling scheme is a powerful alternative to the conventional population-based sampling scheme, that
104 proved to be particularly efficient at detecting recent barriers to gene flow when designed as a targeted
105 sampling scheme, that is when sampled sites are localized in the direct vicinity of a putative barrier
106 (Anderson et al. 2010; Prunier et al. 2013).

107

108

109 **Materials and methods**

110 **Study area**

111 The study was carried out in eastern central France (Bourgogne), over an area of approximately 60 x
112 55 km (Fig. 1a), with elevation ranging from 138 to 857 m (mean elevation: 399 m). This area comprised
113 several water catchments and three distinct landscape entities (Fig. 1b): (1) In the south-west lies *Le*
114 *Morvan*, a massif comprising low elevation fragmented wooded patches and extensively pastured
115 meadows for livestock farming, with a dense hydrographic network (minimum elevation: 400 m). (2) In
116 the east, *L'Auxois* is a cultivated plateau in the continuity of *Le Morvan*, interrupted by two parallel
117 geologic depressions (minimum elevation: 300m). (3) In the north, *Terre-Plaine* is a vast low-elevation
118 plain (minimum elevation: 187m). Both *Terre-Plaine* and geologic depressions in *L'Auxois* are covered
119 with cultivated fields (mainly cereal crops), extensively pastured meadows and wooded patches.

120 The study area is crossed by two large-scale transportation infrastructures (LTIs) localized along
121 the Paris-Lyon axis: (1) the A6 highway, a divided highway in use since 1969, crosses *Terre-Plaine* and
122 the eastern geologic depression in *L'Auxois*; (2) the LGV-PSE high-speed railway, in use since 1981, runs
123 along *Le Morvan* eastern foothills. These infrastructures were respectively 41 and 29 year-old when the
124 field study was initiated in 2010. For comparison, the oldest road sections studied in Van Buskirk (2012)
125 dated back to 1962, that is, 38 years before the beginning of sampling.

126 Both LTIs are approximately 25 m wide (50 m wide when considering the fenced area). They are
127 regularly crossed by country roads, tracks and natural streams, with approximately one crossing structures
128 every kilometer. They intersect in *Terre-Plaine* and delimit four distinct sectors (*A*, *B*, *C* and *D*, Fig. 1a).
129 Except for the two LTIs, artificial areas are mainly composed of farms, villages and a few small towns, all

130 connected by roads with low to medium traffic. The study area includes high densities of aquatic sites,
131 with one pond per km² on average.

132

133 **Biological model**

134 The alpine newt is a widespread species in central Europe that strongly depends on permanent
135 fish-free ponds or smaller aquatic sites such as ruts, surrounded by suitable terrestrial habitat such as
136 woods and semi-natural grasslands (Joly et al. 2001). In Bourgogne, this amphibian benefits from
137 extensive livestock farming that allows the preservation of many natural or artificial ponds in pastures.
138 The alpine newt is an annual breeder, with reported longevity up to 10 years below 1000m of altitude
139 (Miaud et al. 2000; Perret et al. 2003), although skeletochronology of phalanges, the most widely used
140 method of ageing amphibians, has been shown to underestimate the true age of long-lived amphibians
141 (Wagner et al. 2011): in our study area, we may thus expect individuals to live up to 15 or 20 years. Age
142 at maturity is estimated at three year-old at low altitudes (Miaud et al. 2000). Dispersal movements may
143 range from several hundred meters to one kilometer (Joly and Grolet 1996; Denoel 2005; Kovar et al.
144 2009), although movements up to four kilometers have been reported (Schäfer 1993; Schmidt et al. 2006).
145 While juvenile dispersal is rather the doing of male juveniles (Joly and Grolet 1996), several capture-
146 mark-recapture studies also reported high rates of breeding dispersal in both sexes, ranging from 4 to 48%
147 of dispersal adults per population (depending on landscape configuration; Perret et al. 2003; Schmidt et
148 al. 2006; Kopecky et al. 2010, 2012). This suggests that habitat supplementation is probably as important
149 as habitat complementation in this species (Dunning et al. 1992; Kopecky et al. 2010). This highly
150 nomadic behaviour may lead to strong gene flow and weak genetic differentiation among subpopulations
151 despite distances of up to several kilometers (Emaresi et al. 2011; Van Buskirk 2012; Prunier et al. 2013),
152 especially when high densities of aquatic sites are available, which was the case in our study. The alpine
153 newt thus constitutes a pertinent biological model for the study of barrier effects, since individuals with
154 high dispersal abilities may encounter roads at higher rates than less mobile species, thus shortening the
155 time-lag before barrier detection (Landguth et al. 2010; Prunier et al. 2013).

156

157 **Genetic sampling and genotyping**

158 Fieldwork was conducted during the 2010 and 2011 breeding seasons, and 339 aquatic sites
159 (artificial or natural ponds, flooded ruts, swamps, etc.) were prospected with a dip net for thirty to sixty
160 minutes (depending on site size and configuration) or until a male and a female were captured. Using
161 individuals rather than populations as the operational unit proved to be quite efficient at detecting recent
162 barriers to gene flow in previous studies (Landguth et al. 2010; Prunier et al. 2013). It notably allows the
163 number of sampled genotypes per aggregate to be reduced in favour of a better coverage of landscape in
164 the vicinity of both LTIs, thus minimizing the number of unsampled populations (Beerli 2004; Lowe and
165 Allendorf 2010).

