Conservation genetics of a wide-ranged temperate snake: same species, different locations, and different behaviour

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Abstract
Even though reptiles are threatened worldwide, few studies address their conservation, especially snakes. The goal of our study was to measure the genetic structure of a widely distributed temperate reptile, the smooth snake *Coronella austriaca* using microsatellite markers in two different areas at the core (Alsace, north-eastern France) and at the edge (Wallonia, southern Belgium) of its range. We sampled 506 individuals in 38 localities (respectively 10 and 28). Analysis of genetic structure conducted with a clustering method detected three clusters in Alsace, one group gathering all populations but two. In Wallonia, differentiation was observed on both sides of the Meuse River and in the Southern Ardenne region (southernmost sampling sites). Spatial autocorrelation analysis showed that statistically more related individuals occur together up to a distance of 2.8 km in Alsace and up to 10 km in Wallonia. Isolation by distance was detected in Wallonia but the distance explained a very limited part of the differentiation (r = 0.033), whereas no isolation-by-distance pattern was detected in Alsace. Even though genetic differentiation between populations separated by large rivers, highways, or crop fields was detected, dispersal between populations seem currently sufficient to avoid any kind of genetic drift in both regions. These results are similar to a previous study conducted in Poland, but strongly contrast with another analysis held in England which detected a sharp genetic structuring among populations that are geographically close. We consequently suggest that discrepancies could be related to the ecology of island populations and smaller densities.

Keywords Ectotherms · Population genetics · Gene flow · Connectivity · Fragmentation · Reptile conservation

Introduction
In the scope of the current biodiversity crisis, most studies on animal conservation focus on rare and highly threatened species (Foden et al. 2013; Wagner et al. 2014; Howard et al. 2020; Loiseau et al. 2020). Still, common and widespread species can also be under threat, especially populations at the edge of the range, and consequently, they should not be overlooked in conservation studies (Kyek et al. 2017; Sánchez-Bayo and Wyckhuys 2019; Rosenberg et al. 2019). For example, investigating how populations of widespread species react to changes induced by human activities on their environment such as habitat fragmentation, pollution, introduction of pathogens, might be crucial in identifying populations under threat and therefore formulate relevant conservation practices (Gill et al. 2007; Dudaniec et al. 2012; Peterman et al. 2013; Orme et al. 2019; Weihmann et al. 2019). One of the key aspects of understanding the potential of adaptation to changes in the environment is genetic diversity (Pauls et al. 2013). It has been shown for example that
populations with higher genetic diversity can better adapt to changes in the environment than populations with lower genetic diversity (Desvars-Larrive et al. 2017). Populations at the edge of their range are likely to have a reduced genetic diversity, and therefore are more prone to decline (Bohme et al. 2007; Taubmann et al. 2011). In an ever and fast changing world, including common species in the conservation considerations becomes increasingly important, as some can rapidly become endangered especially at the edge of their range (Carrier and Beebee 2003; Gill et al. 2007; Studds et al. 2017; Kyek et al. 2017; Reading and Jofré 2020).

According to the IUCN Red List, one of the major threats to terrestrial wildlife is the destruction of habitats (Pimm et al. 2014; IUCN 2020), which induces fragmentation and loss of connection among populations. Small vertebrates such as reptiles are particularly sensitive to such disturbances, and it has been shown that some populations of common species exposed to fragmentation can become severely threatened (Driscoll 2004; Guiller 2009).

