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 eight 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 individuals share parental relationship 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 strongly contrasting with a previous study in England, suggesting sharp local variation of genetic structuring and diversification between location within the same species, probably related to the position in the distribution area and different densities.

Introduction

In the scope of the current biodiversity crisis, most studies on animal conservation focus on endangered and highly threatened species (Foden et al. 2013; Wagner et al. 2014; Howard et al. 2020; Loiseau et al. 2020). Still, getting to know how more common and widespread species react to different threats remains challenging (Kyek et al. 2017; Sánchez-Bayo and Wyckhuys 2019; Rosenberg et al. 2019). For example, comparing how populations at the core and at the edge of their distribution area manage changes induced on their environment such as habitat fragmentation, pollution, introduction of pathogens, might nonetheless be crucial in understanding how biodiversity can adapt to changes. And consequently how to adjust conservation practices that can also benefit to putative non-endangered species, or to threatened populations of wide-ranged species that occur at the edge of the range (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 a higher genetic diversity can adapt better to changes in the environment than populations with a lower genetic diversity (Desvars-Larrive et al. 2017). At the same time, populations at the edge of their range are likely to have a weaker genetic diversity, and therefore are more prone to decline (Böhme et al. 2007; Taubmann et al. 2011). In an ever and fast changing world, including common species in the conservation considerations becomes more and more important, as some of them can rapidly become endangered (Carrier and Beebee 2003; Gill et al. 2007; Studds et al. 2017; Kyek et al. 2017; Reading and Jofré 2020). Thus, it is crucial to understand the evolutionary mechanisms involved in such adaptations. 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 connexion between populations. Small vertebrates such as reptiles are particularly sensitive to such disturbances, and it has been shown that some populations of common species are particularly exposed to fragmentation, and thus can become severely threatened (Driscoll 2004; Guiller 2009).

As amphibians, non-avian reptiles face declines at a global scale (Gibbons et al. 2000). For example, in Europe, about 20% of species are listed in the threatened categories of the IUCN (Cox and Temple 2009). All the more, 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). These processes lead to isolation of populations and rupture in gene flow (Frankham et al. 2002; Dixo et al. 2009), and are often associated with a reduction of population size, genetic drift, and loss of genetic diversity (Frankham et al. 2002). Therefore, adaptation capacities of populations faced with biotic or abiotic events such as diseases, unusual climatic events, or pollution, become reduced and thus can speed up the decline process at a local level (Frankham et al. 2002). The cumulative effects of these factors can lead to extinctions of populations and, in the case of species with a rather limited range, to the extinction of species. In vertebrates, such rapid extinctions have been observed, for example within amphibians (Pounds and Crump 1994; Stuart et al. 2008; Collins and Crump 2009). Few studies have been carried out in order 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 (Guiller and Legentilhomme 2006). Moreover, a limited number of studies were conducted on the same species but in different locations, in order to determine if species can 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 could provide valuable information on the biology of the species, but also on how species behave in different environments.

Snakes are particularly sensitive to habitat fragmentation and direct destruction (Bonnet et al. 1999). Additionally, reptiles, and particularly snakes, are not often used as model species in conservation biology (Bonnet et al. 2002; Mullin and Seigel 2009). Therefore, we decided to examine a cryptic and rather poorly studied species given its wide range, 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). The knowledge on its ecology and population dynamics increases during the last decades (Völkl and Käsewieter 2003; Dick and Mebert 2017; Graitson et al. 2020). For instance, a previous genetic study carried out on *Coronella austriaca* in England has demonstrated a strong impact of the geographic distance at a local scale (Pernetta et al. 2011), suggesting rather low dispersal capacities of the species.

In this context, our study aimed at evaluating through genetic structure the magnitude of fragmentation on *Coronella austriaca* in two mainland environments with similar population densities but in different climates and 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). The main questions we tackled were to assess the genetic relations of different population patches distributed throughout Alsace and Wallonia using microsatellite markers and evaluate if, as in England, the genetic isolation is clear between proximate populations. We hypothesised that as the smooth snake seems to be a poor disperser (Völkl and Käsewiter 2003; Pernetta et al. 2011; Dick and Mebert 2017), genetic structure should be marked even between geographically close populations. Also, we expect that even if the habitats and climate are somewhat different, the general pattern of genetic differentiation should be quite similar between regions.