166 Alpine newts were found in 225 sites (66% of prospected sites). The median distance between
167 neighbouring sites (following a Delaunay triangulation) was 2.88 km, ranging from 184 m to 10.79 km.
168 Non-destructive genetic samples using buccal swabs were taken from each captured individual (Broquet
169 et al. 2007). Samples were stored at ambient temperature in an air-tight container with silica gel providing
170 total desiccation. DNA extraction, PCR amplifications and genotyping were performed as described in
171 Prunier et al. (2012), using 14 autosomal microsatellite loci: *CopTa1*, *CopTa2*, *CopTa3*, *CopTa4*,
172 *CopTa7*, *CopTa8*, *CopTa9*, *CopTa10*, *CopTa11*, *CopTa12*, *CopTa13* and *CopTa14* (Prunier et al. 2012),
173 *TalCa1* and *TalCaga4* (Garner et al. 2003). Significant gametic disequilibrium was detected between
174 *CopTa7*, *CopTa9* and *CopTa10* in a previous study realized at the population-level (Prunier et al. 2012).
175 *CopTa7* and *CopTa9* were thus discarded from all further analyses except spatial principal component
176 analyses (sPCA; Jombart et al. 2008), as this method is not contingent on any particular genetic model.
177 Neither null alleles nor linkage disequilibrium were detected with this new combination of 12 markers
178 (Prunier et al. 2012). To assess the reliability of our genetic data, we estimated the mean error rate per
179 locus e_l (Pompanon et al. 2005) by blind replication of 45 out of 1081 samples (4.2 %) collected from
180 2009 to 2011 as part of a general research program on the alpine newt.

181 In order to take into account possible sex-biased dispersal in the alpine newt (Joly and Grolet
182 1996; Kopecky et al. 2012), all the following analyses were performed using males (dataset *M*) and
183 females (dataset *F*) separately, except when specified. Indeed, provided that only adults are sampled, the
184 use of autosomal nuclear markers does not prevent the detection of sex-specific differences in dispersal
185 (Goudet et al. 2002).

186

187 **Isolation-by-distance, isolation-by-resistance and isolation-by-barrier**

188 Nearest neighbour mating, the standard process driving genetic differentiation among organisms and
189 leading to patterns of isolation-by-distance *IBD* (Broquet et al. 2006), and landscape fragmentation,
190 leading to patterns such as isolation-by-barrier *IBB* or isolation-by-resistance *IBR*, may occur
191 simultaneously. It is therefore of crucial importance to test for *IBD* prior to any other analysis (Schwartz
192 and McKelvey 2009). We performed spatial autocorrelation analyses with non-directional Mantel
193 correlograms (Smouse and Peakall 1999; Borcard and Legendre 2012) to determine the scale *S* at which
194 *IBD* patterns occur in each dataset (Epperson 2003). For this purpose, we computed inter-individual
195 pairwise genetic distances using the Bray-Curtis percentage dissimilarity measure as described in
196 Cushman et al. (2006). Euclidean distance classes were defined every 3000 m (up to 30 km), resulting in
197 10 binary matrices representing the membership of individuals to the distance class being tested (with 0
198 for pairs of individuals belonging to the same distance class and 1 otherwise). Each binary matrix was
199 compared to the genetic distance matrix using a simple Mantel test with 1000 permutations. We then
200 plotted Mantel correlation values over distance classes, with a 95% confidence interval determined by
201 bootstrap resampling (1000 iterations).

202 The two LTIs were expected to act as barriers to gene flow, and the spatial individual-based
203 sampling scheme was specifically designed to test this hypothesis. We thus tried to detect such a signal
204 with *a priori* knowledge on the location of each putative barrier, using correlative analyses and spatial
205 subsets of datasets *M* and *F* with selected genotypes located at least *S* km from each LTI. This targeted
206 sampling scheme was designed to avoid large gaps between sampled points and putative barriers to gene
207 flow (Anderson et al. 2010; Prunier et al. 2013). This procedure resulted in the design of four new
208 datasets: M_{AG} , F_{AG} , $M_{LGV-PSE}$ and $F_{LGV-PSE}$. For each dataset, we computed a pairwise genetic distance
209 matrix using the Bray-Curtis percentage dissimilarity measure. The use of inter-individual measures of
210 genetic relatedness is expected to substantially enhance the detection of recent genetic patterns when
211 compared to the use of conventional inter-population genetic distances, as allelic frequencies may suffer
212 from a loss of resolution due to the averaging of genetic information at the population level (Landguth et
213 al. 2010; Prunier et al. 2013): occurrence of alleles in individual genotypes may actually evolve at higher
214 rates than allelic frequencies. For each dataset, we also computed a pairwise Euclidean distance (*IBD*)
215 matrix and a pairwise *IBB* distance matrix, coded with 0 when two individuals were located on the same