Non-avian reptiles face declines at a global scale (Gibbons et al. 2000). For example, in Europe, about 20% of reptile species are listed in the threatened categories of the IUCN (Cox and Temple 2009). Moreover, several species of snakes are facing decline at a global scale, among which some are found in Europe (Reading et al. 2010). Fragmentation, alteration, and destruction of habitats are highlighted as the main threats to reptile populations in Europe (Corbett 1989; Cox and Temple 2009; Reading et al. 2010) through isolation of populations and rupture in gene flow (Frankham et al. 2002; Dixo et al. 2009). Such processes are often associated with aggravating factors such as reduction of population size, genetic drift, and loss of genetic diversity (Frankham et al. 2002). When populations face such constraints, their adaptation capacities can be highly reduced and they can face a speeding up of the decline process at a local level (Frankham et al. 2002). The cumulative effects of these factors can lead to extinctions of populations (Pounds and Crump 1994; Stuart et al. 2008; Collins and Crump 2009; Ceballos et al. 2015; Zipkin et al. 2020). Few studies have been carried out to assess the conservation status and the genetic structure of populations of snake species (Mullin and Seigel 2009). Still, snakes can be indicators of the level of fragmentation in a given landscape as their poor dispersal abilities render them highly sensitive to loss of connexion and reduction of habitat sizes (Madsen et al. 1996; Bonnet et al. 1999; Guicking et al. 2004; Guiller and Legentilhomme 2006; Mullin and Seigel 2009). Moreover, a limited number of studies have been conducted on the same species but at different locations to determine if populations react differently in different habitats or if their dispersal behaviour is fixed (Lane and Shine 2011). Consequently, evaluating the genetic structure within different regions in the same species may provide valuable information on the biology of the species, but also on how species behave in different environments.

For these reasons, we decided to examine an elusive species with a wide range in Europe, the smooth snake, Coronella austriaca. Its distribution is rather well defined (Sillero et al. 2014), and a recent study suggest that the widely distributed taxon currently assigned to Coronella austriaca throughout Europe, western Asia and the Middle East might in fact be a complex of species with narrower distributions (Jablonski et al. 2019). Our main goal was to assess the magnitude of fragmentation on Coronella austriaca in two mainland environments with similar population densities but in different locations, within the core of the range of the species (Alsace, north-eastern France), and at the northern edge of the range (Wallonia, southern Belgium). We assessed the genetic relations of different population patches distributed throughout Alsace and Wallonia using microsatellite markers and evaluate if genetic isolation is clear between proximate populations. We predicted that as the smooth snake seems to be a poor disperser (Völlk and Käsewieter 2003; Pernetta et al. 2011; Dick and Mebert 2017) and as it occurs in fragmented landscapes, genetic structure should be marked even between geographically close populations due to low dispersal possibilities. Also, we expected that even if the habitats are somewhat different, the general pattern of genetic differentiation should be quite similar between regions as they both display a rather dense network of roads and intensive agriculture.

**Material and methods**

**Study area**

The study area was comprised of two densely inhabited (roughly 220 inhabitants per km²) regions within the range of the Western 1 Coronella austriaca clade described by Jablonski et al. (2019): Alsace and Wallonia (Fig. 1). The sampling area in Alsace (north-eastern France) was located between 47°44′ N and 48°3′ N and 7°E and 7°27′ E, at elevations ranging from 200 to 500 m a.s.l (Fig. 1A). The sampling area in Wallonia (southern part of Belgium) covered a territory of about 10,000 km². The latitude was comprised between 49°32′ and 50°38′ N, and the longitude between 4°26′ and 5°93′ E (Fig. 1B). Elevations of the studied habitats ranged from 70 and 400 m a.s.l. Both regions contain several environmental elements that could induce habitat fragmentation, such as networks of motorways, roads, railways, wide surfaces of crop fields and vineyards (Alsace), and large surface of coniferous forest culture (Wallonia).
Sampling design

We collected a total of 506 DNA samples of *Coronella austriaca* throughout the study area in 38 different localities through buccal swabbing (Beebee, 2008; Pidancier et al., 2003). We collected 108 samples from 10 localities in Alsace in 2009 and 2010 (Fig. 1A), and 398 samples in 28 localities in Wallonia between 2009 and 2011 (Fig. 1B). Within both regions, the two closest localities were separated by 2.6 km, and the two most distant were separated by 85 km. Snakes were found either by sight or with the use of artificial shelters (70 cm × 70 cm dark rubber plates in Alsace and of various materials in Wallonia). In order to avoid double sampling, a photograph of the pileus (dorsal side of the head) of each specimen captured was taken on the field and printed in a catalogue that was regularly updated after each field session. This technique enabled individual recognition by eye directly on the field, as no sampling site contained more than 35 individuals (Sauer 1994, 1997).