Material And Methods

Study area

The study area 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°56'E (Fig. 1B). Altitudes of the studied habitats were comprised between 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 whole study area in 38 different localities through buccal swabbing (Beebee, 2008; Pidancier et al., 2003). We collected 108 samples from 10 localities in Alsace (Fig. 1A) in 2009 and 2010 (Fig. 1A), and 398 samples in 28 localities in Wallonia between 2009 and 2011 (Fig. 1B). The two closest localities were separated by 2.6 km, and the two most distant ones were separated by 85 km. Snakes were found either by sight or with the use of artificial shelters (70cm x 70cm 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 enabled individual recognition on the field (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 then read with the software PEAK SCANNER v.1.0 (Applied Biosystem).

Data analysis

Each locus was first 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 from the dataset. For each retained locus, we estimated allele frequency, observed and expected heterozygosity (H_O , H_E) with GeneAEx v6.503 (Peakall and Smouse 2006), whereas allelic richness (A_R), intrapopulation structuration (F_{IS}) and population differentiation (F_{ST}) 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 gene diversity (AMOVA) to assess the molecular variance among sampling sites, among individuals and within individuals with GenAEx, again for each region separately. We evaluated population subdivision for 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 MCMC runs with K ranging from 1 to 10 for the Alsace region 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. Then, 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%. The number of pixels was set to 100 for the X axis and 350 for the Y axis for the Alsace region and respectively 350 and 350 for Wallonia, in order to avoid having two sampling sites in the same pixel. Finally, we computed posterior probability of population membership for each pixel of the spatial domain and the modal population of each individual. We then ran again standard population genetic analysis based on the number of populations inferred with GENELAND by calculating pairwise F_{ST} and F_{IS} with FSTAT, and tested the significance of the inferred structure by performing an AMOVA with GenAEX.

For each region, we tested isolation of sampling sites by distance (IBD) with a Mantel test (Mantel 1967) by confronting corrected genetic differentiation [$F_{ST}/(1-F_{ST})$] with 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 with the data included in the article of Pernetta et al. (2011). 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 genetic relatedness measured by Moran's I-statistic (Moran 1950; Sokal and

Wartemberg 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, due to an excess of homozygosity. Therefore, Ca30 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, genetic 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, the allelic richness seems a bit 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 the Alsace region, even if the observed heterozygosity is similar. However, A_R , H_O or H_E were not significantly different between regions when considering the five similar loci in both populations, even when considering locus or populations as cofactors (for all $P > 0.189$).

Table 1: Genetic variation across the sampled populations of *Coronella austriaca* in Alsace. n : number of snakes genotyped per locus; A_r : allelic richness based on min. sample size of 4 diploid individuals; H_O : observed heterozygosity; H_E : expected heterozygosity; F_{ST} : mean genetic differentiation; F_{IS} : heterozygote deficit within populations. Bold indicates that the value is significant.

Population	n	Ar	H_O	H_E	F_{ST}	F_{IS}
Pop1	6	3.35	0.54	0.56	0.100	0.044
Pop2	17	3.60	0.69	0.67	0.088	-0.048
Pop3	19	3.43	0.58	0.65	0.093	0.086
Pop4	14	3.46	0.64	0.69	0.066	0.072
Pop5	12	3.56	0.59	0.64	0.055	0.060
Pop6	6	3.45	0.54	0.64	0.080	0.156
Pop7	8	4.00	0.64	0.73	0.042	0.114
Pop8	11	3.36	0.57	0.64	0.095	0.112
Pop9	10	3.57	0.51	0.67	0.028	0.210
Pop10	5	3.56	0.50	0.71	0.062	0.292

Table 2: Genetic variation across the sampled populations of *Coronella austriaca* in Wallonia. n : number of snakes genotyped per locus; Ar : allelic richness based on min. sample size of 4 diploid individuals; H_O : observed heterozygosity; H_E : expected heterozygosity; F_{ST} : mean genetic differentiation; F_{IS} : heterozygote deficit within populations. Bold indicates that the value is significant.