216 side of a barrier, or 1 when they were separated by a barrier (Epps et al. 2005). *IBD* being regarded as the
217 null hypothesis, competing hypotheses can also be proposed to determine whether genetic structure may
218 be influenced by additional landscape features (e.g. Cushman et al. 2006; Goldberg and Waits 2010), as
219 controlling for *IBR* may indeed reveal LTI influences otherwise undetected. For each dataset, we thus
220 computed three pairwise *IBR* distance matrices based on the potential influence of slope (IBR_{slope} ;
221 Murphy et al. 2010; Emel and Storfer 2012), land cover (IBR_{LC} ; Spear et al. 2005; Goldberg and Waits
222 2010) or a combination of these two features ($IBR_{slopeLC}$). Slope, measured in percent and ranging from 0
223 to 72% in the study area, was derived from a 50-m SRTM-Digital Elevation Model (Shuttle Radar
224 Topography Mission). Wood patches and urbanized areas, respectively known to improve and to impede
225 gene flow in the alpine newt (Emaresi et al. 2011), were extracted from national maps (BD Topo from
226 National Geographic Institute, France, 1/25 000). We used ARCGIS 9.3 and its extension SPATIAL
227 ANALYST to manage and rasterize these features. We then used the MATLAB software coding
228 environment (Mathworks, Inc.) to compute least-cost-paths between pairwise individuals (Adriaensen et
229 al. 2003) over three distinct resistance rasters representing: (1) landscape resistance due to slope (IBR_{slope})
230 with grid cell values parameterized according to a linear function ($y = (2/72)x + 1$) and ranging from
231 1 to 3; (2) landscape resistance due to land cover (IBR_{LC}) with grid cell values set to 1 for wooded
232 patches, 3 for urbanized areas and 2 otherwise; (3) landscape resistance due to a combination of these two
233 features ($IBR_{slopeLC}$) and obtained by addition. Using simple and partial Mantel tests in a causal modeling
234 framework (Cushman et al. 2006; Cushman et al. 2013), we first tested for *IBD* by comparing each
235 pairwise genetic distance matrix to the corresponding Euclidean distance *IBD* matrix. We then tested for
236 *IBR* by comparing each pairwise genetic distance matrix to the corresponding IBR_{slope} , IBR_{LC} and
237 $IBR_{slopeLC}$ distance matrices after controlling for the effect of the *IBD* matrix. Finally, we tested for *IBB* by
238 comparing each pairwise genetic distance matrix to the corresponding *IBB* distance matrix after
239 controlling for the effect of the *IBD* or the *IBR* distance matrices. Given the objective of our study and the
240 current controversy regarding the use of Mantel tests in model selection (Graves et al. 2013; Guillot and
241 Rousset 2013), *IBR* matrices were not used for the selection of alternative scenarios but only to improve
242 barrier detection.

243 All Mantel tests were performed with 1000 standard permutations using the MATLAB software
244 coding environment (Mathworks, Inc.). All spatial variables (Euclidean distances and least-cost-paths)

245 were log-transformed following the $D=\ln(d+1)$ formula and standardized to meet linearity assumptions.
246 In order to ensure that *IBB* detection power was not restricted by a limited number of genotypes, we also
247 performed the same analyses with both males and females located at less than *S* km from each LTI using
248 Mantel tests with 1000 restricted permutations (Prunier et al. 2013).

249

250 **Genetic structure analyses**

251 The combination of overlay clustering methods with correlative analyses proved to be particularly
252 powerful in detecting recent barriers to gene flow (Landguth et al. 2010; Safner et al. 2011; Blair et al.
253 2012). Bayesian clustering algorithms were successfully used in several studies aiming at detecting
254 barriers to dispersal (e.g. Frantz et al. 2012; Hepenstrick et al. 2012). We used TESS 2.3.1 (Chen et al.
255 2007), a spatial Bayesian clustering method, to identify clusters of individuals. This program computes
256 probabilities that each individual genotype originates from one of *K* panmictic populations. Accounting
257 for both the putative barrier effect of LTIs segregating the study area in four distinct sectors (Fig. 1a) and
258 the extent of our study area, we did not expect to detect more than ten distinct genetic clusters (one cluster
259 in sector *D* and three clusters in each sector *A*, *B* or *C*). Genetic data were analyzed using correlated allele
260 frequencies and other parameters were set to default values. We used the admixture CAR model starting
261 from a neighbour-joining tree based on a Delaunay triangulation (Fig. 1c). Preliminary analyses indicated
262 that setting the spatial interaction parameter to its default value (0.6) was well supported by data. To
263 estimate the true number of genetic clusters *K*, we made five runs per *K* for *K* = 2 to 10, with 100000
264 sweeps and a burn-in period of 10000 sweeps. We then identified which value of *K* produced the highest
265 likelihood runs (lowest values of DIC), and made 100 runs for K_{max} , with the same parameters. The 20
266 best results (lowest values of DIC) were averaged with CLUMPP (Jakobsson and Rosenberg 2007). To
267 visualize the results, we used the kriging function provided in the *R*-package 'gstat' (Pebesma 2004; R
268 Development Core Team 2011) to represent the estimated membership of each individual on a grid with a
269 resolution of 500m, following an inverse distance weighted interpolation function.

270 We also performed a spatial principal component analysis (sPCA; Jombart et al. 2008), a spatially-
271 explicit multivariate method using individual genotypes to investigate the spatial patterns of genetic
272 variability. This method, seeking principal components that optimize the variance of individual allelic
273 frequencies while taking spatial autocorrelation of data into account, does not require Hardy-Weinberg or

274 linkage equilibrium: sPCA analyses were thus performed using all 14 loci, including *CopTa7* and
275 *CopTa9*. This explanatory tool provides maps of individual sPCA scores, allowing a visual assessment of
276 the spatial genetic structures. It disentangles global structures, i.e. strong genetic similarity or positive
277 autocorrelation between neighbors, from local ones, i.e. strong genetic differences or negative
278 autocorrelation between neighbors. For each dataset, we used a distance-based neighborhood network
279 with a distance threshold S consistent with Mantel correlograms. A global and a local Monte Carlo test
280 were carried out with 10000 permutations to evaluate the significance of detected patterns (Jombart et al.
281 2008).

282 In addition, we evaluated the presence of sharp genetic boundaries with no *a priori* knowledge on
283 the location of putative barriers using the Monmonier's Maximum Difference Algorithm (Monmonier
284 1973) implemented in AIS (Miller 2005). This tool did not provide additional information about genetic
285 structure in *I. alpestris*: methods and results are thus presented in Appendix A.