Laboratory methods

DNA was extracted using the QIAGEN DNeasy Blood & Tissue kit (QIAGEN®). Then, the following microsatellite loci specifically developed for the smooth snake were amplified by PCR: Ca16, Ca19, Ca30, Ca40, Ca43, Ca45, Ca612, Ca63 and Ca66 following the PCR conditions suggested by Bond et al. (2005). Forward dyed primers were used in order to analyse them with an automatic sequencer (AB3130xl Applied Biosystem). Allele lengths were read with the software PEAK SCANNER v.1.0 (Applied Biosystem).

Data analysis

Each locus was examined for null allele occurrence with MICRO-CHECKER v.2.2.3 (Van Oosterhout et al. 2004) for each sampling site and both regions separately. Loci showing a high probability (p > 0.05) of null alleles were discarded. For each retained locus, we estimated allele frequency, observed and expected heterozygosity ($H_O$, $H_E$) with
GeneAlEx v6.503 (Peakall and Smouse 2006). Allelic richness (AR), intrapopulation structuration (FIS) and population differentiation (FST) were evaluated with FSTAT v.2.9.3.2 (Goudet 1995). Genetic comparison between sampling sites were conducted with an ANOVA (with and without locus or population as factor) in R v3.6.3 (R Development Core Team 2016). We also performed a hierarchical structural analysis of genetic diversity (AMOVA) to assess the molecular variance among sampling sites, among individuals and within individuals with GeneAlEx, again for each region separately. We evaluated population subdivision in both region with a Bayesian clustering approach implemented in the software GENELAND v4.0.3 (Guillot et al. 2005). This method can be used to infer the number of genetic clusters (K) from the individual genotype distributed dataset in a spatial framework. We first performed five independent Markov chain Monte Carlo (MCMC) runs with K ranging from 1 to 10 for Alsace and from 1 to 30 for Wallonia, with the following parameters: 500 000 MCMC iterations, 5 000 thinning, maximum rate of Poisson process fixed at 100, uncertainty attached to spatial coordinates fixed to 0.2 km. We ran the MCMC model 100 times with the same parameters, five times first to determine the best K value and the sixth simulation was conducted with the best K value only as suggested by Guillot et al. (2005). From the last simulations, we selected the 10 runs with the highest mean logarithm value of posterior probability and calculated the posterior probability of population membership for each pixel of the spatial domain for each of these 10 runs, using a burnin of 10%. To avoid having two sampling sites in the same pixel, the number of pixels was set to 100 for the x-axis and 350 for the y-axis in Alsace, and 350 and 350 in Wallonia (each pixel corresponds to about 3.5 × 3.5 km). Finally, we computed posterior probability of population membership for each pixel of the spatial domain and the modal population of each individual. We then reran standard population genetic analysis based on the number of populations inferred with GENELAND by calculating pairwise FST and FIS with FSTAT and tested the significance of the inferred structure by performing an AMOVA with GenAlEx.

For each region, we tested isolation by distance (IBD) of sampling sites with a Mantel test (Mantel 1967) between corrected genetic differentiation [FST/(1 – FST)] and the log values of the geographic distances between each sampling site (Rousset 1997). This test was implemented in R with the mantel.rtest function from the ade4 package (Dray and Dufour 2007) and 10 000 repetitions. We combined the results of the IBD for both regions and also from the UK (Pernetta et al. 2011) and from Poland (Sztencel-Jablonka et al. 2015). In addition, we performed a spatial autocorrelation analysis separately for both regions using SPAGeDI v.1.3 (Hardy and Vekemans 2002) in order to determine correlation between geographic distances and pairwise relationship coefficient measured by Moran’s I-statistic (Moran 1950; Sokal and Wartenberg 1983). We assigned geographic coordinates for each locality. Distance classes were chosen in order to provide similar numbers of pairwise comparisons for each class, separately for each region.