Population	<i>n</i>	<i>Ar</i>	<i>H_O</i>	<i>H_E</i>	<i>F_{ST}</i>	<i>F_{IS}</i>
Pop1	10	3.26	0.55	0.64	0.056	0.182
Pop2	5	3.49	0.60	0.60	0.066	0.106
Pop3	5	3.61	0.77	0.66	0.038	-0.057
Pop4	7	3.17	0.49	0.54	0.127	0.166
Pop5	15	2.88	0.60	0.59	0.112	0.009
Pop6	17	3.62	0.68	0.67	0.071	0.008
Pop7	21	3.31	0.64	0.64	0.083	0.021
Pop8	14	3.44	0.59	0.67	0.069	0.152
Pop9	13	3.17	0.50	0.58	0.127	0.174
Pop10	9	3.19	0.45	0.57	0.096	0.271
Pop11	28	2.95	0.49	0.54	0.157	0.113
Pop12	13	3.23	0.66	0.61	0.089	-0.032
Pop13	21	3.14	0.67	0.64	0.093	-0.027
Pop14	10	3.22	0.68	0.62	0.061	-0.04
Pop15	11	2.94	0.41	0.56	0.112	0.312
Pop16	12	2.92	0.60	0.57	0.073	-0.014
Pop17	9	2.51	0.42	0.49	0.143	0.213
Pop18	19	3.19	0.54	0.63	0.083	0.178
Pop19	6	2.87	0.50	0.58	0.098	0.231
Pop20	24	3.34	0.53	0.61	0.090	0.159
Pop21	33	3.35	0.53	0.64	0.113	0.184
Pop22	21	3.24	0.60	0.63	0.062	0.102
Pop23	7	3.80	0.46	0.67	0.044	0.398
Pop24	15	2.76	0.43	0.56	0.100	0.275
Pop25	11	2.78	0.57	0.55	0.097	0.031
Pop26	8	3.31	0.60	0.61	0.071	0.087
Pop27	16	3.25	0.73	0.63	0.111	-0.133
Pop28	18	3.30	0.64	0.62	0.078	-0.002

The FSTAT analysis revealed an overall mean F_{ST} value of 0.075 in Alsace (pairwise comparisons min: -0.002; max: 0.15, Supplementary Table S1) and 0.114 in Wallonia (pairwise comparisons min: -0.044; max: 0.234; Supplementary Table S2). The AMOVA analysis revealed that only 7% ($p = 0.001$) of the variance was explained by the differentiation among populations (variation among individuals = 11%, $p = 0.001$; variation within individuals = 82%, $p = 0.001$) in the Alsace region, whereas the respective proportions were 9%, 72% and 19% (all with a $p = 0.001$) in Wallonia.

Genetic structure

GENELAND always inferred three groups in Alsace: cluster 1 comprised 72 individuals and corresponded to sampling sites 1, 4, 5, 6, 7, 8, 9, and 10; cluster 2 contained 17 individuals and corresponded to sampling site 3; and eventually cluster 3 contained 19 individuals and corresponded to sampling site 2 (Figs. 1A, S1, Table S1).

In Wallonia, GENELAND suggested five clusters as the best grouping number, but gathered all populations in only four groups: cluster 4 contained 203 individuals and corresponded to sampling sites 1–6, 10, 12–16, 18, 23–25, and 27; cluster 5 containing 94 samples and grouping sampling sites 7–9, 11, and 28; cluster 6 containing 68 individuals and corresponding to sampling sites 17, 19, 20, 22, and 26; cluster 7 containing 33 samples and only sampling site 21 (Figs. 1B, S2, Table S2).