286

287 **Simulations**

288 One of the main issues when trying to detect the impact of roads on gene flow is the time-lag
289 between the creation of infrastructures and the ensuing genetic response (Anderson et al. 2010; Landguth
290 et al. 2010). Simulations were thus performed to determine whether the two LTIs were old enough for
291 possible genetic discontinuities to be detected in our study. For this purpose, we used CDPOP (Landguth
292 and Cushman 2010), a spatially explicit, individual-based cost distance genetic program, to simulate
293 realistic gene flow among aggregates over 200 overlapping generations.

294 We considered a total of 450 localities, including the 225 sites where alpine newts were actually
295 sampled along with 225 additional fictive sites (Fig. 1a). Resulting densities of simulated sites were four
296 to five times lower than those encountered in the field, but were optimized according to software memory
297 limitations. Each locality was initiated with 10 individuals and kept at a constant size over generations.
298 This low number of individuals per aggregate is realistic when high densities of breeding sites are
299 available. For instance, only six aquatic sites out of 72 allowed the capture of more than 20 individuals in
300 a previous study in Bourgogne (Prunier et al. 2013). The age structure was defined according to a normal
301 distribution with a mean $\mu = 0$ and a standard deviation $\sigma = 5$ so that the maximal longevity of individuals
302 was 18 years (Appendix B). Age at maturity was fixed at three year-old (Miaud et al. 2000) and both

303 males and females were allowed to mate with replacement (Garner and Schmidt 2003; Hoeck and Garner
304 2007). The simulated number of offspring per mating event was drawn from a Poisson distribution with
305 mean $\lambda = 1$, resulting in an average of 4.2 offspring per female and per generation. Although below the
306 reported number of eggs laid by alpine newt females during a single breeding season (between 200 and
307 300 eggs; Osikowski 2007), this parameter avoided an overproduction of offspring in regard to the total
308 number of available localities at each generation. Offspring sex was randomly assigned following a
309 binomial distribution and an unbiased sex ratio. Genetic polymorphism was defined according to real
310 genetic data, with 12 microsatellite loci and 9 alleles per locus (mean number of alleles per locus in
311 empirical data: 8.67). Genotypes were randomly assigned at the beginning of simulations and the
312 mutation rate was set to 0.0005, as in Blair et al. (2012).

313 We simulated mating and dispersal movements so that the resulting scale of *IBD* matches with the
314 observed scale *S* of *IBD* in empirical datasets. According to preliminary analyses using non-directional
315 Mantel correlograms as described above, travelled distances were drawn from a probability distribution
316 inversely proportional to a linear function, with the maximal dispersal cost distance that may be travelled
317 (associated with a null probability; Landguth and Cushman 2010) set to 3000 m (Fig. 2c).

318 Cost distances were based on Euclidean distances between pairwise aggregates. We investigated the
319 time-lag between the creation of both LTIs and the ensuing simulated genetic response by respectively
320 placing the A6 highway and the LGV-PSE railway as impermeable barriers to dispersal at generations
321 100 (thus corresponding to year 1969) and 112 (thus corresponding to year 1981). We replicated the
322 simulation 10 times to account for demographic stochasticity.

323 For each replicate, individual genotypes were collected from generation 100 to 200 with an
324 increment of 5. Using only the 225 aggregates corresponding to real aquatic sites in the empirical dataset,
325 we randomly subsampled two individuals per aggregate and computed pairwise genetic distances using
326 the Bray-Curtis percentage dissimilarity measure, as described above. We repeated this subsampling
327 procedure 10 times to account for sampling stochasticity, resulting in a total of 100 genetic matrices per
328 generation. We then used partial Mantel tests with 1000 restricted permutations (Prunier et al. 2013) to
329 test for the effects of the highway and the railway while controlling for *IBD*. Mantel correlations were
330 averaged across all replicates and all subsampling schemes. We finally plotted the averaged Mantel
331 correlation values over generations, with a 95% confidence interval determined by bootstrap resampling

332 (1000 iterations). We considered that a barrier effect was detectable when at least 95 % of partial Mantel
333 tests were significant (P-value ≤ 0.05).

334

335 **Results**

336 **Simulations**

337 Simulations were performed with 3000 m as the maximal distance that may be travelled by
338 juveniles for dispersal and adults for mating. On average, this parameter led to dispersal and mating
339 movements of 1238 m long (± 1000 m) and to annual mean dispersal rates of 14.5% (1.45 dispersal
340 events per year and per population). Mantel correlograms showed significant genetic relatedness between
341 pairwise individuals for the first 12 km (first 4 bins), and either no or negative autocorrelation as distance
342 increased (Fig. 2c). When simulated as totally impermeable, the A6 highway and the LGV-PSE railway
343 were respectively detected as significant barriers to gene flow after 35 and 58 years, that is approximately
344 11 and 19 generations (Fig. 5). Interestingly, time-lag before detection of a significant barrier effect was
345 longer for the railway than the highway, suggesting that the spatial distribution of sampling points
346 directly influences the detection power of genetic discontinuities in correlative analyses. According to
347 these results, only the highway was old enough for a significant barrier effect to be detected in our study
348 (Fig. 5).

349

350 **Genetic data**

351 A total of 417 individuals (206 Males, dataset *M*, and 211 females, dataset *F*) were sampled in the study
352 area. Both a male and a female were captured in 192 ponds, from a total of 225 ponds where *I. alpestris*
353 was detected. The mean number of genotypes per site was 1.85. The genotyping error rate, estimated by
354 blind replications as the mean error rate per locus e_l , was less than 2.4 % in *CopTa1*, *CopTa4*, *CopTa14*
355 and *Ta1Caga4*, and 0% for the 10 other loci.