**Results**

**Null alleles and genotypic disequilibrium**

We detected the presence of a probable null allele in Alsace for Ca30 (detected in 6/7 sampling sites), due to an excess of homozygosity. Therefore, this locus was discarded from our following analyses that were consequently conducted with eight microsatellites markers (Ca16, Ca19, Ca40, Ca43, Ca45, Ca612, Ca63 and Ca66).

We detected an excess of homozygosity in Wallonia in Ca40 and Ca45, suggesting the presence of null alleles for these two markers. Moreover, genotypic disequilibrium was significant between Ca43 and Ca612, as well as between Ca43 and Ca16. We consequently used only six microsatellite markers (Ca16, Ca19, Ca30, Ca612, Ca63 and Ca66) for this dataset.

**Genetic variation and diversity**

The number of alleles varied from 2 (Ca63) to 17 (Ca40) in Alsace (Table 1) and from 3 (Ca63) and 19 (Ca34) in Wallonia (Table 2). Globally, allelic richness seems higher in Alsace, even if four markers were different between both dataset (Ca30, Ca40, Ca43 and Ca45). The expected heterozygosity was also slightly higher in Alsace, even if the observed heterozygosity is similar. However, AR, HO or HE were not significantly different between regions when considering the five shared loci in both populations, even when considering locus or populations as cofactors (for all p > 0.189).

In Alsace, the genetic differentiation between sampled localities was moderate, with a mean FST value of 0.075 [min. – 0.002 to max. 0.15] (Supplementary Table S1). In Wallonia, genetic differentiation was high, with a mean FST value of 0.014 [min. – 0.044; max.0.234] (Supplementary Table S2). The AMOVA analysis showed that 7% (p = 0.001) of the variance was explained by the differentiation among sampling sites (variation among individuals = 11%, p = 0.001; variation within individuals = 82%, p = 0.001) in Alsace, whereas the respective proportions were 9%, 72% and 19% (all with a p = 0.001) in Wallonia.
**Table 1** Genetic variation across the sampled sites of *Coronella austriaca* in Alsace

| Population | n  | Ar    | $H_O$ | $H_E$ | $F_{ST}$ | $F_{IS}$ |
|------------|----|-------|-------|-------|----------|----------|
| A1         | 6  | 3.35  | 0.54  | 0.56  | 0.100    | 0.044    |
| A2         | 17 | 3.60  | 0.69  | 0.67  | 0.088    | -0.048   |
| A3         | 19 | 3.43  | 0.58  | 0.65  | 0.093    | 0.086    |
| A4         | 14 | 3.46  | 0.64  | 0.69  | 0.066    | 0.072    |
| A5         | 12 | 3.56  | 0.59  | 0.64  | 0.055    | 0.060    |
| A6         | 6  | 3.45  | 0.54  | 0.64  | 0.080    | 0.156    |
| A7         | 8  | 4.00  | 0.64  | 0.73  | 0.042    | 0.114    |
| A8         | 11 | 3.36  | 0.57  | 0.64  | 0.095    | 0.112    |
| A9         | 10 | 3.57  | 0.51  | 0.67  | 0.028    | 0.210    |
| A10        | 5  | 3.56  | 0.50  | 0.71  | 0.062    | 0.292    |

Bold indicates that the value is significant

$n$ number of snakes genotyped per locus, $Ar$ allelic richness based on min. sample size of four diploid individuals, $H_O$ observed heterozygosity, $H_E$ expected heterozygosity, $F_{ST}$ mean genetic differentiation, $F_{IS}$ heterozygote deficit within populations.