Population genetic analysis on the inferred populations

In Alsace, we ran standard population genetic analysis based on the results of GENELAND, on the three groups inferred. The overall F_{ST} value was 0.070 ($p < 0.05$). In the first group (including 8 sampling sites), F_{IS} was 0.163 ($p < 0.05$). In the second group (sampling site 2), F_{IS} was -0.048 ($p > 0.05$). In the third group (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 population genetic analysis based on the four groups of populations gathered by GENELAND indicated an overall F_{ST} value of 0.050 ($p < 0.05$), with respectively F_{IS} values of 0.147, 0.148, 0.176 and 0.190 (all $p < 0.05$) within the different groups. Following this grouping, 72% of the variance was explained by the differentiation within individuals and only 3% among groups.

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 the Alsace region, but the distance was negatively correlated with the distance at the margin of statistical significance when tested within the first group 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 groups identified with GENEALX, a significant signal of IBD was only present in the group5 (Mantel test: group4 $r = -0.007$; $P = 0.730$; group5: $r = 0.389$; $P = 0.032$; group6: $r = 0.121$; $P = 0.173$).

The spatial autocorrelation between Euclidian distance and relatedness (measured as Moran's index) was significant for the distance classes between 0 and 2.8 km for the Alsace region. This indicated that smooth snakes in Alsace are more related to each other within a distance of 2.8 km. For Wallonia, this distance is even bigger, as a significant autocorrelation was detected up to a distance of 10 km.

Discussion

Genetic differentiation in Alsace and Wallonia

Globally, the genetic differentiation between populations in both Alsace and Wallonia is limited or follow 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; 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.

In some specific cases, populations are more differentiated than expected based on the genetic pattern observed in both regions. In Alsace, the south-westernmost group (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 of the size of this population, due to a recent isolation event, and thus conduction to some genetic drift. Indeed, this sampling site is located west and north of two main highways. These elements might represent recent physical barriers to gene flow. Similarly, other sub-populations 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 would then be possible that populations of *C. austriaca* located at the southwest of Alsace are isolated from the rest due to the 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 it should be the case for *C. austriaca*, as highways could indeed constitute a strong barrier to gene flow if no underpasses are found along large sections of such roads.

In Wallonia, a significant but weak ($r = 0.033$) signal of isolation by distance was detected. Indeed, it could be related to the global structure detected with GENELAND, with the occurrence of four clusters, three of them representing 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 realised with GENELAND is not based on distances as sampling sites of clusters 4 and 5 are sometimes very close. This splitting is probably more related to historical reasons. For instance, the group6 gathered all the populations from the southern part of the Ardennes, where the species is not very common as habitats are cold and mainly composed of forests. We can hypothesise the differentiation between clusters 4 and 5 is resulting from the occurrence of the Meuse River. Indeed, all but one (sampling site 28) sampling sites from the group5 is on the north-western part of the Meuse River. Within group4, all sampling site except sampling site 3 are on the shore or south-eastern part of this river. The Meuse River is the largest river in Belgium; it probably acted as a barrier to the movement of *C. austriaca* for several centuries. Cluster 7, that gathered individuals from the single sampling site 21, does not present particular geographic barriers with other populations of the group5 that could explain its genetic differentiation. Local monitoring in the 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 undergone a strong founder effect with the colonisation by a very limited number of individuals only 2–3 generations ago, which could explain the significant F_{IS} value. Contrary to what is observed in Alsace, the difference between the groups does not seem to be explained by a barrier effect induced by motorways, which are nevertheless present in the sampled area, but more by geographical elements. We believe that the hilly terrain in Wallonia offers more possibilities to cross the motorways through underpasses.