356

357 **Isolation-by-distance, isolation-by-resistance and isolation-by-barrier**

358 In both empirical datasets, Mantel correlograms showed significant genetic relatedness between
359 pairwise individuals for the first 12 km (first 4 bins), and either no or negative autocorrelation as distance

360 increased (Fig. 2a-b), as in simulated data (Fig. 2c). These results indicate the existence of a significant
361 *IBD* process over the study area. However, correlation values in the first distance classes were not as high
362 and did not show any regular decrease as in simulated data. This may be due to the presence of higher
363 densities of aquatic sites in the study area than simulated in CDPOP, allowing a better spread of alleles at
364 close distances. The spatial scale of genetic autocorrelation ($S = 12$ km) was similar in males and females.
365 Selecting individuals located at less than $S = 12$ km from each infrastructure allowed the design of four
366 new datasets (M_{A6} , F_{A6} , $M_{LGV-PSE}$ and $F_{LGV-PSE}$) with at least 153 genotypes each (Table 1). A significant
367 *IBD* was detected in each dataset (Table 1). Except in M_{A6} dataset, in which case the effect of land cover
368 as described in Emaresi et al. (2011) was close to significance, neither *IBR* nor *IBB* was ever detected
369 whatever the dataset or the considered *IBR* scenario (Table 1). In the case of *IBB* detection, Mantel
370 correlation values were always negative in M_{A6} and F_{A6} datasets and positive in $M_{LGV-PSE}$ and $F_{LGV-PSE}$
371 datasets. The same results were obtained when using both males and females data (data not shown).

372

373 **Genetic structure**

374 When the analyses were performed in males or in females separately, TESS systematically failed
375 to detect any genetic structure (data not shown), which may be explained by the low number of genotypes
376 in each dataset. We thus combined males and females data. The best estimate of the number of clusters
377 using the averaged DIC criterion over the five runs performed per K was 3 (Fig. 3a). However, runs
378 performed for $K_{max} = 3$ showed high variability in DIC values. Furthermore, for $K_{max} > 2$, the posterior
379 estimates of cluster membership for each individual systematically displayed only one or two clusters, the
380 additional clusters containing negligible proportions. We thus performed 100 runs with $K_{max} = 2$. This
381 number of inferred clusters allowed the use of a simple kriging interpolation function. The 20 best runs
382 averaged using CLUMPP led to the progressive discrimination of individuals in two clusters (Fig. 3b):
383 according to figure 3c, individuals with an estimated membership in cluster 1 higher than 40% were
384 located in *Terre-Plaine*, while those with a lower estimated membership were located in *Le Morvan* and
385 *L'Auxois*. Although most individuals had high probability of assignment to one cluster or the other, high
386 levels of admixture were encountered at the interface of these two clusters, confirming the existence of an
387 *IBD* pattern. The detected boundary was not related to the presence of LTIs.

388 All global Monte-Carlo tests performed in sPCA were significant (dataset *M*, $\max(t) = 0.0109$, $p <$
389 10^4 ; dataset *F*, $\max(t) = 0.011$, $p < 10^4$), indicating the presence of significant global genetic structures in
390 all datasets. On the contrary, local Monte-Carlo tests did not detect any significant local structure (dataset
391 *M*, $\max(t) = 0.007$, *NS*; dataset *F*, $\max(t) = 0.006$, *NS*). The analyses revealed four kinds of global genetic
392 patterns (Fig. 4e). In both datasets, scores of individuals along the first sPCA axis distinguished *Terre-*
393 *Plaine* from the rest of the study area, as did TESS (Fig. 4a, Boundary *I*). A second pattern was revealed
394 by second sPCA scores in dataset *M* (Fig. 4b): this structure clearly segregated individuals located in *Le*
395 *Morvan* from the rest of the study area (Boundary *II*); a third group, identified in the west of *Terre-Plaine*
396 (Boundary *III*) corresponded to the westernmost water catchment in *Terre-Plaine*, although further
397 analyses, performed over a more extended study area, may be required to confirm this pattern. Scores of
398 females along the second sPCA axis (dataset *F*) revealed a slightly different pattern in the south, with
399 higher genetic similarity among individuals located in southern hilly landscapes from *Le Morvan* and
400 *L'Auxois* (Fig. 4c, southern white squares, Boundary *IV*). Finally, third sPCA scores in females (Fig. 4d)
401 displayed a pattern highly similar to the one obtained from the second sPCA scores in dataset *M*
402 (Boundary *II*). However, females from *Terre-Plaine* located next to the highway showed higher scores
403 than females located more distantly (area delimited with white dashes), indicating higher genetic
404 similarity among individuals in the direct vicinity of the highway. Boundaries *II* and *IV* in females were
405 also identified as sharp boundaries by Monmonier's algorithm (see Appendix A). LTIs never separated
406 inferred groups.

407

408

409 Discussion

410 Our study was based on an individual-based targeted sampling scheme in a highly mobile species
411 and was thus designed to optimize barrier detection. However, while simulations provided good insight
412 into the temporal scale before barrier detection in our study and suggested that the A6 highway was old
413 enough for a significant barrier effect to be detected, inferred spatial genetic patterns did not support this
414 hypothesis. Indeed, the LTIs were never detected as barriers to gene flow, observed genetic boundaries
415 rather coinciding with transition zones between major landscape entities.

416 Simulations were based on parameters reflecting the alpine newt life history and led to realistic
417 *IBD* patterns in simulated data, with dispersal rates and travelled distances in the range of empirical
418 observations reported in the literature (Joly and Grolet 1996; Perret et al. 2003; Schmidt et al. 2006;
419 Kopecky et al. 2010; Fig. 2c). Despite the use of overlapping generations, both simulated LTIs were
420 detected as significant barriers to gene flow in less than 20 generations, as already observed in Landguth
421 et al. (2010) with correlative analyses (Fig. 5). However, considering the time-lag between the creation of
422 LTIs and the beginning of genetic sampling, the LGV-PSE high-speed railway was probably too recent
423 for a significant barrier effect to be detected in our study.