**Table 2** Genetic variation across the sampled sites of *Coronella austriaca* in Wallonia

| Sampling site | n  | Ar    | $H_O$ | $H_E$ | $F_{ST}$ | $F_{IS}$ |
|---------------|----|-------|-------|-------|----------|----------|
| W1            | 10 | 3.26  | 0.55  | 0.64  | 0.056    | 0.182    |
| W2            | 5  | 3.49  | 0.60  | 0.60  | 0.066    | 0.106    |
| W3            | 5  | 3.61  | 0.77  | 0.66  | 0.038    | -0.057   |
| W4            | 7  | 3.17  | 0.49  | 0.54  | 0.127    | 0.166    |
| W5            | 15 | 2.88  | 0.60  | 0.59  | 0.112    | 0.009    |
| W6            | 17 | 3.62  | 0.68  | 0.67  | 0.071    | 0.008    |
| W7            | 21 | 3.31  | 0.64  | 0.64  | 0.083    | 0.021    |
| W8            | 14 | 3.44  | 0.59  | 0.67  | 0.069    | 0.152    |
| W9            | 13 | 3.17  | 0.50  | 0.58  | 0.127    | 0.174    |
| W10           | 9  | 3.19  | 0.45  | 0.57  | 0.096    | 0.271    |
| W11           | 28 | 2.95  | 0.49  | 0.54  | 0.157    | 0.113    |
| W12           | 13 | 3.23  | 0.66  | 0.61  | 0.089    | -0.032   |
| W13           | 21 | 3.14  | 0.67  | 0.64  | 0.093    | -0.027   |
| W14           | 10 | 3.22  | 0.68  | 0.62  | 0.061    | -0.04    |
| W15           | 11 | 2.94  | 0.41  | 0.56  | 0.112    | 0.312    |
| W16           | 12 | 2.92  | 0.60  | 0.57  | 0.073    | -0.014   |
| W17           | 9  | 2.51  | 0.42  | 0.49  | 0.143    | 0.213    |
| W18           | 19 | 3.19  | 0.54  | 0.63  | 0.083    | 0.178    |
| W19           | 6  | 2.87  | 0.50  | 0.58  | 0.098    | 0.231    |
| W20           | 24 | 3.34  | 0.53  | 0.61  | 0.090    | 0.159    |
| W21           | 33 | 3.35  | 0.53  | 0.64  | 0.113    | 0.184    |
| W22           | 21 | 3.24  | 0.60  | 0.63  | 0.062    | 0.102    |
| W23           | 7  | 3.80  | 0.46  | 0.67  | 0.044    | 0.398    |
| W24           | 15 | 2.76  | 0.43  | 0.56  | 0.100    | 0.275    |
| W25           | 11 | 2.78  | 0.57  | 0.55  | 0.097    | 0.031    |
| W26           | 8  | 3.31  | 0.60  | 0.61  | 0.071    | 0.087    |
| W27           | 16 | 3.25  | 0.73  | 0.63  | 0.111    | -0.133   |
| W28           | 18 | 3.30  | 0.64  | 0.62  | 0.078    | -0.002   |

Bold indicates that the value is significant

$n$ number of snakes genotyped per locus, $Ar$ allelic richness based on min. sample size of four diploid individuals, $H_O$ observed heterozygosity, $H_E$ expected heterozygosity, $F_{ST}$ mean genetic differentiation, $F_{IS}$ heterozygote deficit within populations.
GENELAND always inferred three clusters in Alsace: cluster 1 comprised 72 individuals and 8 sampling sites; cluster 2 contained 17 individuals and one sampling site; and eventually cluster 3 contained 19 individuals and one sampling site (Figs. 1A, S1, Table S1).

In Wallonia, GENELAND gathered all sampling sites in four clusters: cluster 4 contained 203 individuals and corresponded to 16 sampling sites; cluster 5 contained 94 samples and 5 sampling sites; cluster 6 contained 68 individuals and 5 sampling sites; cluster 7 contained 33 samples and one sampling site (Figs. 1B, S2, Table S1).

Population genetic analysis on the inferred populations

In Alsace, we ran standard population genetic analysis based on the results of GENELAND, on the three clusters inferred. The overall $F_{ST}$ value was 0.070 ($p < 0.05$, Table S3). In cluster 1 (including 8 sampling sites), $F_{IS}$ was 0.163 ($p < 0.05$). In cluster 2 (sampling site 2), $F_{IS}$ was −0.048 ($p > 0.05$). In cluster 3 (sampling site 3), $F_{IS}$ was 0.086 ($p > 0.05$). With this grouping, the AMOVA revealed that most of the variance was explained by the differentiation within individuals (82%, $p = 0.001$).