Such a low genetic differentiation was unexpected, as strong differentiation was detected in several species of snakes with similar ecology requirements, even within putative interconnected habitat: for example, ecologically interconnected populations of *Nerodia sipedon*, an aquatic colubrid from North America, which has a similar home range as *C. austriaca* (between 1 and 4 ha), showed a marked genetic differentiation, maybe resulting from a high degree of philopatry (Prosser et al. 1999). For species with different life-history traits ('sit and wait' predators and strict capital breeders) but similar in size and shape, a high genetic structure was also observed within three viperids: *Vipera berus* in Western Europe (Ursenbacher 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). On the other hand, the genetic structure of a terrestrial elapid, *Hoplocephalus bungaroides*, in Australia, demonstrated a low genetic structure (Dubey et al. 2011), like for *C. austriaca* in Alsace. So we can assume that, if the barriers are limited, the smooth snake keeps populations interconnected over long distances, 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 more to the core of the range, and the other to the edge of the range. Moreover, Pernetta et al. (2011) have demonstrated that isolation by distance is marked for smooth snake between population patches distributed in a small forest area in Southern England (highest distance between two patches <6km; Pernetta et al., 2011). In this case, isolation by distance might rather be the result of the low dispersal capacities 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, the density is probably lower in Southern England, conducting to a higher genetic drift and thus higher F_{ST} values. This situation is rather different from what was observed in Alsace and in Wallonia. In Alsace where the overall F_{ST} values were similar (0.075 vs. 0.078 in England), but where the sampled area was much wider (maximum distance between sampled sites $\approx$ 85 km in Alsace vs. <6 km in England) (Pernetta et al. 2011). In Wallonia, the F_{ST} value is higher (0.114), but over an even larger distance (maximum distance between sampled sites $\approx$ 125 km) and with the occurrence of at least four genetic groups. It is to note that the F_{ST} 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 or Wallonia 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 between regions have already been demonstrated in other groups, with a more marked genetic structure and lower diversity at the edge in comparison 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 investigate the genetic structure of populations of *Coronella austriaca* in other parts of its distribution limits and in similar habitats (*i.e.*, lowlands), in Scandinavia or in Western France for example, to detect if the dispersal behaviour varies in function of the positions within the distribution of the species or more due to local geographical elements.

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 (Table 1 and 2), contrary to the populations studied in England (Pernetta et al. 2011). 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 Käsewiter 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).

Smooth snakes have been found in a rather wide range of habitats and microhabitats in Alsace (Thiriet and Vacher 2010) and in Wallonia (Jacob et al. 2007; Graitson et al. 2020), such as dry grassland, heathlands within forests, peat bogs, rocky elements along roads, tracks, railways and dykes, river embankments, stone walls in vineyards, old buildings, and even in gardens. Even though the smooth snake is an elusive species and we certainly tend to underestimate population sizes, our results also suggest that dispersal might also be underestimated, at least for some individuals (males or juveniles) and in mainland Europe, even if, locally, when the density is low or at the edge of the distribution area, the diversification could be strong. We think that further studies on its ecology, distribution, and population dynamics should be carried out to better understand the use of the landscape, the importance of dispersal on the population dynamics of this species, as well as the position in the distribution area or the density on its genetic diversification.

Declarations

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Author's 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.

Availability of data and material: All DNA samples from Alsace are deposited in the BEV collection in Montpellier (#recoding in progress, accession numbers will be provided later for the publication#). The DNA samples from Wallonia are deposited in the Natural History Museum Bern (#recoding in progress, accession numbers will be provided later for the publication#).

Animal research (ethics): 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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Figures

Figure 1

Sampling sites of the different populations of *Coronella austriaca* and GENELAND assignment of individuals in modal populations in (A) Wallonia and (B) Alsace. (C) Adult *Coronella austriaca* in Alsace (photograph by J.P. Vacher). Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Figure 2

Isolation by distance (m) for *Coronella austriaca* in Alsace (black dots; $r = -0.024$; $P = 0.57$), Wallonia (white squares; $r = 0.033$; $P = 0.0003$) and Southern England (grey dots; $r = 0.511$, $P = 0.003$; data from Pernetta et al. 2011). Analyses for Alsace and Southern England were conducted with eight microsatellite markers, and with six microsatellites in Wallonia; only two of them were common between the three studies (Ca19 and Ca66).

Figure 3

spatial autocorrelation in of *Coronella austriaca* a) in Alsace; b) in Wallonia calculated with SPAGEDI. The black lines represent the 95% confidence interval and * are present for significant autocorrelation.

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