424 A significant *IBD* pattern was detected in both sexes in empirical data, with higher genetic
425 relatedness among individuals less than 12 km apart. The detection of this long-distance *IBD* pattern in *I.*
426 *alpestris* is consistent with previous studies (Perret et al. 2003; Pabijan and Babik 2006; Emaresi et al.
427 2011; Prunier et al. 2013) and suggests that genes spread out according to a ‘stepping-stone’ model
428 (Kimura and Weiss 1964), high densities of aquatic sites in the study area allowing alpine newts to
429 perform several discrete dispersal movements between neighbouring ponds throughout their lifetime
430 (Semlitsch 2008). This pattern also reinforced the expectation of significant barrier detection in this
431 species. Highly nomadic individuals may actually encounter roads at higher rates than less mobile ones,
432 thus possibly shortening the period of time until barrier detection (Landguth et al. 2010; Blair et al. 2012;
433 Prunier et al. 2013). Indeed, simulations showed that the A6 highway may have been detected as a total
434 barrier to gene flow in no more than 35 years (approximately 11 generations), despite high longevity and
435 overlapping generations in the alpine newt. However, this LTI was never detected as a barrier to gene
436 flow, whatever the approach.

437 Four main genetic boundaries were detected. The major genetic boundary (*I*), detected with the
438 sPCA (Fig. 4a) as well as with TESS (Fig. 3), explained most of the spatial and genetic variability in all
439 datasets (Fig. 4e). Boundaries *II* and *IV* (Fig. 4b, c and d) were not detected by TESS but were identified
440 as sharp boundaries by Monmonier’s algorithm applied to females (see Appendix A). These patterns
441 coincided with transition zones between major landscape entities in the study area (Fig. 1b and Fig. 4),
442 while the A6 highway and the LGV-PSE high-speed railway were always embedded in inferred clusters.
443 Boundaries *I* and *II* respectively distinguished *Terre-Plaine* and *Le Morvan* as two distinct homogeneous
444 clusters. In females, boundary *IV* assigned individuals located in southern higher elevation areas from *Le*

445 *Morvan* and *L'Auxois* to the same group. The observed genetic patterns may be explained by *IBD*, along
446 with subtle modifications in landscape characteristics such as elevation (Boundaries *II* and *IV*), land use
447 (Boundaries *I*, *II* and *IV*), proportions of wooded patches (Boundary *II*), hydrologic networks (Boundary
448 *III*), or most probably a combination of these factors. This complexity, as well as the use of a targeted
449 sampling scheme designed to specifically assess the influence of LTIs on gene flow (Prunier et al. 2013),
450 may explain why *IBR* scenarios, based on simple hypotheses regarding the influence of landscape on gene
451 flow, were not better supported than *IBD* scenario. More complex scenarios and alternative statistical
452 approaches (Selkoe et al. 2010; Van Strien et al. 2012) may be needed to confirm the observed genetic
453 structure and identify all its possible drivers. Nevertheless, controlling for *IBD* or *IBR* did not improve
454 *IBB* detection.

455 The absence of any detectable barrier effect may be first explained by a lack of statistical power
456 due to the use of too few (or too little variable) microsatellite markers (Landguth et al. 2012). However,
457 simulation results suggested that this number of markers was not a limiting factor in our case. A second
458 explanation may be the time-lag between the processes that caused the formation of spatial genetic
459 structure and the observed spatial genetic structure itself (Landguth et al. 2010). This time-lag may be
460 lengthened by the presence of large effective population sizes on each side of infrastructures (Gauffre et
461 al. 2008), as populations separated by a barrier may still be functionally connected with populations in
462 their hinterland, thus rendering genetic barrier effects difficult to detect. Our study was precisely designed
463 to reduce this time-lag: 1) by considering a species with relatively long range mating and dispersal
464 (Landguth et al. 2010), 2) by using an individual-based sampling scheme and by computing genetic
465 distances based on inter-individual measures of genetic relatedness rather than on conventional inter-
466 population measures of genetic differentiation (Landguth et al. 2010; Prunier et al. 2013), and 3) by using
467 a targeted sampling design so as to avoid large gaps between sampled points and putative barriers to gene
468 flow (Anderson et al. 2010; Prunier et al. 2013). Our simulation results, showing that no more than 35
469 years would be necessary for the A6 highway to be detected as totally impermeable to dispersal (Fig. 5)
470 despite the simulation of gene flow among 450 highly connected fictive aggregates (Fig. 1a), suggest
471 indeed that our study was not susceptible to this issue, at least in the case of the A6 highway.

472 Neither simulations nor empirical results allowed us to conclude about the possible barrier effect
473 of the railway (although field observations suggested that newts may be able to move underneath rail

474 tracks). But interestingly, although both LTIs were similarly simulated as total barriers to gene flow, 58
475 years (approximately 19 generations) were necessary for the detection of the railway as a barrier, as
476 opposed to 35 years in the case of the highway (Fig. 5). This result suggests that the spatial distribution of
477 aggregates in the vicinity of LTIs, and possibly the layout of LTIs itself, may directly influence the time-
478 lag before barrier detection, highlighting the need for a case-by-case study of roads effects on wildlife.