In Wallonia, the genetic analysis based on the four clusters gathered by GENELAND indicated an overall mean $F_{ST}$ value of 0.041 ($p < 0.05$, Table S4), with respectively $F_{IS}$ values of 0.147, 0.148, 0.176 and 0.190 (all $p < 0.05$) within the different clusters. Following this grouping, 72% of the variance was explained by the differentiation within individuals and only 3% among clusters.

Isolation by distance and spatial autocorrelation

Mantel’s correlation test did not reveal any effect of IBD between the studied sites (Mantel test: $r = −0.023; p = 0.86$; Fig. 2) in Alsace, but the distance was negatively correlated with the distance at the margin of statistical significance when tested within the first cluster obtained with GENELAND (Mantel test: $r = 0.079; p = 0.080$). In Wallonia, IBD was detected at the whole scale (Mantel test: $r = 0.032; p = 0.0003$; Fig. 2), but within the clusters identified with GENELAND, a significant signal of IBD was only present in the cluster 5 (Mantel test: cluster 4: $r = −0.007; p = 0.730$; cluster 5: $r = 0.389; p = 0.032$; cluster 6: $r = 0.121; p = 0.173$).

The spatial autocorrelation between Euclidian distance and relatedness was significant for the distance classes between 0 and 2.8 km in Alsace (Fig. 3A). This indicated that smooth snakes in Alsace are more related to each other within a distance of 2.8 km. In Wallonia, this distance was greater, as a significant autocorrelation was detected up to a distance of 10 km (Fig. 3B).

Discussion

Genetic differentiation in Alsace and Wallonia

Globally, the genetic differentiation between populations in both Alsace and Wallonia is limited and mainly follows natural, geographical or historical isolation (River Meuse in Wallonia, or the southern part of the Ardennes). Indeed, as no or only weak isolation by distance was detected (opposite to the results in UK, Pernetta et al., 2011, but similarly to the results of Sztencel-Jablonka et al. 2015; see below), it...
is likely that other processes are influencing the genetic differentiation between populations, such as some landscape elements (e.g., rivers) or historical events (e.g., climate fluctuations) that we could not detect with this study.

Our results indicated that some populations are more differentiated than expected. In Alsace, the south-westernmost cluster 3 (Fig. 1A) seems to be isolated from the others, according to GENELAND, and presenting a significant pairwise $F_{ST}$ with all other sampling. Also, it has a negative $F_{IS}$ value ($-0.048$), which, though not significant, indicates a propensity to outbreeding. This result could be caused by a reduction in size of this population that conducted to some genetic drift. This sampling site is located west and north of two main highways, both landscape elements that represent recent physical barriers that could prevent gene flow. Similarly, other sampling sites included in cluster 1 separated by major highways or by large areas of crop fields also show high and significant $F_{ST}$ values (e.g., highways between pop4 and pop8; crop fields between pop4 and pop5; see Table 2). Therefore, it could be possible that populations of *Coronella austriaca* located at the southwest of Alsace are isolated due to fragmentation caused by highways. It has been shown that average sized and small species of snakes tend to avoid crossing roads (Andrews and Gibbons 2005) or are killed when trying to cross roads (Bonnet et al. 1999). Our results tend to suggest that this may be the case for *Coronella austriaca*, as highways could constitute a strong barrier to gene flow when underpasses are lacking. No strong signal of IBD has been detected, even though the two most distant sampling sites are 85 km apart in this region. By contrast, a low but significant IBD has been revealed in Poland, but at a higher
spatial scale, with sampling sites distant to up to 600 km (Sztencel-Jablonka et al. 2015). We also observed a strong differentiation between cluster 2 and cluster 1. Cluster 2 is represented by sampling site 3, which is geographically close to sampling site 10 that belongs to cluster 1. FST value between these two sampling sites is 11% although not significant (suppl. table S2), indicating a rather strong rupture in gene flow. No obvious geographical barrier has been observed in this area, all the more that both sites are separated by 2.8 km, a distance up to which gene flow should still be possible (Fig. 3A). One explanation could be an effect of small population size, that would cause drift in cluster 2, but this remain to be explored with more samples.