479 Contrary to the LGV-PSE high-speed railway, several clues apart from simulations allow us to
480 reasonably assume that the A6 highway may simply be permeable to gene flow. Firstly, investigating the
481 barrier effect of the A6 highway using correlative analyses led to systematic negative Mantel correlations
482 (Table 1). This pattern may need further investigations to be fully explained, although such negative
483 Mantel correlations were already observed between independent variables in Dutilleul et al. (2000). In
484 any case, observed correlations in *IBB* analyses were far from being positive, suggesting that transverse
485 gene flow was definitely not affected by the A6 highway. Secondly, the detection of a longitudinal
486 genetic pattern in sPCA results, with higher genetic similarity among females located in the direct vicinity
487 of the highway (Figure 4d), suggests that this infrastructure is not only permeable to gene flow, but also
488 that it may serve as a longitudinal dispersal corridor in a low-quality matrix such as *Terre-Plaine*,
489 characterized by high proportions of fragmented wooded patches and cultivated crops (Fig. 1a; Meunier
490 et al. 1999; Tikka et al. 2001). This counterintuitive genetic pattern was only detected with the third sPCA
491 axis, suggesting that it may be the result of recent landscape changes when compared to the main genetic
492 structure coinciding with major historical landscape entities. Direct approaches such as telemetry or
493 capture-mark-recapture protocols will be necessary to confirm this hypothesis. However, this pattern is
494 consistent with sex-biased dispersal abilities observed in the alpine newt: females being less mobile than
495 males at the juvenile stage (Joly and Grolet 1996; Perret et al. 2003), they may benefit from the existence
496 of both evenly-spaced transverse passages (e.g. culverts, extended stream crossings or dirt roads;
497 Lesbarreres et al. 2004; Lesbarreres and Fahrig 2012) and favorable roadside structures (e.g. ditches for
498 rainwater drainage or stormwater retention ponds; Le Viol et al. 2012) to disperse through and along the
499 highway. This phenomenon may be facilitated by the water filling of such structures during rainfall
500 events (Schalk and Lühring 2010), notably in sectors that are highly resistant to movements such as
501 agricultural plains (Joly et al. 2001; Janin et al. 2009; Cosentino et al. 2011).

502 Roads are usually considered as barriers to the mobility of native species (Epps et al. 2005;
503 Hopenstrick et al. 2012) and corridors for alien ones (Trombulak and Frissell 2000; Tikka et al. 2001;
504 Von der Lippe and Kowarik 2007). For instance, drainage ditches along highways may provide suitable
505 conditions for the establishment and the spread of aquatic invaders such as the common reed *Phragmites*
506 *australis* in North America (Jodoin et al. 2008; Brisson et al. 2010). Road verges may also facilitate
507 dispersal in ground-dwelling exotic species such as the cane toad *Bufo marinus* in Australia (Brown et al.
508 2006). However, roads may also facilitate dispersal of native species, as recently shown in the lizard
509 *Gallotia galloti* in Canari islands (Garcia et al. 2007). Although we still have to demonstrate that
510 transverse and roadside structures are effectively used by dispersing individuals, our results revealing that
511 the alpine newt is not affected by and may even benefit from the presence of the A6 highway suggest that
512 LTIs do not necessarily lead to systematic conservation issues, as an increase in dispersal abilities of
513 native species is a key parameter in the context of landscape fragmentation and climate change (Bonte et
514 al. 2012). However, these results are specific to the highway under study, as any road in a different
515 environmental context may hinder genetic exchanges in the alpine newt (e.g. Van Buskirk 2012):
516 Considering local interactions between species, infrastructures and landscape-specific characteristics
517 proves to be essential for a better understanding of the potential impacts of LTIs on movement patterns in
518 terrestrial organisms (Balkenhol and Waits 2009; Knapp et al. 2013). Further investigations are thus
519 required to define species-specific benchmarks in terms of permeability of road structures (and road
520 design) to movements (Mata et al. 2008; Woltz et al. 2008; Lesbarreres and Fahrig 2012), and to clarify
521 the impact of land use conversion in the vicinity of various LTIs (or sections of LTIs).

522

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533

534 **Electronic supplementary material**

535 Supplementary data associated with this article can be found in the online version.

536

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757

758 **Table 1. Correlative analyses on pairwise data.** Simple and partial Mantel correlations (r) between
759 pairwise genetic distance matrices (G) and various effective distance matrices among males and females
760 located at less than $S = 12\text{km}$ from the A6 highway (M_{A6} and F_{A6}) or from the LGV-PSE high-speed
761 railway ($M_{LGV-PSE}$ and $F_{LGV-PSE}$). Effective distance matrices were based on the null model of isolation-by-
762 distance (IBD), on the model of isolation-by-barrier (IBB), and on three models of isolation-by-resistance
763 according to the putative effects of slope (IBR_{slope}), land cover (IBR_{LC}) or both ($IBR_{slopeLC}$). Significant
764 Mantel correlations at $\alpha = 0.05$ are in bold.

Dataset	n^a	Investigated pattern	Mantel test ^b	Mantel correlation r	P-value		
M_{A6}	153	IBD	$G \sim IBD$	0.0893	0		
			$G \sim IBR_{slope} / IBD$	-0.0066	0.5854		
			$G \sim IBR_{LC} / IBD$	0.0534	0.0569		
		IBR	$G \sim IBR_{slopeLC} / IBD$	0.0089	0.3896		
			$G \sim IBB / IBD$	-0.0388	0.962		
			IBB	$G \sim IBB / IBR_{slope}$	-0.0421	0.981	
		$G \sim IBB / IBR_{LC}$		-0.0379	0.965		
		$G \sim IBB / IBR_{slopeLC}$		-0.041	0.977		
		F_{A6}	154	IBD	$G \sim IBD$	0.0725	0
					$G \sim IBR_{slope} / IBD$	-0.0178	0.7213
					$G \sim IBR_{LC} / IBD$	0.0069	0.4146
				IBR	$G \sim IBR_{slopeLC} / IBD$	-0.0099	0.6024
$G \sim IBB / IBD$	-0.0285				0.9351		
IBB	$G \sim IBB / IBR_{slope}$				-0.0304	0.959	
	$G \sim IBB / IBR_{LC}$			-0.0277	0.9291		
	$G \sim IBB / IBR_{slopeLC}$			-0.0298	0.957		
$M_{LGV-PSE}$	160			IBD	$G \sim IBD$	0.0441	0.009
					$G \sim IBR_{slope} / IBD$	-0.0037	0.5335
					$G \sim IBR_{LC} / IBD$	-0.0051	0.5794
				IBR	$G \sim IBR_{slopeLC} / IBD$	-0.0076	0.5804
		$G \sim IBB / IBD$	0.0119		0.2697		
		IBB	$G \sim IBB / IBR_{slope}$		0.0128	0.2258	
			$G \sim IBB / IBR_{LC}$	0.0116	0.2727		
			$G \sim IBB / IBR_{slopeLC}$	0.0126	0.2418		
		$F_{LGV-PSE}$	162	IBD	$G \sim IBD$	0.0572	0.003
					$G \sim IBR_{slope} / IBD$	-0.012	0.6533
					$G \sim IBR_{LC} / IBD$	0.0068	0.4046
				IBR	$G \sim IBR_{slopeLC} / IBD$	0.0046	0.4565
$G \sim IBB / IBD$	0.0188				0.1528		
IBB	$G \sim IBB / IBR_{slope}$				0.02	0.1369	
	$G \sim IBB / IBR_{LC}$			0.0185	0.1648		
	$G \sim IBB / IBR_{slopeLC}$			0.0198	0.1189		

765

766 ^a Number n of genotypes in dataset.
767 ^b $G \sim A$ corresponds to a simple Mantel test between the genetic distance matrix G and the matrix A ; $G \sim$
768 A/B corresponds to a partial Mantel test between the genetic distance matrix G and the matrix A
769 partialling out the matrix B . Mantel tests were performed with 1000 restricted permutations.
770

771 **Fig. 1** Characteristics of the study area and sampling scheme in Bourgogne (France). The A6 divided
772 highway and the LGV-PSE highspeed railway are in thick and dotted thick lines respectively. (a)
773 Localisation of the 225 ponds where alpine newts were sampled, of the 225 additional fictive sites used in
774 CDPOP simulations and of main habitat features in the study area. The intersection of the two
775 infrastructures delimits four distinct sectors (A, B, C and D). (b) Relief and main landscape entities in the
776 study area. Western and eastern geologic depressions in *L'Auxois* are respectively numbered 1 and 2.
777

778 **Fig. 2** Mantel correlograms showing the relationships between inter-individual genetic distances and
779 Euclidean distance classes (defined every 3000 m) in (a) males, (b) females and (c) one of the ten
780 simulated datasets at generation 100. r : standard Mantel correlation with 1000 permutations. Error bars
781 bound the 95% confidence interval about r as determined by bootstrap resampling. Upper and lower
782 confidence limits (dotted line) bound the 95% confidence interval about the null hypothesis of no spatial
783 structure as determined by permutation.

784 *: p -value < 0.05; **: p -value < 0.01; ***: p -value < 0.001.

785

786 **Fig. 3** Spatial genetic structures inferred from TESS. (a) Estimates of the true number K of clusters using
787 the DIC criterion averaged over each five runs (mean \pm SD). (b) Estimated membership (y-axis) of each
788 individual (x-axis) in clusters 1 and 2. The 40 % threshold (dotted line) approximately delimits
789 individuals from cluster 1 (*Terre-Plaine*) according to figure 3c. (c) Spatial distribution of clusters 1 and 2
790 in the study area. Black dots stand for the sampling points. Contour lines stand for the interpolated
791 membership of individuals in cluster 1, using a kriging function.

792

793 **Fig. 4** Analyses of alpine newt data using sPCA. Large white and black squares stand for highly negative
794 and positive scores respectively. Small squares stand for low sPCA scores. White arrows and
795 corresponding roman numerals indicate various inferred boundaries (see text for details). (a) Map of the
796 first global sPCA scores in dataset M ; analyses led to highly similar patterns in dataset F (data not
797 shown). (b) Map of the second global sPCA scores in dataset M ; (c) Map of the second global sPCA
798 scores in dataset F . (d) Map of the third global sPCA scores in dataset F ; white dashes delimit an area in
799 which females located in the direct vicinity of the highway show high genetic similarity. (e) Screeplots of

800 sPCA eigenvalues in datasets *M* and *F*; retained structures are filled in black; letters in italics refer to
801 maps a to d.

802

803 **Fig. 5** Detection of the A6 highway (circles) and the LGV-PSE high-speed railway (diamonds) simulated
804 as total barriers to gene flow, using partial Mantel tests with 1000 restricted permutations in 100
805 simulated datasets (see text for details). Analyses were performed from the placement of each
806 infrastructure to the generation 200. The placement of the fictive highway and railway occurred
807 respectively at generations 100 (standing for the year 1969) and 112 (standing for year 1981), while the
808 real empirical sampling occurred at generation 141 (year 2010). Black arrows indicate for each
809 infrastructure the elapsed time until first significant barrier detection ($\alpha = 0.05$). *r*: partial Mantel
810 correlation values. Black symbols: significant partial Mantel tests at $\alpha = 0.05$. Gray symbols: significant
811 partial Mantel tests at $\alpha = 0.1$. Error bars bound the 95% confidence interval about *r* as determined by
812 bootstrap resampling.

813

814