In Wallonia, a significant but weak (r = 0.033) signal of isolation by distance was detected, that could be related to the global structure found with GENELAND. Among the four clusters recovered in this region, three of them represent geographically well-separated regions [southern region (cluster 6); central region (cluster 4) and the edge of the central region (cluster 5)]. Even if some signal of IBD could be detected within clusters 5 and 6, it was significant only for cluster 5. Moreover, the grouping yielded by GENELAND is not based on geographic distances as sampling sites within clusters 4 and 5 are sometimes very close. This splitting is probably more related to historical reasons. For instance, cluster 6 gathered all the populations from the southern part of the Ardennes, where the species is not very common as most habitats there are cold and mainly composed of forests which is not favourable for the smooth snake. We can hypothesise the differentiation between clusters 4 and 5 is the result of a barrier effect induced by the Meuse River. Indeed, all but one (sampling site 28) sampling sites from group 5 is on the north-western part of the Meuse River. Within group 4, all sampling sites except sampling site 3 are on the shore or on the south-eastern part of this river. The Meuse River is the largest in Belgium; it probably acted as a barrier to the movement of C. austriaca. Cluster 7, that gathered individuals from the single sampling site 21, does not present particular geographic barriers with other populations of cluster 5 that could explain its genetic differentiation. Local monitoring in sampling site 21 highlighted a strong increase of individuals during the last years, with the lack of smooth snake 30 years ago (Graitson et al. 2012). We can consequently hypothesise that this population has undergone a strong founder effect with colonisation by a limited number of individuals only 2–3 generations ago, when considering a generation time of 6 to 8 years (Völkl and Käsewieter 2003), which could explain the significant FIS value. Contrary to what was observed in Alsace, the difference between clusters does not seem to be explained by a barrier effect induced by highways. Further studies would be necessary to infer which spatial features alter gene flow of the smooth snake in Wallonia.

Low genetic differentiation was unexpected, as strong differentiation was detected in several species of snakes with similar ecological requirements, even within putative interconnected habitat. For species with different life-history traits (‘sit and wait’ predators and strict capital breeders) but similar in size and shape, genetic structure was observed within three vipers: Vipera berus in Western Europe (Ursembacher et al. 2009), Vipera ursinii in Southern France (Ferchaud et al. 2011), and Sistrurus catenatus in the north of the United States and South of Canada (Gibbs et al. 1997). The genetic structure of a terrestrial elapid, Hoplocephalus bungaroides, in Australia, demonstrated weak genetic structure (Dubey et al. 2011), similar to what is observed for C. austriaca in Alsace. We can assume that if the barriers are reduced, populations remain interconnected over long distances, in part due to underestimated individual movement, but also a larger and more diffuse presence of the species in between recognised populations (see below).

**Comparison between regions**

Our study reveals a contrasting pattern of genetic structure among the same species in different studied regions, one located in the core of the range, and the other along the edge. Moreover, Pernetta et al. (2011) demonstrated that IBD is marked for smooth snake between population patches distributed in a small forest area in Southern England (highest distance between two patches <6 km; Pernetta et al., 2011), whereas in Poland, IBD measured at a large scale was significant but distance had a limited impact on genetic differentiation (Sztencel-Jablonka et al. 2015), similarly to what we observed in Wallonia. In England, isolation by distance may be the result of the low dispersal capacity of the species, rather than the fragmentation of habitat, as the authors mentioned the occurrence of suitable habitats that could be used as corridors between population patches (Pernetta et al. 2011). Moreover, higher FST values observed in Southern England probably result from a lower density. This situation is rather different from what was observed in Alsace and in Wallonia. In Alsace where the overall FST values were similar (0.075 vs. 0.078 in England), but where the sampled area was much wider (maximum distance between sampled sites ≈ 85 km in Alsace vs. <6 km in England) (Pernetta et al. 2011). In Wallonia, the FST value is higher (0.114), but over an even larger distance (maximum distance between sampled sites ≈ 125 km) and with the occurrence of at least four genetic groups. It is of note that the FST values between the three studies did not result from the exact same set of genetic markers (2/8 were similar for the three studies; 3/8 between Alsace and Wallonia, Poland, and England), but the genetic diversity, number of alleles and allelic richness are
similar between all loci, suggesting that the use of different markers would have only a limited impact on the comparison. Also, the effect of isolation by distance was significant in Southern England (r = 0.511, p < 0.05), whereas no effect was detected in Alsace and only a weak significant signal in Wallonia (Fig. 2). We expected to find a stronger effect of isolation in both Alsace and Wallonia due to the larger distances between populations if a similar genetic pattern as in England has been detected, which was not the case. This observed discrepancy obtained at a different scale should lead to further studies at the same spatial scale and with the same set of microsatellites in order to avoid artefacts due to large variation in distance between populations. However, the comparison of Pernetta et al. (2011) and this project clearly suggests that, within a species, genetic structure can strongly vary between habitats or regions. Such differences of genetic structure and diversity have been demonstrated in other groups, with more marked genetic structure and lower diversity at the edge compared to the core of the distribution (Munwes et al. 2010; Dudaniec et al. 2012; Meeus et al. 2012; Ursenbacher et al. 2015). Therefore, it would be interesting to broaden investigations on the genetic structure of populations of Coronella austriaca to other parts of its distribution limits and in similar habitats (i.e., lowlands), in Scandinavia or in Western France for example, to test if a strong genetic structure at a small scale is a common feature of edge populations.

Moreover, the studied populations in both regions are still large enough and rather widespread to avoid a strong genetic drift, as shown by the similar level of genetic diversity and limited F_{ST} values (Tables 1 and 2), contrary to the populations studied in England that are patchy and strongly associated to one type of habitat (Pernetta et al. 2011) or even in Poland where genetic structure, even though not as marked as in England, has been observed at a high geographical scale (Sztencel-Jablonka et al. 2015). Though our sampling pattern shows populations that are geographically separated (Fig. 1), our results suggest that the dispersal capacities are underrated for this species. Preliminary capture-recapture data suggest that smooth snakes are rather philopatric in Alsace (J.P. Vacher, unpublished data), which is in accordance to what is known in the literature for this species and other temperate snakes (Völkl and Käsewieter 2003; Pattishall and Cundall 2008; Pernetta et al. 2011). Still, further studies on dispersal would be necessary to assess this question (Keogh et al. 2007), as most information on movement behaviour within this species concern movement within the home range and not actual dispersal (Clobert et al. 2012).

Even though the smooth snake is known to be a species with low vagility (Völkl and Käsewieter 2003; Pernetta et al. 2011), our results suggest that dispersal might actually be underestimated, at least for some individuals (males or juveniles). We think that further studies on ecology, distribution, and population dynamics should be carried out at different positions (i.e., core vs. edge) of its range to better understand how this species uses landscape features.

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Author contributions JPV, EG, JC, and SU designed the study and wrote the manuscript. JPV did the field work in Alsace and the subsequent lab work. EG and JC did the field work in Wallonia. JPV, JC, and SU analysed the data.

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Data availability All DNA samples are deposited in the Natural History Museum Bern, Switzerland.

Declarations

Conflict of interest The authors declare that they have no conflict of interest in the scope of this study.

Consent to participate All authors approved to participate to this study.

Consent to publish All authors approved to the publication of this study.

Research involving in animal rights Permits (no reference number provided) were delivered by the Prefect of Alsace to J.-P. Vacher, and by the department of Nature and Forest from Wallonia Public Service to E. Graitson for capture and handling of Coronella austriaca. Snakes were captured and released on spot right after sampling, no other tissue/blood collection has been performed other than buccal swabbing. A permit to drive on forest tracks in the Haut-Rhin department was issued to J.-P. Vacher by the National Forest Department (Mr. Pierrat, Mulhouse, France).

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